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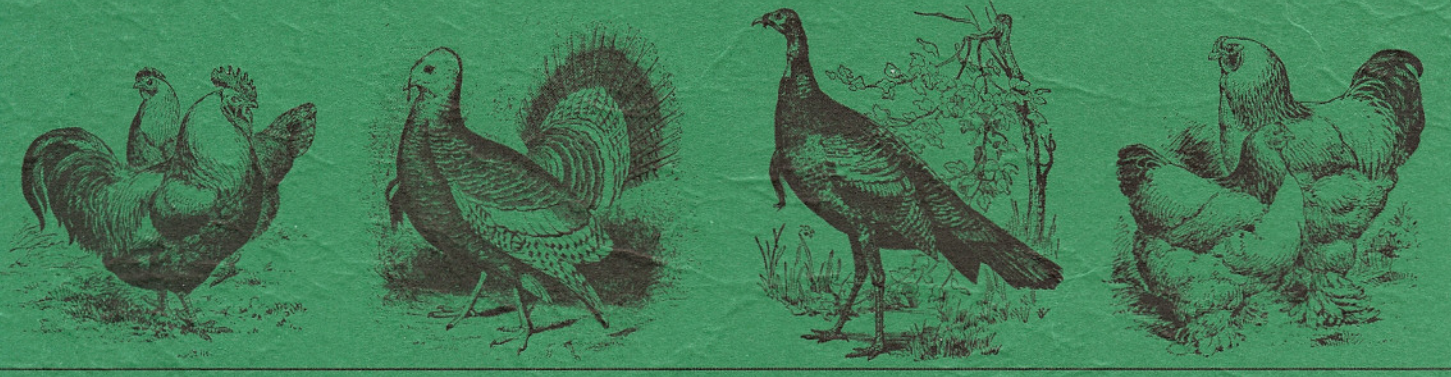

A REVERSE TRANSCRIPTION – POLYMERASE CHAIN REACTION FOR THE DETECTION OF PNEUMOVIRUSES

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Turkey rhinotracheitis (TRT) is a disease of the upper respiratory tract of turkeys and is caused by avian pneumovirus (APV). The virus was first reported in the U.S. in 1997 in Colorado (3).

Since then the virus has been reported from other states. The U.S. isolate (Colorado strain) of APV is different both antigenically and genetically from the European isolates of APV. A reverse transcription - polymerase chain reaction (RT-PCR) assay was developed previously for the detection of the Colorado strain of APV (APV-Col) using the information from the matrix (M) protein gene (1). In later studies, this assay was found to be highly specific for APV-Col since it did not detect the subgroup A and B of APVs. A RT-PCR assay that could detect all pneumoviruses would be highly desirable as a diagnostic screening tool. This study describes a RT-PCR assay for the detection of pneumoviruses from different species (avian and mammalian).

The primers were designed from the consensus sequence of the polymerase (L) protein gene of the pneumoviruses. Using a single tube RT-PCR assay, two sets of primers amplified products of predicted sizes from bovine respiratory syncytial virus (BRSV), APV-Col, human respiratory syncytial virus (HRSV), pneumonia virus of mice, APV-subtype-A (European isolate), APV-subtype-B (European isolate) and caprine pneumovirus. A multiplex RT-PCR assay was also optimized for the simultaneous amplification of the L gene and M gene to detect pneumovirus and/or APV-Col. Using primers for the APV-Col M protein gene and L protein gene in a multiplex RT-PCR assay, the predicted multiplex products were amplified from the APV-Col, however single product resulted when other pneumoviruses were assayed. With this multiplex RT-PCR assay, the APV-Col as well as other pneumoviruses could be detected in turkeys. This assay could potentially be used as a screening aid in the detection of pneumoviruses from turkeys.

REFERENCES

1. Ali, A., and, D. L. Reynolds. A Reverse Transcription -Polymerase chain reaction for the detection of avian pneumovirus (Colorado strain). Avian Dis. 43:600-603. 1999.

2. Pederson, J. C., D. L. Reynolds, and A. Ali. The sensitivity and specificity of a RT-PCR assay for the avian Pneumovirus (Colorado strain). Avian Dis. (accepted). 1999.

3. Senne, D. A., R. K. Edson, J. C. Pederson, and B. Panigrahy. Avian Pneumovirus update. In: Proc. 134th American Association of Avian Pathologists/American Veterinary Medical Association., Reno, NV. p 190. 1997.

A REVIEW OF RECENT INFECTIOUS BRONCHITIS VIRUS ISOLATIONS IN CALIFORNIA

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Since 1994 the majority of infectious bronchitis virus (IBV) isolates have been further characterized to some extent by a variety of methods. These methods include:

1. The use of monoclonal antibodies to the Arkansas 99 (Ark), Massachusetts 41 (Mass) and Connecticut (Conn) serotypes in either an indirect fluorescent antibody assay on frozen sections of the chorioallantoic membrane from inoculated embryonating eggs or a dot blot assay on the allantoic fluid from inoculated embryonating eggs.
2. Polyacrylamide gel electrophoresis (PAGE) and Western blot on the allantoic fluid from inoculated embryonating eggs (1). This method has proven useful for the identification of the California variant of IBV.
3. Reverse transcription polymerase chain reaction (RT-PCR) and restriction fragment length polymorphism (RFLP)
4. Virus neutralization in embryonating eggs, using serotype specific antisera.

Since 1994, 1396 positive isolations of IBV have made. Analysis of these results is currently under way. A striking observation is the rise and decline in the presence of the California variant, which peaked in 1995/96. In 1995, 128 of 320 (40%) isolations were Cal and in 1996, 95 of 190 (50%) isolation were Cal. Isolations of Cal have since steadily declined so that in 1999 only 4 (1.5%) isolations were made. In contrast the number of isolates that cannot be typed as Ark, Conn or Mass with monoclonal antibodies has risen dramatically from 19 (6%) in 1997, to 32 (15%) in 1993 and 184 (69%) in 1999. These changes in the IBV population in California chickens will be discussed together with other observations on variations in the results obtained.

REFERENCE

1. Case, J. T., K. W. Sverlow, and B. J. Reynolds. A novel protein polymorphism differentiates the California serotype of infectious bronchitis virus from other serotypes common to California. J. Vet. Diagn. Invest. 9:149-155. 1997.

A SEROLOGICAL EVALUATION OF A POLYVALENT VACCINE CONTAINING NDV, IB, EDS, AND HPS VIRUS IN LAYER HENS

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INTRODUCTION

The hydropericardium syndrome (HPS) appeared at end of the 80s (1), it was detected first in Pakistan and later in other countries. Since HPS appeared in 1989 in Mexico (3) several vaccines have been used to control the disease mainly in broiler and broiler breeders. Until 1995 there were no reports about the disease in commercial laying hens. In 1996 Aguirre et al. (2) reported some cases of hepatitis in layer hens in Mexico. Many birds presented signs and symptoms characteristic of inclusion body hepatitis (IBH) with the presence of hydropericardium in 40 % of the flock. However they did not find pulmonary edema or hydrothorax and the mortality was from 0.433 to 8.17 %

The objective of this work was to evaluate an experimental oil-emulsion polyvalent vaccine containing Newcastle disease virus (NDV), infectious bronchitis virus (IBV), egg drop syndrome (EDS) virus, and hydropericardium syndrome (HPS) / inclusion body hepatitis (IBH) virus for protecting layer hens against these agents.

MATERIALS AND METHODS

Virus. Newcastle disease virus La Sota strain, infectious bronchitis Massachussetts strain, and EDS PM2804 were grown in amnion-allantoic fluid (AAF) of 9-day-old SPF chicken embryos while HPS virus, SHP-IAP/92, SHP-IAP/94, and SHP-IAP/95 strains were grown on monolayers of SPF chicken embryo liver cultures. All viruses were inactivated with 0.1 % Formol after growth.

Vaccine. An oil emulsion killed vaccine containing NDV, IBV, EDS, and HPS was made. A 60/40 oil-water ratio was used.

Birds. Eight 16-week-old layer hens (Babcock B300) were vaccinated with a dose of the vaccine (0.5 ml) subcutaneously in the neck. All birds were maintained in wire cages for the experiment. Food and water were provided ad libitum. Every week all birds were bled by wing vein and antibodies to each virus were measured by HI (NDV, EDS) and micro virus neutralization test (IBV, HPS). After ten weeks all birds were sacrificed.

RESULTS AND DISCUSSION

The serological results are shown in Table 1. A good response to all agents was found with this experimental vaccine. It is considered that 5 log 2 is a minimal protective titer to NDV and the vaccine produced an average titer of 9.2 log 2. Concerning HPS, a titer of 4.3 log 2 was produced in the first week post-vaccination and good titers were maintained during the experiment. Before vaccination all birds were bled and their titers to HPS antibodies were negative. This vaccine could be a good alternative to protect against HPS in layer hens.

Table 1. Serological response in layer hens vaccinated at 16 weeks of age with an experimental polyvalent vaccine containing NDV, IBV, EDS and HPS agents. Results are expressed as 2 log geometrical mean.

REFERENCES

1. Afzal, M., R. Muneer, and G. Stern. Studies on the aetiology of hydropericardium syndrome (Angara disease)in broilers. Vet. Rec. 128:591. 1991.

2. Aguirre, E.J., R.C. Santos, V.J. Enrigue y B.J. Simenta. Reporte de casos de hepatitis con cuerpos de inclusion en aves de postura comercial. Memorias de la XXI Convencion Anual ANECA. Cacun, Q.R. Mexico, pp175-177. 1996.

3. Altamirano, R., H. Ramirez, A. Retana y J. Zurita. Hepatitis con cuerpos de inclusion y su relacion con altas mortalidades en el pollo de engorda en Mexico. Memorias XV Convencion Anual ANECA. Cancun, Q.R. Mexico. 1990.

Table 1. Serological response in layer hens vaccinated at 16 weeks of age with an experimental polyvalent vaccine containing NDV, IBV, EDS and HPS agents. Results are expressed as 2 log geometrical mean.

W.P.V.*	AGE	NDV	EDS	HPS/92	HPS/94	HPS/95
1	17	11.1	4.7	4.8	4.7	4.7
2	18	10.0	5.0	4.8	4.8	4.7
3	19	9.1	7.1	6.9	6.9	5.7
4	20	7.8	6.1	11.1	11.1	8.9
5	21	9.7	6.3	10.5	10.5	10.1
6	22	10.5	6.7	9.3	7.5	9.3
7	23	8.7	8.7	9.7	8.3	9.7
8	24	8.7	7.9	10.1	8.3	9.9
9	25	8.5	7.7	9.1	7.5	10.1
10	26	7.9	7.3	8.9	6.9	8.5

* WPV= Weeks post-vaccination

ADENOVIRUS GROUP II-LIKE INFECTION IN CHUKAR PARTRIDGES

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Seven live 5- to 6-week-old chukar partridges from a flock of 500 were examined. The flock had a history of increased mortality and ocular problems. The ocular lesions consisted of lacrimation, swollen eyelids and scab formation. Intestines contained watery contents. Two spleens were mildly enlarged and mottled white. Microscopically, there was severe blepharoconjunctivitis, enteritis associated with coccidia, and splenitis in a few birds. The thymus had lymphoid depletion in the cortex but the bursa of Fabricius was normal. Two spleens had homogenous faintly-staining bluish intranuclear inclusion bodies in mononuclear cells. Transmission electron microscopy of the spleen revealed virus particles consistent with the size and morphology of an adenovirus.

Three out of 7 birds were positive for hemorrhagic enteritis virus (HEV) by serology. All the birds were negative for Newcastle disease virus, avian influenza, *Mycoplasma gallisepticum* and *Mycoplasma synoviae* by serology. *Staphylococcus aureus* and *Escherichia coli* were isolated from the conjunctiva. The conjunctiva were negative for *Mycoplasma* sp. and intestines were negative for salmonella by culture. Three birds tested for *Chlamydia psittaci* were negative by fluorescent antibody staining.

Attempts are being made to extract and amplify viral DNA from the spleens using specific primers for group II aviadenovirus. Sequencing will be done on the PCR-amplified products and these sequences will be compared to published sequences for HEV. Also, monoclonal antibody 10B403, which is specific for the hexon of HEV, will be used to positively identify group II aviadenovirus intranuclear inclusion bodies in tissue sections.

AIR QUALITY IN COMMERCIAL BROILER HOUSES DURING THE SUMMER MONTHS

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Air quality issues are perceived by the poultry industry as being an item for concern only in the winter time and much data exists on winter-time ammonia levels. In order to more accurately assess the impact of air quality during the warm months of the year, a five-state survey of 240 commercial broiler houses was conducted representing 10 broiler complexes. Ammonia, fan time, relative humidity, and floor temperature were recorded. Flock age ranged from day 1 to day 14. The majority of the houses had used litter. Less than 5% of the houses had been cleaned out and less than 5% had applied a litter treatment on the flock evaluated. Intermittent fan times were recorded in 128 of the 240 houses. Continuous fans were being run at the time of sampling in 112 houses. Seventy-three percent of the houses running fans intermittently had ammonia levels above 20ppm, levels high enough to cause decreased weights, increased feed conversions, and respiratory damage. These readings were taken on days when the average temperature was 89 degree F. Even with maximum fan utilization, many growers were not able to provide appropriate air quality. Of the houses running fans continuously, 37% of the houses were above 20ppm of ammonia. The highest ammonia readings were normally taken within 48 hours of chick placement but had dropped below 30ppm by the time birds were 5-7 days old.
These findings suggest that short down times in the summer can be detrimental and that birds are not routinely being observed on the days of the worst air quality. Relative humidity and floor temperature were in the appropriate range in the majority of houses observed.

(A full paper will be submitted to *Avian Diseases*.)

AN EVALUATION OF MYCOPLASMA ANTIBODY ELISAS FOR MONITORING TURKEY POPULATIONS

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Mycoplasma antibody ELISA test kits have recently been developed for use on turkey samples. The performance characteristics of the tests are described in the following study. For the detection of *Mycoplasma gallisepticum* (Mg) and *Mycoplasma synoviae* (Ms) antibody titers in turkey serum, the current IDEXX chicken mycoplasma ELISA test kits were updated. Specifically, the conjugate for the test kit was modified to include the addition of goat anti-turkey immunoglobulin in addition to the existing goat anti-chicken immunoglobulin. The modified Mg and Ms ELISA test kits can be used for either chicken or turkey serum samples. For the detection of antibody to *Mycoplasma meleagridis* (Mm) antibody a new test kit was developed. The procedure for all three test kits is consistent with the standard Flockchek indirect format.

The *M. meleagridis* antibody ELISA was evaluated on hemagglutination inhibition (HI) negative and positive field turkey flocks. From a sample set of 205 positive samples, the Mm ELISA had a 96.6% correlation to HI. On the mycoplasma negative field population, the specificity was 99.5%.

In the evaluation of the chicken/turkey Mg and Ms ELISA test kits, samples from HI negative turkey flocks were tested for assessing specificity. The Mg ELISA had a specificity of 99.7%, while the Ms ELISA had a specificity of 99.6%. On a set of samples from Mg HI positive turkey flocks, there was a 95% correlation for HI titers greater than 1:20. There was 92% agreement between ELISA and HI (>1:20) for samples from Ms positive flocks. The modified M gallisepticum and M. synoviae antibody ELISAs were also validated on chicken populations to confirm that sensitivity and specificity were equivalent to the original chicken test kits.

Collectively, the data summarized in this paper support the use of the modified chicken/turkey conjugate in Mg and Ms ELISA test kits for testing either turkey or chicken samples. In addition, a *M. meleagridis* antibody test has been developed which exhibits excellent specificity and good correlation to HI.

ANTIBACTERIAL ACTIVITY, PHARMACOKINETICS AND THERAPEUTIC EFFICACY IN POULTRY OF A NEW CEPHALOSPORIN-FLUOROQUINOLONE (CEFALONE) MOLECULE

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INTRODUCTION

Cephalosporin and fluoroquinolone agents are two of the most potent and prolific antibacterial groups available. Substitution of almost any group in the carboxylic group at position 3 of the quinolone nucleus would reduce its affinity towards the bacterial topoisomerase II enzyme giving place to inactive compounds (5). However, a successful coupling of a 7-aminocephalosporanic acid (7-ACA) molecule, a precursor with no antibacterial activity, to the carboxylic group in position 3 of a 6-fluor-l ciclopropyl 7-(4-ethyl- 1 -piperazinyl)-3-quinolin carboxylic acid rendered a new molecule named, cefalone. This paper describes some of the pharmacological features as well as some evidence of the clinical efficacy of this compound in broilers (Under the Patent Cooperation Treaty, P.C.T., W.O., 95-23153).

MATERIAL AND METHODS

Minimal inhibitory concentrations were determined by the broth macro dilution method using Muller-Hinton medium at a pH of 7.2-7.4 with 60 µ/ml of ionic calcium and 30 µ/ml of ionic magnesium, and a standard inoculum of bacteria calculated in 1 x 10 12 microorganisms per tube.

Three ATCC bacteria and six clinical isolates of 4 bacteria were used, namely: *Pseudomonas aeruginosa* ATCC-25619, *Streptococcus faecalis* ATCC-10791 and Escherichia coli ATCC-10536, *Salmonella pullorum, Pasteurella multocida, Escherichia coli,* and *Haemophillus gallinarum*. Double dilutions, of cefalone (CFL) and other antibacterial drugs were prepared, reaching a lowest dilution limit of 0.0 18 µ/ml. In order to compare the antibacterial activity of cefalone, the following antibacterials were also tested: enrofloxacin (EFX), cefotaxime (CFT), and amoxicillin (AMX).

Pharmacokinetics of the cefalone in poultry was assessed in 72 broilers (Rhode Island) weighing an average of 500 g. They all received a single W (through the radial vein) or oral-bolus dose of 10 mg/kg. Later, blood was obtained through radial vein puncture from 4 birds per time, at 5, 10, 20, 40 minutes and 1, 2 ,4, 8, 12 hours. Plasma was obtained by spinning blood at 6000 rpm for 10 minutes, the plasma pipetted into small vials and frozen at -4 degree C until analyzed.

Concentrations of the cefalone in plasma were determined using the plasma diffusion method designed by Bennett et al. (1), standardized with chicken plasma and a particular strain of Escherichia coli as the test organism. The lowest limit of detection for our study was 0.01 µg /ml. Pharmacokinetic data was obtained using compartmental analysis (PKAnalyst - MicroMath

Scientific Software, Salt Lake City, Utah), and the general formula: Concentration = Ae - time + Be - time (4).

Comparative clinical efficacy was done in a field outbreak of chronic-complicated respiratory disease, within the facilities of a commercial farm in two poultry houses. Birds were 21 days old, having an accumulated mortality of 3 and 3.1%, respectively. A total of 28,000 birds were treated.

An untreated control group was regarded as redundant. Each house was divided into two groups, one treated with 10 mg/kg of enrofloxacin in the drinking water and the other group treated with cefalone at a dose of 10 mg/kg, also in the drinking water. Both treatments were given daily during three days. Mortality, initial and final weight at the slaughter house, and lesion scores in fatalities were recorded (2) on day 24. Statistical analysis included survival analysis, Mann-Whitney and Fisher exact test for lesion scores.

RESULTS AND DISCUSSION

Table 1 shows the MIC values obtained. Minimal inhibitory concentrations for cefalone were consistently the lowest of all antibacterial agents tested excepting enrofloxacin, in which case no statistical differences were obtained. Statistical differences ($P< 0.05$) were seen when comparing MIC values of the cefalone with those of cefotaxime, and amoxicillin. These results show that this particular cefalone possesses an outstanding antibacterial activity against both Gram positive and, particularly against, Gram negative bacteria. The highest MIC value was 0.068 µ/ml for S. faecalis, while the lowest MIC value was 0.017 µ/ml for E. coli. No bacterial resistance for this cefalone was detected in this limited screening.

Table 1. Mean +/- SD minimal inhibitory concentration of cefalone (CFL), enrofloxacin (EFX), cefotaxime (CFT), and amoxicillin (AMX) for ATCC and field strain bacteria (each value is the mean of 6 determinations).

The analytical method chosen, evaluates bioactive forms of a drug; thus giving a more meaningful clinical application to the pharmacokinetics observed. Pharmacokinetics of cefalone was best described by a two open compartment model after IV administration. Oral bioavailability was calculated to be 72%. Results are shown in Table 2. Figure 1 shows the mean (+/- 1 standard deviation) plasma concentrations profiles of cefalone.

Table 2. Pharmacokinetic variables of cefalone after a single oral or IV bolus dose of 10 mg/kg of the drug.

Figure 1. Plasma concentrations of cefalone after a single oral or IV bolus administration of 10 mg/kg of the drug to 500 g, 18-day-old, chicken.

Lipid-solubility may have contributed to the high apparent volume of distribution obtained, and to the outstanding apparent volume of distribution at steady state calculated. Mean values for apparent volumes of distribution can be regarded as above average, and perhaps limited by a very efficient clearance and short elimination half life. However, this fast rate of disappearance from the body must be further confirmed through metabolic and residue-elimination studies. An estimated 8 hour duration of effective antibacterial action is calculated using the oral route. Good

therapeutic efficacy was observed. In this respect, no statistical differences in clinical efficacy could be detected due to the higher survival rate registered for enrofloxacin and the cefalone (3). However, clear statistical differences ($P< 0.05$) were seen in favor of cefalone when end-meat production at 6 weeks was compared between these two groups: 1,761 g for cefalone and 1,706 g for enrofloxacin, with an approximate surplus of 770 kg of meat produced in the groups treated with cefalone.

Further studies must be carried out to elucidate the mechanism of action of this novel antibacterial agent and its bioavailability at various target tissue levels. These findings could be interpreted to indicate that this experimental antibacterial has a considerable potential in veterinary medicine.

REFERENCES

1. Bennett, J. B., J. L. Brodie, E. J. Benner, and W. M. Kirby. Simplified accurate method for antibiotic assay. Clinical specimens. Am. Soc. Microbiol, 14:170-177. 1966.

2. Kogut, M. H., G. I. Tellez, D. E. McGruder, B. M. Hargis, J. D. Williams, D. E. Corner, and J. R. DeLoach, Heterophils are decisive components in the early responses on chickens to Salmonella enteritidis infection. Microb. Pathogen 16:141-151. 1994.

3. Mausner, J. S., and S. H.. Kramer. Selected statistical topics. In: Mausner & Balm Epidemiology. An introductory text. 2nd ed. J. S. Mausner and S. H. Kramer, eds. W. B. Saunders Co., Philadelphia, PA. pp. 329-353. 1985.

4. Rowland, M., and T. N. Tozer. Variability. In: Clinical Pharmacokinetics: Concepts and Applications 2nd ed. Lea & Febiger, Philadelphia, PA. pp. 197-211. 1989.

5. Sumano, L. H. Quinolonas y Fluoroquinolonas en Medicina Veterinaria. Vet. Mex. 24:83-92. 1993.

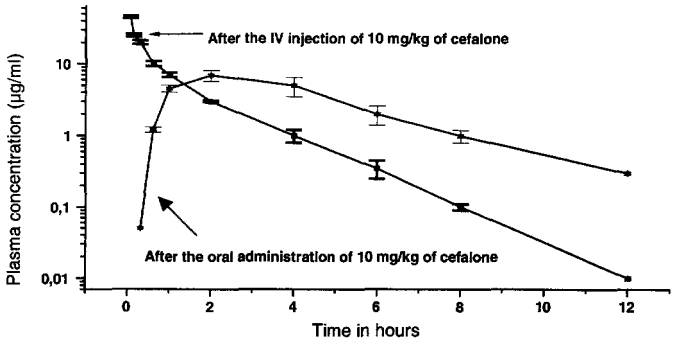

Figure 1. Plasma concentrations of cefalone after a single oral or IV bolus administration of 10 mg/kg of the drug to 500 g , 18-day-old, chicken.

Table 1. Mean ± SD minimal inhibitory concentration of cefalone (CFL), enrofloxacin (EFX), cefotaxime (CFT), and amoxicillin (AMX) for ATCC and field strain bacteria (each value is the mean of 6 determinations).

BACTERIA	EFX	CFT	AMX	CFL
Pseudomona aeruginosa ATCC-25619	0.081 ± 0.055	0.350 ± 0.122	2.916 ± 1.445	0.076 ± 0.056
Streptococcus faecalis ATCC-10791	0.175 ± 0.102	0.650 ± 0.294	0.866 ± 1.110	0.462 ± 0.572
Escherichia coli ATCC-10536	0.043 ± 0.015	1.750 ± 0.602	2.916 ± 1.445	0.068 ± 0.043
Salmonella pullorum	0.212 ± 0.099	7.033 ± 6.320	11.750 ± 5.756	0.237 ± 0.099
Pasteurella multocida	0.175 ± 0.102	7.050 ± 2.574	3.500 ± 1.314	0.150 ± 0.082
Escherichia coli	0.250 ± 0.077	1.033 ± 0.717	3.500 ± 1.314	0.112 ± 0.041
Haemophillus gallinarum	0.112 ± 0.041	2.916 ± 1.445	5.866 ± 2.890	0.275 ± 0.175

Table 2. Pharmacokinetic variables of cefalone after a single oral or IV bolus dose of 10 mg/kg of the drug.

PHARMACOKINETIC VALUE	ROUTE	
	IV	ORAL
α (hr^{-1})	47.74 ± 2.3	
β hr^{-1})	1.57 ± 0.8	1.05 ± 0.20
T½ α (hr)	0.01	
T½ β (hr)	0.44	1.5 ± 0.5
A (µg/ml)	77.4 ± 12.2	
B (µg/ml)	32.4 ± 3.8	
AUC (mg·h/ml)	36.42 ± 7.3	
AUMC (mg·h/ml)	13.39 ± 2.9	
K_{10} (hr^{-1})	22.13 ± 4.6	
K_{12} (hr^{-1})	23.79 ± 10.2	
K_{21} (hr^{-1})	3.38 ± 0.8	
Cl_B (ml/min//kg)	3.01 ± 0.8	
Vd_{AUC} (L/kg)	1.92 ± 0.5	
Vdss (L/kg)	2.45 ± 0.6	
Vdc (L/kg)	45.87 ± 22.5	
Cp_0 (µg/ml)	106.5 ± 15.2	
Cpmax (µg/ml)		8.9 ± 1.1
Tmax (hr)		2.1 ± 0.4

α = overall distribution constant; β = overall elimination constant;T½ α = distribution half life; T½ β = elimination half life; K_{10}, K_{12} and K_{21}= hybrid micro-rate constants; AUC = area under the curve by trapezoidal integral; AUMC = area under the moment curve; Cl_B = total body clearance; Vd_{AUC} = apparent volume of distribution considering the elimination constant; Vdss = apparent volume of distribution at steady state; Vdc = apparent volume of distribution of the central compartment; CP_0 = theoretical maximum concentration extrapolated to zero time; Cpmax = maximum concentration obtained after oral administration; T_{max} = time at which that concentration is achieved; T½ abs = absorption half life.

ANTIBIOTIC SENSITIVITY OF CAMPYLOBACTER ISOLATES FROM THE CENTRAL VALLEY OF CALIFORNIA

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Increased regulation of antibiotic use in food animals is currently under debate at the state, national, and worldwide levels. There is growing concern that bacterial resistance to antibiotics in food animal populations will spread to the human population. The objective of this study was to compare Minimal Inhibitory Concentrations (MIC) of Campylobacter jejuni isolates from chickens to that of beef and human isolates all collected within a small geographic area.

Thirty isolates were collected from chicken carcasses at processing or from retail markets. Thirty-one fecal isolates from healthy beef cattle and thirty-two fecal isolates from humans with diarrheal illness were also collected and tested. We developed a microbroth dilution procedure using commercially prepared micro-titer plates (Trek Diagnostics, Westlake, OH) to test the antibiotic resistance patterns of these isolates. The antibiotic panel contained Enrofloxacin, Ceftiofur, Erythromycin, Gentamycin, Tetracycline, Ampicillin, Penicillin, Amikacin, Sulfdimethoxine, and Spectinomycin. These antibiotics are approved for use in food animals. Drugs not intended for use in food animals were also tested and included Orbifloxacin and Chloramphenicol.

For each antibiotic, we compared the mean MIC value for the group of isolates from beef, chicken, and human using the Kruskal-Wallis Test. The mean MIC was significantly different between host species for Amikacin, Ampicillin, Cefliofur, Chloramphenicol, Enrofloxacin, Gentamycin, Orbifloxacin, Penicillin, Spectinomycin, Sulphadimethoxine, and Tetracycline. The mean MIC was not significantly different between host species for Erythromycin.

(A full length article will be submitted for publication in the *Journal of Clinical Microbiology*.)

AVIAN INFLUENZA DUE TO SEROVAR H7N1 IN LIGHT LAYERS IN ITALY

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ABSTRACT

At the end of March, 1999, a syndrome characterized by severe depression, anorexia, fever, and respiratory and enteric symptoms appeared suddenly in many flocks of turkeys and turkey breeders and, to a lesser extent, in chicken breeders, layers and broilers, in a very crowded area of North Italy. It was due to serotype H7N1 of avian influenza. Concerning the light layers, the mortality was variable from 1.7 to 9.5%, whereas decrease in laying was variable from 10 to 40%, with slightly lower egg quality. According to the epidemiological data, the layers seemed less sensitive to avian influenza virus than the turkey and broiler breeders.

INTRODUCTION

After the discovery of Perroncito (15) there were numerous reports on avian influenza (AI) particularly in Europe and in North America (3, 4) and the disease started to assume a consistent economic importance, mainly in turkeys and chickens 40 years ago, likely as a consequence of intensive poultry production.

Outbreaks of AI were reported in 1959 in chickens in Scotland and in 1963 and 1979 in turkeys in England: the highly pathogenic virus isolates belonged respectively to serotypes H5N1, H7N3 and H7N7 (1, 13). Isolations of different serotypes of AIV with low pathogenicity were reported in Minnesota, both in turkeys (17, 18) and in layers (11). In 1982, Alexander & Stuart (2) isolated from chickens an AIV isolate with low virulence belonging to serotype H7N1. The most serious AI epizootics in chickens broke out in Pennsylvania and neighbouring States (USA) in 1983 (8) and more recently in Mexico (20) and in Pakistan (12); the serotype involved in the first two epizootics was H5N2 and in the third H7N3, highly pathogenic.

Concerning Italy, AI affected in the last 40 years various avian species but particularly turkeys (6, 7, 10, 14, 16, 19); the isolates belonged mostly to low pathogenic serotype H6N2, less frequently to H9N2, H10N8 and only in one case H5N2 (7). The last episode of AI, due to highly pathogenic H5N2 serotype, happened in 1997 exclusively in rural flocks in Veneto region; the 8 outbreaks reported were stamped-out according to EU Council Directive 92/40, so avoiding the eventual spread of disease to commercial poultry in a very intensive production area (6). The infection in Italy, at least in turkeys, was well controlled for many years with an inactivated vaccine in oil emulsion (22).

Since March of this year, a serious AI epizootic, due to serotype H7N1 occurred in Italy in an area limited to the very crowded provinces of Verona, Brescia and Mantova, causing heavy losses, particularly in turkeys, most concentrated in this area, but also in breeders and layers (6). The purpose of the present paper is to report on some episodes of AI in light layers in cages.

CASE DESCRIPTIONS

Case 1. Four groups each of 80,000 layers, from 19 to 60 weeks old, were affected over 15 days by pronounced depression, fever, respiratory and enteric symptoms. At necropsy, catarrhal-fibrinous tracheitis, lung congestion, more or less diffuse hemorrhages, particularly in cecal tonsils, splenomegaly and hepatomegaly, and egg peritonitis were observed. Escherichia coli was frequently isolated. The hemagglutination inhibition test (HI), carried out 20 days after the beginning of signs, gave positive results for AIV serotype H7, with titres variable from 1:8 to 1:256. The morbidity was rather high, the mortality varied from 5.5 to 9.5% in different flocks, but it was higher in birds at the peak of laying. The egg production declined 10% in birds at the beginning of lay and 25% in birds at more advanced stage, with slightly lower egg quality; it did not recover to meet the standard curve.

Case 2. Two groups, each of 45,000 layers, 53 weeks old, were affected by the same symptoms and lesions of the previously reported outbreak. The HI test, carried out after 21 days, gave positive results for AIV serotype H7, with values varying from 1:32 to 1:512. The mortality was 5%, the egg production declined 10%.

Case 3. Three groups, of 18,000 layers each, from 36 to 46 weeks old, were affected with the same symptoms and lesions previously reported. The mortality was respectively 1.7-3.0-3.2% above the standard, the egg production declined respectively 615-40%. The diagnosis of AI on this farm was made both by virus isolation and the HI test (values from 1:32 to 1:512). A fourth group of pullets, 15-weeks-old, housed contiguously, and kept in good health, entered lay normally and remained serologically negative.

Case 4. One group of about 100,000 pullets, were affected at 11 weeks of age over 15 days, with the same symptoms and lesions previously reported; the recovery was quite rapid, but the mortality reached 3%. The diagnosis of AI was made by virus isolation from intestines and confirmed 20 days later by the HI test, which varied from 1:8 to 1:256.

Most of farms affected were very close to turkey farms affected a few days before by AI, with high mortality. Another three episodes of AI in layers were diagnosed serologically; the data on mortality and egg laying drops were not available.

After a remission period of two months, during the summer, the disease started again to affect mainly turkeys. Also official serological monitoring showed that apparently the layers were marginally involved with scarce or no losses, related to both mortality and egg production. But about the middle of December, outbreaks became much worse. Many outbreaks of AI took place in the same area both in turkeys and in chickens, particularly in egg layers, characterized by a sudden and very high mortality (over 50 - 80% in 57 days). In ten days at least 10 layer farms, each with over 100,000 birds, were affected. It appears that the virus may have changed to high pathogenicity and concerns for the future are very worrying.

DISCUSSION

The episodes of AI in light layers in the area affected by the recent epizootic in North Italy as determined from epidemiological surveys were relatively few in the past 9 months, in comparison with those in turkeys. The reason is not known, but it could be due to a less sensitive bird type or to efficient isolation of many layer houses. The blood monitoring, was carried out in compliance with instructions of Regional Veterinary Service. The disease was rare in pullets.

The pathological picture and the alteration of production parameters agreed with that observed elsewhere: mortality variable from 2 to 10% and the decline of egg production from 5 to 30% (8, 11, 21, 23). The serotype, H7N1, cause of the epizootic, in spite of high damage caused in turkeys, was classified as low pathogenicity, according to the aminoacid sequence of the region coding for the cleavage site and the lack of basic aminoacids (sequence PEIPKGR*GLF) and to intravenous pathogenicity injection (IPVI) in SPF 6-week-old chickens, carried out at the Central Veterinary Laboratory, Weybridge (UK). Such characteristics were maintained for 9 months.

Very recently the pathological picture of disease changed suddenly; the pathogenicity tests done on the virus isolates from some very acute outbreaks of disease, both in turkeys and in chickens, proved that the virus changed to a high pathogenicity, even if maintaining apparently the same antigenic characteristics. The IVPI was > / = 3 with deaths of 6-week-old chickens in less than 18 hours; the aminoacid sequence in the cleavage site of haemoagglutinin changed with acquisition of other basic aminoacids (PEIPKGSRVRR*GLF).

The high mortality (sometimes to 100% and also in old birds) and the reappearance of the disease after a period of subsidence, in spite of the application of emergency measures, according to the Directive CEE 92/40, makes one wonder how the pathogenicity of the isolates was defined at their first appearance. In our opinion, using only the characteristics of hemagglutinin aminoacids and the IVPI in chicken (why not in turkey?) appear rather limiting, especially with respect to the high mortality observed in the field, even if the mortality was sometimes due to bacterial complications. The poor efficacy of emergency measures, in an area with a very high concentration of farms (more than 150 million birds) where it is not possible to depopulate, suggests the benefit of rapid vaccination should not be underestimated.

REFERENCES

1. Alexander D.J., and D. Spackman. Characterization of influenza A viruses isolated from turkeys in England during March-May 1979. Avian Pathol. 10:281-293. 1981.

2. Alexander D.J. and J.C. Stuart. Isolation of an influenza A virus from domestic fowl in Great Britain". Vet. Rec. 111:416. 1982

3. Anonymous. In: Proc. 1st Int. Symp. on Avian Influenza, Beltsville MD, USA. 1981.

4. Anonymous. In: Proc. 2nd Int. Symp. on Avian Influenza, Athens GA, USA. 1986.

5. Capua I., S. Marangon, L. Selli, D.J. Alexander, D.E. Swayne, M. Dalla Pozza, E. Parenti, and F.M. Cancellotti. Outbreaks of highly pathogenic avian influenza (H5N2) in Italy during October 1997 to January 1998. Avian Pathol. 28:455-460. 1999.

6. Capua I., M. Dalla Pozza, G. Ortali, A. Moreno Martin, and A. Zanella., Low pathogenicity avian influenza (H7N1) outbreaks in Italy in different avian species". 57th Fachgesprach uber Geflugelkrankheit, Hannover, (in press). 1999.

7. D'Aprile P.N., M. Petek, C. Franciosi, and L. Sperati. L'influenza aviare: esperienza

maturata in Italia". Rivista di Avicoltura n degrees 9, 27-30. 1985.

8. Eckroade R.J., L.A. Silverman, and H.M. Acland. Avian influenza in Pennsylvania". Proc. 33rd Western Poultry Disease Conference. pp. 1-2. 1984.

9. Eckroade R.J. and L.A. Silverman-Bachin. Avian influenza in Pennsylvania: the beginning. Proc. 2nd Int. Symp. on Avian Influenza, Athens, GA, USA. pp.22-32. 1986.

10. Franciosi C., P.N. D'Aprile, D.J. Alexander, and M. Petek. Influenza A virus infections in commercial turkeys in North East Italy". Avian Pathol. 10:303-311. 1981.

11. Halvorson D.A., D. Karmakaran., and J.A. Newman. Avian influenza in caged laying chickens. Avian Dis. 24:288-294. 1980.

12. Naeem K. The avian influenza H7N3 outbreak in South Central Asia. Proc. 4th Symp. on Avian Influenza, Athens, GA, USA. pp. 31-35. 1998.

13. Pereira H.G., B. Tumova, and V.G. Law. Avian influenza A virus". Bull. Wld. H1th. Org. 32:855-860. 1965.

14. Pereira H.G., A. Rinaldi, and L. Nardelli. Antigenic variation among avian influenza A viruses". Bull. Wld. H1th. Org. 37:553-567. 1967.

15. Perroncito E., Epizoozia tifoide nei gallinacei. Annali Accademia Agricoltura, Torino; 21:87-126. 1878.

16. Petek M., Current situation of avian influenza in Italy. Proc. 1St Symp. on Avian Influenza. Beltsville, Md, USA. pp. 31-34. 1981.

17. Pomeroy B.S., Avian influenza in US 1964-1981. Proc. 1st Int. Symp. on Avian Influenza, Beltsville, Md, USA. pp. 13-16. 1982.

18. Poss P.E., D.A. Halvorson, and D. Karmakaran. Economic impact of avian influenza in domestic fowl in the United States. Proc. 1st Int. Symp. on Avian Influenza, Beltsville, Md, USA. pp. 100-111. 1982.

19. Rinaldi A., L. Nardelli, H.G. Pereira, G. Mandelli, G. Cervio, and D. Cessi. Ulteriori osservazioni sull'Influenza del tacchino in Italia". Atti VII Convegno SIPA. pp. 115-121. 1968.

20. Senne D.A., E. Rivera, B. Panigraphy, M. Frare, Y. Kawaoka, and R.G. Webster. Characterization of avian influenza H5N2 isolates recovered from chickens in Mexico. Proc. 45th
Western Poultry Diseases Conference, Cancun, Mex., 1995.

21. Tumova B., Avian influenza and paramixoviruses in Central and Eastern Europe: a review.

Proc. 2nd Int. Symp. On Avian Influenza, Athens, GA, USA. pp. 84-88. 1985.

22. Zanella A., G. Poli, and M. Bignami. Avian influenza: approaches to the control of disease with inactivated vaccine in oil emulsion". Proc. 1st Symp. on Avian Influenza, Beltsville, Md, USA. pp. 180-183. 1981.

23. Ziegler A.F., S. Davison, H. Acland, and R.J. Eckroade. Characteristics of H7N2 (nonpathogenic) avian influenza virus infections in commercial layers, in Pennsylvania 1997-98. Avian Dis. 43:142-149. 1999.

CHARACTERIZATION AND ADHERENCE TESTS OF *ORNITHOBACTERIUM RHINOTRACHEALE* ISOLATES FROM MEXICO

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A total of eight isolates were obtained from broiler (7 strains) and laying hen (1 strain) flocks showing a coryza-like respiratory disease. They were identified as gram-negative pleomorphic bacteria. Biochemical and enzymatic tests, using both commercial (API 20 NE) and laboratory-produced reagents were used. The hemagglutination activity was tested using both chicken-fresh and glutaraldehyde-fixed erythrocytes. All strains were identified by routine laboratory procedures and could be differentiated from other pathogenic bacteria for poultry. Seven strains showed an API 20 NE code of 0-2-2-0-0-0-4 as described (1). All strains showed hemagglutinating activity with glutaraldehyde-fixed but not fresh erythrocytes. Based on this fact, rabbit raised antiserum to a selected strain was elaborated, and two hemagglutination-inhibition antibody titer patterns were identified and related to geographical origin. The pathogenicity of the isolates was tested on chickens raised in our laboratory, and mild respiratory signs and lesions were observed. As adherence of bacteria to mucosal surfaces is considered the initial event in the pathogenesis of most infectious diseases due to bacteria in animals and humans, an in vitro adherence test to chicken tracheal epithelial cells was carried out as previously described (2). All strains showed ability to adhere to tracheal epithelial cells.

In conclusion, all strains were identified as *Ornithobacterium rhinotrachelae* according with biochemical and enzymatic tests as reported (1). Challenge of chickens and in vitro adherence tests demonstrated pathogenicity for poultry. Hemagglutinating activity and the hemagglutination-inhibition test can be used as an additional tool in the characterization and confirmation of suspected *O. rhinotracheale* isolates.

REFERENCES

1. Empel, P.V., H.V.D. Bosh, P. Loeffen, and P. Storm. Identification and serotyping of Ornithobacterium rhinotracheale. J. Clin. Microbiol. 35:418-421. 1997.

2. Fernandez, R. P., G.A. Garcia, G.P. Ochoa, and V.E. Soriano. Adherence of Haemophilus paragallinarum to chicken tracheal epithelial cells. Proc. 48th West. Poult. Dis. Conf., Vancouver, British Columbia, Canada. pp. 111-112. 1999.

CHICKEN INFECTIOUS ANEMIA VIRUS IN REPRODUCTIVE TRACT CELLS IN INFECTED HENS AND IN THEIR EMBRYOS

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Chicken infectious anemia virus (CIAV) was first isolated in 1979 in Japan (7). Since its first description, CIAV has been detected in chickens all over the world and is considered to be ubiquitous (1). The development of CIAV antibodies has been reported to prevent vertical transmission of the virus by both males and females. Hoop reported that infected males transmitted CLAV to 6-9% of their progeny for one week, until they developed humoral immunity to CIAV (5). Similarly, infected hens transmitted CIAV to 7-10% of their offspring until the hens developed CIAV antibodies, 8-14 dpi (4). In natural outbreaks, vertical transmission may occur from 3-9 weeks after exposure to CIAV (2, 3, 6). None of the organs, tissues, or cells involved in the vertical transmission of CIAV has previously been identified.

CIAV was detected in the reproductive tracts of chronically infected chickens from an infected specific pathogen-free flock. In hens from four flocks, ovaries were 37.5 to 71.9 percent positive, oviducts were 16.1 to 40 percent positive, and 33.3 to 60 percent of hens were positive in ovaries only. Individual cells in the theca externa and rare epithelial cells in the infundibular epithelium were positive for CIAV with in situ PCR. Testes were 10 to 60 percent positive, vas deferentia were 30 to 78.9 percent positive, and 31.6 to 60 percent of roosters were positive in the vas deferens only. No positive cells were found in testes or vas deferentia with in situ PCR. Spleens were positive in 31.6 to 60 percent of all chickens and rare cells in the white pulp were positive by in situ PCR. Embryos from the mating of chronically infected dams and a sire had CIAV positive cells in mesenchyme near the forming vertebral column. CIAV persists in the reproductive tissues far longer than previously thought, and can be vertically transmitted from chronically infected chickens.

REFERENCES

1. Bulow, V.V., and K.A. Schat. Infectious anemia. In: Diseases of Poultry, 10th ed., B. W. Calnek, H.J. Barnes, C.W. Beard, L.R. McDougald, and Y.M. Saif eds. Iowa State University Press, Ames, Iowa. pp. 739-756, 1997.

2. Chettle, N.J., R.K. Eddy, P.J. Wyeth, and S.A. Lister. An outbreak of disease due to chicken anemia agent in broiler chickens in England. Vet. Rec. 124:211-215. 1989.

3. Engstrom, B.E., and M. Luthman. Blue wing disease of chickens: Experimental infection with a
Swedish isolate of chicken anaemia agent and an avian reovirus. Avian Pathol. 17:23-32. 1984.

4. Hoop, R.K. Persistence and vertical transmission of chicken anaemia agent in experimentally infected laying hens. Avian Pathol. 21:493-501. 1992.

5. Hoop, R.K. Transmission of chicken anaemia virus with semen. Vet. Rec. 133:551-552. 1993.

6. Vielitz, E., and H. Landgraf. Anaemia-dermatitis of broilers: Field observations on its occurrence, transmission and prevention. Avian Pathol. 17:113-120. 1988.

7. Yuasa, N., T. Taniguchi, and I. Yoshida. Isolation and characteristics of an agent inducing anemia in chicks. Avian Dis. 23:366-385. 1979

COMPARISON OF GLYCOPROTEINS ENCODED BY DIFFERENT PATHOTYPES OF MAREK'S DISEASE VIRUS

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ABSTRACT

Hypervirulent (vv+) strains of Marek's disease virus (MDV) have been isolated from poultry farms throughout the US and the world. These strains are associated with high mortality, stunting, profound thymic and bursal atrophy, and with spleen tumors in layer and breeder flocks. Given our findings that some of these strains: (i) are able to co-exist with and confer horizontal transmission to vaccine viruses; (ii) induce tumors in vaccinated, immune-competent older chickens, and (iii) induce tumors comprised of immature T-cells (as determined by surface antigen expression); we believe that at least part of the phenotype of these viruses may be due to changes in surface glycoproteins. Consequently, we have examined the genes encoding the major glycoproteins encoded by MDVs of different pathotypes. We have found no consistent mutations among the gB, gC, gD, gE, gH and gI genes of vMDV (GA), vvMDV (RB1B), and vv+ MDVs (TK, MK, CD) which could be associated with a shift in pathotype. We have recently, however, found mutations in the gL-encoding genes of vv+ strains. Given the importance of gL expression in the proper surface presentation of herpesvirus gH genes, these mutations could possibly be associated with the evolution of the vv+ (hypervirulent) pathotype of MDV.

INTRODUCTION

Marek's disease is a T-cell cancer of chickens caused by a highly cell-associated herpesvirus, Marek's disease virus (MDV) (3). MDV is an acute-transforming alphaherpesvirus prevalent in commercial poultry production. MDV is shed from the skin of infected chickens and remains infectious for months to years. Currently, losses due to MD are controlled via vaccination using serotypically-related, apathogenic viruses (MDV-2 and herpesvirus of turkeys [HVT]) (14, 15). Despite control through vaccination and management practices, MDV strains have continued to evolve in virulence and several pathotypes of MDV are recognized (12, 13). The most recent pathotype of MDV (very virulent +, vv+, or hypervirulent, hv) has been isolated from broilers, layers and breeding flocks worldwide (13). This pathotype induces tumors in older, vaccinated chickens and induces stunting and profound thymic and bursal atrophy in broiler chickens (7).

To identify the molecular basis for MDV pathotypic differences, we have examined the genes encoding the major surface glycoproteins. In other viral systems, changes in pathogenicity have been associated with genetic mutations in the genes encoding the glycoproteins. The suggestive evidence for a possible role of MDV-encoded glycoproteins in pathotypic differences was threefold: (i) several hvMDVs are able to co-exist with and confer horizontal transmission to vaccine viruses; (ii) hvMDVs can induce tumors in vaccinated, immune-competent older chickens, and (iii) cell lines established using two different hvMDV strains have an immature T-cell phenotype, suggesting that pre-T-cells were accessed by these viruses during infection.

Given these observations, the hypothesis that we have developed is that the hvMDV (vv+) pathotype has evolved as a result of a change or changes in the glycoproteins encoded by the virus. To test this hypothesis, we have amplified each of the currently known glycoprotein genes encoded by MDV using the polymerase chain reaction, cloned these into plasmid vectors and determined the DNA sequence of each gene. The deduced amino acid sequence for each gene product was then compared to the published sequence.

MATERIALS AND METHODS

Cells and viruses. For propagation of MDVs, secondary chicken embryo fibroblasts were used as described previously (11). The strains of MDV used were: (1) the GA strain (a vMDV) obtained as a cellculture passaged strain from Dr. Robin W. Morgan, University of Delaware, (2) the RB1B strain (a vvMDV), initially described elsewhere (9) and obtained from Drs. John K. Rosenberger and Sandra S. Cloud, University of Delaware, and (3) three hvMDV (vv+) strains obtained from Dr. John K. Rosenberger, University of Delaware. These hvMDV strains were designated N, X and TK and were originally isolated from 37-week-old layers (from NH/ME), 8-week-old broilers (from DE/MD) and 4-week-old broilers (from NC), respectively (7).

Cell lines. To serve as sources for template DNA, cell lines established from N, X and TK strain MDV-induced tumors were used (MDCC-UD31, UD32, UD33, respectively). In addition, N, X and TK strains were reactivated from the above cell lines, passaged 2-3 times on CEF, and their DNAs were purified to also be used as PCR templates.

Flow cytometry. For surface antigen expression analysis, a FACSort flow cytometer was used (Becton-Dickinson, San Jose, CA). Antibodies to chicken lymphocyte antigens CD3, CD4, CD8, CD8B, CD45, TCR-1, TCR-2, TCR-3 and IgM were obtained commercially (Southern Biotech Assoc., Birmingham, AL). Antibodies to chicken MHC-II, Bu (pan-B-cell antigen), and K55 (pan-leukocyte antigen) were a gift from Dr. Hyun Lillehoj, USDAARS, Beltsville, MD. Cell staining was performed essentially as described (5).

Polymerase chain reaction (PCR) of Glycoprotein Genes. PCR primers were designed to span translational start and stop sites for each glycoprotein. Amplification conditions varied with primer Tm and product size. Essentially, 35 to 45 cycles of amplification were employed with extension times varying from 1 min. to 3 min. The genes were amplified, cloned and sequenced. The expected product sizes, and references are shown in Table 1. PCR products were cloned into vectors pZero (or pCR-TOPO-TA (InVitrogen, Carlsbad, CA).

DNA Sequence Analysis. Automated DNA sequencing was performed using an ABI 377 sequencer at the University of Delaware. Sequencing primers were designed for every 350 to 450 bp on each strand. DNA sequences were assembled, translated and aligned using DNAStar Seqman, EditSeq, and MegAlign software.

RESULTS

We have been able to PCR amplify and clone 9 glycoprotein genes from 5 different strains of MDV (Table 1). None of the PCR amplification products from any of the amplified genes showed any gross change in size, suggesting that there were no major deletions or insertions into any of the glycoprotein genes.

Table 1. MDV glycoprotein genes examined.

To serve as templates for PCR amplification, we were able to establish a number of cell lines from hypervirulent (hv) MDV-induced tumors (Table 2).

Table 2. HvMDV-transformed cell line immunophenotypes.

Two of these cell lines, MDCC-UD32 and UD33, were of a premature T-cell phenotype (CD3-, TCR-) and were also negative for B-cell markers (IgM-, Bu-), suggesting that these hvMDVs were able to transform premature lineages of T-cells. Given the severity of thymic and bursal atrophy associated with these strains, it seems likely that these hvMDVs may have an expanded tissue tropism in relationship to the vMDV and vvMDV pathotypes. Alternatively, these viruses may: (i) alter the expression of immune cell antigens as a consequence of transformation, or (ii) allow the expansion of aberrant cell populations as a consequence of destruction of the thymus and subsequent loss of the ability to delete these populations.

In our examination of the major glycoproteins encoded by MDV (gB, gC, gD, gH, gI, gE), all are essentially identical among the pathotypes examined (Table 3). In general, point mutations were randomly distributed among the hvMDV strains suggesting that no single mutation was a common feature of pathotypic difference. A series of conserved point mutations were noted between the gI genes of GA and RB1B strains, suggesting a possible link between these mutations and the evolution of the vvMDV strains in the early-1980s (12). The RB1B mutations in gI were also present in the hvMDV strains, supporting the notion of a continuum of viral evolution in virulence (13).

Of the minor glycoprotein genes (gK, gL, and Orf 1), we have only finished assembling the sequence of the gLs from all of the tested strains. The gLs however were the first genes noted to have mutations in one region of the protein that was consistent among all of the hvMDV srains and absent in the vMDV and vvMDV strains (Figure 1). We are currently expanding the number of hvMDV isolates in our study to see if this mutation or region of mutation is conserved among the hvMDVs. The region associated with mutation in the gL coding sequences spans the signal peptide cleavage site, reported to be at the lysine(K) at position 19 (16). Consequently, the significance of the deletion and point mutation observed in this region is that these mutations could profoundly affect the processing of the gL protein. We are currently exploring this hypothesis by immunoblotting.

Table 3. Deduced amino acid sequence identity among glycoproteins

Figure 1. Glycoprotein L (gL) amino acid sequence alignments

DISCUSSION

To date, we have found a remarkable amount of conservation among the major glycoprotein genes, i.e., glycoproteins B, C, D, E, H, I and L. Most mutations that were detected were not consistently represented among the hvMDV strains. We have, however, detected mutations in the gene encoding glycoprotein L (gL) in several hvMDV strains. The mutations that we have seen result either in a four amino acid deletion at the signal cleavage portion of the protein (strains X and TK, aa 19 to 22 deleted) or in a non-conserved point mutation adjacent to this residue (strain N, Asn to Asp, position 21) with the addition of two amino acids at the carboxy

terminus. We are currently examining the gL and gH genes of additional hvMDV strains to determine if this mutation is a common feature of these strains. Moreover, we plan to insert a copy of the mutated gL gene into a less pathogenic strain of MDV to test whether the observed mutations play a role in conferring the hvMDV pathotype. To date, this is the first report of a possible mutation associated with the increase in virulence demonstrated by this pathotype of MDV.

REFERENCES

1. Becker, Y., Y. Asher, E. Tabor, I. Davidson, and M. Malkinson. Open reading frames in a 4556 nucleotide sequence within MDV-1 BamHI-D DNA fragment: evidence for splicing of mRNA from a new viral glycoprotein gene. Virus Genes 8:55-69. 1994.

2. Brunovskis, P., and L. F. Velicer. The Marek's disease virus (MDV) unique short region: alphaherpesvirus- homologous, fowlpox virushomologous, and MDV-specific genes. Virology 206:324-38. 1995.

3. Calnek, B. W. and R. L. Witter. Marek's Disease. In: Diseases of Poultry, 10th ed. B.W. Calnek, H. John Barnes, C.W. Beard, L.R. McDougald, and Y.M. Saif eds. Iowa State University Press, Ames, IA. pp. 367-413. 1997.

4. Coussens, P. M., and L. F. Velicer. Structure and complete nucleotide sequence of the Marek's disease herpesvirus gp57-65 gene. J. Virol. 62:2373-2379. 1988.

5. Parcells, M. S., R. L. Dienglewicz, A. S. Anderson, and R. W. Morgan. Recombinant Marek's disease virus (MDV)-derived lymphoblastoid cell lines: regulation of a marker gene within the context of the MDV genome. J. Virol. 73:1362-1373. 1999.

6. Ren, D., L. F. Lee, and P. M. Coussens. Identification and characterization of Marek's disease virus genes homologous to ICP27 and glycoprotein K of herpes simplex virus-l. Virology 204:242-250. 1994.

7. Rosenberger, J. K., S. S. Cloud, and N. Olmeda-Miro. Epizootiology and adult transmission of Marek's disease. Avian Tumor Virus Symposium, Reno, NV. pp.30-32. 1997.

8. Ross, L. J., M. Sanderson, S. D. Scott, M. M. Binns, T. Doel, and B. Milne. Nucleotide sequence and characterization of the Marek's disease virus homologue of glycoprotein B of herpes simplex virus. J. Gen. Virol. 70:1789-1804. 1989.

9. Schat, K. A., B. W. Calnek, and J. Fabricant. Characterization of two highly oncogenic strains of Marek's disease virus. Avian Pathol. 11:593-605. 1982.

10. Scott, S. D., G. D. Smith, N. L. Ross, and M. M. Binns. Identification and sequence analysis of the homologues of the herpes simplex virus type 1 glycoprotein H in Marek's disease virus and the herpesvirus of turkeys. J. Gen. Virol. 74:1185-1190. 1993.

11. Solomon, J. J., R. L. Witter, K. Nazerian, and B. R. Burmester. Studies on the etiology of Marek's disease. I. Propagation of the agent in cell culture. Proc. Soc.. Exp. Biol. Med. 127:173-177. 1968.

12. Witter, R. L. Characteristics of Marek's disease viruses isolated from vaccinated commercial chicken flocks: association of viral pathotype with lymphoma frequency. Avian Dis. 27:113-132. 1983.

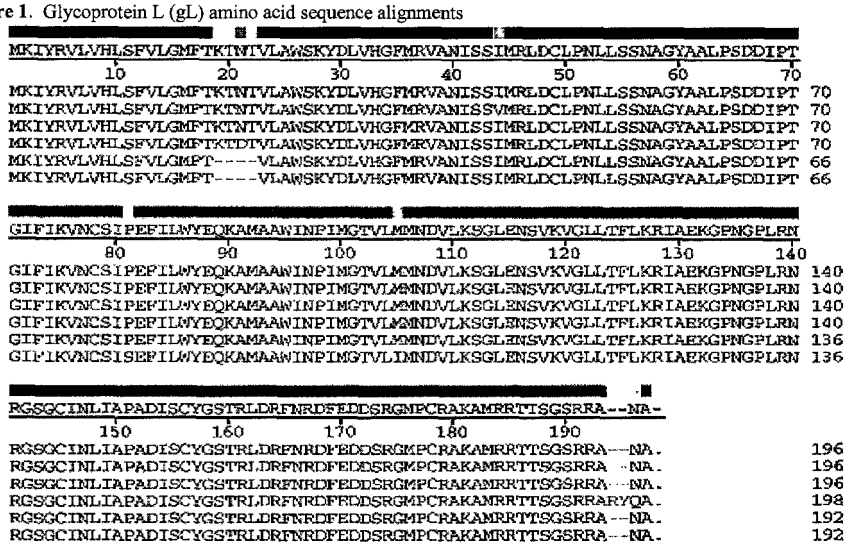
13. Witter, R. L. Increased virulence of Marek's disease virus field isolates. Avian Dis. 41:149-163. 1997.

14. Witter, R. L., J. M. Sharma, W. B. Chase, D. A. Halvorson, and V. Sivanandan. Field trials to test the efficacy of polyvalent Marek's disease vaccines in layer and broiler breeder chickens. Poult. Sci. 64:2280-2286. 1985.

15. Witter, R. L., J. M. Sharma, L. F. Lee, H. M. Opitz, and C. W. Henry. Field trials to test the efficacy of polyvalent Marek's disease vaccines in broilers. Avian Dis. 28:44-60. 1984.

16. Yoshida, S., L. F. Lee, N. Yanagida, and K. Nazerian. Identification and characterization of a Marek's disease virus gene homologous to glycoprotein L of herpes simplex virus. Virology 204:414-419. 1994.

Figure 1. Glycoprotein L (gL) amino acid sequence alignments



Con: consensus sequence
Pub: published sequence

Table 1. MDV glycoprotein genes examined.

Glycoprotein	GA strain	RB strain	N strain	X strain	TK strain	Product size	Ref.
gB	A,C,S[1]	**A,C,S**[2]	A,C,S	A,C,S	A,C,S	2615 bp	(8)
gC	**A,C,S**	A,C,S	A,C,S	A,C,S	A,C,S	1558 bp	(4)
gD	**A,C**	A,C,S	A,C	A,C,S	A,C,S	1239 bp	(2)
gE	**A,C,S**	A,C,S	A,C,S	A,C,S	A,C,S	1591 bp	(2)
gH	A,C,S	**A,C,S**	A,C,S	A,C,S	A,C,S	2457 bp	(10)
gI	**A,C,S**	A,C,S	A,C,S	A,C,S	A,C,S	1109 bp	(2)
gK	**A,C**	A,C	A,C	A,C	A,C	1161 bp	(6)
gL	**A,C,S**	A,C,S	A,C,S	A,C,S	A,C,S	673 bp	(16)
Orf1	**A,C,S**	A,C	A,C	A,C	A,C	2360 bp	(1)

[1] A = amplified, C = cloned, S = sequenced
[2] Bold letters indicate strain from which sequence was reported

Table 2. HvMDV-transformed cell line immunophenotypes.

Cell Line	Transforming MDV	Immunophenotype
MDCC-UD14[1]	RB1B	CD3+, CD4+, TCR2+, TCR3+, CD28+, K55+, MHC-II+, CD8-, CD8ß-, TCR1-, CD45-, IgM-, Bu-
MDCC-UD31[2]	N strain	CD3+, CD4+, TCR2+, TCR1, 3-, CD28+, K55+, MHC-II+, CD8-, CD8ß-, CD45-, IgM-, Bu-
MDCC-UD32[3]	X strain	CD3-, CD4-, CD8-, CD8ß-, (TCR1, 2, 3)-, CD28+, K55-, MHC-II+, CD45-, IgM-, Bu-
MDCC-UD33[4]	TK strain	CD3-, CD4-, CD8-, CD8ß-, (TCR1, 2, 3)-, CD28-, K55-, MHC-II+, CD45-, IgM-, Bu-

[1] described elsewhere (5).
[2] established from N-strain induced spleen tumor (7).
[3] established from X-strain induced spleen tumor (7).
[4] established from TK-strain-induced spleen tumor (7).

Table 3. Deduced amino acid sequence identity among glycoproteins.

Glycoprotein	GA strain	RB strain	N Strain	X strain	TK strain
gB	99.3%	99.5%	99.5%	99.4%	99.5%
gC	99.6%	100%	99.2%	99.8%	98.5%
gD	NA	98.5%	NA	98.0%	98.5%
gE	98.5%	99.8%	100%	100%	99.3%
gH	99.7%	99.5%	99.8%	100%	99.6%
gI	NA	97.5%	98.0%	98.6%	98.0%
gK	NA	NA	NA	NA	NA
gL	99.4%	100%	98.4%	97.9%	96.8%
Orf1	NA	NA	NA	NA	NA

NA – Sequences not assembled

COMPETITIVE PATHOGENICITY OF LITTER *E. COLI* TO INDUCE CELLULITIS LESIONS IN A SCRATCH MODEL EXPERIMENT

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Cellulitis in broiler chickens is caused primarily by *Escherichia coli*. In order to initiate a lesion, there must be a breach in the integument of the broiler that subsequently becomes contaminated with environmental E. coli. We have observed that within a single flock, there are typically one to three predominant DNA fingerprints of E. coli associated with the cellulitis lesions. It is unknown, however, if these E. coli DNA fingerprints are the most common in the litter or if the isolates of E. coli associated with cellulitis have a unique set of virulence factors that make them more capable of inducing lesions. The objective of this study was to design an experimental trial in which we could compare the distribution of specific E. coli DNA fingerprints in the lifter to the distribution of E. coli DNA fingerprints isolated from cellulitis lesions.

Four isolates of *E. coli* that had previously been cultured from cellulitis lesions were DNA fingerprinted. Antibiotic resistant mutants of these isolates were then distributed in a floor pen with rice hull litter. Forty 14-day-old broilers were added to the pen, and at 42 days of age, the birds were given an abdominal scratch and inoculated with a swab that had been dragged across the litter. At this time, drag swabs were taken from five areas in the pen, and 150 E. coli colonies were randomly selected and saved. Seven days post inoculation, the birds were necropsied, and all lesions were cultured. The *E. coli* colonies from the lesions and 60 randomly selected *E. coli* colonies from the litter were DNA fingerprinted.

Twenty-eight birds survived until necropsy, and 21 of these had cellulitis lesions (75%). Twelve of the lesions contained identical E. coli based on DNA fingerprinting (57%). Five additional *E. coli* DNA fingerprint patterns were found in the remaining nine lesions. Only one of the *E. coli* isolates associated with a cellulitis lesion was one of the antibiotic resistant mutants that we had inoculated onto the litter. There were at least 15 different fingerprint patterns present in the *E. coli* from the litter. The predominant E. coli DNA fingerprint cultured from the lesions was only observed in 1 of the 60 *E. coli* colonies isolated from the liter. The predominant litter isolates were not observed in the lesions. In this experiment, we assessed the competitive pathogenicity of liter *E. coli* isolates to induce cellulitis lesions. The results suggest that the isolates of *E. coli* associated with cellulitis in a flock are not necessarily the isolates present in the greatest numbers within the litter. On the contrary, it appears that specific virulence factors are required for an isolate of *E. coli* to induce a cellulitis lesion.

(A full-length article will be submitted for publication in *Avian Diseases*.)

CONSTRUCTION OF RECOMBINANT MAREK'S DISEASE VIRUSES USING A SOLUBLE-MODIFIED GREEN FLUORESCENT PROTEIN (smGFP) EXPRESSION CASSETTE

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Marek's disease is a T-cell cancer of chickens caused by a cell-associated herpesvirus called Marek's disease virus (MDV). Key aspects of MDV cell-to-cell spread, as well as the function of many MDV-encoded genes, remain unknown. To study cellular aspects of MDV cell-to-cell spread, and to study the function of MDV-encoded genes in the infection of cells in culture and in vivo, we have constructed several recombinant MDVs (1, 3, 4, 5, 6). For recombinant construction, we have previously used lacZ (3, 4, 6), lacZ/gpt (1), and GFP (S65T) (5) expression cassettes.

These expression cassettes have had drawbacks such as: (i) they required the use of toxic substrates (lacZ, lacZ/gpt), (ii) they were sensitive to fixation (GFP-S65T), or (iii) they became insoluble at high levels of expression (GFP-S65T). As a result, we are currently using a soluble-modified form of GFP (smGFP) developed for enhanced GFP analysis in plant cells (2). The smGFP is tolerated well by chicken cells and retains a high level of fluorescence after fixation with paraformaldehyde or EtOH. We report the construction of several mutant MDVs tagged with this cassette. We have also fused this cassette to several MDV-encoded genes in the context of the viral genome. These viruses will prove invaluble to the functional study of MDV-encoded genes, and will allow the tracing of MDV infection through different cell types in infected chickens.

REFERENCES

1. Anderson, A. S., M. S. Parcells, and R. W. Morgan. The glycoprotein D (US6) homolog is not essential for oncogenicity or horizontal transmission of Marek's disease virus. J. Virol. 72:2548-2553. 1998.

2. Davis, S. J., and R. D. Vierstra. Soluble, highly fluorescent variants of green fluorescent protein (GFP) for use in higher plants. Plant Mol. Biol. 36:52 1-528. 1998.

3. Parcells, M. S., A. S. Anderson, J. L. Cantello, and R. W. Morgan. Characterization of Marek's disease virus insertion and deletion mutants that lack US1 (ICP22 homolog). US10, and/or US2 and neighboring short-component open reading frames. J. Virol. 68:8239-8253. 1994.

4. Parcells, M. S., A. S. Anderson, and R. W. Morgan. Characterization of a Marek's disease virus mutant containing a lacZ insertion in the US6 (gD) homologue gene. Virus Genes 9:5-13. 1994.

5. Parcells, M. S., A. S. Anderson, and R. W. Morgan. Rapid construction of mutant Marek's disease viruses using a green fluorescent protein (GFP) expression cassette. Current

Research on Marek's Disease, Michigan State University, East Lansing, MI. pp. 284-289. 1996.

6. Parcells, M. S., A. S. Anderson, and T. W. Morgan. Retention of oncogenicity by a Marek's disease virus mutant lacking six unique short region genes. J. Virol. 69:7888-7898. 1995.

CONTROL OF SALMONELLA AND OTHER ENTEROPATHOGENS IN ANIMAL FEED

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In the United States and Europe, the risk of Salmonella contamination of poultry has been a continuous concern. Recent changes in food safety regulations and consumer awareness have prompted the animal industry to implement Hazard Analysis Critical Control Programs (HACCP) for controlling human food borne pathogens, such as Salmonella and Escherichia coli. Although, most of this emphasis has focused on the processing plant level, many companies are examining methods to reduce bacterial contamination at the farm. Today, this includes reducing environmental contamination through intensified rodent/pest control, implementing stricter biosecurity, purchasing Salmonella negative breeding stock, and feeding Salmonella negative feed.

Animal feed was first recognized as a vector for Salmonella in 1948 by researchers at the University of Kentucky Agricultural Research Station. Since the original isolation of the organism, the animal industry has attempted to reduce the incidence in feed by imposing more stringent requirements on ingredient suppliers, omitting high risk ingredients from diet formulations, and decontaminating feed by heat or chemical treatment.

Animal producers initially placed most of the emphasis on controlling Salmonella in feed on the selection of low risk feed ingredients. Early studies on the incidence of Salmonella in feed ingredients indicated that animal protein, such as meat meal, poultry meal and fishmeal were a major source of Salmonella contamination, Contamination rates for animal protein meals ranged from 33-87% (1,2,3,4). With the exception of cottonseed meal, (51.7% incidence) cereal grains and vegetable protein were considered low risk ingredients due to the lower contamination rates (<10%) (4). As a result of these early studies, some animal producers formulated their diets to exclude animal protein meal, in an attempt to reduce the incidence of Salmonella in complete feed. This approach overlooked the importance of other feed ingredients as potential sources of Salmonella contamination of the finished feed. Recent surveys conducted by the Food and Drug Administration confirm that animal protein meal is a high-risk ingredient, however, vegetable protein meals can pose the same risk (36.0 and 36.7% incidence) (5,6).

Exclusion of animal protein meals in feed formulation, based on the Salmonella risk factor, does not appear to be effective in controlling Salmonella in feed and may not be cost effective. Houston (7) calculated that exclusion of animal protein meals in feed in favor of an all-vegetable protein diet increases feed cost by $3.42/ton. Thus, efforts on controlling Salmonella in feed ingredients should be placed on purchasing feed ingredients from reputable suppliers who implement Salmonella control programs and verifying the ingredient quality upon receipt. At the feed mill, it is not feasible to assay each lot of feed ingredient received for the presence of Salmonella. The expense of sampling and laboratory analyses would be cost prohibitive and in most eases the ingredient would be utilized for feed manufacture days before the analytical results are available. A more practical approach to controlling feed ingredient quality would be to pre-approve vendors prior to purchase and monitor quality on a less frequent basis. This practice has already been implemented by the National Poultry Improvement Plans for rendered animal protein and

fishmeal. Suppliers of animal proteins utilized in primary breeder feed must be certified by the Animal Protein Products Industry (APPI) Salmonella Education/Reduction Program or the Fishmeal Inspection Program of the National Marine Fisheries Service.

Heat treatment, such as the cooking of protein meals or the pelleting/extrusion of feed, can be effective means of reducing or eliminating Salmonella in raw materials and finished feed. The efficiency of these processes is dependent on time, temperature, and the moisture content of the feed or ingredient (8) (Table 1). It should be realized that heat treatment does not provide residual activity and the ingredient or feed can become recontaminated post-process.

Table 1. Effect of time, temperature, and moisture on the reduction of Salmonella enteritidis in animal feed from Reimann (8)

Recontamination can occur through contact with residues in transport systems or storage bins, dust, domestic and wild animals, rodents, equipment, worker clothing/footwear, and transport trucks. Critical control points include the transport equipment from the press to the drier/mill, the hammer mill, the storage silo and loadout.

The first critical control for recontamination is the transport system from the press to the drier/mill. When moist warm air from the protein meal, exiting the press, contacts the cooler metal surfaces of the transport system, moisture condensation may occur. This excess moisture causes small particles of protein meal to adhere to the cover and upper portion of the transport auger. This residue may serve as a medium for Salmonella growth and recontamination of the meal. In a survey conducted by Nape (9), residue adhering to the sides and tops of conveying equipment was observed to be contaminated with Salmonella at an 18% incidence rate. Other researchers have also reported high incidences of Salmonella from samples (scrapings and dust) obtained from conveying equipment (10,11,12,13). As expected, condensation and residue problems may occur more frequently during the winter months. Periodic inspection and cleaning of the transport augers may reduce the incidence of recontamination.

The second critical control point is the hammer mill. During the grinding process, dust particles become suspended in air and, when not contained, may result in Salmonella recontamination of the finished product. This vector of recontamination is well recognized in the feed industry where the incidence of Salmonella in dust samples has been reported to be 10-50% (9,14,15). Dust generated at the hammer mill may be controlled through equipment design, such as an air scrubber. Air scrubbers also remove excess moisture generated during the grinding process.

Residue and dust problems can also occur in the transport systems exiting the grinder and during storage. Covering transport augers can reduce the potential of dust recontamination prior to storage. In facilities utilizing open bulk storage, the storage area must be separated from the grinding area. Residue build-up in transport systems and storage silos can be reduced by periodic inspection and cleaning.

Another critical point for Salmonella control is at the bagger or bulk load-out site. As feed is being packaged in bags or bulk trucks, dust is generated. When the next batch of feed is bagged or loaded, the dust from the prior batch can cross-contaminate. In the survey conducted by Nape (9), dust at the bagging operation was observed to be 10% positive for Salmonella. No data on the contamination rate of dust at bulk load-outs are available. Cross-contamination of finished product by dust and ingredient spillage can be reduced by routine cleaning. This also reduces the

risk of attracting insects, rodents and wild birds. The load-out area should be covered to minimize moisture penetration.

Other vectors of contamination at the feed mill can not be ignored. Insects, such as cockroaches and beetles, have been reported to be possible sources of Salmonella contamination. Kopanic (16) reported that five of 45 cockroaches obtained from a commercial poultry feed mill were positive for Salmonella (11.1% incidence). Cockroaches and beetles are considered mechanical vectors of Salmonella contamination (9,15). Particles of meal or dust adhere to their bodies that can recontaminate meal. At the farm level, rodents have been identified as sources of Salmonella contamination of feed (17,18).

Chemical preservatives, such as organic acids or buffered organic acids, have been used for several decades as mold inhibitors. Research has indicated that many of these compounds exhibit bactericidal activity (19,20,21). Organic acids, which have been evaluated for their effectiveness in eliminating Salmonella, include formic, acetic, propionic and lactic acid. Vanderwal (21) published a comparison of the effectiveness of each acid. In this study, it was observed that effectiveness of the acid was dependent on treatment dose and exposure time (Table 2). Zaldivar (22) reported similar findings with commercial products that were composed of mixtures of these acids. These included buffered propionic acid, a mixture of buffered propionic, acetic, benzoic and sorbic acid, a mixture of formic and propionic acid, and a mixture of formaldehyde and propionic acid (Termin-8(R)). The effectiveness of these commercial products was also dependent on dose and exposure time. The propionic acid based products required a minimum of 1-7 days (Table 3). Termin-8 was effective at 3 kg/ton after just one hour, while the formic: propionic mixture required a dose level of 10 kg/ton to be effective in one hour.

Table 2. Level of organic acids required for a 90% reduction of enterobacteriaceae (including Salmonella) in feed from Vanderwal(21)

Table 3. Time required for organic acids to eliminate Salmonella senftenberg in fishmeal from Zaldivar (22)

Additional research was conducted on the ability of these commercial products to prevent recontamination (provide residual activity). It was observed that greater than 10kg/ton of propionic acid (or mixture of organic acids) was required to prevent recontamination of protein meal by Salmonella (<1000 colonies/gram). The effective level of propionic acid required to prevent recontamination differs from the level reported by Rouse (23). This difference in effective dose may be due to the media (non-sterile versus sterile, feed versus fishmeal) or the Salmonella serotype. Jones (24) observed that different serotypes required different levels of bactericides.

In Zaldivar's initial research, the residual activity of Termin-8 was not determined. This study was later reported at the 1992 International Association of Fishmeal Manufacturers Annual Meeting in Hamburg, Germany (25) (Table 4). Termin-8 was found to be effective in preventing recontamination of fishmeal at 2 kg/ton when challenged with 1000 colonies of S. senflenberg/gram.

Recontamination studies have been conducted with other serotypes of Salmonella and similar results obtained (Richardson, unpublished data).

Table 4. Recontamination of Termin-8 treated fishmeal by Salmonella senftenberg from Kaiser (25)

Field data on Termin-8 was presented at the 1998 APPI meeting in Irving, TX. Under field conditions, the product was shown to be effective (Table 5 and 6). Treatment level and method of application were determined to influence effectiveness. Termin-8 has been approved for use in Europe to treat Salmonella contaminated protein meals since 1992 and is now considered a technological processing aid for feeds and feed ingredients under a 1998 EC Directive. In the United States, Termin-8 was approved in 1996 to maintain complete poultry feed Salmonella negative in 1996. This approval was expanded to all animal feeds and ingredients in 1998.

Table 5. Incidence of Salmonella in poultry feeds treated with Termin-8 from APPI meeting in Texas, 1998.

Table 6. Incidence of Salmonella in animal protein meal treated with Termin-8 from APPI meeting in Texas, 1998.

It should be realized that Salmonella is only one of the microbial contaminants in animal feed. Feed can contain other gram-negative bacteria, such as E. coli, Clostridia, Pseudomonas and Pasteurella, as well as gram-positive bacteria (i.e. Streptococcus, and Staphylococcus. In most instances these bacteria occur in feed at higher levels and greater frequency than Salmonella. The implementation of a HACCP program designed to control Salmonella will also have a significant impact on the levels of bacteria and molds found in feed.

REFERENCES

1. Morris, G. K. et al. A study of the dissemination of Salmonellosis in a commercial broiler chicken operation. Am. S. Vet. Res. 30(8):1413-1421. 1969.

2. Mackenzie, M. A. and B. S. Bains. Dissemination of Salmonella serotypes from raw feeds ingredients to chicken carcasses. Poultry Sci. 57:957-960. 1976.

3. Shrimpton, D. H. The Salmonella of Britain. Milling Flour and Feed (Jan.).p. 16-17. 1989.

4. Smith, P. Salmonella in proteins. In: Proc. International Association of Fishmeal Manufacturers Annual Conference, Reykjavik, Iceland. 87:12-21. 1990.

5. McChesney, D. G. FDA survey results: Salmonella contamination of finished feed and the primary meal ingredient. 99th Annual Meeting of the US Animal Health Association. Grand Rapids,
Michigan (presentation). 1995.

6. McChesney, D. G. FDA survey results on Salmonella contamination of animal and vegetable
protein products. 98th Annual Meeting of the US Animal Health Association. Reno, Nevada

(presentation). 1994.

7. Houston, J. E., et al. Georgia Agricultural Experiment Stations, Research Bulletin: Number 409. 1992.

8. Reimann, H. Report of the Feed Safety Committee. In: Proc. 100th Annual Meeting of the US Animal Health Association. Little Rock, Arkansas. pp. 178-179. 1996.

9. Nape, W. F. 72nd Annual Meeting of the U.S. Livestock Sanitary Assoc., New Orleans, LA. P. 1. 1968.

10. Malmqvist, M., et al. Acta Vet. Scand. 32:21. 1995

11. Eld, K., et al. Acta Vet. Scand. 32:261. 1991.

12. Martensson, L., et al. Nord Vet.-Med, 36:371. 1984.

13. Sandstedt, K., et al. Nord Vet.-Med, 32:57. 1980.

14. Van Schothorst, M. and J. Oosteram. Anton. Van Leeuwenhoek, 50:1. 1984.

15. Jones, F., et al. In: Colonization Control of Human Bacterial Enteropathogens in Poultry, 3. 1991.

16. Kopanic, R. J., et al. J. of Food Prot. 57:125. 1994.

17. Henzler, D. J., et al. Avian Dis. 36:625. 1992.

18. Davies, R. H., and C. Wray. Vet. Rec. 137:137. 1995.

19. Westerfield, B. L. et al. Effect of a chemical additive on Salmonella in poultry feed and host birds. Poultry Sci. 49:1319-1323. 1970.

20. Hinton, M. A., et al. Vet. Rec. 116:502. 1985.

21. Vanderwal, P. Salmonella control of feedstuffs by pelleting or acid control. J. World Poultry Sci. 35:70-78. 1979.

22. Zaldivar, J. Use of chemical products in sterilizing Salmonella in fishmeal. In: Proc. International Association of Fishmeal Manufacturers Annual Conference, Reykjavik, Iceland 87:22-34. 1990.

23. Rouse, J. et al. Effect of a chemical treatment of poultry feed on survival of Salmonella. Poultry Sci. 67:1225-1228. 1988.

24. Jones, F. T., and S.C. Ricke, Poultry Sci., 74 (suppl):50. 1995.

25. Kaiser, S. The use of Salmex in the control of Salmonella in fishmeal. International Association of Fishmeal Manufacturers Research Report 4. 1992.

Table 1. Effect of time, temperature, and moisture on the reduction of Salmonella enteritidis in animal feed from Reimann (8)

TEMPERATURE (°F)	TIME (SECONDS)	PERCENT REDUCTION AT DIFFERENT MOISTURE LEVELS		
		5%	10%	15%
160	20	68.28	83.44	90.06
	40	73.50	86.35	97.43
	80	83.57	90.80	99.70
170	20	87.36	92.36	98.24
	40	80.93	96.91	98.91
	80	91.61	93.49	99.73
180	20	76.92	98.09	99.80
	40	89.14	99.02	99.99
	80	91.62	99.19	99.98

Table 2. Level of organic acids required for a 90% reduction of enterobacteriaceae (including Salmonella) in feed from Vanderwal (21)

ORGANIC ACID	DAYS REQUIRED FOR 90% REDUCTION AT DIFFERENT TREATMENT LEVELS			
	5 KG/TON	8 KG/TON	10 KG/TON	12 KG/TON
FORMIC	12	4	1.5	0.8
ACETIC	>35	>35	16	12
PROPIONIC	>35	>35	14	8
LACTIC	>35	35	17	10

Table 3. Time required for organic acids to eliminate Salmonella senftenberg in fishmeal from Zaldivar (22)

TREATMENT	SALMONELLA (ABSENCE OR PRESENCE) AT DIFFERENT TIMES AFTER TREATMENT				
	1 HR	24 HRS	48 HRS	72 HRS	168 HRS
CONTROL	+	+	+	+	+
PROPIONIC (pH 7) 4 kg/ton	+	+	+	+	+
PROPIONIC (pH 7) 6 kg/ton	+	+	+	+	-
ORGANIC ACID MIX(pH 6) 10 kg/ton	+	+	+	+	+
ORGANIC ACID MIX(pH 6) 20 kg/ton	+	-	-	-	-
FORMIC: PROPIONIC MIX 10 kg/ton	-	-	-	-	-
FORMALDEHYDE:PROPIONIC MIX 2 kg/ton	+	+	-	-	-
FORMALDEHYDE:PROPIONIC MIX 3 kg/ton	-	-	-	-	-

Table 4. Recontamination of Termin-8 treated fishmeal by Salmonella senftenberg from Kaiser (25)

TREATMENT	SALMONELLA (ABSENCE OR PRESENCE) AT DIFFERENT DAYS AFTER TREATMENT						
	1	7	15	21	30	60	90
CONTROL	+	+	+	+	+	+	+
TERMIN-8 - 1 KG/TON	+	+	+	+	+	+	+
TERMIN-8 - 2 KG/TON	-	-	-	-	-	-	-

Table 5. Incidence of Salmonella in poultry feeds treated with Termin-8 from APPI meeting in Texas, 1998.

LOCATION	NO. OF LOCATIONS	SAMPLES ASSAYED	POSITIVE SAMPLES
MILL	24	759	0
FARM	6	177	1[a]

[a] Sample of poultry feed found to contain less than 1.6 kg/ton of Termin-8.

Table 6. Incidence of Salmonella in animal protein meal treated with Termin-8 from APPI meeting in Texas, 1998.

YEAR	TONS TREATED	SAMPLES ASSAYED	POSITIVE SAMPLES
1992	4800	240	0
1993	2400	120	0
1994	3700	185	0
1995	6300	315	0
1996	7200	360	0
1997	2000	100	0

DETECTION OF AEROSOLIZED FLUORESCENT MICROSPHERES IN THE LUNG PARENCHYMA AND AIR SACS OF PIGEONS (*Columbia livia domestica*)

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The objective of this study was to examine the feasibility of utilizing aerosolized fluorescent microspheres to examine particle distribution in the respiratory tract of birds following aerosol exposure. Five adult domestic pigeons (*Columbia livia domestica*) were utilized for this study. The birds were obtained from a local commercial squab producer and were found to be in good health and body condition. This study utilized the previously described technique for aerosolizing a monodisperse population of commercially available latex beads (1). The microsphere suspension was prepared utilizing 3 micron yellow-green Fluoresbrite™ carboxylate microspheres (Polysciences Inc., Warrington, PA) which were obtained in a 2.5% solids suspension. All microspheres were coated with albumin prior to aerosolization to reduce the loss of microspheres from the lung parenchyma and air sacs during fixation and processing of tissues. The latex bead solution was aerosolized utilizing a compressed air nebulizer (Acorn II Nebulizer System, Model No. 124015, Marquest Medical Products, Inc., Englewood, CO) set at 30 p.s.i. The aerosol was then passed through a heated discharging column to vaporize water from microspheres and reduce static charge and a diffusion drier to remove water vapor. For aerosol exposure purposes, the birds were anesthetized with a ketamine/xylazine combination, intubated, and placed on positive pressure ventilation utilizing a Mark 7 respirator (Byrd Corporation, Palm Springs, CA). The birds were exposed to the aerosol for 30 min. Immediately following aerosol exposure, the cutaneous ulnar veins were catheterized utilizing 26 gauge catheters, 2,000 units of heparin were administered intravenously, then the birds were humanely euthanized with an intravenous overdose of pentobarbital. Immediately following euthanasia, approximately 500 ml of 0.9% sodium chloride was intravenously infused utilizing a peristaltic perfusion pump at a flow rate of approximately 90 ml per mm. The avian carcasses were then fixed utilizing intravenous infusion of Karnofsky's fixative at a pH of 7.4 and 340 mOsm. The carcasses were stored at 4 degrees C for 24 hours then the air sacs and lung parenchyma were carefully dissected out. Initial examination of the dissected air sacs and ostia was performed using an Leica MZ12 dissecting microscope (Heerbrugg, Switzerland) with a fluorescent module. Confocal imaging was then performed utilizing a Bio-Rad 1024 confocal microscope with a krypton argon laser (Watford, England). Examination of the air sacs was confined to the cranial and caudal thoracic and abdominal air sacs. This study revealed that positive pressure ventilation resulted in even distribution of fluorescent beads in the cranial and caudal thoracic and abdominal air sacs of the five birds. Examination with dissecting and confocal microscopes allowed visualization of the beads in all of the airs sacs. The beads were in highest concentration in the region of the ostia for all of the examined air sacs. The results from this study allow for a better understanding of particle deposition following positive pressure ventilation and aerosol exposure in birds. Ultimately, we hope to be able to utilize this technique to evaluate aerosol/nebulization regimes for treating respiratory disease in avian species.

REFERENCE

1. Pinkerton, K., J. Gallen, R. Mercer, V. Wong, C. Plopper, and B. Tarkington. Aerosolized fluorescent microspheres detected in the lung using confocal scanning laser microscopy. Microsc. Res. Techniq. 26:437-443. 1993.

DIAGNOSTIC CHALLENGES OF ALV-J

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Primary breeders have spent millions and millions of dollars in an effort to reduce/eradicate serotype J Avian Leukosis Virus (ALV). The biggest challenge being faced in addressing this problem is the lack of a single, rapid, cost effective test to accurately diagnose birds carrying exogenous ALVs. Laboratory methods used for diagnosis include direct enzyme linked immunosorbent assay (Elisa), virus isolation (VI), polymerase chain reaction (PCR), and antibody testing. No method is perfect, and no single method can make a 100% accurate diagnosis. In order to accurately define the status of a flock, one must use multiple tests and on multiple occasions.

Direct Elisa has the advantage of being a very inexpensive and quick test that can be applied to a variety of samples. Samples that have been used with some degree of success include albumen, vaginal swabs, meconium, and cloacal swabs. In meat-type chickens, feather pulp and serum have not proven to be useful samples because of a very high level of false positives from endogenous virus.

There are several drawbacks to direct Elisa. The most important drawback is that results of the test are often misinterpreted. Because direct Elisa detects one protein of the virus, glycoprotein 27 (gp27), a positive test only indicates that gp27 is present. GP27 is a common protein product of both exogenous and endogenous viruses and a positive result from direct Elisa in no way indicates the source of the protein. Thus, a certain number of false positive results are expected when testing for the presence of exogenous virus.

Nevertheless, Elisa has been widely used. This has caused much controversy because virtually all lines of chickens will test positive to some degree on direct Elisa. This does not always mean that the birds are infected with J or any other exogenous virus. If direct Elisa is to be used as the diagnostic test, the flock must be tested on multiple occasions. A true exogenous infection will show an increasing prevalence of gp27 positive birds over time while background from endogenous virus should remain relatively stable (usually at a low percentage).

Recent reports of multiple copies of "J-like" endogenous viruses found in virtually all lines of chickens (1,2), including specific pathogen free, have shed some light on the confusing results from PCR and antibody testing. Commercial antibody test kits are being marketed as anti-ALV-J antibody kits. These kits are designed to detect antibodies to another viral envelope protein, glycoprotein 85 (gp85). If ALV-J were the only source of gp85, the nomenclature of anti-ALV-J antibody kit would be appropriate. Since we now know that ALV-J is not the only source of gp85, the same problem exists for antibody testing as exists for direct Elisa; a positive result only means that the immune system was exposed to gp85 at some time. It says nothing about the source of the gp85. Commercial manufacturers of kits for detection of antibodies against gp85 now acknowledge that a positive antibody result in 10% of the samples is considered to be normal background. As with direct Elisa, the appropriate use of this test is as a screening tool applied to populations of birds sampled over time and should not be used to determine the status of individual birds.

The gold standard of tests, and the test that is most sensitive, still continues to be virus isolation. As with all the tests, there are disadvantages. For virus isolation, the biggest

disadvantages are cost and turnaround time. Even under the best conditions, the time from taking a sample until results are reported is usually a minimum of 10 days. In the world of viruses moving around in a flock of chickens, this can potentially be a long time. This fact has important implications when designing an eradication program.

One often-overlooked advantage of virus isolation is that it is not specific for J virus. The test is done on a specific type of cell from a line of chickens developed at the Avian Disease and Oncology Laboratory. These cells, called C bar E (C/E), allow exogenous ALV's to grow, but do not allow endogenous viruses to grow. Antigen capture Elisa is the technique used to diagnose whether viral growth has occurred. A positive result when C/E cells are used means an exogenous ALV is present. It does not tell you whether this is A, B, J, or any other avian leukosis virus that might affect chickens. This means that, if virus isolation is used as the basis for the eradication program, the program is directed at not only J virus, but also all exogenous viruses.

Most people believe that primary breeders have been working hard on eradication of exclusively J virus when, actually, they have been working on eradication of all exogenous avian leukosis viruses. If it is desirable to confirm the presence of ALV-J, a PCR test can be used in virus isolation positive samples.

There are still challenges ahead. Given aggressive eradication programs using all the tools available and comprehensive testing schemes, it is conceivable that exogenous ALV's could eventually be eliminated. Scientists need to continue to develop better and more reliable diagnostic techniques. Ideally, a quick and reliable test that could differentiate between exogenous and endogenous virus can be developed. The war is not over. No one is sure what freedom really is and how to get there. Are birds that are virus negative on multiple tests using multiple techniques really free? As we head into the new millennium, we should learn the answer.

REFERENCES

1. Benson, S. J., B. L. Ruid, A. M. Fadly, and K. F. Conklin. The unique envelope gene of subgroup J avian leukosis virus derives from ev/J proviruses, a novel family of avian endogenous viruses. J. Virol. 72:10157-10164. 1999.

2. Ruis, B. L., S. J. Benson, and K. F. Conklin. Genome structure and expression of the ev/J family of avian endogenous viruses. J. Virol. 73:5345-5355. 1999.

DIFFERENCES IN PATHOGENICITY OF ISOLATES USED IN A MODEL FOR GANGRENOUS DERMATITIS

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Gangrenous dermatitis is a disease of chickens and turkeys that has been associated with a wide variety of bacteria including, *Clostridium perfringens*, *C. septicum*, *C. novyi*, *C. sordellii* and *Staphylococcus aureus* (1). The disease, although never disappearing from commercial poultry, became less frequent in many poultry producing areas of the United States. Recently, gangrenous dermatitis has re-emerged and now ranks as the second most common skin related disease, behind cellulitis. The disease most commonly occurs in broilers from 4 to 8 weeks of age, but has also been reported in commercial layers, broiler breeders and turkeys. Birds affected by the disease are characterized by pronounced depression, anorexia and incoordination. Morbidity is generally high and mortality can range from low to very high (2). Lesions generally are very dark and moist and can occur in many areas, including the wings, abdomen, and legs. A unique feature of the disease observed in some cases is the presence of air in the subcutis, which when present causes a crackling sound when the skin is rubbed. Underlying musculature is often necrotic, congested and hemorrhagic. Affected birds rapidly deteriorate after death and when opened are frequently characterized by a foul stench due to anaerobic gas production and fatty acid metabolism.

Many investigators have reported a correlation between immune suppression and gangrenous dermatitis. Immunosuppressive agents, such as infectious bursal disease virus (IBDV), chick anemia agent, reticuloendotheliosis virus and inclusion body hepatitis virus have all been associated with outbreaks of the disease (1).

The present preliminary study was designed to test the pathogenicity of different *Staphylococcus aureus* and *Clostridium septicum* isolates and isolate combinations in birds that had been purposely immunosuppressed by the introduction of IBDV vaccination at an inappropriate time (14 days). The purpose of the experiment was to facilitate the design of a reproducible model for gangrenous dermatitis, so that pharmaceuticals and vaccine regimens can be tested.

MATERIALS AND METHODS

Bacteria. The isolates used in this experiment had been previously isolated from commercial cases of gangrenous dermatitis in broiler chickens. Anaerobic bacteria were collected, purified, identified by cellular fatty acid analysis using gas chromatography (Hewlett Packard 6890 series GC equipped with Sherlock Microbial Identification Software, MIDI, Inc.) and stored in cryoprotectant media at -80 degree C until needed. Two days prior to challenge, the appropriate isolates were thawed, used to inoculate pre-reduced Brain Heart Infusion Broth (Difco) and grown up anaerobically, (5%CO2, 5% H, 90% N) overnight (37 degree C) in an anaerobic chamber (Bactron IV, Sheldon Manufacturing, Inc.) and verified for purity. The procedures were repeated a second time and the resulting culture was diluted 1:10 with pre-

reduced sterile saline. Aerobic bacteria were treated in much the same manner except that after storage at -80 degree C, the isolates were grown aerobically.

Broiler chickens. Newly hatched broiler chicks were obtained from a commercial hatchery, placed in battery cages and provided food and water ad libitum. The specific chicks were chosen on the basis of the breeding flock being near completion of theft egg production cycle and having the lowest available titers for IBDV. At 14 days of age the chicks were water vaccinated with S doses of BURSA-BLEN M (R) (Select Laboratories, Inc.). Birds were then raised to four weeks of age and challenged with a combination subcutaneous and intra-muscular injection (1 ml total = 0.5m1 sub-Q, 0.5 ml IM) of an overnight culture of either single or multiple isolate combinations of bacteria (Table 1), so that each bird received a total of approximately 1 x l0 7 cfu C. septicum or 1 x 10 9 cfu S. aureus. Birds were kept for one week and surviving birds were euthanized and scored for the presence of gangrenous dermatitis or other lesions.

Table 1. Bacterial isolates and isolate combination used for challenging broiler chickens in a gangrenous dermatitis model.

RESULTS AND DISCUSSION

Table 1 summarizes the bacterial isolates and used for challenging the broilers. Lesions were not produced by *Staphylococcus aureus*, isolate L101-l either alone (Treatment 8) or in combination with *Clostridium septicum*, isolates 5-2r or 2-4r (Treatments 1, 13). Lesions were also not produced in birds injected with *Clostridium septicum* isolate 5-2r (Treatment 4) or in the uninoculated control birds. The highest frequency of lesion production was in birds injected in Treatments 3 (*S. aureus* isolate 1158), 6 (*C. septicum* isolate 2-4r + *S. aureus* isolate Giambrone), 7 (*C. septicum* isolate 2-4r + *C. septicum* isolate 1158), 9 (*S. aureus* isolate Giambrone), 11 (*S. aureus* isolate Giambrone + *C. septicum* isolate 3-4x), 12 (*S. aureus* isolate 1158 + *C. septicum* isolate 5-2r), 14 (*S. aureus* isolate Giambrone + *C. septicum* isolate 5-2r), 15 (*S. aureus* isolate 1158 + *C. septicum* isolate 3-4x). Mean lesion score severity results of the experiment are given in Figure 1.

Figure 1. Mean gangrenous dermatitis lesion score of broiler chickens injected with various bacterial isolates and isolate combinations.

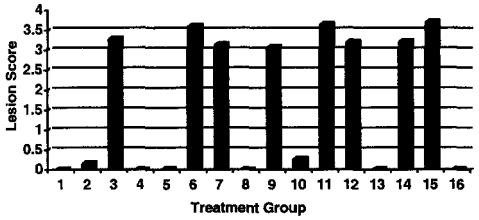
Severity of gangrenous dermatitis lesions was closely correlated with the frequency of lesions being produced. In other words, isolates or isolate combinations that caused gangrenous dermatitis most frequently were also isolates or isolate combinations that produced the most severe lesions. Those isolates or isolate combinations that produced gangrenous dermatitis less frequently were those that produced the least severe lesions. It is not clear, within this experimental design (IBDV titers not measured, bursae not weighed or assessed for atrophy, etc.) what effect individual differences in the immune status of the birds had on the frequency or severity of gangrenous dermatitis lesions or whether the lack of gangrenous dermatitis lesions was due to the lack of an initial infection or due to the lesions resolving more quickly than with other bacterial isolates or isolate combinations. Further research will concentrate on answering these questions and specifically determining details as to the pathogenesis of the disease and the effect of the immune status of the challenged birds in the resolution of lesions.

REFERENCES

1. Ficken, M.D., and D. P. Wages. Gangrenous dermatitis. In:. Diseases of Poultry. 10th ed. B.W. Calnek, H.J. Barnes, C.W. Beard, L.R. McDougald, and Y.M. Saif, eds.. Iowa State University Press, Ames, IA. pp. 265-268. 1997.

2. Fowler, N. G., and S. N. Hussaini. Clostridium septicum infection and antibiotic treatment in broiler chickens. Vet. Rec. 96:14-15. 1975.

Figure 1. Mean gangrenous dermatitis lesion score of broiler chickens injected with various bacterial isolates and isolate combinations.



(1= mild lesions, 4 = severe lesions).

Table 1. Bacterial isolates and isolate combination used for challenging broiler chickens in a gangrenous dermatitis model.

Treatment	S. aureus isolate	C. septicum isolate
1	L101-1	5-2r
2	-	2-4r
3	1158	-
4	-	5-2r
5	L101-1	3-4x
6	Giambrone	2-4r
7	1158	2-4r
8	L101-1	-
9	Giambrone	-
10	-	3-4x
11	Giambrone	3-4x
12	1158	5-2r
13	L101-1	2-4r
14	Giambrone	5-2r
15	1158	3-4x
16	Uninoculated Control	-

DNA FINGERPRINTING OF TWELVE *BORDETELLA AVIUM* STRAINS ISOLATED FROM BORDETELLA OUTBREAKS IN TURKEY FLOCKS

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INTRODUCTION AND SUMMARY

Bordetella avium causes an acute tracheal disease in birds, especially turkeys, known as turkey coryza or bordetellosis. Morbidity is high (80-100%), especially in turkeys 2 to 6 weeks of age. Secondary infections dramatically increase the mortality, which is generally less than 10%.

A chemically mutated strain of *B. avium* has been used as a vaccine since 1980. In turkey farms in southern Utah, there have been several outbreaks of the disease since use of the vaccine began.

This study is to determine the origin of the outbreaks. One of our hypotheses is that the vaccine strain has back mutated, and is now causing outbreaks. To analyze this we applied a pulsed-field gel electrophoresis (PFGE) analysis and randomly amplified polymorphic DNA (RAPD) analysis to determine similarities or differences between the vaccine and the outbreak strains.

MATERIALS AND METHODS

Pulsed Field Gel Electrophoresis. PFGE was performed by the method of Barrett et al. (1), with minor modifications. Strains were grown in brain-heart infusion (BHI) media (Difco) at 37 degrees C with shaking to an optical density between 0.8 and 1.0 at 610 nm. One ml samples of the bacterial strains were centrifuged and washed once with 75 mM NaC1 25 mM EDTA (pH 8.0) and were resuspended in the original volume. Cell suspensions and 2% clean cut agarose (Bio- Rad) were warmed to 55 degrees C in a waterbath for 5 minutes. Equal volumes of the cell suspension and agarose were mixed, dispensed into 1.5-mm-thick disposable plug molds (Bio-Rad) and allowed to solidify at 4 degrees C for 10 minutes. After solidification, the plugs were transferred to tubes containing lysis buffer (50 mM Tris (pH 8.0), 50 mM EDTA (pH 8.0), 1% Sarkosyl (Sigma), and 1 mg/ml proteinase K (Sigma)) and incubated overnight at 50 degrees C. After lysis, the plugs were washed twice for 15 minutes in 10 mM Tris- 1 mM EDTA (pH 8.0) (11) containing 1.5 mM phenyl methylsulfonyl fluoride (PMSF) (Sigma) and four times in TE without PMSF. Plugs that were not used immediately were stored at 4 degrees C in TE. Plugs used for digestion with restriction enzymes were washed twice for 15 mm each in lx universal buffer (1:10 dilution of 10x universal buffer: 1 M KOAc, 250 mM Tris Acetate (pH 7.6), 100 mM MgOAc, 5 mM B-mercaptoethanol, 100 ig/m1 BSA (Sigma)) before replacement with fresh 1x universal buffer and 80U of XbaI (Stratagene). Restriction took place overnight at 37 degrees C. After restriction, plugs were washed again in TE buffer. Restriction fragments were separated by electrophoresis through 2% Pulsed Field Certified Agarose (Bio-Rad) in 0.5x Tris-borate-EDTA (TBE) buffer at 14 degrees C in a CHEF DR III apparatus (Bio-Rad). The run time was

15 hours at 7V/cm with a 120 degrees included angle and a linearly ramped pulse time of 5 to 50 sec.

Randomly Amplified Polymorpic DNA. Genomic preps were prepared using the protocol "large-scale CsC1 prep of bacterial genomic DNA" in Current Protocols in Molecular Biology with modifications: in Step 7 the phenol/chloroform/isoamyl alcohol wash, and an additional chloroform/ isoamyl alcohol wash were done. At Step 9 the DNA was left over night to resuspend in the TE buffer. We did not follow the protocol any longer but digested the RNA with 50 il RNAse A and incubating for 1.5 h at 37 degrees C, after which 0.4 ml 3 M sodium acetate was added. After mixing, 0.6 vol isopropanol was added to reprecipitate the DNA and the solutions were mixed until a DNA precipitate formed. Centrifugation was done at 10000x g for 5 minutes, and the isopropanol was removed with a vacuum pump. One ml 70% ethanol was added to wash the DNA and sucked off with a vacuum pump. 1.5 ml TE buffer was added before storage at 4 degrees C. RAPD analysis was done using the Ready-To-Go kit from Amersham Pharmacia Biotech, Piscataway, NJ, following the manufacturer's instructions.

RESULTS AND DISCUSSION

We will present data from pulsed field gel electrophoresis of the genomes from each strain, as well as RAPD data using the 6 different primers for each strain. We had two different researchers (F.R. and T.T.) run all of the strains through both types of analysis. As will be shown from the gels, with both the pulsed-field gel electrophoresis and the RAPD analysis, although we could demonstrate some difference between the clinical isolates and the vaccine strain, there was some variation in the results from the two different researchers. We are currently carrying out amplified fragment length polymorphism analysis (AFLP) on all of the strains in a effort to eliminate the variation in results. From our results to date, we have identified at least five different RAPD patterns in the clinical isolates, and the vaccine strain gives a pattern that is different from any of the clinical isolates.

REFERENCE

1. Barrett, T. J., H. Lior, J. H. Green, R. Khakhria, J. G. Wells, B. P. Bell, K. D. Greene, J. Lewis, and P.M. Griffin. Laboratory investigation of a multistate food-borne outbreak of Escherichia coli 0517:H7 by using Pulsed-field gel electrophoresis and phage typing. J. Clin. Microbiol. 3013-3017. 1994.

EFFECT OF THE MAJOR HISTOCOMPATIBILITY COMPLEX (MHC) ON INHIBITING CELLULITIS DEVELOPMENT FROM VARIOUS *ESCHERICHIA COLI* ISOLATES

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INTRODUCTION

Resistance to several diseases in chickens has been shown to be associated with the major histocompatibility complex (MHC or B) (1). The majority of diseases studied are caused either by viruses or parasitic nonbacterial pathogens. Comparatively there have been only a few studies in which the MHC's role in bacterial infections has been performed. Some of the bacterial isolates that have been studied include *Pasteurella multocida*, *Staphylococcus aureus*, and *Salmonella* spp.

Cellulitis was initially observed in Great Britain seventeen years ago, since that time it has spread to other countries worldwide to become one of the leading causes of carcass condemnation. Cellulitis is a diffuse, spreading edematous, suppurative inflammation of the deep subcutaneous tissues, sometimes extending into the muscle and frequently associated with abscess formation, often seen as a caseous mat (2). The bacterium most often associated with cellulitis lesions is *Escherichia coli*. The majority of the *E. coli* isolated from cellulitis cases are often nonserotypable. Of the serotypable isolates O1, O2, and O78 are frequently observed (3).

Previous work has shown that the pathogenicity of *E. coli* in relation to avian cellulitis is variable, depending on the strain of *E. coli*. The purpose of this research was to determine if the MHC confers resistance to avian cellulitis, this was performed by challenging a broiler chicken line with defined MHC haplotypes to five avian cellulitis *E. coli* strains.

MATERIALS AND METHODS

Experimental animals. Eggs were obtained from a commercial flock and incubated at Auburn University's research farm. At day of hatch the chicks were wingbanded and vaccinated against Marek's disease. The birds were then randomly placed into battery cages and given feed and water ad libitum. At three weeks of age each bird was bled and a serological test performed to determine the birds B haplotype. Any moribund birds observed during the experiments were killed either by cervical dislocation or by carbon dioxide asphyxiation.

Serotyping. Approximately 0.1 ml of blood was collected from the wing vein of every bird used in these trials at three weeks of age. The blood was then tested using reagents made in this broiler bird line that were specific to B21 or B13 (4).

Experimental design. Do to the limited number of eggs available from the commercial broiler company, this study was composed of three hatches with each treatment being composed of at least 100 birds. This designated bird number was necessary to provide sufficient data points for a meaningful evaluation of the effects of the MHC on inhibiting cellulitis. Table 1 summarizes the total number of birds utilized in these five experiments.

Bacteria. Five different *E. coli* isolates were utilized in this experiment. All five isolates had been isolated from field cases that had typical cellulitis lesions. One of the isolates (EC-AR1) has

been used extensively in this lab in characterizing cellulitis 5. Two of the five isolates were serotypable their serotypes being O2 and O78, the other three isolates used were unserotypable. Storage and culture methods of each isolate has been previously described (5).

Disease challenge. At four weeks of age the birds were injected subcutaneously with one of a possible of five E. *coli* isolates. Each bird was injected on the ventral side parallel to the keel with 1.0 ml of a solution containing approximately 1.0x10 6 cfu/ml of *E. coli*. One week post challenge the birds were euthanized by carbon dioxide asphyxiation and necropsied (5). Lesion presence was noted.

Statistical analysis. The data were analyzed using the General Linear Model procedure, with MHC as the fixed factor and lesion presence as the dependent variable. The means were separated using Duncan's multiple range test.

RESULTS AND DISCUSSION

Tables 1 and 2 summarize the number of birds of each haplotype used with each isolate and the overall incidence of cellulitis observed from five different avian cellulitis causing *E. coli* isolates. Isolate EC-AR1 gave a statistically significant difference ($P<0.05$) between the homozygous haplotype B 21/B 21 and the other two possible haplotype combinations of B 13/B 21 and B 13/B 13. This difference is noteworthy since B 21 in either homozygous or heterozygous combinations has been shown by several researchers to confer resistance to Marek's disease, while B 13 has been shown to be susceptible to Marek's disease (6, 7). Isolate EC-CA462, though not statistically significant, does exhibit the general trend observed in birds challenged with isolate EC-AR1 of B 21/B 21 being slightly more susceptible to cellulitis lesion development then either B 13/B 21 or B 13/B 13. Additionally the O2 isolate had B21/B21 birds being more susceptible than B13/B13 however, the heterozygous B 13/B21 birds had a higher incidence of cellulitis than either of the two homozygous haplotypes. The other two isolates (O78 and EC-AL51) caused opposite results than were observed with EC-AR1 in that B 21/B 21 birds has a lower incidence of cellulitis than either B 13/B 21 or B 13/B 13 birds.

Table 1. Number of birds used grouped according to the *E. coli* isolate used and MHC (B) haplotype.

Table 2. The percentage of birds that were positive for cellulitis lesions, comparing each of the five *E. coli* isolates to the three possible MHC (B) haplotypes. Differences in superscript letters denote statistical difference at $P<0.05$.

The non-statistical differences observed in birds challenged with isolates O78, O2, EC-CA462 and EC-AL51 versus EC-AR1 may be explained by dose differences. Isolates O78, O2, EC-CA462, and EC-AL51 were exceptionally pathogenic towards the birds at the dose given (approximately 1x10 6 cfu/ml), while isolate EC-AR1 was noticeably less pathogenic. The overall incidence of cellulitis for isolates O78, O2, EC-CA462, and EC-AL51, as can be observed in table 2, is at least 90%. This differed markedly from the bird's, challenged with isolate EC-AR1, overall cellulitis incidence (approximately 45%). This high overall incidence of cellulitis lesion development in birds challenged with O78, O2, EC-CA462, and EC-AL 51 may have diminished the statistical differences that had been observed with isolate EC-AR1.

Currently work is being performed to determine the optimal bacterial concentration to administer the birds in order for the overall cellulitis incidence to be around 50%. It is hypothesized that if the overall incidence of cellulitis is approximately 50% it may be possible to statistically separate any differences conferred by the MHC. In conclusion, there appears to be a correlation between the MEW haplotype and the incidence of cellulitis at least for isolate EC-AR1. The other four isolates utilized in these trials were inconclusive, possibly due to the high degree of pathogenicity that was associated with them.

REFERENCES

1. Lamont, S. J. The chicken major histocompatibility complex and disease. Rev. Sci. Tech. Off. Int. Epiz. 17:128-142. 1998.

2. Norton, R. A. Avian cellulitis. World's Poult. Sci. J. 53:337-349. 1997.

3. Peighambari, S. M., J. P. Vaillancourt, R. A. Wilson, and C. L. Gyles. Characteristics of Escherichia coli isolates from avian cellulitis. Avian Dis. 39:116-124. 1995.

4. Li, L., L. W. Johnson, E. J. Livant, and S. J. Ewald. The MHC of a broiler chicken line: serology, B-G genotypes, and B-F/B-LB sequences. Immunogenetics. 49:215-224. 1999.

5. Norton, R. A., S. F. Bilgili, and B. L. McMurtrey. A reproducible model for the induction of avian cellulitis in broiler chickens. Avian Dis. 41:422-428. 1997.

6. Bacon, L. D., and R. L. Witter. Influence of turkey herpesvirus vaccination on the B-haplotype effect on Marek' s disease resistance in 15 B-congenic chickens. Avian Dis. 36:378-385. 1992.

7. Schat, K. A., R. L. Taylor Jr., and W. E. Briles. Resistance to Marek's disease in chickens with recombinant haplotypes of the major histocompatibility (B) complex. Poult. Sci. 73:502-508. 1994.

Table 1. Number of birds used grouped according to the *E. coli* isolate used and MHC (B) haplotype.

	B^{21}/B^{21}	B^{13}/B^{21}	B^{13}/B^{13}
EC-AR1 (nonserotypable)	81	104	55
O78	37	68	36
O2	33	38	24
EC-CA462 (nonserotypable)	25	53	31
EC-AL51 (nonserotypable)	26	51	36

Table 2. The percentage of birds that were positive for cellulitis lesions, comparing each of the five *E. coli* isolates to the three possible MHC (B) haplotypes. Differences in superscript letters denote statistical difference at $P<0.05$.

	B^{21}/B^{21}	B^{13}/B^{21}	B^{13}/B^{13}
EC-AR1 (nonserotypable)	56.41[a]	39.60[b]	38.89[b]
O78	86.49	89.29	90.48
O2	96.97	97.37	95.83
EC-CA462 (nonserotypable)	100	96.23	93.55
EC-AL51 (nonserotypable)	92.31	92.16	100

EFFECT OF TWO INJECTABLE ANTIBIOTICS ON MAREK'S VACCINE

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INTRODUCTION

Marek's disease is a contagious disease with lymphoid tumors in chickens. The disease is caused by a cell-associated herpesvirus with lymphotrophic properties similar to those of gamma herpesviruses. Marek's disease exists in poultry producing countries throughout the world. The Marek's disease virus (MDV) group includes three serotypes: serotype 1, serotype 2 and serotype 3 (1). Marek's disease virus is the prototype and is designed as serotype 1, non-oncogenic chicken herpesvirus belongs to serotype 2, and non-oncogenic turkey herpesvirus belongs to serotype 3.

Many different kind of vaccines have been used to protect against MDV. Vaccines containing herpesvirus of turkeys are widely used to protect both layer and broiler flocks. For economic reasons many farmers would like to incorporate some additives like antibiotics and buffering compounds into diluents and inject them concurrently with MDV to prevent early mortality in chicks. In this study different diluents mixed with Gentaidi Plus 10% (gentamicin sulfate) and Fosfotrim 10% (a mixture of two antibiotics, Fosfomycin and Trimethoprim) were used to study the effect of the antibiotics on the titer and pH of Marek's HVT cell-associated vaccine.

MATERIALS AND PROCEDURES

Vaccines. Commercial HVT vaccines were used in this study.

Diluents. Diluents from Fort Dodge, Intervet and Merial were used. They were designed as A, B and C respectively.

Antibiotics. Gentaidi Plus 10% (Investigacion Aplicada) contains gentamicin sulfate and was added to the diluent at a final concentration of 0.2 mg/dose. Fosfotrim 10% (Investigacion Aplicada), a synergistic mixture of Fosfomycin and Trimethoprim was added to the diluent at a final concentration of 0.2 mg/dose of Fosfomicyn and 0.05 mg/dose of Trimethoprim.

Tissue cultures. Monolayers of chicken embryo fibroblast cell culture were used to determine the titer of Marek's vaccine with and without antibiotic.

pH determination. The pH of the diluents was measured with a pocket pH meter (Corning).

Trial 1. 2 ml of one antibiotic was added to one bottle of diluent; another diluent did not have antibiotic. pH was measured before and after antibiotic had been added. This procedure was followed with all diluents and two antibiotics.

Trial 2. An ampule containing HVT vaccine was added into diluent with antibiotic and another ampule was added into diluent without antibiotic and plate-forming units were determined by conventional methods.

RESULTS

No differences in pH with both antibiotics before and after they were added into diluent of Marek's disease were found. Complete results are shown in Table 1. The results of the laboratory trial in which the cell-associate vaccine was mixed with both antibiotics added to diluent and titrated in chick embryo fibroblasts are also summarized in Table 1. It was found that Gentaidi Plus and Fosfotrim did not significantly reduce the titer of the cell-associate HVT vaccine except with diluent C where there was a loss of 15% PFU in the vaccine with Gentaidi Plus. In spite of this drop in titer with diluent C, the titer of the vaccine meets minimal Mexican requirements which are 1000 PFU per dose.

Table 1. Effect of Gentamicin and Fosfomycin on Marek's disease vaccine

DISCUSSION

In Mexico antibiotics are commonly mixed with HVT vaccine to prevent bacterial infections in one day-old chicks at the hatchery. In many cases the results are disastrous because some antibiotics destroy HVT virus and as a result the protection against Marek's disease is inadequate (2, 3). There already are some studies about the effect of gentamicin sulfate on Marek's disease vaccine (4). In some cases laboratories sell special diluents (buffered) to add to the antibiotic which could be important in the antibiotic being effective. In this study different diluents which had not been buffered were tested and no significant differences between these diluents were found. In conclusion it appears that Gentaidi Plus and Fosfotrim can be administered safely with HVT vaccine to prevent infections in one thy-old chicks.

REFERENCES

1. Calnek, B.W. Marek's Disease. In: Diseases of Poultry, 10th ed. B.W. Calnek, H.J. Barnes, C.W. Beard, L.R. McDougald and Y.M. Saif eds. Iowa University Press, Ames, Iowa. pp.
367-413. 1997.

2. Coldwell, W.M., D.G. Simmons, J.R. Harris, T.G. Fulp, J.H. Carrozza, and T.A. Maag. Influence of some physical factors on survival of Marek's disease vaccine virus. Avian Dis.
19:781-790. 1975.

3. Eidson, C.S., S.H. Kieven, and D.P. Anderson. Effect of antibiotics on turkey herpesvirus vaccine. Poult. Sci. 52: 755-760. 1973.

4. Eidson, C.S., R.K. Page, and S.H. Kleven. In vivo and in vitro studies on the effect of gentamicin sulfate on the efficacy of the turkey herpesvirus vaccine. Poult. Sci. 57:1519-1525. 1978.

Table 1. Effect of Gentamicin and Fosfomycin on Marek's disease vaccine

Diluent	Gentaidi Plus				Fosfotrim			
	pH		PFU /dose		pH		PFU /dose	
	Without antibiotic	With antibiotic	Without antibiotic	With antibiotic	Without antibiotic	With antibiotic	Without antibiotic	With antibiotic
A	7.24	6.28	6625	6400	7.01	7.26	8400	8250
B	6.25	6.6	9900	9500	6.76	7.18	7000	7300
C	7.3	6.8	10625	9000	6.90	7.20	7740	7520

ELISA FOR THE DETECTION OF INFECTIOUS BURSAL DISEASE VIRUS

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An antigen capture ELISA was developed and optimized for the detection and differentiation of Infectious Bursal Disease Virus (IBDV) directly from bursa samples using a set of monoclonal antibodies. The antigen capture ELISA was evaluated for sensitivity and specificity. Progeny challenge studies were conducted to evaluate the IBD antigen capture ELISA in vaccine efficacy studies. The specificity of the ELISA was tested using antigens of various avian pathogens and non-infected bursa samples. The data suggested that the ELISA have excellent specificity and sensitivity for the detection of IBDV antigens directly from bursal samples. The progeny challenge studies suggest that the presence of histological lesions in bursa and positive IBD antigen ELISA results have over a 93% agreement. An epidemiological study of various isolates of IBDV suggests that there are five antigenic groups (Classic, E/Del, GLS, RS593 and a new variant) of IBDV viruses circulating in US commercial poultry flocks.

ELISA TEST FOR THE DETECTION OF *ORNITHOBACTERIUM RHINOTRACHEALE* INFECTION IN CHICKENS AND TURKEYS

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A *Pasteurella*-like bacterium, isolated from chickens and turkeys with respiratory symptoms accompanied with mortality and body weight loss, has previously been described (1, 2). The bacterium was named *Ornithobacterium rhinotracheale* (ORT) in 1994 following the genetic taxonomic characterisation of strains isolated from different avian species in different geographies (3). ORT is known as a secondary pathogen causing severe symptoms when triggered by other respiratory pathogens, such as IBV, TRT, ILT and NDV. The diagnosis of ORT infection is usually difficult because of concomitant infectious agents. Bacterial isolation can be done from respiratory tract tissues on sheep blood agar. ORT is a slow growing bacterium, often overgrown by other bacteria. The serological diagnosis is complicated by the different serotypes of the bacteria (4). Today more than a dozen different serotypes have been described. There is a high degree of cross reactivity between serotypes.

IDEXX Laboratories has developed an ELISA test for the detection of antibodies specific for ORT in chicken and turkey serum samples. The test is a member of the IDEXX Flockchek product line, using the same sample dilution and test protocol as the other poultry tests. The test can be performed within two hours and the results can be interpreted qualitatively or quantitatively with the use of the xChek software. The performance of the new test kit was evaluated on a set of samples differing in both geographical origin and the nature of the samples (e.g. naturally infected, vaccinated or negative birds). Test results were compared to current ORT serological methods as well as the available clinical history for some of the flocks.

Sensitivity of the test was assessed on a set of samples including turkey and chicken field samples, samples from one-day-old broilers with maternal antibodies, vaccinated chickens, and flocks with a well-defined clinical history. The results of the ORT ELISA kit were well in concordance with the expected results based on other serological tests and clinical examinations performed at the different laboratories. The specificity of the new IDEXX kit was evaluated on negative samples from both SPF and broiler flocks. The negative population exhibited a normal distribution with 10 standard deviations between the mean S/P for the negative population and the positive cut-off. The new kit was also tested with regard to the ability to detect different ORT serotypes of the bacterium by means of monospecific antisera. End-point titers for serotype A through M antisera demonstrated that the Floekchek ORT ELISA detects antibodies to all serotypes at a similar level.

In conclusion, the new IDEXX ORT Antibody ELISA is a test kit with good sensitivity and excellent specificity, which correlates well with other ORT serological methods and clinical history.

REFERENCES

1. Charlton, B.R., S.E. Channing-Santiago, A. A. Bickford, C. J. Cardona, R. P. Chin, G L. Cooper, R. Droual, J. S. Jeffrey, C. U. Meteyer, H. L. Sivaprasad, and R. L. Walker.

Preliminary characterization of a pleomorphic gram-negative rod associated with avian respiratory
disease. J. Vet. Diagn. Invest. 5:47-51. 1993

2. Hafez, H. M., W. Kruse, J. Emele, and R. Sting. Eine Atemwegisinfektion bei Mastputen durch Pasteurella-ahnliche Erreger: Klinik, Diagnostik unf therapie. In: Proc. International Conference on Poultry Diseases, Postdam, Germany. pp.105-112. 1993

3. Vandamme, P., P. Segers, M. Vancanneyt, K. Van Hover, R. Mutters, J. Hommez, F. Dewhirst, B. Pastert, K. Kersters, E. Falsen, L. Devrieze, M. Bisgarrd, K.-H. Hinz, and W. Mannheim. Description of Ornithobacterium rhinotracheale ge. Nov. sp.nov. isolated from the avian
respiratory tract. Int. J. Syst. Bacteriol. 44:24-37. 1994

4. van Empel, P., H. van den Bosch, P. Loeffen, and P. Storm. Identification and serotyping of Ornithobacterium rhinotracheale. J. Clin. Microbiol. 35:418-421. 1996

EMERGING IBV - VACCINE-DERIVED FIELD STRAINS AND NEW SEROTYPES

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Infectious bronchitis (IB) is among the most common and difficult of all poultry diseases to control. IB is highly contagious and causes significant economic losses in commercial broiler, layer and breeder chickens. The causative coronavirus, infectious bronchitis virus (IBV), frequently causes respiratory disease in young chickens and egg production losses in adult hens. In addition, some strains of the virus exhibit a renal tropism and cause up to 25% mortality in affected flocks.

IBV is perhaps best known for its existence as numerous antigenic types or serotypes. Although antigenic variation of IBV has been recognized for years it is only within recent years that the scientific community has had the capability to truly appreciate the genetic diversity of the virus.

IBV is uniquely suited to undergo mutation during its replication/life cycle. Replication of the virus' RNA genome is error-prone and the resulting mutations may alter important antigenic components of the virus and result in new variants. The major target for mutation is the spike (S) envelope protein gene that the virus uses to attach to the host cell. Mutations in S result in antigenic changes and the emergence of variant serotypes as well as subtypes of recognized serotypes. Furthermore, the S protein gene is able to tolerate numerous mutations without compromising the virus' ability to replicate and cause disease.

Newly mutated variant serotypes are subject to immunological selection so that only the most antigenically novel variants persist in a poultry population. A new variant that is not antigenically novel, e.g. one similar to a vaccine strain used on the farm, will not persist in the flock because the vaccine-induced immunity will eliminate it from the population. Conversely, newly mutated variants that are antigenically distinct from vaccine strains will, in essence, have a far greater potential to escape vaccine-induced immunity, persist and potentially cause disease.

The presentation will focus on two ways that IBV causes disease; by the emergence of vaccine-derived field strains and by the development of new variant serotypes.

EMERGENCE OF VACCINE-DERIVED FIELD STRAINS

In recent years, many broiler producing areas of the USA have experienced serious outbreaks of the Arkansas (Ark) serotype, in many instances in spite of the use of Ark vaccination. Investigation in our laboratory (3) of this apparently paradoxical finding demonstrated that Ark pathogenic field isolates represented two distinguishable subtypes of Ark DPI, the reference strain contained in the majority of attenuated vaccines. Additional studies determined that the Ark subtypes were actually a minor subpopulation of the original Ark DPI strain contained in vaccines. RNA viruses, including coronaviruses, commonly exist as multiple subpopulations, also termed quasispecies. The presence of viral quasispecies provides distinct

advantages by affording viruses the potential to adapt to selective pressures, persist in host populations and cause disease.

We theorize that the Ark subtypes, minor subpopulations in the Ark vaccine, emerged from inconsistent and seasonal use of the Ark DPI vaccine over many years. Since it was first commercially available in 1985, Ark DPI vaccine has been used to control IBV, but primarily only on a seasonal basis from October to April when the maximum benefit of reducing airsacculitis condemnations and mortality in broiler flocks were realized. In contrast, the necessity for the immunity provided by Ark DPI vaccination is much reduced in the warmer months, because environmental respiratory challenges of ammonia, dust, and cold housing temperatures are comparatively low and the impact of IBV field infections on broiler performance is diminished. We propose that the Ark subtype viruses, present originally as minor subpopulations in the vaccine underwent back passage in the field and became pathogenic for broilers as a result of the seasonal use of vaccines containing the Ark DPI strain. Moreover, the establishment of a high level of Ark subtype field challenge may likely have been enhanced in recent years by a reduced emphasis on poultry house clean-out and disinfection practices due to economic and environmental constraints in the industry.

Figure 1. Infectious bronchitis virus dendogram depicting S-1 protein similarities based on sequence of the CK2/CK4 PCR fragment (1). Strains with asterisks (*) represent new genotypes characterized by our lab.

DEVELOPMENT OF NEW VARIANT SEROTYPES

It is no coincidence that new variant serotypes arise in commercial layers raised on farms with multiple-aged flocks. These farms provide all the conditions that favor the emergence of new mutant IBV variants. Layer flocks of different ages frequently numbering in excess of a million birds, are housed in close proximity on egg production complexes. Periodic introduction of new pullets, and the continual reinfection and recycling of IBV in the layers, results in a greater opportunity for infection and spread than occurs on farms using an "all in-all out" management system. Novel IBV variants build up in the poultry house environment since the premises are rarely, if ever, cleaned and disinfected. Importantly, immunological mechanisms provide a major selective pressure for the most antigenically novel variants since new variants are continually arising. The presentation will provide an update on the new variant serotypes of IBV in the USA and the procedure used to identify them. Emphasis will be placed on a recently-developed polymerase chain reaction approach (2) that uses analysis of the S-1 gene for identification of unknown/previously unrecognized field serotypes. The procedure, which utilizes direct automated cycle sequencing of S-1, has proven useful for epidemiologic studies. Comparative analysis of IBV S-1 protein sequences (Fig. 1) was performed using the Clustal V package of DNAStar (DNAStar, Inc.; MegAlign; vl.03, 1993; Madison Wisconsin). The current status of nephropathogenic IBV in the state of Pennsylvania will also be discussed.

REFERENCES

1. Keeler, C. L., Jr., K. L. Reed, W. A. Nix, and J. Gelb, Jr. Serotype identification of avian infectious bronchitis virus by RT-PCR of the peplomer (S-1) gene. Avian Dis. 42:275-284. 1998.

2. Kingham, B. F., C. L. Keeler, Jr., W. A. Nix; B. S. Ladman, and J. Gelb, Jr.
Identification of avian infectious bronchitis virus by direct automated cycle sequencing of the S-1
gene. Avian Dis. In press.

3. Nix, W. A., D. S. Troeber, B. F. Kingham, C. L. Keeler, Jr., and J. Gelb, Jr.
Emergence of subtype strains of the Arkansas serotype of infectious bronchitis virus in Delmarva broiler chickens. Avian Dis. Submitted.

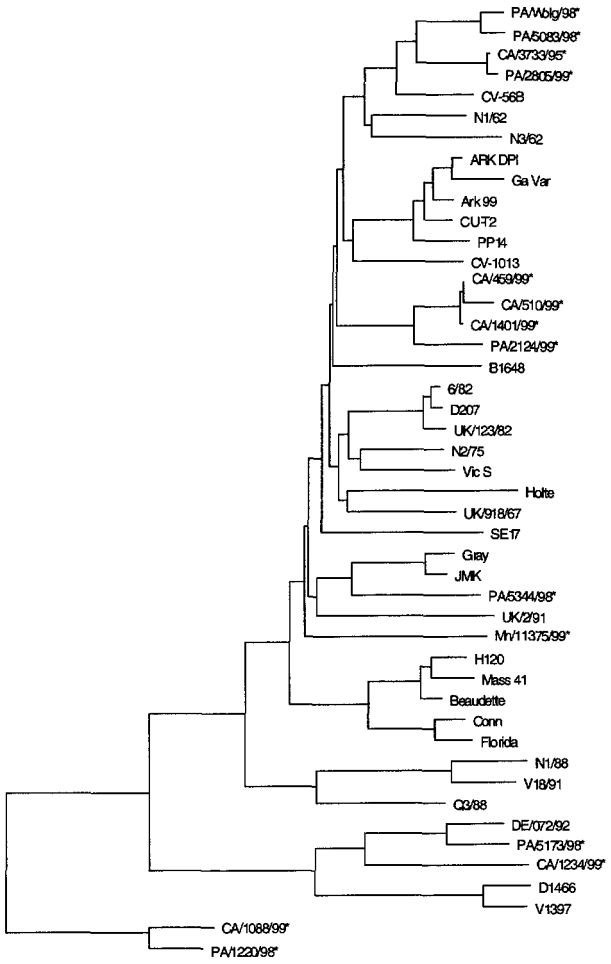

Figure 1. Infectious bronchitis virus dendogram depicting S-1 protein similarities based on sequence of the CK2/CK4 PCR fragment (1). Strains with asterisks (*) represent new genotypes characterized by our lab.

EPIDEMIOLOGY OF E. COLI CELLULITIS IN CALIFORNIA BROILER FLOCKS

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Cellulitis has emerged as an economically important disease of broiler chickens over the last 8 to 10 years. *Escherichia coli* (*E. coli*) has been causally linked to cellulitis. In California, the rate of cellulitis in broiler flocks is generally less than 1 or 2 %, but may reach 8 to 10% in some flocks. The majority of published epidemiologic investigations of cellulitis have been done in Canada where management practices are very different from those in the western USA. Previous risk factor analysis for cellulitis have concentrated at the gross level (e.g. season, flock size, sex). We hypothesized that the environment of the litter would play an important role in the pathogenesis of cellulitis.

A fourteen-month prospective study was performed to investigate the effect of the environment at the floor level and litter parameters on the flock incidence of cellulitis. Five broiler ranches were followed over 5 or 6 flock cycles. Ammonia levels, relative humidity, ambient temperature and wind velocity were measured at nine randomized sites in every house per ranch. Litter samples were also obtained at three randomized and stratified sites per house and then analyzed for dry matter, pH, water activity, gram negative and lactose positive bacteria (cfu per gram of dry matter). Measurements and samples were taken on day 0, day 35, and day 45 for each flock. The incidence of cellulitis by house for each ranch was obtained from processing records.

Preliminary analysis of the data was performed to test the correlation of litter and environment variables to cellulitis. The day 0 data showed a positive correlation between ammonia, pH, dry matter, temperature and cellulitis. Ammonia was negatively correlated with water activity and positively associated with dry matter. The number of lactose positive colonies per gram of litter, with no chicks present, was negatively correlated with the flock cellulitis rate. On days 35 and 45, ambient ammonia, water activity, pH and cellulitis were negatively correlated. Positive associations between cellulitis and dry matter, and cellulitis and temperature were observed. Wind velocity data was inconclusive. Further analysis of the data is in progress.

(A full-length article is being prepared for publication in *Avian Diseases*.)

ESCHERICHIA COLI INFECTION IN LAYING HENS SEROTYPE PREVALENCE AND ANTIBIOTIC SUSCEPTIBILITY OF SEVERAL STRAINS IN ARGENTINA

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ABSTRACT

Several isolations of *Escherichia coli* from outbreaks in laying hens are reported for the period 1998 - 1999. The outbreaks were most severe between 35 - 60 weeks of age and were characterized by fibrinous peritonitis, polyserositis and high mortality. An antibiogram was performed on all isolates and the results are summarized. In some cases treatment was not 100% effective and it was necessary to repeat it with a different antibiotic. In a few cases more than one serotype of *E. coli* was isolated.

Serotyping of several isolates was done and the serotypes found were: O2:K1, O114:K+, O1:Kl, O8:K+, O78:K80, O37:K+, O17:K-, Ol42:K+, O32:K+, Ol7:K+, O115:K+, and Ol8:K+. Seven were untypable. The most frequent serotypes were Ol:Kl and O2:K1.

INTRODUCTION

Colibacillosis of poultry is a common systemic infection caused by *E. coli*. The disease is responsible for significant economic losses in the poultry industry. *E. coli* infection causes colibacillosis, colisepticemia, Hjarre's disease, coligranuloma, peritonitis, salpingitis, synovitis, omphalitis, and air sac disease (6). *E. coli* is a gram negative, nonacid, uniform staining, nonsporeforming bacillus, usually 2-3 x 0.6 um. The organism may be variable in size and shape. Many strains are motile and have peritrichous flagella. Various serotypes of *E. coli* are classified according to the Ewing scheme (4). Many of them have been isolated everywhere in the world (1,5,7,9). The most frequent serotypes in poultry are O1, O2, O8, O35 and O78 (10). *E. coli* may be sensitive to many drugs, but some of them are frequently resistant to one or more antibiotics (3,10). The aim of this paper is to report the serotype and antibiotic susceptibility of several strains of *E. coli* isolated in Argentina during 1998 and 1999.

MATERIALS AND METHODS

Samples. The samples were taken from more than 10 different flocks of caged layers. In almost all flocks the mortality started to increase at around 26 weeks of age. The average mortality was around 0.5% to 0.7% weekly and continued for several weeks. Post-mortem examination revealed regressed ovaries and peritonitis. In some birds, the oviduct was congested and contained fibrinous and caseous exudate. The peritoneal exudate and the affected ovaries and oviducts were cultured for bacteria.

Bacteriology. Peritoneal exudate, ovaries and oviducts were cultured on MacConkey agar (DIFCO). The isolates were typed by biochemical properties.

Antibiotic sensibility. A 12 hours broth culture was swabbed onto the surface of a 150x15 plate with count agar (DIFCO) and paper discs, impregnated with the antibiotics (DIFCO) to be

tested, were laid on the medium. The plates were incubated 24 hours at 37 degrees C and the inhibition area was recorded.

Serotyping. The isolates were submitted to Veterinary Laboratory, Lohmann Tierzucht GmBH, Cuxhaven, Germany, where the serotyping was done.

RESULTS AND DISCUSSION

Twelve typable serotypes of *E. coli* were isolated and 7 untypable isolates were isolated. The serotypes found were O2:K1,. O1l4:K+, Ol:K1, 08:K+, O78:K8O, O37:K+, O17:K-, O142:K+, O32:K+, Ol7:K+, O115:K+, and O18:K+. Serotype 02:K1 was isolated 4 times. Other surveys have reported that the most frequent serotypes in poultry are O1, O2, O8, O35 and O78, and untypable strains (10). In our study we found the mentioned strains. The most frequent was O2:Kl. All the strains were isolated from laying hens with lesions, and we consider it will be necessary in the future to conduct a pathogenicity study. Table 1 shows in vitro antibiotic sensitivity tests of 26 *E. coli* isolates. The results show that a high percent of the isolates were resistant to the most frequently used antibiotics in the poultry industry. It is necessary to perform an antibiotic sensitivity test before treating a flock. The use of *E. coli* inactivated vaccines should be considered.

Table 1. Results of antibiotic sensitivity to different E. coli isolates.

REFERENCES

1. Cloud, S.S., J.K. Roserberger, P.A. Fries, R.A. Wilson, and E.M. Odor. In vitro and in vivo characterization of avian Escherichia coli I. Serotypes, metabolic activity and antibiotic sensitivity. Avian Dis. 29:1084-1093. 1985.

2. Deb, J.R., and E.G. Harry. Laboratory trials with inactivated vaccines against Escherichia coli (O2:Kl) infection in fowls. Res. Vet. Sci. 24:308-313. 1978.

3. Erganis, O., O. Kaya, M. Corlu, and E. Istanbulluoglu. Hemagglutination, hydrophobicity, entero-toxigenicity, and drug-resistance characteristics of avian Escherichia coli. Avian Dis. 33:631-635. 1989.

4. Ewing, W.H., H.W. Tatum, B.R. Davis, and R.W. Reavis. Studies on the serology of the Escherichia coli group. US Dept. Health, Educ., and Welfare, Publ. Health Serv., Atlanta. pp. 42. 1956.

5. Glanz, P.J., S. Narotsky, and G. Bubash. Escherichia coli serotypes isolated from salpingitis and chronic respiratory disease of poultry. Avian Dis. 6:322-328. 1962.

6. Gross, W.B. Colibacillosis. In: Diseases of poultry, 9th ed. B.W. Calnek, H.J. Barnes, C.W. Beard, W.M. Reid, and H.W. Yoder, Jr., eds. Iowa State University Press, Ames, Iowa. pp 138-144. 1991.

7. Hemsley, R.V., D.A. Barnum, and D.G. Ingram. Biochemical and serological studies of

avian strains of Escherichia coli. Avian Dis. 11:90-97. 1967.

8. Panigrahy, B., and L. Yushen. Differentiation of pathogenic and nonpathogenic Escherichia coli isolated from poultry. Avian Dis. 34:941-943. 1990.

9. Sojka, W.J., and R.B.A. Carnaghan. Escherichia coli in poultry. Res. Vet. Sci. 2:340-351. 1961.

10. Zanella, A., G.L. Alborali, M. Bardotti, P. Candotti, P.G. Guadagnini, P.A. Martino, and M. Stonfer. Escherichia coli infection characterized by septicemia and fibrinous polyserositis in egg-type hens at the start of laying. Proc. 48th West. Poult. Dis. Conf., Vancouver, Canada. pp. 142-144. 1999.

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for the serotyping.

Table 1. Results of antibiotic sensitivity to different E. coli isolates.

Antibiotics	Disc Potency (μg)	N° sensitive/total	%
Ampicillin	10	16/26	61.54
Chloramphenicol	30	16/26	61.54
Chlortetracycline	30	5/26	19.20
Gentamicin	10	0/26	0.00
Oxytetracycline	30	3/26	11.53
Streptomycin	30	0/26	0.00
Apramycin	15	1/26	3.84
Fosfomicin	50	24/26	92.80
Tetracycline	30	3/26	11.53
Enrofloxacin	5	19/26	73.07
Florfenicol	30	24/26	92.30
Norfloxacin	10	20/26	76.90
Furazolidone	100	20/26	76.90
Ceftiofur	30	26/26	100.0
Kanamycin	30	2/26	7.69
Amoxicillin	20	24/26	92.30

EVALUATING IBD PASSIVE IMMUNITY USING HISTOLOGY, BURSAL/BODY WEIGHT AND PCR

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Protection for infectious bursal disease (IBD) through passive immunity from broiler breeders, was evaluated in commercial broilers. Broiler breeders were vaccinated at 12, 20 and 38 weeks of age with inactivated IBD vaccine. Progeny hatched from eggs laid when the breeders were 49 to 52 weeks of age were challenged with standard challenge strain and Delaware variant E. Protection was determined by: 1) bursa/body weight ratios and 2) histopathology lesion scores. Protection results, determined by each method, were collected and identified to each individual bird for comparison. Statistical analysis of data collected from 314 challenged birds found that the two methods do not correlate to one another for either standard or Delaware variant E challenges. Another forty birds were challenged in the same manner. Protection was determined as before but also included PCR to determine the presence of IBD virus. All three methods found different birds to be protected or not protected.

EXPERIMENTAL INFECTION OF SALMONELLA ENTERITIDIS PHAGE TYPE 4, 5A AND 8 FROM POULTRY ENVIRONMENT ORIGIN IN ONE-DAY-OLD SPF CHICKS

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Nine groups of twenty-six, one-day-old Specific Pathogen Free (SPF) chicks were inoculated with *Salmonella enteritidis* (SE) Phage Type (PT) 4, 5A, and 8, isolated from the poultry environment. *Salmonella* cultures were inoculated by crop gavage with 1 X 10 6 Colony Forming Units (CFU). One group was similarly inoculated with Salmonella pullorum (S. pullorum). One group of 26 chicks was used as the uninoculated control group. The chicks were observed daily for signs of illness and mortality. Two chicks were randomly selected from each group at 7, 14, 21, and 28 days post-inoculation (DPI) for gross pathology, bacterial culture, and histopathologic evaluations. Body weights were measured at 7, 14, 21, and 28 DPI. The remaining of SPF chicks from each group were euthanized and necropsied at 28 DPI. The mean body weights were lower in SPF chicks inoculated with S. pullorum, SE PT4 (both human and chicken origin) than in uninoculated controls at 7, 14, and 21 DPI. No significance differences in body weights were observed among uninoculated controls, SE P18, SE PT5A and SE P14 (chicken origin) at 28 DPI. High mortality was observed in chicks inoculated with *S. pullorum*, SE PT5A (2), and SE PT4 (chicken origin). No signs of illness or mortality were observed in the uninoculated control group. Bacteriologic evaluations showed that both in acute and chronic infections, the ceca is the best organ from which to culture salmonellae, followed by the crop. Pericarditis, perihepatitis, and peritonitis were present in-groups treated with SE PT5A (2), SE P14 (chicken origin), and SE PT4 (human). Yolk sacs which formed into an abscess often attached to the posterior body wall, were observed in the birds that died between 14, 21, and 28 DPI inoculated with SE PT5A (2), SE P14 (chicken and human origin), and *S. pullorum.*

(A full-length article will be submitted for publication in *Avian Diseases*.)

FACTORS AFFECTING DRAG SWAB EFFICIENCY WHEN SAMPLING CHICKEN MANURE

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Salmonella enteritidis (SE) is a worldwide foodborne health risk often associated with table eggs, that is subject to control and surveillance programs that assume that the risk of SE contaminated eggs can be estimated by detection of SE in chicken manure. Drag swabbing chicken manure piles and culturing for SE is the primary means used in these programs to detect SE. It is the responsibility of veterinarians performing SE surveillance for their egg producing clients to collect and transport the samples collected in such a way as to maximize the probability of detecting SE if it is present on the farm.

This study examined such factors as drag swab distance, media for maintenance of *Salmonella*, and site selection for increasing the probability of successfully determining the presence of *Salmonella enteritidis*, when environmentally sampling a laying facility by drag swabbing manure piles. A laying facility in California's San Joaquin Valley was sampled with drag swabs of manure piles. Samples were cultured with standard laboratory methods, and counts were made by standard means. Numbers obtained were subjected to statistical analysis using "off-the-shelf" computer software. Salmonella spp. counts were expected to be highly variable due to reported clustering. Therefore, total bacteria and *E. coli*, which were assumed to have a more uniform distribution on the surface of the manure, were additionally used as proxies for *Salmonella*. Media for moistening the swabs were compared by seeding post-swabbing samples with *Salmonella typhimurium* (BFP), and culturing at different delay times. Total bacterial counts were compared between samples that were obtained from either wet or dry surfaces.

Results of the studies show that higher bacteria counts were obtained by swabbing wet areas rather than dry areas, but that swab efficiency was degraded in a shorter distance when used in wet areas. Media selected for the swab did not make a practical difference in maintaining *Salmonella* viability over a 48 hour period when the samples were kept at refrigerated temperatures. Once swabs became saturated, bacteria numbers on the swab did not increase with further swabbing. Spatial clustering of SE in chicken manure decreases the probability of identifying its presence once swabs become saturated, so swabs should be changed based on their appearance rather than the length of a cage row.

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FERMENTATION AS A METHOD FOR CARCASS DISPOSAL

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SUMMARY

Every turkey and broiler production facility is faced with the reality of carcass disposal. For a flock of 30,000 turkeys avenging 0.5% mortality weekly (9% total mortality), approximately 16,272 kg (17.9 tons) of carcasses require disposal during an 18 week growing period. For a flock of 50,000 broilers grown to 49 days of age averaging 0.1 % daily mortality (4.9 % total mortality), approximately 2,182 kg (2.4 tons) of carcasses require disposal (1). Nationwide, this represents a tremendous amount of organic matter that requires environmentally and biologically safe disposal or utilization. Poultry carcasses resulting from death by natural occurrences at high levels of production must be disposed of by environmentally acceptable methods. Rendering carcasses into a valued protein by-product meal is an alternative. Removing poultry carcasses from the farm is environmentally acceptable and a valuable feed ingredient results; however, the spread of pathogenic microorganisms during routine pickup and transportation to a rendering facility presents a substantial threat. Lactic acid fermentation of poultry carcasses prior to transportation stabilizes carcass deterioration and minimizes pathogen threat. Unlike routine pickup of "fresh" carcasses, fermentation allows for long-term on-farm storage of poultry carcasses, reduced transportation costs and results in a usable feed ingredient.

CURRENT DISPOSAL METHODS

Burial pits are most commonly used for disposing of poultry carcasses. The decline in ground water quality where pits are located is one concern and residue remaining in pits after years of use is recognized as another concern. In July 1994, the use of burial pits was no longer allowed in the state of Arkansas. In July 2000, burial pits will no longer be permitted for poultry carcass disposal in the state of Alabama. Undoubtedly, other states will eventually follow suit and no longer permit the use of burial as a method for routine poultry carcass disposal. In most cases, catastrophic losses will be allowed to be disposed by burial.

Anaerobic digesters which are designed to promote the microbial breakdown of poultry carcasses and eliminate harmful bacteria have also been tested (2). Results indicated that these systems tend to harbor and maintain potentially pathogenic bacteria. Consequently, the use of digesters is not permitted in Alabama.

Incineration is a biologically safe method of disposal; however, it tends to be slow, expensive, and generates the greatest number of nuisance complaints even when highly efficient incinerators are used. Due to imposing local, state, and federal water and air quality standards, alternative methods of disposal are of interest to the poultry producer. As the poultry industry expands, so also will the amount of waste generated on the farm. The poultry industry must aggressively pursue efforts to protect the environment and maintain a good public image.

RENDERING POULTRY CARCASSES

Rendering can be used as a method for the conversion of poultry carcasses into a valued, biologically safe protein by-product meal. However, the spread of pathogenic microorganisms during routine pick up and transportation of poultry carcasses to a rendering facility is viewed as a potential threat. Removing poultry carcasses from the farm is most acceptable for the environment, and a valuable feed ingredient results.

A major concern with centrally located carcass disposal sites is disease transmission. Sound biosecurity of disposal sites is essential to prevent disease transmission (3). Central carcass disposal sites have been placed on trial in Minnesota and North Carolina with limited success (4,5).

Freezing carcasses for short-term storage prior to transportation to a rendering facility is effective. Heavy loading of the refrigeration unit during periods of high environmental temperature (>20 degree C) with> 35 kg daily may result in carcass decomposition in the bottom and middle layers (6). Carcasses appear to be frozen on top, but heavy loading may create conditions whereby the carcasses cannot eliminate their heat load and thoroughly freeze prior to addition of more carcasses. Large-capacity units are usually required because 90 kg or more of carcasses at near body temperature (41 degree C) may be encountered daily. Refrigeration has potential for the storage of carcasses prior to transportation to a rendering facility, but the costs of operation and transportation need careful consideration. Daily operating expenses for an individual freezer unit can average $0.60 1.00/day assuming an electrical cost of $0.08/kW (5,6,7).

FERMENTATION

Fermentation, a controlled natural process has been successfully used as a preservation method for foods and feeds for millennia, and has become well documented as a scientifically sound method for the preservation of organic materials. Lactic acid fermentation of poultry carcasses prior to transportation inhibits carcass deterioration and minimizes pathogen threat (4,8,9,10).

Initial studies conducted by Dobbins (8) described methods for preserving poultry carcasses by lactic acid fermentation. Successful fermentation is enabled by the combination of prescribed amounts of poultry carcasses with a fermentable carbohydrate source such as sugar, whey, molasses or ground corn (8,9,10,11,12). In order for effective fermentation to occur, carcasses must be ground to 2.5 cm or less particles. Particle reduction is required for tissue acidification. Grinding aids the dispersion and mixing of intestinal anaerobic lactic acid-forming bacteria. Bacteria that produce lactic acid ferment the carbohydrate source resulting in the production of volatile fatty acids and a subsequent decline in pH to below 4.5, which preserves the nutrients in the carcasses.

Pathogenic microorganisms associated with the carcasses are effectively inactivated or inhibited during the fermentation process via the decrease in pH (8,9,10,11,12,13). Presumably, fermented material can be stored and will remain in a stable state for several months. Therefore, fermentation could be initiated and continue on-farm until carcass amounts are sufficient to warrant the cost of transportation for rendering.

Initial investigations were conducted in small-scale vessels to evaluate the appropriate combination of ground poultry carcasses with fermentation carbohydrate and/or other additives

required to assure rapid fermentation and biosecure stabilization that would result in long-term storage on the farm (11,12).

Results from laboratory studies indicated:

* Addition of at least 6 % glucose or whey permeate or 8% whey to ground carcasses promoted fermentation as evidenced by a decline in pH from 5.6 to a range of 4.2 to 4.5 within 7 days.
* Ground corn at a level of 15 % or greater was necessary to support adequate fermentation as indicated by a decline in pH to less than 4.5.
* Addition of an acidulant, bacterial culture, a protease enzyme, or antifungal agents failed to improve the fermentative process.
* Populations of indigenous coliform bacteria and added Salmonella typhimurium were reduced from moderately high (ca. 10 6 colony forming units (CFU)/g) levels to undetectable levels (< 10 CFU/g).

Two experiments were conducted to address the scaling-up of an endogenous fermentation system of carcass stabilization from laboratory to on-farm use. In both experiments, approximately 10 kg of ground carcasses were mixed with an appropriate carbohydrate and placed in a closed container with subsequent additions occurring on four consecutive days, resultant batch size of 50 kg (13).

Results from scale-up studies indicate:

* Batches fermented with sucrose (10%) or whey (10%) and subsequently stored at either 2° C or 25°C exhibited declines in pH from 5.8 to 4.1 (25 degree C) and from 5.8 to 4.8 (2 degree C).
* Batches fermented with whey (10%), whey permeate (10%) or ground corn (20%), stored at 25 degree C for 12 weeks, exhibited pH decreases from 5.8 to 4.6, 4.5 and 5.1 within 7 days. Lowest pH levels for whey (4.0), whey permeate (4.3) and corn (4.8) occurred at 18 days and remained relatively constant.
* Initial coliform levels were greater than 10 6 CFU/g and declined to undetectable levels (< 10 CFU/g) by the 18th day of fermentation.

ON-FARM FERMENTATION

Broilers. Two on-farm fermentation facilities have been installed on contract farms with capacities of 86,000 and 68,000 broilers to demonstrate the feasibility and commercialization of on-farm endogenous fermentation of poultry carcasses (14). A grinding unit was specifically designed and fabricated which allows for the simultaneous addition of a carbohydrate source during the grinding of carcasses (15). Daily, broiler mortality is ground and ground corn added at the 20% level. The mixture (mortality and carbohydrate) was directly fed into a 1,135 liters (300 gallon) capacity enclosed tank (PCO Tank #10951, Raven Industries, Sioux Falls, SD).

Weekly pH measurements were obtained from the fermentation tank(s) at 3 locations approximately 30 cm below the surface. Typically, the pH values of the fermented product decline below 5.0 within a 10-day period. Levels of coliform bacteria that were tested at random

time periods remained non-detectable (<10 CFU/g). All resulting ferment obtained from both farms was transported for rendering at the end of a typical grow-out cycle (4549 days later). Over 13,636 kg of fermented carcasses have been processed into a usable feed ingredient (14). Results from these studies indicated that fermentation can be adapted for the stabilized, pathogen-free storage of broiler carcasses during a typical 7-week growout.

Broiler breeders. A system was designed and installed on a primary breeder farm to demonstrate feasibility and economics of on-farm endogenous microbial fermentation for stabilizing poultry carcasses (16). This system was also utilized for the disposition of selective genetic breeding stock. Dead broiler breeders (males and females) were ground while a calibrated feeder unit added ground yellow corn at approximately 20% mortality weight (15). The mixture (mortality and corn) was fed into a trailer-mounted sealed storage tank (3175 kg capacity). Ferment was monitored periodically to ensure pH remained below 5.5 and was transported to a rendering facility weekly. An economic evaluation of incineration versus fermentation was completed. Prior to installation of the fermentation system, incineration was employed for carcass and genetic stock disposal. From June 1, 1996 to June 30, 1997, 76,166 kg mortality consisting of 27,988 carcasses up to 8 kg in size, required incineration. Propane ($0.67/gal) and labor costs were $11,591 and $7,300, respectively, resulting in a total incineration cost of $18,891. Fuel and labor costs averaged $0.248/kg. In comparison, all mortality (28,077 carcasses totaling 67,039 kg) was ground and fermented from July l to December 30, 1997 at a cost of $7,444. (8,044 kg corn, $3695; labor, $3136; transportation, $463; and utilities, $150). Payback received from the rendering company was $1478. ($0.022/kg). As a result, net disposal costs averaged $0.089/kg, about 1/3 the cost of incineration for large breeder carcasses. Capitalization costs for the systems were equivalent for a ten-year usage period.

Endogenous microbial fermentation represents an economical, feasible, and environmentally safe method for on-farm storage of carcasses prior to transportation to a rendering facility. Unlike routine pickup of "fresh" mortalities, fermentation and subsequent storage of dead poultry reduces transportation costs by 90% and eliminates the potential for transmission of pathogenic microorganisms through poultry via rendered products.

REFERENCES

1. Blake, J. P., M. F. Cook, and D. Reynolds. Dry extrusion of poultry processing plant wastes and poultry farm mortalities. Proc. 6th International Symposium on Agricultural and Food Processing Wastes, American Society of Agricultural Engineers, St. Joseph, MI. pp. 319-327. 1990.

2. Macklin, K. S., R. A. Norton, and J. P. Blake. Current status of dead bird digesters in Alabama. Poult. Sci. 77:123. 1998.

3. Collins, E. R., and W. D. Weaver. Rendering poultry mortalities. American Society of Agricultural Engineers. 1991 International Summer Meeting. Albuquerque, NM. Paper 91-4050. 1991.

4. Parsons, J., and P. R. Ferket. Alternative dead bird disposal methods. Central pickup

and fermentation. Proc. North Carolina State University Poultry Symposium Short Course, Raleigh, NC. pp. 7-20. 1990.

5. Poss, P. E. Central pick-up of farm dead poultry. Proc. National Poultry Waste Management Symposium, Auburn University, AL. pp. 75-76. 1990.

6. Blake, J. P., J. K. Tucker, and J. O. Donald. Operating costs associated with on-farm refrigeration of poultry carcasses. Poult. Sci. 77:97. 1998.

7. Donald, J. O., and J. P. Blake. Comparison of mortality disposal systems. Proc. National Poultry Waste Management Symposium, Auburn University, AL. pp. 56-63. 1992.

8. Dobbins, C. N. Lactobacillus fermentation; A method of disposal/utilization of carcasses contaminated by pathogenic organisms or toxic chemicals. Proc. National Poultry Waste Management
Symposium, Ohio State University, Columbus, OH. pp. 76-80. 1988.

9. Murphy, D. W., and S. A. Silbert. Carcass preservation systems-lactic fermentation. Proc. National Poultry Waste Management Symposium, Auburn University, AL. pp. 56-63. 1990.

10. Blake, J. P., J. O. Donald, and D. E. Conner. On-farm fermentation of broiler carcasses; Proc. National Poultry Waste Management Symposium, Auburn University, AL. pp. 328-334. 1992.

11. Conner, D. E., J. P. Blake, and J. O. Donald. Fern-tentative stabilization of poultry farm mortalities. Poult. Sci. 70:28. 1991.

12. Conner, D. E., J. P. Blake, and J. S. Kotrola. Levels of carbohydrate needed to support endogenous fermentative stabilization of poultry carcasses and the effect of propionic acid
on fungal growth. Poult. Sci. 71:29. 1992.

13. Kotrola, J. S., D. E. Conner, and J P. Blake. Development of a practical fermentative process for stabilization of poultry carcasses prior to rendering: Scale-up of laboratory studies. Poult. Sci. 71:52. 1992.

14. Blake, J. P., and J. O. Donald. An on-farm fermentation system for dead poultry disposal. Poult. Sci. 71:21. 1992.

15. Sellnow, W. Personal communications. Dixie Grinders, Inc., Guntersville, AL. 1992.

16. Blake, J. P., R. M. Roden, and J. T. Scott. Feasibility and economics of on-farm poultry carcass fermentation. Poult. Sci. 77:97. 1998.

FIELD EXPERIENCES IN REDUCING *SALMONELLA* COLONIZATION WITH FLAVOMYCIN® FEED ADDITIVE

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INTRODUCTION

In 1973, Nurmi and Rantala introduced a technique to increase the resistance of baby chicks to salmonella infection by inoculating them orally with adult fowl intestinal contents. This technique was later termed "competitive exclusion" (6). There have been several proposed mechanisms of action with competitive exclusion. However, most scientists agree that a population of normal protective bacteria in the intestines of chickens do provide a benefit in reducing invading pathogenic bacteria. Several early studies (1,2,5) indicate that Lactobacillus spp. is a major component in providing the protective aspect of competitive exclusion. Other protective bacterial species include *Bifidobacterium* and *Bacteroides*. A major component of the protective nature of competitive exclusion is thought to be the production of volatile fatty acids.

Flavomycin® (bambermycins) has been used in broiler diets world-wide for growth promotion and feed conversion improvement since the early 1970's. The spectrum of antimicrobial activity indicates that Flavomycin has little effect against common normal flora bacteria, particularly *Lactobacillus acidophilus* and *Bifidobacterium bifidum* (FOI Summaries, Hoechst Roussel Vet, Clinton, NJ). Other commonly used growth promotant antibiotics may have a significant effect in reducing the Lactobacillus population of the intestine. Free et al. (3) showed that chickens fed Flavomycin at 2 ppm had a significantly greater level of volatile fatty acids in the small intestine than birds fed lincomycin, bacitracin or virginiamycin. Kling and Quarles (4) found that broilers fed with a continuous Flavomycin ration were no more susceptible to necrotic enteritis than birds fed other growth promotants that have a therapeutic claim for the disease. Recent reports by Schleifer (7) showed that *Lactobacillus* and *Bifidobacter* populations of the intestines are generally higher in birds fed a continuous Flavomycin program compared to other treatments, in field trial settings. In another study, broiler house *Salmonella* numbers were found to be lower from houses where birds were fed a continuous Flavomycin program compared to other treatments (8). The theory supported by these works, is that Flavomycin does not negatively affect the protective microflora of the chickens intestine. Therefore, by allowing the normal protective bacteria to live, a secondary competitive exclusion is achieved, compared to birds continuously fed commonly other commonly used growth promotant programs.

The purpose of these studies is to continue the work previously reported by this author. In these studies the possible effects on intestinal bacterial populations by Flavomycin are investigated under normal field conditions and with a same farm comparison. The effects on intestinal *Salmonella* colonization is specifically addressed.

TEST DESIGN

Trial 1. A U.S. integrated operation was identified as the trial location. The operation normally processes approximately 1,200,000 broilers per week. Circa May 5, 1999, the operation initiated a growth promotant program of 2g/ton Flavomycin from one day of age until the birds were processed. The growth promotant program prior to May 5 was 50g/ton BMD(R) from one day of age until approximately 18 days of age and then 2g/ton Flavomycin from 18 days until approximately 42 days of age, when the birds were slaughtered. At the time of processing, one farm was randomly selected for intestinal sampling. Ten birds were randomly selected for intestinal collection. The first sampling occurred on April 6. The follow-up sampling from the same farm was obtained August 4. Intestinal samples were collected sanitarily on the processing line, right after evisceration. The samples were placed in sterile Whirl-pak plastic bags containing 100 ml of glycerin-salt solution. The contents of the bag were thoroughly mixed and quick frozen on dry ice. They were immediately shipped via next day courier to an independent laboratory for microbiological enumeration.

Trial 2. Another US operation that processes over 1 million broilers per week decided to change its growth promotant program circa June 1, 1999, to a continuous Flavomycin program at 2g/ton. This operation had been using BMD at 50g/ton to approximately 35 days of age and then used Stafac to approximately 49 days of age or when the birds are slaughtered On May 18 sampling of ten randomly selected birds from a flock on the previous program was performed. Sampling of the replacement flock was performed on August 9. The intestinal sampling procedure was consistent with that noted in Trial 1.

Enumeration of microbial populations. The contents of each Whirl-pak were weighed and removed from the bags into a blender jar. An appropriate volume of peptone dilution solution was added to the sample and the mixture was blended into a homogenate with as little aeration as possible. A 1:10 dilution sequence was used to prepare dilutions representing 10 -1 to 10 -6 g of the original sample. Dilutions were plated in triplicate on selective media to enumerate each of the specific groups of bacteria. Enrichment tubes were inoculated with material from the initial dilution. *Clostridium perfringens* was enumerated on Perfringens Selective Medium (O.P.S.P.). These plates were incubated at 35 degrees C in an anaerobic chamber for 72 h Coliforms and *E. coli* were enumerated on Violet Red Bile Agar medium containing 4-methylumbelliferyl-beta-D-glucuronide (MUG). Poured plates were overlaid with a second layer of medium to increase the selectivity of the procedure and were incubated at 37 degrees C. Lactobacilli were enumerated on Rogosa SL Agar plates. These plates were incubated at 37 degrees C in an anaerobic chamber to allow for the growth of the oxygen-sensitive Lactobacilli found in the gastrointestinal tract. *Bifidobacteria* were enumerated on BS-LV agar plates which were overlaid with medium containing neomycin sulfate. These plates were incubated for 72 hours at 37 degrees C in an anaerobic chamber. *Salmonella* were enumerated on Hectoen Enteric Agar medium. Plates were incubated at 37 degrees C. Prevalence of *Salmonella* in birds with low concentrations were detected after enrichment in lactose broth and selenite cystine broth. Colonies that gave typical results for *Salmonella* on Hectoen Enteric Agar medium were presumptively identified on Triple Sugar Iron Agar.

Additional testing for trial 2 was performed for the further enumeration of *Salmonella* populations. For all samples, the initial blending and dilution was in 2% buffered peptone. 13.6 ml of the peptone dilution was removed for the standard dilutions, plates, and enrichments. 40 ml of the peptone dilution was quick-frozen and stored at 70 degrees C. The remainder of the

sample was incubated as a pre-enrichment step. After 18-24 hours incubation of the pre-enrichment, 0.5 ml was transferred to TT Hajna broth as an enrichment step. At the end of an 18-24 hr incubation, 10 µl of the enrichment was streaked on a plate of XLT-4 agar. The XLT-4 was incubated at 35 degrees C for 48 hr. Colonies that gave typical results for *Salmonella* were transferred to Triple Sugar Iron Agar for confirmation. Samples positive in this testing had a minimum of one *Salmonella* cell in the entire intestinal tract sample and were tested further. Further testing involved thawing the reserve sample and performing the same basic testing steps, but on three 10 ml samples, three 1 ml samples, and three 0.1 ml samples in the 2% buffered peptone pre-enrichment. TT Hajna tubes followed these and XLT-4 plates were used to verify Salmonella, tube for tube. One colony from each positive plate was confirmed on Triple Sugar Iron Agar. The results of these tube tests were used to calculate most probable number (MPN) values using the tables and instructions in the USDA BAM Manual, 8th edition.

RESULTS

Results are summarized for Trial 1 in Table 1 and for Trial 2 in Table 2.

Table 1. Bacterial enumeration in number of organisms per intestine, recovered from whole intestinal samples from 43 and 42 day old birds (Trial 1)

Table 2. Bacterial enumeration in number of organisms per intestine, recovered from whole intestinal samples from approximately 49 day old birds (Trial 2).

DISCUSSION

In both of these field trials, birds that were started on Flavomycin at 2 g/ton and fed until processing had lower numbers of *Salmonella*-positive birds, than birds exposed to other growth promotant programs. Correspondingly, the birds fed Flavomycin also had higher numbers of *Lactobacillus* spp. and *Bifidobacterium* spp. per gram of intestine than birds fed other growth promotant programs. Additionally, lower numbers of *Clostridium perfringens*, coliforms and *E. coli* were recovered from birds fed Flavomycin. In both of these trials, growth promotion and feed conversion rates for the Flavomycin treated birds were as good as or better than birds on other treatments.

Based on the results of these trials, it can be stated that the field trials do support the theory that Flavomycin does allow higher numbers of *Lactobacillus* spp. and *Bifidobacterium* spp. to populate the gut. This increased population of beneficial bacteria appear to directly correlate with a reduction in *Salmonella*, both qualitatively and quantitatively, compared to other growth promotant programs. These results further support previously reported field data that demonstrated similar results.

REFERENCES

1. Barnes, E. M., 0. C. Mead, D. A. Barnum, and E. G. Harry. The intestinal flora of the chicken in the period 2 to 6 weeks of age, with particular reference to the anaerobic bacteria. Br. Poult. Sci. 13:311-326. 1972

2. Barnes, E. M., C. S. Impey, and B. J. Stevens. Factors affecting the incidence and anti-salmonella activity of the anaerobic caecal flora of the young chick. J. Hyg., Camb. 82:263-283. 1979.

3. Free, S. M., T. O. Lindsey, and R. D. Hedde. Possible mode of action of antibiotics on energy utilization. Zootecnica Int. pp 48-49. December 1986.

4. Kling, H, and C. Quarles. Performance of broilers fed commonly used antibiotics in the presence of necrotic enteritis. Southern Poult. Sci. Abstracts. Atlanta, GA. 1995.

5. Mead, G. C., and B. W. Adams. Some observations on the caecal micro-flora of the chick during the first two weeks of life. Br. Poult. Sci. 16:169-176. 1975.

6. Nurmi, B., and A. M. Rantala. New aspects of salmonella infection in broiler production. Nature 241:210. 1973.

7. Schleifer, J, K. Dawson, and H. King. Field experiences in altering broiler intestinal microflora with the use of Flavomycin feed additive. Proc. Western Poultry Disease Conference. Vancouver, BC. 1999.

8. Schleifer, J, D. Waltman and H. King. Field experiences in changing broiler-house Salmonella populations with the use of Flavomycin feed additive. Proc. Western Poultry Disease Conference. Vancouver, BC. 1999.

Table 1. Bacterial enumeration in number of organisms per gram intestine, recovered from whole intestinal samples from 43 and 42 day old birds (Trial 1)

Bacteria	April 6 (BMD/Stafac treatment)	August 4 (continuous Flavo treatment)
Lactobacilli[A]	1.59	45.2
Bifidobacteria[A]	4.90	22.7
Coliforms[B]	776	200
E. coli	195	11
C. perfringens	852	33
Qualitative Salmonella[C]	7 / 10	0 / 10

[A] Bacterial enumeration times 10^6

[B] Bacterial enumeration times 10^3

[C] Number of birds positive for Salmonella / Number of birds sampled

Table 2. Bacterial enumeration in number of organisms per gram intestine, recovered from whole intestinal samples from approximately 49 day old birds (Trial 2).

Bacteria	May 18 (BMD/Flavo treatment	August 9 (continuous Flavo treatment)
Lactobacilli[A]	7.24	11.15
Bifidobacteria[A]	4.73	9.12
Coliforms[B]	1260	263
E. coli[B]	576	141
C. perfringens	132	1000
Salmonella[C]	0.69	0.14
Qualitative Salmonella[D]	17 / 21	3 / 20

[A] Bacterial enumeration times 10^6
[B] Bacterial enumeration times 10^3
[C] Bacterial enumeration per 100g intestinal contents
[D] Number of birds positive for Salmonella / Number of birds sampled

GENETIC AND GEOGRAPHICAL ANALYSIS OF PASTEURELLA MULTOCIDA CLINICAL ISOLATES FROM TURKEYS IN UTAH USING RANDOM AMPLIFIED POLYMORPHIC DNA ANALYSIS AND AMPLIFIED FRAGMENT LENGTH POLYMORPHISM FINGERPRINTING

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Fowl cholera, a disease caused by *Pasteurella multocida*, continues to be a major concern for turkey growers. Currently, there are two live attenuated vaccines commonly used to immunize birds, the M-9 and CU strains. These vaccines have occasionally been implicated in outbreaks of disease. In the current study, 66 clinical isolates from turkeys that died of fowl cholera on several Utah farms were analyzed and compared to the M-9 vaccine strain. Analysis of the isolates included Random Amplified Polymorphic DNA (RAP])) analysis, Amplified Fragment Length Polymorphism (AFLP) fingerprinting, and serotyping. The results of the two genetic analyses showed that those organisms isolated from vaccinated flocks were significantly closer genetically to the M-9 strain than were the isolates from unvaccinated turkeys. Statistical analysis also revealed that this relationship could not have been determined by serotyping alone. Although both genetic techniques were effective for the purposes of this study, AFLP provided more detail with respect to genetic diversity than RAPD. A strong correlation between genetic profile and serotype was noted. The results showed no relationship between geographic location and genetic composition or serotype.

(The full-length article will be published in the *Journal of Clinical Microbiology*.)

HEXAMITIASIS ASSOCIATED WITH HIGH MORTALITY IN CHUKAR PARTRIDGES IN CALIFORNIA

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An outbreak of hexamitiasis (*Spironucleus meleagridis*) associated with high mortality is described in 6-week-old chukar partridges in California. The principle necropsy findings were catarrhal enteritis, and dermatitis of the feet and skin around the beaks and eyes) presumably due to secondary nutritional deficiency). Possible contributing factors found in some, but not all birds, included tapeworms, rotaviruses, intestinal cryptosporidia, and Salmonellae. On transmission electron micrographs some of the flagellated protozoans were found buried deep in the intestinal mucosa, and in some instances appeared to be intracellular.

HYDROPERICARDIUM SYNDROME. A REVIEW

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The first outbreaks of hydropericardium syndrome (HPS) were at Angara Goth in Pakistan, in late 1987 (1). In fact it was described as Angara disease because it was recognized in this region first. After 1987 the disease was recognized in other countries including Mexico, India, Chile, Iraq and Peru. The disease affects broilers and occasionally broiler breeders. Outbreaks occur in broilers of 3 - 7 weeks of age and last for approximately two weeks. HPS is characterized by a high mortality (60 % in some cases) (5), general congestion, pulmonary edema, enlarged, pale and friable liver and kidney, and the presence of clear transudate in the pericardial sa (1). In Mexico, HPS was recognized in 1989 in the high-density poultry producing state of Queretaro by Altamirano (2) with mortalities ranging between 17.5 and 34.5 %. An inquiry in 1990 revealed outbreaks in the Mexican states of Aguacalientes, Hidalgo, Jalisco, Estado de Mexico and Morelos. At this time there were two hypotheses about the etiology of HPS. The first one was that the disease was caused by a high pathogenic Type 1 adenovirus serotype 8, subtype E. (3,4). The second one was that two viruses were involved, an adenovirus and another small virus unidentified (possibly a RNA virus), which grew well on monolayers of liver or kidney chicken embryo tissue cultures (7).

In 1992 Morales (6) isolated and identified an adenovirus serotype 4 from liver homogenate in liver chicken embryo tissue cultures (LCETC). In 1994 (7) he isolated another virus which was not inhibited by 5 bromodeoxiuridine and which was cytopathogenic in tissue cultures. It was determined that the virus contained RNA. Lucio et al. in 1992 (5) produced high mortality and lesions of HPS in one day-old chicks when they were inoculated orally with a liver homogenate containing Adeno 4 and the RNA virus. The pathology was modified when the homogenate was heated at 70 degree C for 15 minutes. This suggested that the adenovirus alone did not cause high mortalities in one day-old chicks. In 1995 Morales et al. (7) tried to identify the RNA virus involved in HPS. They separated the virus using serological methods with Adeno 4 - specific antiserum and found an apparently unknown RNA which was 37-45 mm in diameter, nonenveloped and showed no cross-reaction with RNA antiserum against other RNA viruses. Since 1992 at least adenovirus serotypes 4 and 8 have been reported to cause outbreaks of HPS in Mexico. Some of these viruses have been identified as SHP-IAP/92, SHP-IAP/94, and SHP-IAP/95.

Many methods to control HPS have been described in Mexico. Because at first there were no commercial vaccines against HPS many farmers made formolized-liver homogenates and inoculated birds. The protective response was irregular because the amount of antigen was not standardized. Furthermore, there was not enough information about the agent or agents present in the region, the immunogenicity and the pathogenicity of the viruses involved. Several commercial vaccines to prevent HPS in broilers and broilers breeders, alone or combined with Newcastle or other agents, now exist. These produce good results in the field. Another way to prevent HPS during an outbreak is the use of specific immunoglobulins (Igs) against HPS. In 1995 Morales et al. (8) reported several experiments with Igs applied at 0.5 and 1.0 ml per dose subcutaneously and found complete protection. In another assay they challenged birds with

Gumboro disease before an Igs treatment. Birds that received Igs and were challenged with HPS were 100% protected. This indicates that Igs can be used in immunosuppressive birds

REFERENCES

1. Afzal, M., R. Muneer, and G. Stern. Studies on the aetiology of hydropericardium syndrome (Angara disease) in broilers. Vet.Rec.128:591. 1991.

2. Altamirano, R., H. Ramirez, A. Retana y J. Zurita. Hepatitis con cuerpos de inclusion y su relacion con altas mortalidades en el pollo de engorda en Mexico. Memorias XV Convencion Anual ANECA, Cancun Mex. 1990.

3. Christensen, N.H., and Md Saifuddin. A primary epidemic of inclusion body hepatitis in broilers. Avian Dis.33:622-630. 1989.

4. Grimes, T.M. Causa y control de una forma sobreaguda de hepatitis por cuerpos de inclusion. Acivultura profesional 10:64-68. 1992.

5. Lucio, D.E., M.B. Lucio y G.A. Morales. Reproduccion del sindrome del hidropericardio en Mexico.Informe preliminar. Memorias de la XVII Convencion anual ANECA.Puerto Vallarta Jal.Mexico. pp.175-180. 1992.

6. Morales, G.A., and D.E. Lucio. Algunas caracteristicas de los agentes presentes en el sindrome del hidropericardio en Mexico. Memorias de la XVII Convencion anual ANECA.Puerto Vallarta Jal.Mexico. pp.198-201. 1992.

7. Morales, G.A., V.V. Valle, y D.E. Lucio. Identificacion de los agentes etiologicos del sindrome del hidropericardio. Memorias de la XX Convencion Anual ANECA Acapulco, Gro.Mexico. pp225-227, 1995.

8. Morales, G.A., V.V. Valle y D.E. Lucio. Evaluacion de la proteccion al desafio conferida por immunoglobulinas contra el sindrome del hidopericardio en pollo de engorda. Memorias de la XXI Convencion Anual ANECA,Cancun Q.R.México. pp.172-174. 1996.

IN OVO NDV VACCINATION IN COMBINATION WITH INTERFERON- TYPE I IS SAFE AND EFFICACIOUS

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SUMMARY

In ovo administration of chicken interferon type I (IFN-I) has been tested to determine its ability to render a commercially available live viral LaSota strain of Newcastle disease vaccine (NDV) safe and efficacious for in ovo use. Data from our laboratory indicates both improved hatch and survivability of chicks vaccinated in ovo with NDV+IFN-I compared with in ovo NDV alone vaccinated animals. Furthermore, birds vaccinated in ovo with NDV+IFN-I develop protective antibodies when immunized at day-18 and these birds are protected from lethal challenge with Texas GB strain of Newcastle disease virus. The studies reported here indicate safety and efficacy can be achieved by in ovo vaccination with NDV+IFN-1 in both SPF birds and in maternal antibody positive broilers.

INTRODUCTION

Newcastle disease (ND) is an economically important respiratory disease of poultry. Typically broilers are vaccinated at hatch for ND followed by a booster during growout. The development of an in ovo vaccine which proves safe and protective for the life of the bird would be highly advantageous. The objective of the two studies described here was to determine whether a combination of the anti-viral cytokine IFN-I can render an otherwise harmful live ND vaccine safe and effective for in ovo use in both specific pathogen free (SPF) leghorns and broilers.

MATERIALS AND METHODS

Reagents and bird strains. Yeast-expressed, HPLC purified IFN-I was combined with a commercially available LaSota strain of ND vaccine at indicated concentrations in both studies. Protein determinations were made using the Lowry method and a bovine serum albumin standard. A vial of commercially available, live LaSota strain ND vaccine was reconstituted in PBS and concentrated aliquots stored frozen at -70 degrees C until time of use. Prior to egg injections, IFN-I and ND vaccine were combined. SPF leghorns were obtained from Hy-Vac, Adel, IA, and commercial broilers from Green Forest, AR.

Vaccinations and viral challenge. All vaccinations were performed in ovo by manual injection of day 18 embryos, unless otherwise noted. In Experiment 1 (SPF), one group of control birds was vaccinated intranasally/ intraocularly post hatch with a commercially available B1 vaccine. The Texas GB strain of NDV was administered for challenge at day 21 in Experiment 1 (SPF) and at day 28 in Experiment 2 (broilers). The challenge was 10 2 EID 50 given intramuscularly.

Experimental procedures. At least 50 day-18 embryonated eggs were inoculated per treatment group for each experiment. Separate hatchers were used where appropriate, and experimental treatment groups housed separately. Percent hatch of live was recorded on day of hatch by determining the number of normal chicks hatched and by conducting an egg necropsy to confirm that inoculated embryos were alive and normal at day 18 of incubation. After hatch, each treatment group was separated into two replicates. A biosecure traffic pattern was followed for daily bird observations to minimize cross contamination risk. Mortality was determined by dividing the number of dying chickens by the number of chickens placed. Body weights and sera for antibody titers were collected on days indicated in each experiment. Blood was collected from 10 birds per group and these same 10 birds were monitored throughout the study unless death occurred. Titers were determined by hemagglutination inhibition.

RESULTS

Experiment 1. NDV+IFN-I protection study in SPF birds. Previous data from studies performed in our laboratory indicate SPF chicks can develop protective titers after in ovo vaccination with NDV+IFN-I. However, treatment groups in these previous studies were not kept in isolation. The objective of this experiment was to determine whether NDV+IFN-I vaccination would be efficacious under isolation conditions as measured by protection from Texas GB Newcastle challenge. Treatment groups and safety data are shown in Table 1. After hatch, treatments were separated into two replicates. Birds surviving to three weeks of age were challenged with Texas GB (10 2 EID50, intramuscularly). Antibody titers and protection data are shown in Table 2.

Table 1. Treatment groups and safety results for SPF birds.

Table 2. Antibody titers and % protection for SPF birds.

Experiment 2. NDV+IFN-I protection study in broilers. The objective of this experiment was to determine whether NDV+IFN-I vaccination would be efficacious in maternal antibody positive broilers. Safety data are shown in Table 3. Birds surviving to four weeks of age were challenged with Texas GB (10 2 EID50, intramuscularly). Antibody titers and protection data are shown in Table 4.

Table 3. Treatment groups and safety results for broilers.

Tables 4. Antibody titers and % protection for broilers.

DISCUSSION

Data presented here indicate IFN-I has the potential for rendering an otherwise harmful dose of NDV vaccine safe and efficacious for in ovo use. Hatchability and pre-challenge livability of NDV+IFN-I groups was always improved in comparison to NDV groups not receiving IFN-I. At some doses of NDV+IFN-I, hatch and pre-challenge mortality was comparable to vehicle controls. Efficacy of vaccination with NDV+IFN-I was demonstrated by protection of

NDV+IFN-I vaccinates when challenged with a lethal dose of the NDV challenge virus, Texas GB. It was also shown here that the combination of NDV+IFN-I doses is critical, with too much or too little of either component offsetting the efficacy or safety of the vaccine. However, it should be noted that the same combination dose of NDV and IFN-I (NDV at 10 2.5 EID50 and 20 ug of IFN-I) was shown to be safe and efficacious in both SPF birds and in broilers. Taken together, these data indicate in ovo vaccination with NDV+IFN-1 is technically feasible.

Table 1. Treatment groups and safety results for SPF birds.

	Treatment Group	% Hatch	% Mortality to 21d (pre- challenge)
1	PBS	98	0
2	PBS B1,B1 post hatch	74[a]	0
3	$10^{2.5}$ B1 LaSota	55	69
4	$10^{1.5}$ B1 LaSota	94	26
5	$10^{2.5}$ B1 LaSota, 20 ug IFN-I	96	6
6	$10^{2.5}$ B1 LaSota, 40 ug IFN-I	86	2
7	$10^{1.5}$ B1 LaSota, 20 ug IFN-I	89	2

[a] % hatch was not as expected for treatment 2; the two sets of *in ovo* PBS injected groups (groups 1 and 2) had a large disparity in % hatch. These two groups were incubated in same incubator (Jamesway 252) in the center of incubator.

Table 2. Antibody titers and % protection for SPF birds.

	Treatment Group	HI (D21)	HI (D35) survivors	% Protection
1	PBS	0.8^{C}	NS	0%
1a	PBS	0.8^{C}	NS	0%
2	PBS B1,B1post hatch	7.1^{AB}	8.8	100%
2 a	PBS B1,B1 post hatch	7.3^{AB}	6.1	100%
3	$10^{2.5}$ NDV	8.1^{A}	7.1	89%
3a	$10^{2.5}$ NDV	7.8^{A}	7.4	100%
4	$10^{1.5}$ NDV	7.6^{AB}	6.4	100%
4a	$10^{1.5}$ NDV	8.0^{A}	7.1	100%
5	$10^{2.5}$ NDV 20 ug IFN-I	7.3^{AB}	7.4	100%
5a	$10^{2.5}$ NDV 20 ug IFN-I	7.7^{A}	8.5	100%
6	$10^{2.5}$ NDV 40 ug IFN-I	1.0^{C}	NS	0%
6a	$10^{2.5}$ NDV 40 ug IFN-I	1.0^{C}	12.0	4%
7	$10^{1.5}$ NDV 20 ug IFN-I	1.1^{C}	NS	0%
7a	$10^{1.5}$ NDV 20 ug IFN-I	6.1^{B}	8.6	83%

^{A,B,C} Different superscripts within a column indicate statistically significant differences.

Table 3. Treatment groups and safety results for broilers.

	Treatment Group	%Hatch	% Mortality to 28d (pre- challenge)
1	PBS	97^{A}	$0\%^{A}$
2	$10^{2.5}$ NDV 20 μg IFN-I	97^{A}	$4\%^{A}$
3	$10^{2.5}$ NDV 10 μg IFN-I	98^{A}	$0\%^{A}$
4	10^{3} NDV 20 μg IFN-I	95^{A}	$4\%^{A}$
5	$10^{3.24}$ NDV 20 μg IFN-I	98^{A}	$0\%^{A}$
6	$10^{3.5}$ NDV 20 μg IFN-I	97^{A}	$17\%^{B}$
7	$10^{2.5}$ NDV	87^{B}	$18\%^{B}$

^{A,B} Different superscripts within a column indicate statistically significant differences.

Table 4. Antibody titers and % protection for broilers.

	Treatment Group	Log 2 HI (D 0)	Log 2 HI (D 28)	Log 2 HI (D 42) survivors	% Protection
1	PBS	3.5^{A}	0.9^{A}	9.0	5^{A}
1a	PBS		0.7^{A}	NS	0^{A}
2	$10^{2.5}$ NDV 20 µg IFN-I	3.5^{A}	6.9^{B}	7.8	100^{B}
2a	$10^{2.5}$ NDV 20 µg IFN-I		6.6^{B}	7.1	100^{B}
3	$10^{2.5}$ NDV 10 µg IFN-I	3.5^{A}	6.4^{B}	7.5	100^{B}
3a	$10^{2.5}$ NDV 10 µg IFN-I		6.2^{B}	6.8	100^{B}
4	10^{3} NDV 20 µg IFN-I	3.4^{A}	6.2^{B}	8.0	100^{B}
4a	10^{3} NDV 20 µg IFN-I		6.3^{B}	5.5	100^{B}
5	$10^{3.24}$ NDV 20 µg IFN-I	3.2^{A}	6.2^{B}	7.3	100^{B}
5a	$10^{3.24}$ NDV 20 µg IFN-I		6.2^{B}	6.3	100^{B}
6	$10^{3.5}$ NDV 20 µg IFN-I	3.6^{A}	6.6^{B}	6.9	100^{B}
6a	$10^{3.5}$ NDV 20 µg IFN-I		7.5^{B}	5.9	100^{B}
7	$10^{2.5}$ NDV	3.1^{A}	7.3^{B}	6.9	100^{B}
7a	$10^{2.5}$ NDV		6.6^{B}	7.0	100^{B}

^{A,B} Different superscripts within a column indicate statistically significant differences.

IN OVO PASSIVE ANTIBODY ADMINISTRATION FOR THE CONTROL OF AVIAN LEUKOSIS VIRUS - J

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It has been understood for some time that Avian Leukosis Virus (ALV) elicits a humoral response that may result in development of neutralizing antibody (1). Typically these birds are then able to eliminate the viral infection and not develop ALV symptoms or lesions. Current infections of ALV strain J have been shown to develop antibody responses but may remain shedders of ALV-J virus (2). The use of anti-ALV-J antibodies in ovo in attempts to reduce the incidence or effects of infection have indicated potential benefits (3). One of the considerations for the administration of anti- ALV-J antibodies is whether or not they will neutralize all isolates of ALV-J due to genetic and/or antigenic variation. Initial tests, using six different isolates of ALV-J characterized by the Avian Disease and Oncology Laboratory, have shown that polyclonal antiserum developed in immunization of Specific Pathogen Free (SPF) chickens is capable of in vitro neutralization not only the immunizing strains of ALV-J but six other isolates of this virus as well. Careful characterization of antiserum produced for these assays has been performed so that in vivo studies utilizing this reagent may be evaluated for efficacy and reproducibility.

REFERENCES

1. Payne, L. N., and A. M. Fadly. Leukosis/sarcoma group. In: Diseases of Poultry, 10th ed. B.W. Calnek, H.J. Barnes, C.W. Beard, L.R. McDougald, and Y.M. Saif eds. Iowa State University Press, Ames, Iowa. pp. 414-466. 1997.

2. Payne, L.N., K. Howes, L.M. Smith, and K. Venugopal. Current Status and Diagnosis, Epidemiology and Control of ALV-J. In: Avian Tumor Viruses Symposium. A.M. Fadly, K.A. Schat, and J.L. Spencer, eds. American Association of Avian Pathologists, 40th Annual Meeting, Reno, Nevada. pp. 58-62. 1997.

3. Singbeil, B., A.P. Avakian, T. Girshick, A.L. Harrison, M. Hudock, D. Grosse, B. Aid, J. Schaeffer, L. Murray, A. Carter, and J. Tyczkowski. Immunotherapeutic Approaches to Avian Leukosis Virus. Poster presentation. American Association of Avian Pathologists, 42nd Annual Meeting, New Orleans, Louisiana. 1999.

IN VITRO ADHERENCE NEUTRALIZATION OF HAEMOPHILUS PARAGALLINARUM TO CHICKEN TRACHEAL EPITHELIAL CELLS BY HEMAGGLUTINATION-INHIBITION ANTIBODIES

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The adherence of bacteria to mucosal surfaces is the initial event in the pathogenesis of most infectious diseases due to bacteria in animals and humans (1). Recently, the adherence ability of *Haemophilus paragallinarum* to chicken tracheal epithelial cells was demonstrated (2). In chickens, the hemagglutination-inhibition (HI) antibody titers produced by *H. paragallinarum* immunogens have been correlated with protection against clinical signs and nasal clearance of the challenge organism (3).

The purpose of the present work was to determine the effect of the HI antibodies on the in vitro adherence of *H. paragallinarum* to chicken tracheal epithelial cells. Rabbit-raised antiserum and sera of vaccinated chickens were used. Strain 221 (serovar A-1) of *H. paragallinarum* was used as immunogen and hemagglutinating antigen. The in vitro adherence test was carried out as previously reported (3). Briefly, the HI titers of the antiserum and sera were determined and a 1:80 antibody titer obtained in PBS. The hemagglutinating (HA) units of washed cultures of *H. paragallinarum* were determined and 8 HA units adjusted in PBS with 0.01% thimerosal. The chicken tracheal epithelial cells were obtained from 7-week-old chickens raised in our laboratory. Cells were suspended in erythrocyte lysis buffer with 1% bromhexine. After three hours storage at 4 degrees C, cells were resuspended in Hank solution. Equal volumes of each 1:80 HI dilution and 8 HA units of antigen were mixed at 37 degrees C for 1 hr. Centrifuged bacteria were washed and resuspended at the same volume and HA activity tested. Equal volumes of both bacteria and tracheal cells suspensions were mixed at 37C for 1 hr. After collection and washing three times by centrifugation at 200 r.p.m., tracheal cells were fixed to slides and stained with Giemsa-Maygrunwald. All bacteria adhered to thirty tracheal epithelial cells were counted and the adherence means compared by the Tukey test (0.01 significance).

All bacteria with HA activity adhered to tracheal epithelial cells. Bacteria exposed to 1:80 HI dilution antibodies lacked HA activity and no adherence to epithelial cells was observed. There were statistical differences ($p<0.0\ 1$) in the HA activity and adherence ability of the groups tested. Results obtained indicate that HI antibodies may have a protective role by blocking the adherence of *H. paragallinarum* to respiratory epithelial cells.

REFERENCES

1. Beachey, E. H. Bacterial adherence: adherence: adhesinreceptor interactions mediating the attachment of bacteria to mucosal surfaces. J. Infec. Dis. 143:325-345. 1981.

2. Fernandez, R. P., G. A. Garcia, G. P. Ochoa, and V. E. Soriano. Adherence of Haemophilus paragallinarum to chicken tracheal epithelial cells. Proc. 48th West. Poult. Dis. Conf., Vancouver, British Columbia, Canada. pp. 111-112. 1999.

3. Kume, K., A. Sawata. and T. Nakai. Clearance of the challenge organisms from the upper respiratory tract of chickens injected with an inactivated Haemophilus paragallinarum vaccine. Jpn.
J. Vet. Sci. 46:843-850, 1984.

MYCOBACTERIUM GENAVENSE INFECTION IN A PSITTACINE AVIARY

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Psittacines from an aviary in the Pacific Northwest have been necropsied in the Avian Health Laboratory during past 16 years; several have had a history of weight loss and anorexia. The most significant gross findings have included distended intestines appearing like a rope with thickened walls and mucosa being whitish granular in appearance. The livers were pale and enlarged with a hard texture. The spleens were enlarged. The kidneys appeared to be congested with some enlargement. The histopathologic lesions were severe granulomatous enteritis, splenitis, and hepatitis. The presence of acid fast positive rod-shaped bacteria was consistent with avian tuberculosis. Tissues submitted on several occasions to the National Veterinary Services Laboratory were found to be negative for the isolation of *Mycobacterium avium*. PCR analysis performed on the tissues from one of the parakeets was found to be positive for *Mycobacterium genavense*; this is considered to be the first outbreak caused by *M. genavense* in the Pacific Northwest.

(The full-length article is to be published in *Avian Diseases*.)

MYCOPLASMOSIS: SOMETHINGS OLD AND SOMETHINGS NEW

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INTRODUCTION

Mycoplasmas are cell wall-less bacteria. They are small in size and also have minimal genomes; the functional result of these features means that these organisms do not survive for long outside a host, they are nutritionally fastidious, and may be difficult to isolate but do grow on cell-free media (24). The mycoplasma species that we are concerned with as poultry pathogens are *M. gallisepticum* (MG), M synoviae (MS), M meleagridis (MM), and M iowae (M.I) (24). There is a growing body of evidence that not all members of a mycoplasma species are the same (3, 30). Dr. Stan Kleven has used the example of a normal distribution or bell-shaped curve to illustrate the potential range of key attributes of strains such as pathogenicity, immunogenicity and ability to grow in culture. The majority of strains have been relatively easy to identify, either by clinical disease or routine diagnostic tests. However, there are strains that may be difficult to recognize because they are less pathogenic, less immunogenic, and may even be difficult to culture.

DIAGNOSTIC TESTS

Diagnostic tests for avian mycoplasmosis can be divided into two categories, 1) antibody detection or serology, and 2) organism detection. Serologic tests commonly used include: serum plate agglutination (SPA), hemagglutination inhibition (HI), and enzyme-linked immunosorbent assay (ELISA) (14). SPA tests are highly sensitive, detect antibodies made early in response to infection, are relatively inexpensive and easy to perform, and very rapid. The major disadvantage of these tests is lack of specificity, i.e. false positive results are common. HI tests are highly specific (too specific?) and have been used to confirm SPA results. HI tests are not very sensitive, they detect antibodies 2 to 4 weeks (or longer) post-infection. There is no commercial source for reagents, these tests require a moderate to high level of technical time and skill, and a test time of 1 to 1.5 days. ELISAs have sensitivity and specificity somewhere between SPA and HI tests. Commercial kits are available, and they are moderately expensive and labor intensive. Test time is approximately 4 hr., the results are read by spectrophotometer, and titers calculated by computer. ELISAs are especially useful for high volume testing due to automation of steps and excellent data handling ability of the associated computer software.

Organism detection tests include: culture and identification of the organism, and polymerase chain reaction (PCR)-based tests which detect DNA of the organism (14). Culture and identification of mycoplasma organisms has been the Gold Standard of diagnosis. This method can be very sensitive, theoretically requiring very few organisms, but they do have to be viable and able to grow in the media provided. Methods of identification, such as immunofluorescence, can distinguish among species, but not strains within a given species. The antisera used for identification are not commercially available, and this method requires moderate technical skill and equipment. The major disadvantage of culture is the time that it takes to obtain results, which

could range from 3 to 30 days. Polymerase chain reaction (PCR)-based tests can be highly sensitive (detecting DNA from as few as 100 organisms, living or dead) and specific. Ideally, these tests are not affected by contamination with other organisms. Another major advantage of PCR over culture is the test time; 1.5 days for results, negative or positive. There are commercial kits for MG, and MS; and various laboratory tests for MG, MS. MM, and MI (18). PCR requires high technical skill, moderate technician time, and expensive specialized equipment, all of which makes the per test cost moderate to high.

A PCR-based test using random rather than specific primers is termed random amplification of polymorphic DNA (RAPD). RAPD is a PCR-based method of DNA fingerprinting that can be used for subspecies or strain identification of MG, MS, MM and MI (4, 5, 10, 20). Subspecies or strain identification gives us the ability to confirm the identities of reference strains. These are strains that are used by researchers for experimental purposes or as reagents in diagnostic tests. We can also use this capability to identify vaccine strains, and distinguish them from field isolates. This ability is especially important in the case of MG for which three different live vaccine strains are available, and for MS which has one. Strain identification can also be used for outbreak investigation: for mapping the range of a given outbreak, tracking its spread, and possibly identifying its origin.

In the very near future we may see the development and more common use of multiplex-type PCR tests. These tests will be based on the use of several organism-specific primers in a single test reaction, so that, for example, a tracheal swab from a turkey could be tested for MG, MS, and MM simultaneously (28). In fact this can already be accomplished; however, the PCR products still have to be separated by gel electrophoresis to be identified. The real innovations are going to come with improved and easier methods to detect specific PCR products. This technology is already becoming available, and will allow for PCR reactions be done in a multiple well format (like a 96 well microtiter plate) for high volume testing. Multiple PCR products will be detected directly in each well, without gel electrophoresis, e.g., using a luminescence spectrometer.

PREVENTION STRATEGIES AND VACCINATION

Strategies to reduce the adverse impact of mycoplasma infections in commercial poultry include, 1) surveillance and eradication programs, 2) use of antimicrobials, and 3) vaccinations (24). Surveillance and eradication programs, such as that administered by the National Poultry Improvement Plan (NPIP), have produced enormous benefits where they have been successfully implemented. These programs rely on establishing mycoplasma-free primary breeder flocks, and then maintaining primary and multiplier breeder flocks free of infection by biosecurity under surveillance by serology. When outbreaks occur, flocks are quarantined and may be depopulated. Generally, this program has been very effective. However, in spite of continued efforts at mycoplasma eradication in the US and other highly developed poultry industries, endemically infected flocks remain (multi-age layer complexes) and outbreaks in other poultry continue to occur. Therefore, where mycoplasma outbreaks continue to occur regardless of eradication programs, or where these programs cannot be implemented, there is increasing interest in new paradigms for mycoplasma control which may include the use of vaccines (13.16.29).

Changes in the poultry industry have contributed to the challenges faced by surveillance and eradication programs. For example, in many areas we see increased densities of poultry and close

proximity of various poultry types. Some broiler and turkey producers have moved toward more multiple-age, continuous production systems, with decreased down times between placements. Additionally, good management practices and biosecurity have not been optimally and consistently enforced. So at the commercial (meat) level there are many examples of broiler and turkey production becoming more like layer complexes. Therefore, factors contributing to the re-evaluation of the role of mycoplasma vaccines include, 1) changing production systems, 2) failure of surveillance and eradication programs in some area, 3) the availability of new vaccines, and 4) the possibility that vaccination could be used as a step toward eradication (13, 16, 29).

An ideal *Mycoplasma gallisepticum* (MG) vaccine should be safe and effective for all types of poultry. It should be capable of displacing field strains, but should not persist when vaccination stops, thereby allowing a return to MG-free status. And there should be vaccine-specific detection methods, i.e. to easily identify vaccinated flocks by detection of the organism or antibody (serology). Unfortunately, none of the available MG vaccines meet all of these criteria.

F strain-vaccinated pullets do better than unvaccinated pullets when both are placed in MG-infected laying complexes, but not as well if placed in MG-free laying complexes (1, 2, 27). Continued use of F strain may displace more virulent field strains of MG (15, 17). However, there is also evidence that F strain persists in laying complexes even after pullet vaccination stops; and F strain is pathogenic for turkeys (19) and to a lesser degree for broilers. It is this last point that has been the most serious problem for the F strain, at least in terms of the broiler and turkey industries.

Given our experience with F strain vaccine in the US, it is appropriate to pose some questions regarding the use of the new vaccines, strains 6/85 and ts-11, which were also developed and tested for use in layers. Primary concerns are for safety and efficacy, both of which need to be determined for various types and ages of poultry. We need to know if we can detect vaccinated or exposed flocks by serology or organism identification. It would be very useful to know if these vaccines could displace field strains of MG from infected complexes, or F strain from vaccinated complexes (where applicable); and whether they will persist in a complex if vaccination was stopped. Finally, what are the regulatory or marketing considerations impacted by vaccination?

In the US, 6/85 and ts-11 vaccines were both licensed for use in layer pullets (29). They both have a record of low virulence in layers, which attests to their safety in this type of poultry. There is much less knowledge of their safety in other poultry, especially under field conditions. Another aspect of safety is transmissibility, do these vaccines remain only in vaccinated flocks, or can they be transmitted to 'non-target' poultry. Under experimental conditions, transmissibility of both vaccines from layers appears to be negligible: ts-11 does persist in vaccinates but only transmits to birds in direct contact: 6/85 is difficult to recover from vaccinates and is apparently even less transmissible (15, 23, 29). But would the same be true under field conditions and for other types of vaccinated poultry?

The same types of questions need to be asked in terms of vaccine efficacy for other types of poultry, especially under field conditions. Regarding detection of vaccinated flocks, data relate to layers and indicate that 6/85 does not induce a detectable antibody response while ts-11 does (23, 29), but there is no vaccine-specific serologic test for either. Furthermore, neither strain has a pre-determined or designed vaccine-specific marker for organism identification. They can be differentiated by RAPD (4, 10, 20), but this requires growth and isolation of the organism in pure culture which can be difficult.

Some information has been developed on ability of the various MG vaccines to displace other strains from layers (15, 17). In pen studies, F strain displaced challenge strains better than 6/85 or ts-11; and in field trials, ts- 11 displaced F strain. This suggests that in layers, it may be possible to eradicate MG by progressive vaccination, using F strain to displace the field strain, followed by ts-11 to displace F strain, and finally to stop vaccination and achieve MG-free status. This concept needs to be proved in commercial layer complexes, and the question asked could this approach work for other poultry?

There are still critical questions that need to be answered before initiating use of live MG vaccines. Of primary concern is the potential for virulence in non-target poultry, especially under field conditions where there may be multiple stresses and interactions that are not duplicated under controlled experimental conditions. With live vaccines, there is also a concern for reversion to or acquisition of virulence. And finally, there is the possibility that vaccines may lose effectiveness, perhaps due to the evolution of new or variant field strains.

In the US it is clear that various segments of the poultry industry are open to new paradigms for mycoplasma control, and that vaccination may have a role to play. It seems equally clear that programs will have to be carefully crafted and tailored to regional circumstances.

MYCOPLASMOSIS IN SONGBIRDS

Reports of house finches (*Carpodacus mexicanus*) with conjunctivitis were first made in February 1994 (6, 21). Since that time, numerous affected house finches have been observed at feeders or submitted to wildlife care facilities in the Middle Atlantic and Southeastern regions of the US (7, 11, 12, 20, 25, 26). Clinical signs and gross lesions were characterized by mild to severe unilateral or bilateral conjunctival swelling with serous to mucopurulent drainage and nasal exudate. Typical microscopic lesions in affected house finches consisted of chronic lymphoplasmacytic conjunctivitis, rhinitis, and sinusitis.

Notably slow-growing mycoplasmas (mean incubation time = 25 days) were isolated from lesions in clinically affected birds and identified as MG by direct immunofluorescence (20.21). These findings suggested that MG was the likely etiology for this epidemic of conjunctivitis in house finches. MG was also isolated from a blue jay (*Cyanocitta cristata*) that developed conjunctivitis after being housed in a cage previously occupied by affected house finches. This observation suggested that house finches infected with MG may be capable of transmitting the infection horizontally, and that avian species other than house finches are susceptible to infection and disease. More recently conjunctivitis was observed in American goldfinches (*Carduelis tristis*) and isolates of MG have been made from this species. This finding suggested that the infection could be transmitted naturally to additional host species.

MG is a well-known cause of economically important diseases of domesticated chickens and turkeys worldwide, most notably of chronic respiratory disease of chickens and infectious sinusitis of turkeys. Although MG is a reported pathogen for other gallinaceous birds, and has been isolated from ducks and geese, it has not been considered a naturally occurring pathogen of wild birds including songbirds.

In order to examine the epidemiological relationships among MG isolates from this epidemic, and compare them with vaccine strains and isolates from poultry, we employed random amplification of polymorphic DNA (RAPD), a polymerase chain reaction (PCR)-based method of DNA fingerprinting. RAPD analyses indicated the following results and conclusions

(20). MG isolates from songbirds (house finches, goldfinches, and blue jay) had essentially identical RAPD banding patterns. Therefore, the epidemic in songbirds is caused by the same strain of MG suggesting a single point source for this outbreak, probably first involving house finches and more recently goldfinches (infection in the blue jay was apparently acquired while the bird was in captivity). The MG "finch isolate" RAPD banding patterns differed from RAPD patterns of reference strains, vaccine strains, and isolates from commercial poultry. Therefore, the epidemic of MG in songbirds is not caused by the vaccine strains, and has not been linked to the reference strains or poultry isolates tested. Presently, there is no evidence that MG infection has been shared between songbirds and commercial poultry.

In 1994, northern mockingbirds (*Mimus polyglottos*) and blue jays (*Cyanocitta cristata*) in a Florida wildlife care facility developed clinical signs and gross lesions suggestive of the ongoing outbreak of MG conjunctivitis in house finches and American goldfinches (22). Mycoplasmal organisms were cultured from conjunctival/corneal swabs of birds with sinusitis, conjunctivitis, and/or epiphora; but these isolates could not be identified with the available test reagents. However, in 1996-97 a new species, *M. sturni*, isolated in Connecticut from a European starling (*Sturnus vulgaris*) with bilateral conjunctivitis, was described (8, 9). This finding suggested the possibility that the previously unidentified mycoplasmas from Florida northern mockingbirds and blue jays might be *M. sturni*. In November 1996, these isolates were removed from archival storage, cultured on agar, and tested by indirect immunofluorescence with antiserum prepared to *M. sturni*. All of the isolates tested were identified as *M. sturni* (22). Therefore, *M. sturni* as well as MG should be considered in the differential diagnosis of songbirds with conjunctivitis.

The significance of these emerging diseases in songbird populations, and potential impact to other avian populations, including poultry, remains to be determined. However, the emergence of these avian diseases provides a strong reminder that it is important to maintain the highest possible standards of biosecurity for commercial poultry flocks.

REFERENCES

1. Carpenter, T. E., E. T. Mallinson, K. F. Miller, R. F. Gentry, and L. D. Schwartz.

Vaccination with F-strain Mycoplasma gallisepticum to reduce production losses in layer chickens.
Avian Dis. 25:404-409. 1981.

2. Carpenter, T. E., K. F. Miller, R. F. Gentry, L. D. Schwartz, and E. T. Mallinson. Control of Mycoplasma gallisepticum in commercial laying chickens using artificial exposure to Connecticut F strain Mycoplasma gallisepticum. Proc. Annual Meeting of the United States Animal Health Association. pp. 364-370. 1979.

3. Dingfelder, R. S., D. H. Ley, J. M. McLaren, and C. Brownie. Experimental infection of turkeys with Mycoplasma gallisepticum of low virulence, transmissibility, and immunogenicity. Avian Dis. 35:910-919. 1991.

4. Fan, H. H., S. H. Kleven, and M. W. Jackwood. Application of polymerase chain reaction with arbitrary primers to strain identification of Mycoplasma gallisepticum. Avian Dis.

39:729-735. 1995.

5. Fan, H. H., S. H. Kleven, and M. W. Jackwood. Studies of intraspecies heterogeneity of mycoplasma synoviae, M. meleagridis, and M. iowae with arbitrarily primed polymerase chain reaction. Avian Dis. 39:766-777. 1995.

6. Fischer, J. Conjunctivitis in house finches. Proc. 98th Annual Meeting of the USAHA. pp. 530-531. 1994.

7. Fischer, J. R., D. B. Stallknecht, P. Luttrell, A. A. Dhondt, and K. A. Converse. Mycoplasmal conjunctivitis in wild songbirds: the spread of a new contagious disease in a mobile host population. Emerg. Infect. Dis. 1:69-72. 1997.

8. Forsyth, M. H., J. G. Tully, T. S. Gorton, L. Hinckley, S. Frasca, Jr., H. J. van Kruiningen, and S.J. Geary. Mycoplasma sturni sp. nov., from the conjunctiva of a European starling (Sturnus vulgaris). Int. J. Syst. Bacteriol. 46:716-719. 1996.

9. Frasca, S., Jr., L. Hinckley, M. H. Forsyth, T. S. Gorton, S. J. Geary, and H. J. Van Kruiningen. Mycoplasmal conjunctivitis in a European starling. J Wildl. Dis. 33:336-339. 1997.

10. Geary, S. J., M. H. Forsyth, S. Aboul Saoud, G. Wang, D. E. Berg, and C. M. Berg. Mycoplasma gallisepticum strain differentiation by arbitrary primer PCR (RAPD) fingerprinting. Mol. Cell. Probes. 8:311-316. 1994.

11. Hartup, B. K., and G. V. Kollias. Field investigation of Mycoplasma gallisepticum infections in house finch (Carpodacus mexicanus) eggs and nestlings. Avian Dis. 43:572-576. 1999.

12. Hartup, B. K., H. O. Mohammed, G. V. Kollias, and A. A. Dhondt. Risk factors associated with mycoplasmal conjunctivitis in house finches. J. Wildl. Dis. 34:28 1-288. 1998.

13. Kleven, S. H. Changing expectations in the control of Mycoplasma gallisepticum. Acta. Vet. Hung. 45:299-305. 1997.

14. Kleven, S. H. Mycoplasmosis. In: A laboratory manual for the isolation and identification of avian pathogens. D. E. Swayne, J. R. Glisson, M. W. Jackwood, J. E. Pearson, and W. M. Reed, eds. American Association of Avian Pathologists, Kennett Square, Penn. pp.74-80. 1998.

15. Kleven, S. H., H. H. Fan, and K. S. Turner. Pen trial studies on the use of live vaccines to displace virulent Mycoplasma gallisepticum in chickens. Avian Dis. 42:300-306. 1998.

16. Kleven, S. H., J. R. Glisson, M. Y. Lin, and F. D. Talkington. Bacterins and vaccines for the control of Mycoplasma gallisepticum. Isr. J. Med. Sci. 20:989-991. 1984.

17. Kleven, S. H., M. I. Khan, and R. Yamamoto. Fingerprinting of Mycoplasma gallisepticum strains isolated from multiple-age layers vaccinated with live F strain. Avian Dis. 34:984-990. 1990.

18. Lauerman, L. H. Mycoplasma PCR assays. In: Nucleic acid amplification assays for diagnosis of animal diseases. L. H. Lauerman, ed. American Association of Veterinary Laboratory Diagnosticians, Turkock, Calif. pp. 4 1-42. 1998.

19. Ley, D. H. A. P. Avakian, and J. B. Berkhoff. Clinical Mycoplasma gallisepticum infection in multiplier breeder and meat turkeys caused by F strain: identification by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, restriction endonuclease analysis, and the polymerase chain reaction. Avian Dis. 37:854-862. 1993.

20. Ley, D. H., J. E. Berkhoff, and S. Levisohn. Molecular epidemiologic investigations of Mycoplasma gallisepticum conjunctivitis in songbirds by random amplified polymorphic DNA analyses. Emerg. Infect. Dis. 3:375-380. 1997.

21. Ley, D. H., J. E. Berkhoff, and J. M. McLaren. Mycoplasma gallisepticum isolated from house finches (Carpodacus mexicanus) with conjunctivitis. Avian Dis. 40:480-483. 1996.

22. Ley, D. H., S. J. Geary, J. E. Berkhoff, J. M. McLaren, and S. Levisohn. Mycoplasma sturni from blue jays and northern mockingbirds with conjunctivitis in Florida. J. Wildl. Dis. 34:403-406. 1998.

23. Ley, D. H., J. M. McLaren, A. M. Miles, H. J. Barnes, S. H. Miller, and G. Franz. Transmissibility of live Mycoplasma gallisepticum vaccine strains ts-11 and 6/85 from vaccinated layer pullets to sentinel poultry. Avian Dis. 41:187-194. 1997.

24. Ley, D. H., and H. W. Yoder, Jr. Mycoplasma gallisepticum infection. In: Diseases of Poultry. B. W. Calnek, H. J. Barnes, C. W. Beard, L.R. McDougald, and Y. M. Saif, eds. Iowa State University Press, Ames, Iowa. pp. 194-207. 1997.

25. Luttrell, M. P., J. R. Fischer, D. E. Stallknecht, and S. H. Kleven. Field investigation of Mycoplasma gallisepticum infections in house finches (Carpodacus mexicanus) from Maryland and Georgia. Avian Dis. 40:335-341. 1996.

26. Luttrell, M. P., D. E. Stallknecht, J. R. Fischer, C. T. Sewell, and S. H. Kleven. Natural Mycoplasma gallisepticum infection in a captive flock of house finches. J. Wildl. Dis. 34:289-296. 1998.

27. Mohammed, H. O., T. E. Carpenter, and R. Yamamoto. Economic impact of Mycoplasma
gallisepticum and M. synoviae in commercial layer flocks. Avian Dis. 31:477-482. 1987.

28. Wang, H., A. A. Fadl and M. I. Khan. Multiplex PCR for avian pathogenic mycoplasmas. Mol. Cell. Probes. 11: 211-216. 1997.

29. Whithear, K. G. Control of avian mycoplasmoses by vaccination. Rev. Sci. Tech. 15:1527-1553. 1996.

30. Yoder, H. W., Jr. A historical account of the diagnosis and characterization of strains of Mycoplasma gallisepticum of low virulence. Avian Dis. 30: 510-518. 1986.

MYOPATHY IN PELICANS ASSOCIATED WITH CONSUMPTION OF RANCID FEED

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Three adult brown pelicans with a 2- to 3-week history of weakness and anorexia, unresponsive to antibiotics, anti-inflammatories, vitamins, and steroids were submitted to the California Veterinary Diagnostic Laboratory System, Fresno Branch. Blood biochemistry analysis showed high levels of AST, CK, and LDH even after treatment. Radiographically leg muscles were radiodense, suggesting that they were calcified. Pallor of thigh and wing muscles with white streaks was the most striking gross change. Histologically, most of the skeletal muscles had various degrees of degeneration, characterized by loss of striation, variation in fiber size, eosinophilia, necrosis, vacuolation, mineralization and proliferation of mononuclear cells. Multifocal myofiber degeneration also was observed in the heart. No significant bacteria or viruses were isolated. Liver analysis for heavy metals, selenium and vitamin E were unremarkable. The normal concentration of vitamin E in the liver of these pelicans probably is related to treatment with vitamin E for one-week prior to presentation for necropsy.

Peroxide levels in feed (capelin) were 69 meq/Kg. The high level of peroxide in feed is consistent with rancidity (normal < / = 10 to 15 meq/Kg). In addition, vitamin E concentration in the feed was much lower (0.5ppm) than the 20 to 30ppm recommended in avian rations (5). Since the feed that these pelicans received was rancid and had high peroxide levels, it is hypothesized that most of the vitamin E in the feed was depleted during storage, in the process of constraining free radical formation (6).

Rancid diets may induce myopathy, poor performance, or even death of the animals due to refusal of feed, presence of toxic products that can decrease the digestibility of nutrients (2), and damage of cell membranes (4). Because of the high concentrations of long-chain poly-unsaturated fatty acids, fish are very susceptible to oxidation during storage. Vitamin E is a natural scavenger antioxidant that is used in the process of inhibition of the formation of lipid-derived oxidation products. Consequently, high dietary intakes of polyunsaturated dietary fats would increase the vitamin E requirement because of their eventual deposition on cell membranes and higher susceptibility to oxidation (6). Nutritional degenerative myopathy due to vitamin E deficiency has been recognized for many years in many animal species, including birds (1, 3).

This case demonstrates that nutritional myopathy may occur when feeding rancid feed. Myopathy may develop by interaction of vitamin E depletion and toxic products in rancid feed. This case also suggests that estimation/determination of peroxides in the feed is important as vitamin E and selenium in the liver can be normal.

REFERENCES

1. Austic, R. E., and M. Scott. Nutritional diseases. In: Diseases of Poultry, 10th ed.

B. W. Calnek, H. J. Barnes, C. W. Beard, L. R. McDougald and Y. M. Saif, eds. Iowa State University Press, Ames, Iowa. pp. 47-73. 1997.

2. Borsting, C. F., R. M. Engberg, K. Jakobsen, S. K. Jensen, and J. O. Andersen. Inclusion of oxidized fish oil in mink diets: 1. The influence on nutrient digestibility and fatty-acid accumulation in tissues. J. Anim. Physiol. Anim. Nut. 72:132-145. 1994.

3. Campbell, G., and R. J. Montali. Myodegeneration in captive brown pelicans attributed to vitamin E deficiency. J. Zoo An. Med. 11:35-40. 1980.

4. Comporti, M. Lipid peroxidation. Biopathological significance. Mol. Asp. Med. 14:199-207. 1993.

5. National Research Council U. S. Subcommittee on Poultry Nutrition. Nutrient requirements of poultry. In: Nutrient requirements of domestic animals. 9th rev. ed. National Academy Press, Washington, D.C. 1994.

6. Rucker, R. B., and J. G. Morris. The vitamins. In: Clinical Biochemistry of Domestic Animals, 5th ed. J. J. Kaneko, J. W. Harvey and M. L. Bruss, eds. Academic Press, San Diego, California. pp. 703-739. 1997.

NEUROLOGICAL SIGNS AND ACUTE DEATHOBSERVED IN A FLOCK OF RING-NECKED PHEASANTS WITH A HISTORY OF UNDIAGNOSED CHRONIC UPPER RESPIRATORY DISEASE

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The threat of a reportable disease found in a poultry flock has significant repercussions on the flock evaluated as well as on the poultry industry as a whole. The adverse effect on poultry trade with the diagnosis of velogenic viscerotropic Newcastle disease virus in a backyard game bird flock in May, 1998, showed that California is not immune from reportable diseases that can adversely effect our industry. A flock of birds showing clinical signs including respiratory signs, neurological signs, or a rapid increase in mortality is grounds for concern. Evaluation of any of these problems is necessary to determine if the case is reportable.

A privately owned flock of approximately 15,000 ring-necked pheasants was evaluated in September, 1999, for an increase in mortality and periorbital swelling. Live birds were submitted to the California Veterinary Diagnostic Laboratory Service (CVDLS) at Davis and were found to have severe chronic sinusitis without a specific infectious agent identified. The owner opted to self-treat the flock with unspecified over-the-counter antibiotics.

At the beginning of October, 1999, the owner of the pheasant flock found approximately 40-60 birds dead in the morning. There were several live birds showing neurological signs including ataxia and presenting obtunded. Blood was observed around the ear region of some of the live and dead birds. The owner submitted a second group of three live birds to CVDLS at Davis for evaluation of the neurological signs. Birds were found to have significant cranial trauma with penetrating puncture wounds through the skull and into the brain. Trauma was most likely from a wild mammal, which had invaded the flock pen.

Initially, the cause of the rapid neurological signs and death in the case were not apparent, but with thorough gross necropsy, it was easily diagnosed as a mammalian attack. Although it would have been preferable to view the facilities where the birds were showing signs, evaluation of the problem was made without going out in the field at the request of the owner. In this case, the respiratory signs and the signs of neurological disease with death were unrelated and there were no grounds for reporting the case.

NEWCASTLE DISEASE: PROTECTION AND IMMUNITY

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INTRODUCTION

Newcastle disease virus (NDV) is a membraned virus with a genome possessing single-stranded, nonsegmented, RNA with negative polarity (3). NDV was previously classified in the virus family Paramxyoviridae and the genus paramxyovirus. In fact, it was once considered the prototype virus for the paramxyovirus genus (4). Currently, NDV is classified into the genus rubulavirus (3) but recent reports suggest this classification may not be appropriate (7).

With only minor variation, NDV isolates are all of the same antigenic type. That is, there is only one serotype of NDV. However, NDV isolates do differ with respect to their pathogenicity and have been differentiated accordingly. Pathotypes of NDV isolates having low, or no, virulence are termed lentogenic; medium pathogenic strains are termed mesogenic; and highly virulent strains are termed velogenic. The genome of NDV codes for 6 viral polypeptides as follows: L protein (polymerase), HN (hemagglutinin-neuraminidase), F (fusion), NP (nucleoprotein), P (phosphorylated nulear protein) and M (matrix). There is a molecular basis to pathogenicity that is related to the amino acid sequence of the F protein. The HN and F protein have been found to be the neutralizing epitopes of NDV with the F protein generating a greater neutralizing response than the HN (3).

HOST RESPONSE TO NDV

When birds are infected with NDV many organ systems may be affected including the respiratory, neurologic, reproductive, and digestive systems. The respiratory tract and the intestinal tract are common sites of NDV replication and are also convenient locations for sampling / swabbing when obtaining samples for virus detection. Birds vaccinated with NDV vaccines subsequently develop neutralizing antibodies and are protected from challenge with virulent virus. The HN and F proteins have been shown to be the viral antigens responsible for inducing neutralizing antibodies (3, 27, 35).

Cell mediated immunity (CMI) in response to vaccination with Newcastle disease (ND) vaccines has been shown to develop earlier than humoral responses (1, 14, 17). It has been reported that there is no correlation with CMI and humoral immunity nor does CMI response correlate with protection (1, 14). However, studies using bursectomized birds in which antibody responses were very low following vaccination with ND vaccines (and presumed to be biologically insignificant), found that the bursectomized vaccinated birds demonstrated CMI responses and were protected from challenge as were nonbursectomized ND vaccinated birds. The protection from challenge was attributed to the antiNDV specific CMI response since both bursectomized and nonbursectomized birds had demonstrable CMI whereas, only the nonbursectomized birds had high levels of antibody (17). Additionally, CMI responses have often been attributed to the protective effect of administering ND vaccines by spray administration to birds with high levels of pre-existing (maternal) antiNDV antibodies. These

seemingly conflicting reports and beliefs concerning CMI and protection prompted our laboratory to initiate studies to more clearly define the roles of humoral immunity, CMI and protection. We began by depleting birds of their immune cells by in ovo injection of cyclophosphamide (28, 29). The cyclophosphamide treated (CY) birds were reared in SPF conditions and by 2 weeks of age had regenerated their T cells but not theft B cells. This allowed a time period where CY birds were T cell positive and B cell negative. The birds were closely monitored for T and B cell responses and were vaccinated with live and inactivated ND vaccines. It was found that birds having antiNDV CMI and antiNDV antibody responses were protected from challenge. However, birds that had positive antiNDV CMI responses without antiNDV antibody were not protected. This strategy employed ND vaccines generated from unaltered virus and used birds that had their immune systems compromised; that is, they were unable to produce antibodies. We also used another strategy in which the birds were not altered (i.e. they were healthy immunocompetent birds) but we chose to alter the NDV. Polypeptides of NDV were subjected to electrophoresis on SDS polyacrylamide (PAGE) gels. The SDS treatment denatured the conformational epitopes of the NDV. These denatured NDV proteins were used as antigens to vaccinate healthy birds. It was found that birds developed antiNDV specific antibody (as determined by western blotting) and CMI responses against these denatured viral proteins. However, the birds vaccinated with the SDS denatured NDV proteins did not develop HI or VN antibodies to NDV and were not protected from challenge even though they had CMI responses comparable to that of birds vaccinated with intact ND vaccine. These studies clearly show that protection is dependent upon the presence of neutralizing antibodies. Furthermore, although CMI responses may play a role in protection, they are not in themselves (i.e. without neutralizing antibody) protective.

The role of antibody has been recognized to be important in protecting progeny from NDV (11, 35). Maternal antibody has been shown to be protective and breeder vaccination programs have been developed to protect the progeny in broiler operations (36). Passive immunity studies have shown that birds that were provided with neutralizing antibodies against the F and HN NDV proteins were afforded protection (27). However, when passively immunized birds were challenged with velogenic NDV they shed virus from their tracheas even though they did not develop clinical disease. In another study conducted in our laboratory, birds with high levels of maternal antibody were vaccinated by the intranasal / intraocular (IN/IO) route. Although these birds did not develop increased sera antibody titers, they were protected when challenged with virulent NDV whereas, unvaccinated birds from the same hatch were not protected from challenge. The protection was attributed to stimulation of local secretory antibody at the mucosal surface. Similar findings have been previously reported (9).

VACCINES AND VACCINATION

Newcastle disease is an attractive disease for vaccine researchers to investigate for several reasons. First, the disease has a good challenge model using velogenic NDV strains that typically produce either mortality or clearly demonstrable signs of clinical disease. Second, antibody responses (i.e. HI and VN) correlate well with protection and are very useful in determining the onset and duration of immunity. Third, there is much known about NDV and ND. For example, the entire genome of NDV has been sequenced and the molecular basis for pathogenicity is well understood. Fourth, there are many NDV isolates, reagents, and laboratory techniques (e.g. antibody determination and virus isolation) which are readily available for use. Fifth, there is

field application, market opportunity and a potential economic incentive if one is successful. Therefore, it is not surprising to find that nearly every type of vaccine, vaccination program and /or immunoprophylaxis strategy has been attempted for ND. Vaccines such as live, inactivated, subunit, complexed, nucleic acid and genetically engineered have been reported.

For a review of live and inactivated ND vaccines please see the latest edition of Diseases of Poultry (3). In addition to new vaccines there have also been advances in vaccine adjuvants used in conjunction with either inactivated or subunit ND vaccines. One study reports achieving efficacy with an inactivated ND vaccine adjuvanted with cholera toxin subunit B administered by the intranasal route (32). There have been reports of immune-complex NDV vaccines being successfully used to induce protective immunity. In one study, live NDV was propagated in embryonating eggs and the harvested allantoic fluid was treated with Triton-X 100 and then combined with polyclonal antiserum. The resulting immune complex subunit ND vaccine was emulsified with oil and administered intramuscularly to chickens. Chickens receiving the immune complexed vaccine generated antibodies and were protected from challenge (23). Nucleic acid vaccines have also been developed for NDV and have been reportedly efficacious under experimental conditions (30).

The recombinant or genetically engineered NDV vaccines have emerged over the past decade. Nearly all these vaccines have expressed the UN and/or the F protein in a viral vector. Vectors have included fowlpox, pigeonpox, retrovirues, baculovirus and herpesvirus of turkeys (HVT) (8, 12, 16, 19-21, 33, 34). One common feature with all the genetically engineered vaccines is the route of administration. Recombinant vaccines have been used successfully in the live bird when they were parenterally injected. Reports on using recombinant fowlpox vectored ND vaccine (rFPNDV) have shown efficacy in birds that were free of preexisting antibodies (5, 6, 8, 18, 22, 33, 34). However, when the rFPNDV vaccine was used in birds with maternal antibody (either fowlpox or NDV) the effect was less than anticipated (13, 33). This has not been the case with recombinant NDV vaccines using HVT (rHVTNDV) as the expression vector (19). Although protection has been achieved using rHVTNDV typically birds that have been vaccinated with rHVTNDV do not develop high serum titers when compared to birds that have been vaccinated with conventional live vaccines. Additionally, birds vaccinated with rHVTNDV shed virus from their tracheas following challenge (26). In ovo vaccines have been of increasing interest within the last few years. Conventional live ND vaccines can be used in two to immunize embryos at day 18 of embryonation (E18). Experiences in our laboratory have shown that live B1 vaccine delivered from 2 to 4 EID50 can immunize hatchlings without inducing mortality in the embryos. However, doses higher than this can cause embryo mortality. Therefore, the range between efficacious and safe is so narrow that it is not feasible to use live conventional vaccines. However, there are a number of reports of ND vaccines that have been successfully used for in ovo vaccination. Live B1 ND vaccines that have been treated with the alkylating agent ethylmethane sulfate have been reported to be efficacious and safe when administered by the in ovo route (2). Inactivated ND vaccines that had been emulsified in oil were reported to be efficacious in ovo if the antigen was delivered properly and in sufficient quantity (3). Recombinant ND vaccines have been reported to be efficacious when administered by the in ovo route. Both rFPVNDV and rHVTNDV have been used successfully (10, 24, 25). The addition of interferon type I or II appears also to have a beneficial effect on in ovo vaccination of rFPVNDV vaccines (15, 24).

Passive immunity has been shown to be an effective way to protect birds and breeder hen vaccination programs have been proposed as an alternative to vaccination programs involving

the progeny (see above). The use of antiNDV serum antibody to passively vaccinate progeny was demonstrated more than 40 years ago (37). A similar report used egg yolk antibody to protect the progeny (38). These early reports were limited in their effectiveness to provide protection by the amount of antibody material needed to immunize the bird and the duration of immunity. Typically, little or no passive protection remained by 4 weeks of age. However, Wills and Luginbuhl (38) elucidated 4 important aspects of passive immunity as follows: 1. Protection could be obtained without the stress of active immunization. 2. Disease would not be perpetuated by the use of live virus vaccines. 3. Parental and induced immunity would augment one another whereas parental immunity has been shown to interfere with active immunization. 4. The use of effective passive immunization would lend itself to a disease eradication program. More recently our laboratory has been involved in passive immunity studies involving ND. In brief, we have obtained either egg yolk or sera antibody from hyperimmunized birds and superconcentrated it. The superconcentrated antibody was administered to SPF chickens either at day of age or in ovo (E18). Birds immunized in this manner were then challenged with virulent NDV. It was found that birds were protected for periods up to 7 - 8 weeks of age. This corresponds to a period that would fall within the growout period of most broilers.

CONCLUSIONS

Newcastle disease is a disease, that for the most part, is well controlled. Most emphasis in vaccination and control programs has been on developing better, more efficacious vaccines, with less vaccine reaction. There are a number of new vaccines and vaccination methods that may be an improvement over the current and/or traditional vaccination programs. However, the economics of vaccines and vaccination programs is of utmost concern and undoubtedly will be the guiding factor for future vaccines and vaccination strategies.

REFERENCES

1. Agrawal, P. K., and D. L. Reynolds. Evaluation of the cell-mediated immune response of chickens vaccinated with Newcastle disease virus as determined by the under-agarose leukocytemigration-inhibition technique. Avian Dis. 35:360-4. 1991.

2. Ahmad, J., and J. M. Sharma. Evaluation of a modified-live virus vaccine administered in ovo to protect chickens against Newcastle disease. Am. J. Vet. Res. 53:1999-2004. 1992.

3. Alexander, D. J. Newcastle Disease and Other Avian Paramyxoviridae Infections. In: Diseases of Poultry, 10th ed. B.W. Calnek, H. John Barnes, C.W. Beard, L.R. McDougald, and Y.M. Saif, eds. Iowa State Press: Ames, IA. pp. 541-570. 1997.

4. Alexander, D.J. Newcastle Disease and Other Paramyxovirus Infections. In: Diseases of Poultry, B.W. Calnek, H.J. Barnes, C. W. Beard, W. M. Reid, and H. W. Yoder, Jr. eds. Iowa State Press: Ames, IA. pp. 496-519. 1991.

5. Boursnell, M. E., P. F. Green, I. I. Campbell, A. Deuter, R. W. Peters, F. M. Tomley, A. C. Samson, P. T. Emmerson, and M. M. Binns. A fowlpox virus vaccine vector with insertion sites

in the terminal repeats: demonstration of its efficacy using the fusion gene of Newcastle disease virus. Vet. Microbiol. 23:305-16. 1990.

6. Boursnell, M. E., P. F. Green, A. C. Samson, J. I. Campbell, A. Deuter, R. W. Peters, N. S. Millar, P. T. Emmerson, and M. M. Binns. A recombinant fowlpox virus expressing the hemagglutinin-neuraminidase gene of Newcastle disease virus (NDV). protects chickens against challenge by NDV. Virology 178:297-300. 1990.

7. de Leeuw, O., and B. Peeters. Complete nucleotide sequence of Newcastle disease virus: evidence for the existence of a new genus within the subfamily Paramyxovirinae. J. Gen. Virol. 80:131-6. 1999.

8. Edbauer, C., R. Weinberg, J. Taylor, A. Rey-Senelonge, J. F. Bouquet, P. Desmettre, and E. Paoletti. Protection of chickens with a recombinant fowlpox virus expressing the Newcastle disease virus hemagglutinin-neuraminidase gene. Virology 179:901-4. 1990.

9. Ewert, D. L., C. S. Eidson, and D. L. Dawe. Factors influencing the appearance of antibody in tracheal washes and serum of young chickens after exposure to Newcastle disease virus. Infect.
Immun. 18:138-45. 1977.

10. Gagic, M., C. A. St Hill, and J. M. Sharma. In ovo vaccination of specific-pathogen-free chickens with vaccines containing multiple agents. Avian Dis. 43:293-301. 1999.

11. Giambrone, J. J., and J. Closser. Effect of breeder vaccination on immunization of progeny against Newcastle disease. Avian Dis. 34:114-119. 1990.

12. Heckert, R.A., J. Riva, S. Cook, J. McMillan and R.D. Schwartz. Onset of protective immunity in chicks after vaccination with a recombinant herpesvirus of turkeys vaccine expressing
Newcastle disease virus fusion and hemagglutinin-neuraminidase antigens. Avian Dis. 40:770-777.
1996.

13. Iritani, Y., S. Aoyama, S. Takigami, Y. Hayashi, R. Ogawa, N. Yanagida, S. Saeki, and K. Kamogawa. Antibody response to Newcastle disease virus (NDV) of recombinant fowlpox virus (FPV)
expressing a hemagglutinin-neuraminidase of NDV into chickens in the presence of antibody to NDV
or FPV. Avian Dis. 35:659-61. 1991.

14. Jayawardane, G. W., and P. B. Spradbrow. Cell-mediated immunity in chickens vaccinated with
the V4 strain of Newcastle disease virus. Vet Microbiol. 46:37-41. 1995.

15. Karaca, K., J.M. Sharma, B. J. Winslow, D. E. Junker, S. Reddy, M. Cochran, and J.

McMillen. Recombinant fowlpox viruses coexpressing chicken type I IFN and Newcastle disease virus
HN and F genes: influence of IFN on protective efficacy and humoral responses of chickens following in ovo or post-hatch administration of recombinant viruses. Vaccine. 16:1496-503. 1998.

16. Letellier, C., A. Burny, and G. Meulemans. Construction of a pigeonpox virus recombinant: expression of the Newcastle disease virus (NDV) fusion glycoprotein and protection of chickens against NDV challenge. Arch Virol. 118:43-56. 1991.

17. Marino, O. C., and R. P. Hanson. Cellular and humoral response of in ovo-bursectomized chickens to experimental challenge with velogenic Newcastle disease virus. Avian Dis. 31:293-301.
1987.

18. McMillen, J. K., M. D. Cochran, D. E. Junker, D. N. Reddy, and D. M. Valencia. The safe and effective use of fowlpox virus as a vector for poultry vaccines. Dev. Biol. Stand. 82:137-45. 1994.

19. Morgan, R. W., J. Gelb, Jr., C. R. Pope, and P. J. Sondermeijer. Efficacy in chickens of a herpesvirus of turkeys recombinant vaccine containing the fusion gene of Newcastle disease virus:
onset of protection and effect of maternal antibodies. Avian Dis. 37:1032-40. 1993.

20. Morgan, R. W., J. Gelb, Jr., C. S. Schreurs, D. Lutticken, J. K. Rosenberger, and P. J. Sondermeijer. Protection of chickens from Newcastle and Marek's diseases with a recombinant herpesvirus of turkeys vaccine expressing the Newcastle disease virus fusion protein. Avian Dis. 36:858-70. 1992.

21. Morrison, T., V. S. Hinshaw, M. Sheerar, A. J. Cooley, D. Brown, C. McQuain, and L. McGinnes. Retroviral expressed hemagglutinin-neuraminidase protein protects chickens from Newcastle
disease virus induced disease. Microb. Pathog. 9:387-96. 1990.

22. Ogawa, R., N. Yanagida, S. Saeki, S. Saito, S. Ohkawa, H. Gotoh, K. Kodama, K. Kamogawa,
K. Sawaguchi, and Y. Iritani. Recombinant fowlpox viruses inducing protective immunity against
Newcastle disease and fowlpox viruses. Vaccine. 8:486-90. 1990.

23. Pokric, B., D. Sladic, S. Juros, and S. Cajavec. Application of the immune complex for immune protection against viral disease. Vaccine. 11:655-9. 1993.

24. Rautenschlein, S., J. M. Sharma, B. J. Winslow, J. McMillen, D. Junker, and M. Cochran. Embryo vaccination of turkeys against Newcastle disease infection with recombinant fowlpox virus

constructs containing interferons as adjuvants. Vaccine. 18:426-433. 1999.

25. Reddy, S. K., J. M. Sharma, J. Ahmad, D. N. Reddy, J. K. McMillen, S. M. Cook, M. A. Wild, and R. D. Schwartz. Protective efficacy of a recombinant herpesvirus of turkeys as an in ovo vaccine against Newcastle and Marek's diseases in specific-pathogen-free chickens. Vaccine. 14:46977. 1996.

26. Reynolds, D., J. McMillen, S. Cook, R. Schwartz, and J. Sharma. A recombinant HVT vaccine expressing Newcastle disease virus antigens protects chicks against a lethal Newcastle disease challenge. In: Proc. 42nd Western Poultry Disease Conference, Sacramento, CA. pp. 40-43. 1993.

27. Reynolds, D. L., and A. D. Maraqa. Protective immunity against Newcastle disease: The role of antibodies specific to Newcastle disease virus polypeptides. Avian Dis. Accepted for publication. 2000.

28. Reynolds, D. L., and A. D. Maraqa. Protective immunity against Newcastle disease: The role of cell-mediated immunity. Avian Dis. Accepted for publication. 2000.

29. Reynolds, D. L., and A. D. Maraqa. A technique for inducing B-cell ablation in chickens by in ovo injection of cyclophosphamide. Avian Dis. 43:367-75. 1999.

30. Sakaguchi, M., H. Nakamura, K. Sonoda, F. Hamada, and K. Hirai. Protection of chickens from Newcastle disease by vaccination with a linear plasmid DNA expressing the F protein of Newcastle disease virus. Vaccine. 14:747-52. 1996.

31. Stone, H., B. Mitchell, and M. Brugh. In ovo vaccination of chicken embryos with experimental Newcastle disease and avian influenza oil-emulsion vaccines. Avian Dis. 41:856-63.
1997.

32. Takada, A., and H. Kida. Protective immune response of chickens against Newcastle disease, induced by the intranasal vaccination with inactivated virus. Vet. Microbiol. 50:17-25. 1996.

33. Taylor, J., L. Christensen, R. Gettig, J. Goebel, J. F. Bouquet, T. R. Mickle, and E. Paoletti. Efficacy of a recombinant fowl pox-based Newcastle disease virus vaccine candidate against velogenic and respiratory challenge. Avian Dis. 40:173-80. 1996.

34. Taylor, J., C. Edbauer, A. Rey-Senelonge, J. F. Bouquet, E. Norton, S. Goebel, P. Desmettre, and E. Paoletti. Newcastle disease virus fusion protein expressed in a fowlpox virus recombinant confers protection in chickens. J. Virol. 64:1441-50. 1990.

35. Umino, Y., T. Kohama, M. Kohase, A. Sugiura, H. D. Klenk, and R. Roil. Protective effect of antibodies to two viral envelope glycoproteins on lethal infection with Newcastle disease virus.
Arch. Virol. 94:97107. 1987.

36. van Eck, J. H. Protection of broilers against Newcastle disease by hyperimmunisation of the dams. Vet. Q. 12:139-45. 1990.

37. Vasington, J. J., N. C. Laffer, A. P. Holst, and H. M. DeVolt. Studies on the protective value of Newcastle-immune serum and gamma globulin against artificially induced Newcastle disease
of chickens. Poult. Sci. 39:1418-1427. 1960.

38. Wills, F. K., and R. E. Luginbuhl. The use of egg yolk for passive immunization of chickens against Newcastle disease. Avian Dis. 7:5-12. 1963.

OBSERVATION OF CONGENITAL ABSENCE OF THE WINGS (WINGLESSNESS) IN ASSOCIATION WITH SCOLIOSIS IN 3 COMMERCIAL LAYING HENS AND A REVIEW OF PREVIOUSLY OBSERVED WINGLESS MUTANTS

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ABSTRACT

Five mutations (wg-1, wg-2, jwg, ws, and lb) known to cause wing reduction or absence in chickens have been reported previously in the literature. One is sex-linked and the others are autosomal. Most of these mutations also exhibit leg abnormalities. Organ malformations are observed in three of the autosomal mutants making them lethal. Three "wingless" pullet hens were recently discovered at a grow-out facility for an egg-production ranch. This wingless defect differed from the previously reported mutations in that all three affected birds possessed scoliosis to varying degrees. The inheritance pattern has not yet been determined.

INTRODUCTION AND REVIEW

The first report of a wingless mutant chicken was in 1942 (20). This mutation designated wg (6) (now referred to as wg-1) was a lethal autosomal recessive produced from sibling matings. In addition to the wings, multiple internal organs were affected. All chicks exhibited atresia of the kidneys and lungs. Many chicks also displayed leg and feather defects. Embryos either died in the shell or failed to hatch even if alive. Unlike the first wingless mutant, which originated solely from single comb White Leghorns, the second mutant wingless (wg-2) strain was derived from Rhode Island Reds, White Leghorns, Dorkings and Jungle Fowl (30). The second mutation also differed from the first in that there was no association with lung atresia, although kidney, leg, and feather abnormalities were again observed. The third wingless mutant (jwg) arose from Light Sussex stock, showed variable leg defects, but showed no abnormalities of the feathers or internal organs (12, 5, 7). The fourth reported wingless mutant (ws) is a non-lethal sex-linked trait with manifestation in females (4, 8, 19). Leg defects occurred but there was no observation of internal organ or feathering defects. The fifth reported mutation, limblessness (lb), was observed in chickens derived from the Rhode Island Reds. The lb mutant is due to an autosomal mutation resulting in the absence of wings and legs distal to the pectoral and pelvic girdles. The chicks possess normal organ development (13). Embryonic mortality is high and survivors are unable to hatch without assistance but if assisted the chicks can live for a number of days. No defects of the spine were noted (11).

CASE REPORT

Three commercial Hyline Brown TM layer pullet hens were identified at a grow-out facility in Northern California as missing their wings when the vaccination crew was administering a pox, avian encephalomyelitis, laryngotracheitis combination vaccine to the flock at 4 weeks of age. This vaccine combination was routinely administered in the wing web requiring handling of individual birds and resulted in the observation of the defects in these pullets. At the time the birds were medically examined at 8 weeks of age, all three appeared alert and active and showed no evidence of maladies other than the absence of their wings. No feather, toe, eye or leg deformities were noted. Gait in all birds was within normal limits. It was noted that when startled or trying to rise from lateral recumbency the birds were less coordinated than flock mates. One of the three birds was slightly smaller than the other two, but it was still within the range anticipated for age and strain.

All birds were radiographed. Hen 1 was the least affected. She showed minimal scoliosis of the mid thoracic spine and partial absence of the wings. The humeri were present but greatly shortened. Distally there was another shortened bone presumed to be the ulna. Hens 2 and 3 were more intensely affected both showing complete absence of the wings. A dorsoventral radiograph of Hen 2 showed the absence of the wings, intense scoliosis towards the left side of the bird in the thoracic region, several abnormally shaped vertebrae (hemivertebrae), and some suggestion of pelvic deformity. A lateral radiograph of Hen 3 demonstrated the absence of wings and the absence of kyphosis or lordosis. The intense scoliosis of Hen 3 could not be appreciated on the lateral radiographic projection.

The smallest bird was sacrificed for postmortem examination and histopathologic tissue exam. After reflection of the skin no residual wing stubs could be found on the chest wall. At the time of postmortem exam the radiographic finding of scoliosis was confirmed. The skeleton was then prepared by removal of tissues to confirm the spinal deformity. Histopathologic examination of the heart, lung, kidney, liver, spleen, pancreas, intestines, and sciatic nerves on the sacrificed bird revealed no abnormalities of any structures examined.

Blood samples were collected from the remaining two pullets. The complete blood counts (CBC), differentials, and serum chemistries were within normal limits for both birds. These birds are currently maintained on a commercial lay ration (Layena, Purina Mills, St. Louis, MO) and have grown normally. They came into lay at 20 weeks of age, as projected in the Commercial Management Guide (Hyline, West Des Moines, IA). This was similar to the rest of the flock. The shells on their eggs appeared normal being smooth, resisting breakage, and visually thick shelled when cracked open.

DISCUSSION

We report here the observation of a new wingless mutation and propose its designation as wb. (The ws notation has already been used; wb then standing for wing and back abnormality.) This mutation was not associated with leg, feather, or organ abnormalities but all affected birds exhibited some degree of scoliosis. The combined defect was observed only in females. In layer flocks it is common to eliminate males just after hatch, therefore, only females were present in this flock, obviating speculations regarding sex linkage.

The finding of scoliosis in these pullets makes this abnormality easily recognizable as different from the previous wingless mutations as none of those mutations exhibited axial skeletal deformities. Speculation regarding the possibility of abnormal hatchery profiles of temperature, humidity, or egg position was entertained because incubation irregularities can result in congenital malformations. No citations in the literature or observations by experts could support this speculation (Gadzinski, P., personal communication, 1999).

Scoliosis is well reported in the chicken but not in conjunction with winglessness (9, 2, 16, 17, 18). Scoliosis is not usually found in association with other defects (16). The scoliosis may have been missed because wingless mutants are often embryonically lethal (20, 21, 3) and scoliosis is often not manifest until sexual maturity (10, 14, 15). Thus, an early lethal wingless mutant with the genetics for scoliosis may have not survived long enough to express the second defect.

The incidence and prevalence of the current wingless-scoliotic mutant is unknown and is likely to remain so. Commercial breeders and grow-out facilities are hesitant to share frequency data.

REFERENCES

1. Cain J. R., and U. K. Abbott. Incubation of avian eggs in an inverted position. Poult. Sci. 50:1223-1226. 1971.

2. Fulton, J. R., B. S. Wenger, E. L. Wenger, and R. D. Crawford. Anatomical defects associated with a feathering mutant (Ottawa naked) in domestic fowl. Teratol. 35:137-145. 1987.

3. Grieshammer, U., G. Minowada., J. M. Pisenti, U. K. Abbot, and G. R. Martin. The chick limbless mutation causes abnormalities in limb bud dorsal-ventral patterning: implications for the mechanism of apical ridge formation. Devel. 12:3851-3861. 1996.

4. Hinchliffe, J. R., and D, A. Ede. Cell death and the development of limb form and skeletal pattern in normal and wingless (ws) chick embryos. J. Embryol. Exp. Morph. 30:753-772. 1973.

5. Hirai, Y. Developmental genetic studies on the wingless mutation in the domestic fowl. Thesis. Tokyo University of Agriculture, Tokyo. 1962 (Cited in Ohuchi H, M. Shibusawa, T. Nakagawa, T. Ohata, H. Yoshioka, Y. Hirai, T. Noho, S. Noji, and N. Kondo. A chick wingless mutation causes abnormality in maintenance of Fgg8 expression in the wing apical ridge, resulting in loss of the dorsoventral boundry. Mech. Devel. 62:3-13. 1997.)

6. Hutt, F. B. Genetics of the fowl. New York: McGraw-Hill. p66. 1949.

7. Kondo, N., Y. Hirai, and Y. Miura. Genetical studies on a wingless mutation in the domestic fowl. In: Bulletin of the Institute for Breeding Research, Tokyo University of Agriculture, Tokyo. No. 2 pp. 1-8. 1961. (Cited in Ohuchi, H., M. Shibusawa, T. Nakagawa, T. Ohata, H.

Yoshioka, Y, Hirai, T. Noho, S. Noji, and N. Kondo. A chick wingless mutation causes abnormality in maintenance of Fgg8 expression in the wing apical ridge, resulting in loss of the dorsoventral boundry. Mech. Devel. 62:3-13. 1997.)

8. Lancaster, F. M. Sex-linked winglessness in the fowl. Heredity (London) 23:257-262. 1968.

9. Landauer, W. Recessive rumplessness of fowl with kyphoscoliosis and supernumerary ribs. Genetics, 30:403-428. 1945.

10. McCarrey, J. R., U. K. Abbott, D. R. Benson, and R. S. Riggins. Genetics of scoliosis in chickens. J. Hered. 72:6-10. 1981.

11. Mochida, J., D. R. Benson, U. K. Abbott, R. B. Rucker. Neuromorphometric changes in the ventral spinal roots in a scoliotic animal. Spine 18:350-355. 1993.

12. Ohuchi, H., M. Shibusawa, T. Nakagawa, T. Ohata, H. Yoshioka, Y. Hirai, T. Noho, S. Noji, and N. A. Kondo. A chick wingless mutation causes abnormality in maintenance of Fgg8 expression in the wing apical ridge, resulting in loss of the dorsoventral boundry. Mech. Devel. 62:3-13. 1997.

13. Prahlad K. V., G. Skala, D. G. Jones, and W. E. Briles. J. of Exp. Zool. 209:427-434. 1979.

14. Riggins, K S., U. K. Abbott, C. R. Ashmore, R. B. Rucker, and J. R. McCarrey. Scolisosis in chickens. J. Bone Joint Surg. 59A:1020-1026. 1977.

15. Rucker, R., W. Opsahl, U. K. Abbott, C. Greve, C. Kenney, and R. Stern. Scoliosis in chickens: a model for the inherited form of adolescent scoliosis. Am. J. Path. 123:585-588. 1986.

16. Taylor, L. W. Inheritance of kyphoscoliosis unassociated with other defects in chickens (Abstr). Poult. Sci. 34:1225. 1995

17. Taylor, L. W. Some conditions apparently not associated with kyphoscoliosis (Abstr). Poult. Sci. 45:1131-1132. 1966.

18. Taylor, L. W. Kyphoscoliosis in a long-term selection experiment with chickens. Avian Dis. 15:376-390. 1971.

19. Pease, M. S. Wingless poultry. J. Hered. 53:109-110. 1962.

20. Waters N. F. and J. H. Bywaters. Lethal embryonic wing mutation in the domestic fowl. J. Hered. 34:213-217. 1942.

21. Zwilling, E. Interaction between limb bud ectoderm and mesoderm in the chick embryo: IV experiments with a wingless mutant. J. Exp. Zool. 132:241-251. 1956.

OBSERVATIONS WITH A NOVEL NEWCASTLE DISEASE STRAIN, C2

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INTRODUCTION

Newcastle disease (ND) is a widespread and highly contagious disease of great economic importance to the poultry industry. Live and inactivated vaccines have been developed to provide protection against the disease. In the United States, only lentogenic live NDV vaccines are used. Within the lentogenic group, there is a considerable variation in virulence. The Hitchner B1 type NDV vaccine strains are most commonly used as a primer at one day of age in the United States and are followed in the field by B1 type, LaSota type, or Clone-30 vaccine strains in broilers and inactivated NDV vaccines in breeders and commercial layers. Infectious bronchitis (IB) vaccine strains are typically administered in combination with the NDV vaccines. Undesirable respiratory vaccinal reactions and systemic stress following the administration of the B1 type vaccine strains at one day of age may occur and have a negative affect on growth rates, livability, susceptibility to secondary bacterial infections such as Escherichia coli, and overall performance in broilers (2, 3).

In response to the problems associated with respiratory reactions, producers currently cut the day of age ND/IB vaccines to reduce the systemic distress in broilers. Vaccine manufacturers have tried for many years to develop a live NDV vaccine that would not induce these negative affects. Recently, a new live lentogenic NDV vaccine strain, the C2 strain, has been developed that does not cause undesirable respiratory reactions and systemic stress. In this presentation, the vaccine developed from the C2 strain will be discussed.

C2 STRAIN BACKGROUND

The NDV C2 strain is classified as a lentogenic strain and is derived from the Hitchner B1 strain. In the hemagglutination-inhibition (HI) test using the monoclonal antibody AVS-1, the C2 strain and the B1 strain react similarly. One of the differences between the C2 strain and B1 strain is the elution time. The C2 strain is a slow eluter (>24 hours), and the B1 strain is a rapid eluter (<7 hours) (1).

The NDV C2 strain is distinct from all other live lentogenic NDV vaccines in that it causes practically no vaccination reaction and systemic distress, yet it induces a good immune response. Using a standardized stress model (2), the C2 and B1 strains were compared (1). The comparison was based on body weight gain, mortality rate and susceptibility to colibacillosis following aerosol application of the vaccines at one day of age and subsequent intratracheal inoculation with virulent *E. coli* at 8 days of age. In that study, the NDV C2 strain induced hardly any reaction even in chickens in which the B1 strain caused severe respiratory distress and high susceptibility to colibacillosis (Tables 1&2).

Table 1. Mean weights and colibacillosis scores following aerosol spray of live NDV strains at one day of age and intra-tracheal inoculation of E. coli at 8 days of age.

Table 2. Mortality following aerosol spray of live NDV strains at one day of age and intra-tracheal inoculation of E. coli at 8 days of age.

In one of the efficacy trials conducted in commercial broilers, the C2 strain was compared with the Clone 30 vaccine strain. Broilers were vaccinated at one day of age by coarse spray and then challenged with the LaSota strain NDV at 28 days of age. Very good immunity was achieved by the C2 strain (90%), which was not significantly different from the immunity obtained following vaccination with the Clone 30 vaccine strain (100%). Using SPF chickens the C2 strain induced 100% protection against challenge with the Texas GB strain NDV.

FIELD TRIALS

In the past, newly developed live respiratory vaccines that have been shown to be safe and efficacious under laboratory conditions have not always performed optimally when used in the field. Therefore, it is essential to carry out proper field trials. Extensive field trials using the C2 strain were carried in more than 20 million broilers throughout the U.S. In these field trials, flocks vaccinated with a full dose of the C2 strain at one day of age were compared with flocks receiving each producer's standard dose of B1 type vaccine. In most of the trials, the C2 and B1 strains were combined with IBV strains.

In the field trials, flocks vaccinated at one day of age with the C2 strain had less vaccination reaction that appeared to clear earlier than that of flocks vaccinated with B1 type vaccines. No significant vaccination reactions following the field boosts were observed in flocks vaccinated with C2. Lower early chick mortality and better or equal performance was also observed in broilers vaccinated with the C2 strain compared to flocks vaccinated with B1 type vaccines at one day of age (Table 3).

Table 3. Summary of field trial results.

The field data clearly demonstrates that the C2 strain is unique and different from the B1 strain in that it induced less respiratory reaction, systemic stress and consequently improved the overall performance. Because the C2 strain does not induce the respiratory reactions seen with the B1 strain, producers do not need to cut the vaccine to reduce undesirable field reactions. This is advantageous, particularly in situations where there are problems with IBV challenge in the field, in that the IB vaccine will not be concomitantly cut when administered in combination with the NDV vaccine at one day of age.

REFERENCE

1. Schrier, C. Mild Newcastle Disease Virus Vaccine. U.S. Patent No. 5,750,111 (Akzo Nobel N.V.).

2. Van Eck, J. H. H., and E. Goren. An Ulster 2C strain-derived Newcastle disease vaccine; vaccinal reaction in comparison with other lentogenic Newcastle disease vaccines. Avian
Pathol, 20:497-507. 1991.

3. Westbury, H. A., G. Parsons, and W. H. Allan. Comparison of the residual virulence of Newcastle disease vaccine strains V4, Hitchner B1, and La Sota. Aust. Vet. J. 61:47-49. 1984.

Table 1. Mean weights and colibacillosis scores following aerosol spray of live NDV strains at one day of age and intra-tracheal inoculation of E. coli at 8 days of age.

VACCINE	NO. BIRDS	MEAN WEIGHT ± s.d [1] (g)			Colibacillosis	
		1 DAY OF AGE	8 DAYS OF AGE	15 DAYS OF AGE	Mean Lesion Score[4]	No. of affected chickens (%)
C2 strain	34	32±3	68±5	123±10	0.7	12 (35)
Hitchner B1	30	32±3	37±58	73±0[3]	7.0[3]	1 (100)[3]
Diluent[2]	30	32±3	70±8	130±14	0.0	0(0)

[1] s.d. = standard deviation

[2] Placebo group exposed to an aerosol of peptone at one day of age and E. coli at 8 days of age.

[3] Value of only one bird.

[4] Colibacillosis lesions were scored in the following organs: thoracic air sac, pericardium and liver. The maximum score per bird was 12.

Table 2. Mortality following aerosol spray of live NDV strains at one day of age and intra-tracheal inoculation of E. coli at 8 days of age.

VACCINE	NO. BIRDS	MORTALITY	
		DAYS 1-8	DAYS 9-15
C2 strain	34	0	0
Hitchner B1	30	19	10
Diluent[2]	30	0	0

Table 3. Summary of field trial results.

Trial[1]	Group[2]	Feed Conversion	% Livability	% Mortality	
				1st Week	2nd Week
1	C2	2.087	93.06	1.26	0.51
1	B1	2.088	91.78	1.84	0.72
1	C2	2.321	88.97	1.64	0.63
1	B1	2.335	88.19	2.26	1.21
2	C2	2.03	95.15	1.23	0.50
2	B1	2.03	94.30	1.16	0.66
2	C2	2.01	94.76	0.97	0.45
2	B1	2.06	93.38	1.18	0.77
3	C2	1.90	95.7	n.a.	0.42
3	B1	1.90	95.4	n.a.	0.46
4	C2	2.05	94.94	1.26	0.80
4	B1	2.10	93.44	1.55	0.86

n.a. – data not available

[1] Each number represents a different producer

[2] Group indicates the NDV vaccine administered at one day of age. In majority of cases the B1 type vaccine was cut anywhere from ¼ to ½ dose.

pH MANIPULATION - APPLICATIONS IN LIVE PRODUCTION

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SUMMARY

Selected literature concerning the effects of pH on bacterial populations and potential applications in live production is reviewed. The creation of conditions contrary to bacterial proliferation may benefit control of new infections and related syndromes. Regulatory constraint and increasing scrutiny of veterinary services to the animal industry may be eased by the implementation of less controversial techniques. Potential synergisms with established and evolving disease control measures exist.

INTRODUCTION

As early as the 1960s, concern over antibiotic resistance and possible links to animal feeding practices was voiced in a textbook concerning food safety (8, 17). A December 21, 1980, newspaper article in the New York Times by Seth King outlined poultry industry practices and made very specific mention of antibiotic usage in feed and the then recent introduction of national legislation to ban sub-therapeutic uses. The issue is far from novel and new. Recently, much attention has again been focused on the subject of resistance of bacteria to antibiotics used in human medicine. The emergence of so-called "Superbugs" in human clinical cases has been postulated to be the result of antibiotic use for growth promotion in animal agriculture. As is often the case, hysteria trumps science - some European concerns have banned the use of specified antibiotic groups in feeds. Very recently, the largest chicken company in the United Kingdom voluntarily decided to curtail the use of antibiotics in poultry production based on "focus group" attitudes on the issue. Added scrutiny in the United States has required prescriptions for antibiotic administration via feed and for some recently introduced antimicrobial products. Other approaches to control bacterial populations may be needed.

Gnotobiosis describes the condition of an animal in a biologically-advantaged environment: no bacteria, viruses, fungi or parasites co-exist. Monogastric animals raised under such conditions have been demonstrated to exhibit superior metabolic performance (growth and feed conversion). As might be suspected, removal of the immunologically unchallenged animal from the protected environment leads to their quick demise due to overwhelming infection. It seems plausible that a "middle ground" exists that fosters adequate immunity development without sacrificing superior production performance.

pH AND ANTIMICROBIAL ACTION

Hydrogen ion is a bare proton in theory; it does not exist as such in water or tissue fluids. Hydrogen binds to water to form hydronium (OH_3^+). Hydrogen reacts with ammonia (from deamination of amino acids) and other bases (bicarbonate and phosphate) as well as plasma proteins. The strength of an acid is determined by how fast a hydrogen ion will dissociate from a

bound state of existence. Definition of pH is "the logarithm of the reciprocal of the hydrogen ion concentration". Acid is defined as "a compound that can react with a base to form a salt" (Webster). Acidity is increased by the greater predominance of H+ ions over hydroxyl (-OH) ions in the media. Every numerical drop in pH value denotes a ten-fold increase in hydrogen ion concentration (pH 5 is 10X more acidic than pH 6, etc.). "Optimum" pH is the acid concentration at which an enzyme or catalyst-mediated chemical reaction occurs most rapidly and completely. Riemann (17) postulated that the antimicrobial mechanism of pH activity may be the hydrogen ion or the toxicity of the undissociated acid or the anion. Mineral acids act mainly through pH effects. Organic acids like formate and acetate have a toxic effect, possibly related to structure (stoichiometry). Non-spore forming bacteria are inhibited or destroyed by pH extremes; sporeformers are countered by interference or arrest of the vegetative phase (growth or log phase). Work by Cohen in 1922 (7) heavily emphasized the importance of pH, time and temperature on disinfection of infected materials. The production of enzymes, toxins and immunity factors (antigens) are pH dependent. Very often, optimum conditions for production of certain biologically active substances (enzymes and toxins) are different from those favoring stability.

Toxin producing fungi are reportedly the only food poisoning organisms of importance at pH <4.3 in culture. Bacteriologic populations require a source of nutrients and acclimatization to a satisfactory temperature range to thrive. The availability of nutrients is often dependent on adequate moisture and proper "reactivity" or pH of the media. As determined by the early work of Louis Pasteur and as contemporary microbiologists in a diagnostic lab can attest, there exists a need to properly adjust the acidity of the culture media. Optimum pH for each organism varies within a relatively small range. Some broad ranges of pH favorable to microbial growth are: molds pH> 1.5 to < 8.5, yeasts pH>2.5 to < 8.0 and bacteria pH from >4.0 to < 7.5 (7). The relatively limited range of pH for bacteria allows an opportunity to control proliferation by less expensive and perhaps less controversial means than systemic or intestinal antimicrobials. Alterations in pH can render nutrients unavailable to growing organisms. Minor changes in pH, especially on the edges of tolerable ranges, can have a detrimental effect and render bacteria unable to multiply. Can facilitating the predominance of certain yeasts and desirable molds allow one to take advantage of culture dynamics and production of certain metabolites of microbial growth? Antibiotics are byproducts of fungal or bacterial metabolism. Antimicrobials are agents that "destroy microbes, prevent their development, or to prevent their pathogenic action" (19). The total effect of any control agent is dependent on concentration and availability to vulnerable sites or structures for interaction.

pH AND ANAEROBES

Reddish and Rettger in 1924 (7) studied effects of pH on anaerobes. They investigated the growth characteristics of spore forming bacteria, at that time considered to be the most fastidious organisms. The optimum range for the species studied was a pH of 7.0 to 7.5; a pH of<6.6 completely inhibited growth with predictable results for spore formation. Vegetative cells would likely fade away with no replacement. In work performed with Bacillus subtilis spores, ensitivity to heat and killing effect was enhanced by acidic conditions (12).

pH AND AEROBES

Reed (16) has characterized *Salmonella* spp. growth requirements. The bacterium can grow in a range of 5 to 46 degree C (35-37 degree C optimum), facultative aerobic conditions, pH > 4.0 and water activity >0.945. High protein or carbohydrate substrates are favored; eggs are ideal nutrient sources. Durant, et al. (4) studied the effect of molting (and subsequent crop environment) on the invasive activity of *Salmonella enteritidis* in Leghorn hens. The research group postulated that prolonged feed withdrawal in forced molting may augment SE virulence. The mechanism for this response was proposed to be by facilitating genetic expression for intestinal invasion associated with elevated crop pH and reductions in the amounts of crop volatile fatty acids ("VFA") in the feed-restricted group. Some 0157:H7 strains of *Escherichia coli* demonstrate the ability to tolerate acidity. British researchers investigating acid tolerant characteristics demonstrated that killing of the *E. coli* could be increased by the incubation at a pH of 3:a 4 log decrease could be obtained in 5 minutes. Habituated and stationary-phase bacteria were able to partially offset the effect of lower cytoplasmic pH. Exponential or log-phase bacteria were most susceptible (6). A study conducted by Boyd Hardin and C.S. Roney (unreferenced) measured the effect of pH on bacteria utilizing small changes in pH (0.2-0.3). Growth could be arrested abruptly with a small drop in pH, much like an end-point determination in an acid-base titration. Their investigation concerned pathogens of poultry-*E. coli*, *Clostridium*, *Salmonella* and *Pasteurella*. Isolates of *Pasteurella* were most sensitive to lowered pH (no growth at <5.7). *Clostridium* spp. were hardier, requiring a pH <4.5 to arrest growth.

PRACTICAL APPLICATIONS

Lactic acid was studied by Bulgarian researchers as an aerosol application in a poultry rearing facility. A 20% solution was dispensed at the rate of 20cc/m3 over a twenty-minute period at three intervals daily. Reportedly, a three-to-four fold decrease in air microbes was attained: "coliforms and moulds" most significantly. A 30 gram/day/bird advantage in the test group was attributed to the lactate treatment. Higher serum protein levels, liver protein content, serum tryptophan and liver glycogen were also noted in the test broilers (13). "Acidified water" (9) has been successfully utilized to "sanitize" swine pancreas harvested from abbatoirs for islet xenotransplantation. The solution is made from a preparation similar to physiological saline and subjecting to electrolysis (anode). The resulting solution has a pH in the 2.5 range and 30 ppm+ chlorine and 10 ppm dissolved oxygen. Some tissue deterioration was detected but deemed acceptable. Iodophors have been shown to be ineffective at neutral and alkaline pH. Activity of iodine against *S. typhimurium* was enhanced when pH = 3.5 was maintained, even in the presence of egg solids (10).

Reductions in bacterial populations may be as beneficial as elimination. Altering the animal's environment to maintain a sub-threshold challenge of bacterial pathogens serves to both stimulate natural immunity and minimize the negative effect of excessive immune challenge. Pope and Cherry (14) studied the effect of sodium bisulfate (Poultry Lifter Treatment) applied on lifter at placement and one week prior to processing. Levels of *E. coli* and *Salmonella* were diminished as evidenced on bird carcass rinses. It is assumed that the treated lifter and environment were less contaminated than untreated houses.

pH AND FOOD-BORNE PATHOGENS

A pathogen modeling program (21) for prediction of growth rates of food-borne pathogens has been developed by the U.S. Department of Agriculture. Variables such as pH, temperature and salinity can be modeled to determine associated risks and help adopt an acceptable formulation or scenario. A table of limiting conditions for growth has been developed for a number of pathogens significant in food poisoning cases. Some of these same organisms factor in poultry health issues:

As may be determined from the data, "salting" alone in the proportions shown would require substantial quantities in the media or litter pack. Acidification and temperature can synergistically enhance killing effect.

In a recent trade journal article (1), Kristen Bell, Catherine Cutter and Susan Sumner were cited for work with hydrogen peroxide. As well as being an effective bactericide for use in the food industry, peroxide is a time-honored product for wound management. The addition of acetic acid reportedly augmented the killing action of the peroxide solution. Acetic acid has been approved for sanitization of beef carcasses since 1982 and has been effective in reducing *E. coli*, *Salmonella* and *Listeria* contamination of carcasses.

Food preservation has been practiced for centuries. A substantial aspect of the food preservation procedure hinges on the production of acids from natural processes or via addition to mixtures. Salting, smoking and candying are examples of long-utilized processes. Often, a combination of processes is employed to effect a sustainable, usable food product (i.e. sauerkraut salting, fermentation and thermal processing of cabbage). Alteration of pH is essential to accomplish the desired result.

Salt is a widely employed and poorly characterized technique for food preservation. The combination of salting and pH has selective action on microbial proliferation and survival. Chemical preservation has been a boon to the modem food industry: enhancement of food quality results in many cases by the inclusion of preservation substances (5). Ascorbic acid (vitamin C) is often used to prevent oxidation (browning) of some fruits. Various sugars, vinegars and other chemicals classified as acidulants are utilized in commercial food preparation. Benzoic acid is a natural component of cranberries; native Americans mixed cranberries, currants or blackberries with lean meat for preservation of hunted food. Some synthetic compounds used for food protection are chloroacetates, propionates, glycols and ionic salts of sulfites/sulfates. Sulfur compounds are extensively used in the sausage industry and in thy curing of meats. Pickling depends on the presence of acid and/or salt. Movement of intracellular fluids out of the substrate to the area of higher osmotic pressure ("drawing") in the preservative solution dehydrates the foodstuff and likewise dessicates other structures possessing a cell wall (bacteria). Sodium and hydrogen may exchange across membranes, facilitated by Na+/K+ "pumps" in order to maintain an ionic or osmotic equilibrium in the media. These principles may often be employable in live production.

pH AND CHEMOTHERAPY AND INTERACTIONS

Research in polymer technology has identified mixtures of terpolymer that interact with temperature and pH to release “packaged” pharmaceutical compounds (15). These polymers may be useful in the delivery of acidifying agents to targeted sites without degradation of chemical integrity by the media. Other applications include disinfectant compounds that would activate

after warming of the litter or during the pelleting process in feed manufacturing. Sensitivity to pH for initiation of activity could be very useful in other applications; acidification of water at "harvest" could be used to facilitate the release of effective but unpalatable compounds for gut sanitation administered via feed.

The term "chemotherapy" is typically applied to the usage of internal pharmaceutical agents. The principles of chemotherapy are applicable to environmental management of disease (witness; disinfectants). Valuation of potential therapeutic interventions can be based on four criteria: characterization of the pathogen, adequate and effective concentrations of the antimicrobial agent(s) for sufficient periods of exposure, maintenance of an effective presence in the face of infection and varying situations, and maximizing host biological defenses (2). A fifth parameter for consideration is often a barrier to opportunity: cost. Synergistic relationships are often exploited in the art of therapy. Combinations of agents and approaches are often more effective than one alone. Adverse interactions are a real possibility in internally administered therapies, as are prolonged clearance times (withdrawal). The coherent intertwining of internal and external means of addressing problems may help to avoid some of these issues.

The considered application of pH mediated disease interventions can be effective in preventing new infections or recurrent exposure and minimize the need for expensive antibiotic therapy and related withdrawal periods. Occurrence of drug-related adversity should also be avoidable. Development of resistant strains of bacteria should be lessened, in part due to the small molecular structure and absence of antigenic moieties. Most pH active compounds are simple molecules comprised of atoms commonly found in biochemical or metabolic processes. Failures of intervention may involve: use of an inappropriate chemical, too short a duration / inadequate concentration of the therapeutic agent, reemergence or overwhelming presence of the disease organism (re-infection), inadequate supportive therapy, or persistence of contributing stress or management factors.

REFERENCES

1. Bjerklie, S. Fizzing away pathogens. In: Meat Processing. pp. 36-41. 1999.

2. Chemotherapeutics Introduction. Merck Veterinary Manual, 8th ed. Merck & Co. Inc., Whitehouse Station, N.J. pp. 1693, 1738-45. 1998

3. Currie, W. B. Structure and function of domestic animals. Butterworths Publishers, Stoneham, Massachusetts. pp. 178-80, 399-401. 1988

4. Durant, J. A., D. B. Corner, J. A. Byrd, L. H. Stanker, and S.C. Ricke. Feed deprivation affects crop environment and modulates Salmonella enteriditis colonization and invasion of Leghorn hens. Appl Microbiol (6K6) 65:1919-23. 1999.

5. Food Preservation. In: Encyclopedia Brittanica, William Benton (Publisher), Chicago, Illinois. Volume 9, pp. 543-51. 1965.

6. Jordan, S. L., J. Glover, L. Malcolm, F. M. Thomson-Carter, I. R. Booth, and

S.F.Park. Augmentation of killing of Escherichia coli 0157 by combinations of lactate, ethanol, and
low pH conditions. Appl Environ Microbiol 65:1308-11. 1999.

7. LaMotte, F. L., W. R. Kenny, and A.B. Reed. Bacteriology, Pathology and Titration Procedures in pH and Its Practical Application. Williams and Wilkins, Baltimore. pp. 191-214. 1932.

8. McWhorter, A. C., M. C. Murrell, and P. R. Edwards, Appl. Microbiol. 11:368. 1963.

9. Miyamoto, M., K. Inoue, Y. Gu, M. Hoki, S.Haji, and H. Ohyanagi. Effectiveness of acidic potential water in preventing infection in islet transplantation. Cell Transplant 8:405-411. 1999.

10. Moats, W. A. Antimicrobial activity of compounds containing active chlorine and iodine in the presence of egg solids. Poult. Sci. 60:1834-1839. 1981.

11. Nouman, T. M., M. M. Amer, M. M. Hamby, and A. M. Darwish. Quality of broilers recovered from the chronic respiratory disease. Vet. Med. J. 34:49-60. 1986.

12. Palop, A., F. J. Sala, and B. Condon. Heat resistance of native and demineralized spores of Bacillus subtilis sporulated at different temperatures. Appl Environ Microbiol 65:1316-1319. 1999

13. Petkov, U., P. Stoianov, V. Tsuchumanski, B. Baikov, and L. Borosova. Effect of aerosol disinfection with lactic acid and its effect on the body of poultry. Vet Med Nauki 12:23-28. 1975.

14. Pope, M. J., and T. E. Cherry. An evaluation of the presence of pathogens on broilers raised on sodium bisulfate (SBS) treated litter. Abstract. Southern Conference on Animal Disease. 1999.

15. Ramkissoon-Gzonokar, C., A. Gutoweka, F. Liu, M. Baudys, and S. W. Kim. Polymer molecular weight alters properties of pH-/temperature sensitive polymeric beads. Pharm. Res. (PHS) 16:819-27. 1999.

16. Reed , G. H. Foodborne illness. Salmonellosis. Dairy Food Environ. Sanit. 13:706. 1993.

17. Riemann, H. Food Processing and Preservation Effects. In: Food Borne Infections and Intoxications, Academic Press, New York. pp. 515-24. 1969.

18. Suhkara, G., C. B. Navarre, and U. B. Kompella. Influence of pH and temperature on kinetics of ceftiofur degradation in aqueous solutions. J. Pharm. Pharmacol. 51:249-255. 1999.

19. Stedman's Medical Dictionary, 23rd., Williams and Wilkins Company, Baltimore, 1976.

20. Thompson, J. L., and M. Hinton. Antibacterial activity of formic and propionic acids in the diet of hens on salmonellas in the crop. Brit. Poult. Sci. 38:59-65. 1997.

21. Fish and Fishery Products - Hazards and Controls Guide. U. S. Food & Drug Administration, Center for Food Safety and Applied Nutrition. Appendix 4 in bacterial pathogen growth. January 1998.

Pathogen	Min Aw	Min pH	Max pH	Max % Salt	Min Temp	Max Temp
Campylobacter jejuni	0.99	4.9	9.5	1.5	86	113
Clostridium perfringens	0.93	5.0	9.0	7.0	50	126
Clostridium botulinum	0.93-0.97	4.6-5.0	9.0	5.0-10.0	38	118
Escherichia coli	0.95	4.0	9.0	6.5	44	121
Salmonella	0.94	3.7	9.5	8.0	41	115
Staphylococcus aureus	0.83	4.0	10.0	25.0	44	122

Adapted from Appendix 4 (http://vm.cfsan.fda.gov/~dms/haccp-zw.html)

POULTRY MANAGEMENT AND WELFARE ISSUES

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This topic was discussed at a symposium held in conjunction with the AAAP meeting in New Orleans, LA, in July, 1999. At the symposium Dr. Henry Classen listed five freedoms used to define animal welfare standards of production:

1. Freedom from hunger and thirst
2. Freedom from thermal and physical discomfort
3. Freedom from pain, injury and disease
4. Freedom from fear and distress
5. Freedom to exercise most normal patterns of behavior

A video, "Chickens Under Contract", to be presented at the current meeting, covers item #3. The video is descriptive and shows the need for training and supervision to reduce pain, injury and disease. Other sources of resource materials include:

1. Animal Industry Foundation
P.O. Box 9522
Arlington, VA 22209-0522

2. Farm Animal Welfare Council
Government Buildings
Hook Rise South
Tolworth, Surbiton
Surrey KT67NF (U.K.)

3. University of California - Cooperative Extension
Davis, CA 95616
(Animal Care Series)

4. U.S. Department of Agriculture
Cooperative State Research, Education &
Extension Service (CSREES)/PAS
901 D Street SW
Room 842, Aerospace Center MS220
Washington, DC 20250-2220
rreynnells@reeusda.gov
http://www.reeusda.gov/pas

POULTRY VACCINES "USE AND ABUSE"

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SUMMARY

Billions of doses of live vaccine are administered to US breeder, broiler, commercial layer and turkey flocks each year representing a wide range of antigens which effectively prevent disease and contribute to the profitability of the industry. To quote Dr. C. Beard "most birds are vaccinated - are they immunize?" Abuse resulting in suboptimal protection can occur at all levels and can include:

Poor antigen choices Poor delivery
Inappropriate equipment
Lack of understanding of proportioners and water systems
Incorrect route of administration
Dilution of antigens
Reduction or dropping of antigens in "summer program"
Inadequate staff training
Inadequate "downtime" between flocks Poor biosecurity
High poultry density
Vaccinator compensation based on number of birds vaccinated per hour (piecework) with no connection to accuracy of administration.

INTRODUCTION

Poultry vaccination resorts to many different methods of use for both live or inactivated vaccines prepared from selected vaccinal strains. Vaccination aims at developing protection before the birds are in contact with field infectious agents. The decision to vaccinate must always be made to prevent against a potential and future risk. Unfortunately, the above chronology is far from being systematic in poultry farming. There may be a true race between vaccination and field infection. The best known examples in this respect are: Marek's disease, infectious bronchitis (IB), Pneumovirus infection, and infectious bursal disease (IBD). This requires careful scheduling of vaccination in both breeders and progeny. Vaccination methods recommended aim at developing an immune reaction that involves cellular or humoral immunity mechanisms, which most often are associated. Immune protection requires standard vaccination schemes or schedules which meet the user's need not only to decrease mortality in his flocks of poultry but also to increase performance and obtain the best economic yield. The vaccination schedule should comply with prevention against lethal diseases and also those that have an economic significance. The financial consequences due to lower performances can be more severe than transient mortality.

In industrial poultry farming, vaccinations involve thousands, if not tens of thousands, of birds. The proximity of poultry farms can favor the passage of virus from one building to another, which therefore requires precautions when using spreadable vaccinal strains, especially infectious laryngotsracheitis (ILT) and avian encephalomyelitis (AE).

VACCINE CHOICES

Poultry vaccines manufactured in accordance with USDA Division of Biologics Requirements represent the state-of-the-art in efficacy and safety. Rigorous tests are performed to demonstrate that live agents are stable under simulated field conditions and demonstrate satisfactory antigenicity to stimulate immunity together with low pathogenicity to prevent adverse reactions. A major choice between vaccination methods is between individual vaccination and mass vaccination. The advantages, disadvantages and use of different vaccines are illustrated in Tables 1 and 2.

EFFICACY

Manufacturers of biologicals are required to perform extensive laboratory and field-testing to ensure both the efficacy and safety of their products. The USDA requires specific label instructions relating to storage, reconstitution and administration. In addition to the labels, supplementary package inserts provide detailed information on the characteristics of the vaccine and procedures and precautions relating to administration and disposal of containers are detailed. It is noted that some live vaccines including ILT and AE will spread from recipients to susceptible contact flocks, especially if transfer of flocks is within two weeks of vaccination or if deficiencies in biosecurity exist. The latter situation is more severe when unauthorized routes of administration are used. Adherence to manufacturer's recommendations will avoid potential problems. Deviation from label instructions is not only not recommended but users have a statutory obligation to comply with USDA approved label and instruction leaflets. Company veterinarians may decide to use their judgement on occasions but consultation with the technical staff of the biological manufacturer is highly recommended. It is the author's experience that on many occasions bad practice may not show up because, on that occasion, field challenge may be absent. Normally if combinations or alternative routes of administration are desired by the customer, attempts may well have been made by manufacturers to meet these desires but the results may have not been up to USDA or company requirements. (examples include: unlicensed multiple respiratory vaccines containing three to four strains of IBV, ILT/IBV/NDV, and Pneumovirus/NDV/IBV). Licensed combination vaccines have been "balanced" to ensure non-interference. Mixing of different products may, in itself, cause such interference.

Table 1. Advantages, disadvantages and indications for use of live vaccine.

LIVE VACCINES

Table 2. Advantages, disadvantages and indications for use of inactivated vaccine.

INACTIVATED VACCINES

Dilution of vaccines may, under field conditions, remove the insurance that is built into the release titer and result in an ineffective product before vaccination is even attempted. Effective immunization programs require constant review to ensure that appropriate strains are administered by methods that optimize protection with minimal stress. Removal of strains in the

summer period may allow aggressive components within mixed populations to establish dominance. New facilities are often staffed by inadequately trained or supervised workers. Detailed field studies have shown that adverse reactions often follow replacement of ventilation or drinking systems. Retrofitting a nipple drinking system in a farm requires a complete evaluation and modification of the method of reconstitution and administration of vaccine by the water route. Failure to modify a vaccination program consistent with new facilities and equipment may result in either under- or over-vaccination with potentially serious consequences.

The factors influencing the response of flocks to vaccines include the type, age and strain of bird, age and route of administration, parental immunity, current health status, nutrition, and the level of environmental stress. Appropriate vaccination programs designed to optimize protection and prevent undesirable reactions require evaluation of the vaccine and host factors in relation to the dynamics of the complex or operation over successive seasons. The proof of programs has to be based on birds in actual specific field conditions due to the vast numbers of birds involved. The routine review of production results should consider the efficacy and response to vaccination. Processing data including condemnation by category or egg quality should be viewed in relation to prevention programs, which incorporate both biosecurity and vaccination.

It is necessary to constantly validate standard vaccination procedures to ensure that vaccines are appropriately stored, reconstituted and administered.

* Vaccine sprayers, proportioners, in ovo and automatic injectors should be frequently calibrated to insure correct rates of delivery. Embrex provides a test pattern for the Inovoject (R) unit. Spray cards are available to confirm that droplet size from aerosol sprayers are consistent with specifications. The rate of delivery from water systems can be measured and the distribution of vaccine can be monitored using dye additives.

* Equipment used to reconstitute and administer vaccines should be examined 24 to 72 hours prior to commencement of each vaccination session for cleanliness. If equipment is transferred from farms, appropriate decontamination is required. Extreme care should be exercised to ensure that all possible disinfectant residues are removed and do not contact live vaccine.

Vaccine inventory should be consistent with the shelf life and use pattern of vaccines in a given complex or operation. Unauthorized substitutions or using expired vaccine is inconsistent with acceptable practice. A rotation system of first in first out should be initiated. Generally, vaccines should be stored under refrigeration at a central facility, frequently a hatchery, or live bird production office. The various products are distributed to farms or issued to vaccination crews by company service personnel.

For efficacy of vaccination, proper handling and administration are essential. Defects in these procedures can include any of the following:

1. Poor water quality (mineral content, pH extremes)
2. Water lines contaminated with bacteria and carrying heavy biofilm loads.
3. Blocked needles, tubing and defective vaccine reservoirs.
4. Use of contaminated diluents.
5. Re-use of containers with viable vaccine residues.
6. Mixing of oil emulsion with aqueous aluminum hydroxide inactivated vaccines.
7. Use of hot water to reconstitute freeze-dried vaccine pellets.

8. Mixing heat labile and time sensitive vaccines for a complete day of vaccinations.
9. Incorrect volumes for reconstitution and dilution.
10. Absence of liquid nitrogen in dewars used to store Marek's and other frozen vaccines.
11. Incorrect spray pressure on nozzles resulting in defective distribution patterns.
12. Too long or too short a time within waterlines.
13. Leaving filters in proportioners used to deliver vaccine.
14. Use of alcohol to flush lines immediately prior to administration of live vaccines.

BIOSECURITY

Biosecurity precautions should be constantly evaluated when using vaccination crews. This should include inspection of vehicles, supplying adequate decontaminated clothing and footwear, specifying procedures to clean, adjust, maintain and calibrate sprayers and injection equipment, and appropriate disposal of packaging, including glass bottles and plastic reservoirs. It is absolutely necessary to completely decontaminate mixing utensils after reconstituting live vaccine to insure that residual virus or the bacterial suspension is not transferred to the subsequent farm or can contaminate a different vaccine. Disposable containers or bags used to administer vaccine should be destroyed by incineration or burial. Flocks should be inspected following vaccination to determine the clinical response, noting the intensity of respiratory or systemic signs. Adverse reactions should be investigated with specific reference to vaccination and recipient factors and environmental conditions.

Vaccination records should include the flock identity, day of administration, the vaccine type, strain and batch number, age of flock, route of administration, and the signature of the responsible vaccinator or crew supervisor. Data should be correlated and stored on a computerized database, which facilitates retrieval for subsequent analysis, forming part of the feedback process.

DISCUSSION

The vaccination of many millions of birds of diverse ages remains a very difficult operation with many potential pitfalls. It is more obvious for mass vaccination by drinking water or spray. Still, the same applies to administration by injection which, repeated thousands of times during a vaccination session, is highly monotonous. Failing precautions and vigilance, deviations occur easily and can quickly, within a few hours, result in a large percentage of badly vaccinated or missed birds. It is the author's experience that 20% "misses", without adequate training and supervision, are quite common.

The objective of vaccination is the administration of the correct dose of vaccinal antigens to a maximum of birds, knowing in advance that it is impossible to immunize 100% of the birds, and this for two essential reasons. There will always be imperfectly immunized birds, even if they have, in theory, received an adequate vaccinal dose. This is due to the biological variation and the general health condition of the birds; in particular chicks which remain fragile during their first days of life; some respond well to vaccination and others do not. There always are some birds which have received an insufficient dose of vaccinal virus, especially by the water route. Trained personnel with sufficient time and suitable equipment are critical to avoid errors in which the results from suboptimal protection may vary from mild clinical problems and down

grades to catastrophic losses. If protection is suboptimal it is clear that on many occasions a significant field challenge may result in a situation that would be, to put it mildly, less than desirable. The licensing of new vaccines is becoming more difficult, time consuming and expensive. We should protect the integrity of the current vaccines. What is the objective? To just "vaccinate" or to "immunize" your flock. To just "vaccinate" will cost more.

Table 1. Advantages, disadvantages and indications for use of live vaccine.

LIVE VACCINES	
Advantages	
-	economical
-	mass vaccination (spray, drinking water)
-	large number of doses in a low volume
-	rapid onset of immunity
-	possible to obtain early local immunity
Disadvantages	
-	risks of adverse vaccinal reactions especially with environmental stress or variable maternal antibody
-	spread of some strains
-	short duration of immunity
-	possible interference by maternally-derived antibodies
-	interference between two viruses with common receptor sites
Indications	
-	economical mass vaccinations
-	early vaccination at a young age to obtain rapid, local and general immunity
-	primary vaccination before booster vaccinations with an oil-adjuvant inactivated vaccine.

Table 2. Advantages, disadvantages and indications for use of inactivated vaccine.

INACTIVATED VACCINES
Advantages
- safe
- nonviable
- absence of vaccinal reaction such as observed with live vaccines
- no spread of vaccinal strains
- protection with extended duration
- possible effective combination of multiple components
- can produce rapidly as autogenous vaccines in emergencies (highly pathogenic avian influenza, Angara disease)
- can be used in conjunction with live vaccines to protect chicks against catastrophic disease (vvNewcastle disease, vvinfectious bursal disease)
Disadvantages
- individual handling of birds
- costly to produce
- large storage volumes
- longer period prior to onset of immunity (especially if priming is inadequate)
- potential lack of secretory or local immunity
Indications
- Essentially intended for booster vaccinations of birds of high economic value (breeders, layers)

PROBLEMS ASSOCIATED WITH THE TECHNICAL SERVICING OF *MYCOPLASMA GALLISEPTICUM* VACCINE (MG TS-11) IN ASIA

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SUMMARY

This paper describes the difficulties associated with the technical servicing of an Australian manufactured live MG TS-11 vaccine following access to markets in Thailand, Japan, South Korea and China. The poultry industry in Asia often lacks infrastructure for vaccine transportation, uses antibiotics routinely for control of many diseases, expects to monitor vaccines by measuring antibody levels and receives low prices for product. Problems encountered in the administration of MG TS-11 vaccine in the face of these difficulties are discussed. The solutions to these problems required close liaison with the company veterinarians or distributors on a case by case basis.

INTRODUCTION

The Australian manufacturers of MG TS-11, Bioproperties (Australia) Pty Limited has provided evidence in its International Registration Dossier of the following safety and efficacy claims:

1. Single dose protection for breeder and layer pullets from 3 weeks of age against field challenge from MG infection
2. Prevention of the adverse respiratory and egg production effects of MG infection
3. Egg production improvement of up to 10 eggs per hen housed over MG infected flocks
4. Blood testing allowing differentiation of vaccine response and field infection
5. Administration by the eye drop for accurate vaccination of each bird
6. Complete safety and maintenance of protection in the presence of other respiratory vaccines

Whilst these claims can often secure a market in many western countries, gaining access to markets in Asia often requires additional effort. Competition from other MG vaccines is significant, but in addition, there is competition from antibiotic manufacturers, limitations to success from husbandry practices and significant price considerations. Distributors often tightly restrict market access and the traditional connections ("quanxi") are often facilitated by bonuses and other forms of inducement to cement relationships. Problems encountered in the marketing and administration of MG TS-11 vaccine in the face of these difficulties is discussed.

TECHNICAL SERVICING OF MG TS-11 IN ASIA

Storage and handling. The administration of live bacterial vaccines has not been commonly practiced in Asia, particularly when supplied as a wet frozen product. Transportation and storage facilities are geared to freeze-dried live viral vaccines and inactivated viral and bacterial vaccines. Hence, how to store and handle MG TS-11 is a frequently asked question.

Transportation to Asia by airfreight has been accomplished in polystyrene containers, well insulated and packed with dry ice. However, the lack of -70 degrees C freezers has necessitated the export of freezers from Australia to service the long-term storage of the product. An attempt to store MG TS-11 in liquid nitrogen was successful but the plastic containers required careful handling due to their brittleness at extreme temperatures. Dry ice is reasonably accessible in Asia. However, on farm storage in domestic freezers (permissible up to four weeks at -20 degrees C) was limited. Farmers were advised that rapid thawing in tepid water was required for maximum potency and that transportation to the farm with wet ice may result in loss of potency due to the slow thawing time. Completion of vaccination within 2 to 3 hours of thawing was the objective and was readily achieved due to the low labour costs. Supply of vaccine within the diluent bottle together with easily inserted droppers as caps greatly facilitated vaccination in Asia.

Age of vaccination. Farming conditions in Asia often encourage early challenge from wild MG due to close proximity of infected flocks. Hence, it is not uncommon to attempt to apply the vaccine at as early an age as possible. Whereas the recommended age for administration is from 6-14 weeks of age, vaccination as early as 2-3 weeks of age is often requested. Farmers often fail to understand that it will take a further 3 weeks before full immunity is developed. Vaccination programs, especially for broiler parents are very crowded with up to 27 separate vaccine administrations being required in some flocks. Choosing a suitable time for administration is not difficult where it can be combined with other bird handling requirements such as weighing or the inoculation of inactivated bacterial vaccines

Serological response. Many Asian poultry companies have developed their own diagnostic laboratories and regularly test for antibody responses following vaccination. However, some diagnosticians fail to understand that MG TS-11 produces a relatively weak serological response compared to field-type MG (I). A poor serological response is often interpreted as poor administration of vaccine or loss of vaccine potency. Variable serological responses, possibly related to epitope switching of the organisms could cause some variability in response to batches of antigens and lead to misinterpretation of results. Convincing diagnosticians that a weak antibody response about 5 weeks post-vaccination is a useful indicator of a strong immune response has proved to be difficult.

Use of antibiotics. The widespread and entrenched use of antibiotics during all stages of production has been the greatest hurdle to adoption of live MG vaccination. Many companies use antibiotics for MG control as well as other bacterial disease such as fowl cholera or fowl typhoid. Withdrawal of these medication programs can leave chickens vulnerable to reinfection. As some antibiotics (e.g. tylosin, tetracyclines, quinolones and spectinomycin) suppress the level of vaccine multiplication in the respiratory tract, withdrawal at least one week prior to vaccination has been recommended. In addition, it is recommended that the use of these mycoplasma-inhibitory antibiotics should be avoided, especially for the month following vaccination and during a period of high field mycoplasma challenge. Following withdrawal of these antibiotics, full protection will return and it is therefore, unnecessary to revaccinate. If antibiotic treatment is essential, it is better to use such antibiotics as amoxycillin, neomycin or bacitracin although they may be less effective against some diseases. It has become necessary to consult closely with company veterinarians to determine where an antibiotic-free window exists to allow vaccination with MG 15-11. When change over from an antibiotic medication program for mycoplasma to a vaccination program is planned, it should be undertaken with care to avoid vaccine suppression.

Biosecurity issues. Whereas the objective of vaccination with MG TS-l1 is to develop said immunity against field MG infection, many Asian farms are located and structured in a manner that allows continual challenge from field MG infection. Strengthening of biosecurity measures has been emphasized to many farmers particularly during rearing and when moving pullet flocks into infected flocks before full immunity from vaccination has been established. Maintaining breeding flocks free of field MG infection through high quality biosecurity programs together with vaccination with MG TS-11 as added insurance should a break in MG biosecurity occur, has been recommended.

Economic benefits. Whilst there are data to indicate that MG TS-11 vaccination can result in the production of up to 26 extra eggs over an infected medicated flock (Y Saito - unpublished), economic benefits have been commonly calculated on an increase of 10 eggs per hen housed from vaccination over that of an MG infected flock. There are clear economic benefits that can be demonstrated in Asia for breeding stock where the value of hatching eggs is relatively high and additional benefits can be derived from savings in medication of progeny and improved broiler quality. However, there is still an attitude that mycoplasma freedom should be achievable at the parent level despite obvious farming practices that make this target unattainable. In commercial egg layers, the farm gate price is often very low and a 15c vaccine cost may only return 45c from the extra eggs. Egg farmers need to be convinced that there arc savings to be made from the reduction in the use of antibiotics. In addition to these economic arguments, distributors expect to receive margins that are comparable to those received from the marketing of antibiotics. This type of price structure places cost pressures on the manufacturer of a vaccine that is regarded by some producers as a significant disease prevention cost.

DISCUSSION

The distribution and adoption of a live mycoplasma vaccine in Asia requires a significant technical servicing input. Specialized knowledge of the stability of the vaccine, immune mechanism, serological response in the face of field MG challenge, antibiotic susceptibility and biosecurity issues are required. Successful implementation of an MG control program based on the use of MG TS-11 vaccine requires careful consultation and planning with the distributor and the farmer. Changes in management and husbandry practices may be required. Economic issues need to be considered in full as a change from a one step vaccination approach combined with upgraded biosecurity requires far more forward planning and quality assurance than the routine use of antibiotics to control MG infection. Fortunately, many Asian poultry enterprises are adopting quality assurance programs that should meet the requirements of undertaking an MG control program involving vaccination with MG IS-11 vaccine.

REFERENCE

1. Scott, P. C., S. F. Markham, A. H. Noormohammadi, and K. G. Whithear. Serological response to laboratory and field vaccination of chickens with two live mycoplasma vaccines, TS-11 (Mycoplasma gallisepticum) and MS-H (Mycoplasma synoviae). Proc. 48th West. Poult. Dis. Conf., Vancouver, Canada. pp. 77-79. 1999.

PROGENY PROTECTION AGAINST INCLUSION BODY HEPATITIS THROUGH MATERNAL IMMUNITY

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SUMMARY

Heavy breeders were vaccinated with a commercial oil emulsion vaccine against inclusion body hepatitis (IBH) / hydropericardium syndrome (HPS) at 18 and 23 weeks of age. The immune response was evaluated by the agar gel precipitin (AGP) test. Serology in breeders was more consistent up to 64 weeks of age with a double administration. Progeny with maternal antibodies were challenged at intervals of 7 days. Immunization of breeders was proved to be highly effective in protecting their progeny against a challenge with a highly virulent IBH virus strain for the first 14 days of life.

INTRODUCTION

IBH / HPS can kill up to 80% of infected broilers in a 2 to 3 week outbreak. The disease can be present as early as 5 days of age. Killed oil emulsion vaccines have proven their efficacy against mortality due to IBH /HPS in broilers vaccinated at 10 days of age and challenged at different ages. The objective of this study was to demonstrate that vaccination of breeders with an oil emulsion vaccine during the growing period can protect progeny against early infection.

MATERIALS AND METHODS

A commercial flock of 18-week-old heavy breeders housed in three separate houses were vaccinated once (group A) or twice (group B) at 18 and 23 weeks of age with 0.5 ml of a commercial oil emulsion vaccine. One house remained unvaccinated as control (group C). Birds were bled every 20 weeks and the agar gel precipitin (AGP) test was performed for IBH / HPS. At 35 weeks of age maternal antibodies were determined and progeny from the unvaccinated breeders (group C) and vaccinated breeders (group A) were challenged at intervals of 7 days. A highly pathogenic strain of IBH / HPS (strain DCV-94) was used with 10 4.0 CLD 50% in 0.2 ml per bird, by the intramuscular route. Birds were observed for 7 days after the challenge.

RESULTS

Breeders that received two oil emulsion vaccines (group B) showed better serological results up to 64 weeks of age than those breeders with a single application (group A) (Table 1).

Table 1. Percentage of antibody-positive breeders by the AGP test.

Maternal antibodies titers showed a good correlation with those of the parent flocks, but declined to almost negative at day 21 (Table 2). Protection against mortality due to challenge was 100% satisfactory in progeny at 1, 7 and 14 days of age, but very poor protection was found

at 21 days of age and later. Group C (control) showed up to 100% mortality for each challenge (Table 3). Protection against mortality was demonstrated even when progeny were 32% seropositive by the AGP test.

Table 2. Percentage of antibody-positive breeders and progeny detected by the AGP test when breeders were 35 weeks of age.

Table 3. Percentage survival of progeny after challenge with a highly pathogenic IBH/HPS strain.

DISCUSSION

The double vaccination program offered a better serology response during the laying period of the breeders. Immunization of breeders proved to be highly effective in protecting their progeny against the challenge with a highly pathogenic IBH / HPS virus strain for the first 14 days of life.

Table 1. Percentage of antibody-positive breeders by the AGP test.

GROUP	18 WEEKS	24 WEEKS	44 WEEKS	64 WEEKS
A	0%	85%	75%	70%
B	0%	100%	100%	96%
C	0%	0%	0%	0%

Table 2. Percentage of antibody-positive breeders and progeny detected by the AGP test when breeders were 35 weeks of age.

		DAYS OF AGE OF PROGENY					
GROUP		1	7	14	21	28	35
A	BREEDER	85%					
	CHICK	75%	52%	32%	12%	1%	0%
B	BREEDER	100%					
	CHICK	95%	85%	54%	13%	1%	0%
C	BREEDER	0%					
	CHICK	0%	0%	0%	0%	0%	0%

Table 3. Percentage survival of progeny after challenge with a highly pathogenic IBH/ HPS strain.

GROUP	DAYS OF AGE OF PROGENY				
	1	7	14	21	28
A	100%	100%	100%	22%	0%
C	0%	0%	0%	0%	0%

DISCUSSION

The double vaccination program offered a better serology response during the laying period of the breeders. Immunization of breeders proved to be highly effective in protecting their progeny against the challenge with a highly pathogenic IBH / HPS virus strain for the first 14 days of life.

QUANTITATION OF THYMIC AND BURSAL LYMPHOCYTES POPULATIONS IN NORMAL AND PEMS AFFECTED TURKEYS

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INTRODUCTION

PEMS is a disease syndrome characterized by stunting, high to very high (spiking) mortality and a collapse of the intestinal immune defense (1). During the occurrence of the syndrome in North and South Carolina, seven clinical types of PEMS were maintained by live bird contact transmission under strict isolation. For this work we selected Type R9/96A, a high mortality type that has remained at all times negative for Turkey Corona Virus (TCV). Earlier contact-exposure studies with other TCV+ PEMS Types (e.g. R27/95E; R3/97-5) indicated that the thymus showed histopathological changes very early after infection. Early changes were also observed in the spleen, although the exact tissue change was difficult to identify by histopathology (2). Other contact exposure experiments pointed to a dysfunction of the macrophage/T-lymphocyte immune defense mechanism (3). These and other observations led to the hypothesis that an agent common to the different types of PEMS alters thymic functions. This results in the deficiency of a thymus-dependent defense function that is important in the immune and/or physiological functions of the intestine.

EXPERIMENTAL DESIGN

Day old turkeys were obtained from a hatchery outside the PEMS affected area (Cuddy Farms, MO). Randomized week-old birds were contact exposed with contact infected (Type R9/96A) or healthy birds. At 2.5-4 days, and 6.5-10 days post-exposure (DPE) two controls and 4 infected turkeys were randomly taken, euthanized, weighed (BW) and the left thymus, bursa and spleen were aseptically removed, organs weighed (OW) and placed in transport medium. Organs were minced and single cell suspensions prepared, using standard methods and the total number of viable cells per organ calculated. Intracellular labeling for apoptosis and labeling for surface immunoglobulin (sIg), CD4 and CD8 were done for flow cytometric analysis.

Apoptosis. The percentage of cells in apoptosis was measured by end labeling with fluorescinated nucleotides of the 3'- OH ends of nucleosomes or "nicks" using terminal deoxynucleotidyl transferase. (TUNEL. Boehringer-Mannheim). Samples and controls were prepared in duplicate. Using a flow cytometer and analytical software (Beckton-Dickinson) 2x 10 4 cells were counted. Gate settings were determined and identical settings were used for all samples of an experiment.

Surface antigen labelling. Labelling for sIg was done using the fluorescinated affinity purified IgG fraction of polyclonal anti-turkey IgG (H+L). Double labeling for CD4 and CD8 was done using fluoresceine labeled monoclonal anti-chicken CD4 and PE red labeled monoclonal anti-chicken CD4 (Southern Biological Assoc., AL). Gate settings were determined to uniformly count CD4 +/CD8, CD8 +/CD4 -, CD4 +/CD8 + and CD4/CD8 - cells. Gate settings were constant for all samples analyzed during an experiment. Thus for each animal the

rate of apoptosis, B-cells and different CD4/CD8 phenotype T-cells were measured for thymus, bursa and spleen.

RESULTS

Live bird contact exposure reproduces the syndrome very well. A major setback, however, is that with this approach the experimenters do not know the quantity and timing of exposure of individual birds. Identical experiments (10 controls 20 contact-exposures) were performed. Of the 28 contact exposed animals that were sampled, 7 showed no signs of PEMS (2 at 4DPE, 4 at 7DPE and one at I4DPE).

Birds 2.5 DPE (Cont =2, Exp = 4)

Thymus. At this early stage no differences were observed. The number of cells/thymus varied widely and % apoptosis ranged from 1.5 to 3.7%. CD4 + cells ranged from 8-21%; CD8 + cells ranged from 15-22% and CD4 +/CD8 + cells were most numerous at 3050%. The CD4 +/CD8 + ratio was always <1.0 except for one exposed bird (CD4 +/CD8 + ratio = 1.3)

Bursa. Total cell numbers/bursa showed no difference as did the rate of apoptosis (2.8 - 5.5%). Ig + cells constituted 75-85% of the bursal cells. CD4 + (0.5%-l.5%), CD8 + (0.7%-2.9%) and CD4 +/CD8 + (0.25 - 1.2%) showed no difference. The CD4 +/CD8 + ratio was 0.8-0.5 and was not different from controls.

Spleen. At this early stage 3 out of 4 exposed birds showed a significant increase in OW/BW ratio (0.06 for cont. and 0.11 for exp). Total number of cells in these birds were 80x10 6 vs. 26x10 6 for controls. The rate of apoptosis was 6.6% (exp) vs. 1.8% (cont), however, these differences were not reflected in splenic cell composition. These were sIg + 25-30%; CD4 +(18-30%) and CD4 +/CD8 +(4-6%).

Birds 4 DPE (Cont =4, Exp =8)

Thymus. Six out of 8 exposed birds had severe decrease in OW/BW ratios and a much lower total number of cells/organ (25-30% of controls). The rate of apoptosis was about 3X higher in these birds (1% in controls vs. 3% in exp.). While in control birds the CD4 +/CD8 + ratio was <1.0, it reached 3.0 in exposed birds. The % of CD4 +/CD8 + double positive cells declined by about 50% in controls and experimentals alike as compared to the % at 2.5 DPE.

Bursa. While 5 of the 8 exposed birds had substantial lower BW (<50%) the OW/BW ratios differed little (0.14) and the rate of apoptosis was not different between exposed and controls (2.2-4.2%). The % of sIg + was around 80% in both groups. The % of CD4 +, CD8 +, CD4 +/CD8 + cells and the CD4 +/CD8 + ratio did not differ between exposed and controls and was similar to the profile obtained at 2.5

DPE.

Spleen. Exposed birds that showed stunting (50% BW) had lower OW, OW/BW ratios and total number of cells/spleen. The rate of apoptosis in exposed birds dropped to 1.3% vs. 5.0% in controls. These rates were quite different from those measured at 2.5 DPE. The % of sIg + cells (35-60%) was not different between exposed and controls but was higher than at 2.5 DPE. No substantial differences were observed in the % and ratio of CD4 +, CD8 +, and CD4 +/CD8 + double positive cells.

Birds 7DPE (Cont =4, Exp =8)

Thymus. Four of the 8 exposed birds showed no or minimal signs of PEMS (2 in each experiment). Affected birds had substantially decreased OW/BW ratios (0.22 vs. 0.13) and much lower total cells/organ (50x10 6 vs. 425x10 6). The rate of apoptosis was much higher in affected thymi (6% exp vs. 1.9% cont). At this time substantial changes were observed in thymocyte phenotypes. CD4 + cells reached 35% in exposed vs. 10% in controls. CD8 + remained constant or decreased resulting in a CD4 +/CD8 + ratio of >2 for exposed vs. <1.0 for controls. CD4 +/CD8 + double positives were much higher in controls than in experimentals. CD4 +/CD8 + double positive cells were high in controls (50%) and low in exposed (l5%).

Bursa. At this time point there is a substantial decrease in OW and total cells/organ. There was an increase in CD8 + cells in affected birds (0.5% cont vs. 15% exp).

Spleen. At 7 DPE there was a substantial decrease in sIg + cells (35% cont vs. 10% exp). There was also a substantial increase in CD4 + cells in the exposed birds (35% exp vs. 17% cont) resulting in a CD4 +/CD8 + ratio of 0.7 for controls vs. 1.8 in exposed.

DISCUSSION

Despite the difficulties associated with contact exposure and the prodigious amount of data generated by flow cytometry, the following sequence is consistent with the observed data. On or before 2 DPE there is a marked increase in spleen cells, and an increased rate of apoptosis. There is no decrease in the % of cell phenotypes measured. It is thus possible that the increased apoptosis takes place in a cell type different from the cells analyzed. On or before 4 DPE substantial changes are observed in the thymus. The rate of apoptosis increases 3x. The CD4 +/CD8 + cell ratio reverses and attains values >1.0. There is a 30% decrease in the CD4 +/CD8 + double positive cells by 7 DPE. The rate of apoptosis remains high (3x) and the CD4 +/CD8 +4 ratio remains >1.0. CD4 +/CD8 + double positive cells remain low in some exposed birds. Although the bursa does not seem affected early after exposure (unlike in TCV + PEMS types) by 7DPE. The functionality of the organ is greatly compromised as reflected by low total sIg + cells and a decrease of sIg + cells in spleen. The data indicate that the CD8 + T cell population is the most affected. It is tempting to speculate that the CD8 + phenotype or a subfraction of the CD4 + - CD8 + double positives is the site of the increased apoptosis. However, the experimental design does not allow such a conclusion. It is likely that a non lymphocytic thymus cell is the target. An improved experimental design is needed to answer this question. Recently, a group of investigators was able to reproduce PEMS with oral administration of a bacteria free filtrate of thymi obtained from PEMS Type R9/96A exposed birds (4). The same investigators later isolated a small virus from such thymi (5). These new findings could facilitate a better experimental design to investigate the early events in thymus and spleen of PEMS affected birds.

REFERENCES

1. Barnes, H. J., and J. S. Guy. Poult Enteritis Mortality Syndrome ("Spiking Mortality ") of Turkeys. In: Diseases of Poultry, 10th ed. B.W. Calnek, H.J. Barnes, C.W. Beard, L.R. McDougald and Y.M Saif, eds. Iowa State University Press; Ames, Iowa. pp 1025-1031, 1997.

2. Barnes, H. J., and J. S. Guy. Sequential pathogenesis study with PEMS TYPE R27/95E. (Personal communication)

3. Heggen, C. L., M. A. Gureshi, F. W. Edens, H. J. Barnes, and G. B. Havenstein. Alterations in the lymphocytic and mononuclear phagocytic systems of turkey poults associated with
exposure to poult enteritis mortality syndrome. Avian Dis. 42:711-720. 1998.

4. Schultz-Cherry, S., D. R. Kapcynski, V. M. Simmons, M. D. Koci, C. Brown, and H. J. Barnes. Identifying agent(s) associated with Poult Enteritis Mortality Syndrome: Importance of the thymus. (Personal communication).

5. Schultz-Cherry, S. Isolation of novel viruses from the thymus of poult-enteritis mortality syndrome infected birds. J.A.V.M.A. 215:1684. 1999.

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QUANTITATIVE RISK ASSESSMENT OF *CAMPYLOBACTER JEJUNI* IN LIVE-POULTRY MARKET

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Despite the high visibility of foodborne outbreaks due to the consumption of contaminated meat and poultry products there exist no accurate estimates of their costs. Published reports have documented that these outbreaks are the major causes of illnesses in the U.S. resulting in estimated annual costs of $7.7 to $8.4 billion (8). There are additional indirect costs to the animal industry associated with recalls of finished food product that are not accounted for in these reports.

Epidemiological studies have demonstrated strong association between the consumption of poultry products and the risk of infection with Campylobacter jejuni (6). Campylobacter jejuni is prevalent in chickens and turkeys (1). In chickens the organism was isolated from 77 (45%) of 170 hens of 19-20 weeks of age, 184 (92%) of 200 of 26-28 weeks of age and 17 (34%) of 50 of 43-46 weeks of age but not from any of their eggs (7). Culture of the organs of 15 hens showed that *C. jejuni* was mainly located in the duodenum, jejunum, ileum, caecum, rectum and cloaca, but was also found in the pharynx, proventriculus, ovary and oviduct of some birds. It was not isolated from the crop. Egg white did not inhibit *C. jejuni* growth in culture. It was concluded that *C. jejuni* is not transmitted through eggs to chicks. This organism has been isolated from several layer flocks in the United States and in countries abroad, including China, Japan, Taiwan, and Australia. *C. jejuni* was isolated from 64 (57%) of 112 broiler flocks (11). Recently, the organism was detected in 67 (74%) of 91 chicken products purchased from 16 retail markets representing 11 franchises in the Minneapolis-St Paul metropolitan area (9). The organism was isolated from the surrounding environment, including; sands, housefly, litters, drinking water containers, water, air, dogs and cats.

It is estimated that the incidence of campylobacteriosis in humans is 2%, which translates into 4,000,000 cases per year with a significant economic consequence (National Food Safety). The mean duration of the disease was reported to be 10 days with 7 lost work days (10). The case-fatality of this disease was estimated to be 1% (10). Thirty percent of the foodborne outbreaks in humans during 1980-1982 that were reported to the Center for Disease Control (CDC) were attributed to the consumption of contaminated poultry products (5).

Although the reported outbreaks of foodborne disease that are caused by *C. jejuni* have different characteristics, there is a clear bimodal seasonality of the disease with peaks in May and October and low incidence in summer (5). The majority of these outbreaks are sporadic in nature and attributed to mishandling or the consumption of undercooked poultry (4, 6). A lesser source of exposure that has been incriminated in these outbreaks is the cross-contamination of foods by raw poultry (2, 6).

The epidemiologic risk analysis has three complementary components: risk identification, risk communication, and risk management. Although the concept of risk analysis has been conceived similarly under different disciplines, e.g., toxicological and environmental (3), the epidemiologic approach differs. The epidemiologic approach not only quantifies the potential risk but also stresses the knowledge regarding the factors which have lead to the introduction,

perpetuation, transmission and control of the hazard in a population. In the process one will learn about both the factors that promote the risk and the factors that modify the potential hazard. Therefore, the epidemiologic risk assessment combines both elements of the risk assessment and the risk management. The risk assessment represents the first step in the risk analysis and deals with hazard identification and exposure assessment.

Our long-term objective is to carry out risk analysis of the hazard associated with the presence of *C. jejuni* in poultry at live-poultry markets in New York State. A first step in the risk analysis is to perform a risk assessment to evaluate the potential hazard this organism poses to human health. The production of safe food is a complex process and the control of food borne contaminants begins at the production unit.

To achieve the stated objective we carried out a risk assessment using a scenario pathway model one of the quantitative risk analysis methodologies. The conceptual framework for the pathway model is presented in Figure 1. This scenario represents a chain of events measured by conditional probabilities (P i). The probability of occurrence of an event is conditional on the occurrence of the previous events. The initial hazard is at the bird level (event 1). The first event in this scenario is that the birds are infected with *C. jejuni* and they have a potential to shed the organism in the environment. If the birds are not infected then there is no risk of shedding in the environment, down arrow (shaded arrow).

However, if the birds are infected they pose a potential hazard to their immediate environment (killing platform) by shedding the organism in their feces (right arrow). Several factors will lead to the survival of the *C. jejuni* in the killing platform. However, if the circumstances are not conducive for the survival, then the organism will die and the killing platform would not pose a risk (right arrow at event 2). If the organism survives in the killing platform it will be transmitted through fomites (utensils, handlers, and other structures in the habitat) and hence, put susceptible birds at risk of infection in the scalding bath (down arrow). If the organism survived the conditions at the scalding bath, the birds in the bath will be contaminated and have a potential to contaminate birds in the defeathering machine. If such scenario holds then the carcasses leaving the live-poultry market would be contaminated with *C. jejuni* and susceptible humans would be at risk of the disease.

Figure 1. Conceptual framework for scenario pathway model for the risk assessment of *C. jejuni* in live-poultry market

Probability values for each event (I). There is a probability associated with each event (1) in the scenario. Estimates for each of these probabilities were obtained from published literature.
The final probability outcome (end-state), risk of presence of *C. jejuni* on finished carcasses, associated with the scenario represents a conditional probability of occurring in all prior events.
The end-state probability can be computed as follows:

Figure 1

P end-state is the final probability outcome, and P 1 represents an estimate of the probability at each event (E 1).

This approach assumes that the probability of each event is completely independent from other events, i.e., the events are mutually exclusive. However, this assumption is not always true

for certain events. For example, there is a high correlation between the presence of the organism in the fomites and in the immediate environment. In the final analysis we adjusted for the degree of dependency using a simulation model in @RISK software (Palisade Corporation, Newfield, New York). Another consideration in the calculation of the P end-state is to capture the potential variability at each P i. Because of the lack of accurate data on estimates of P is, we assumed that these probabilities have triangular distributions. The triangular distribution has three parameters: minimum, most probable, and maximum values. The PDF will be computed as follows:

Figure 2

Estimates for these parameters for each event in the model were obtained from published literature and represented the mode (for most-probable value) and the range (for minimum and maximum values). The variability in P i's was captured by using a range of parameters to simulate the probability distribution function (PDF) for each event in the scenario.

Estimates of the risk in finished carcasses (P end-state). This probability represents the risk of the presence of *C. jejuni* on finished carcasses at the live-poultry market. The calculations of these probabilities for each possible scenario were performed using @RISK software. The analyses and simulations produced a cumulative PDF that reflect the risk of animal's infection from all events of the complete scenario.

Sensitivity analysis. Because of the uncertainty associated with some of the P is several iterations were performed using the Monte Carlo simulations in @RISK software to determine whether the P end-state would be altered if reasonable variations in each of the above parameters were made. These variations were perceived as risk mitigation procedures or hygiene measures.

Our analysis showed that the hazard of the presence of *C. jejuni* in processed carcasses at the live-poultry market depends largely on the initial contamination rate of birds as they arrive in the market. The cumulative probability graph predicted, with 95% confidence, that the risk of carcass contamination is one in a thousand if the prevalence of *C. jejuni* in birds arriving at the market is below 1%. Although our approach is a simulation approach, which favors a worse case scenario, it was evident that strict risk mitigation efforts have significant impact on the hazard of carcass contamination.

To carry out comprehensive risk analysis studies for *C. jejuni* in live-poultry markets requires extensive resources and considerable time. We have chosen the mathematical simulation approach as a first step so that we will be able to reduce the burden on resources and save time. The mathematical approach will allow us to identify significant inputs where data is lacking and setup research priorities. We are in the process of carrying out observational studies to validate this model and provide complementary data for the stakeholders.

REFERENCES

1. Acuff, G.R., C. Vanderzant, M.O. Hanna et al. Prevalence of C. jejuni in turkey carcasses processing and further processing of turkey products. J. of Food Protect. 49:712-717. 1987.

2. Brown, P., D. Kidd, T. Riordan, and R.A Barrell. An outbreak of food-borne Campylobacter jejuni infection and the possible role of cross-contamination. J. Infect. 17:171-176.

1988.

3. Covello, V.T., and M.W. Merkhofer. Risk assessment methods: Approaches for assessing health and environmental risks. Plenum Publishing, New York, 1993.

4. Deming, M.S., R.V. Tauxe, P.A. Blake, S.E. Dixon, B.S. Fowler, T.S. Jones, E.A. Lockamy, C.M. Patton, and R.O. Sikes. Campylobacter enteritis at a university: transmission from eating chicken and from cats. Am J. Epidemiol. 126:526-534. 1987.

5. Finch, M., and P. Blake. Foodborne outbreaks of campylobacteriosis. The United States experience 1990-1982. Am. J. Epidemiol. 122:262-268. 1985.

6. Harns, N.V., N.S. Weiss, and C.S. Nolan. The role of poultry and meats in the etiology of Campylobacter jejuni/coli enteritis. Am. J. Pub. Health 76: 407-411. 1986.

7. Izat, A.L., F.A. Gardner, J.H. Denton, and F.A. Golan, Incidence and level of Campylobacter jejuni in broiler processing. Poult. Sci. 67:1568-1572. 1988.

8. Kvenberg, D.E., and D.L. Archer. Economic impact of colonization control of foodborne diseases. J. Food Technology 41:77-80. 1987.

9. Smith, K.E., J.M. Besser, C.W. Hedberg, F.T. Leano, J.B. Bender, J.H. Wicklund, B.P. Johnson, K.A. Moore, and M.T. Osterholm. Quinoloneresistant Campylobacter jejuni infections in Minnesota, 1992-1998. Investigation Team. N. Engl. J. Med. 340:1525-1532. 1999.

10. Sobel, J, R. Tauxe, A. Reis, et al. The burden of C. jejuni infection: A target for early treatment. 45th Annual Epidemic Intelligence Service (EIS) Conference. Center for Disease Control and Prevention, Atlanta, GA. April 22-26, 1996.

11. van de Giessen, A.W., B.P. Bloemberg, W.S. Ritmeester, and J.J. Tilburg. Epidemiological study on risk factors and risk reducing measures for campylobacter infections in Dutch broiler flocks. Epidemiol. Infect. 117:245-250. 1996.

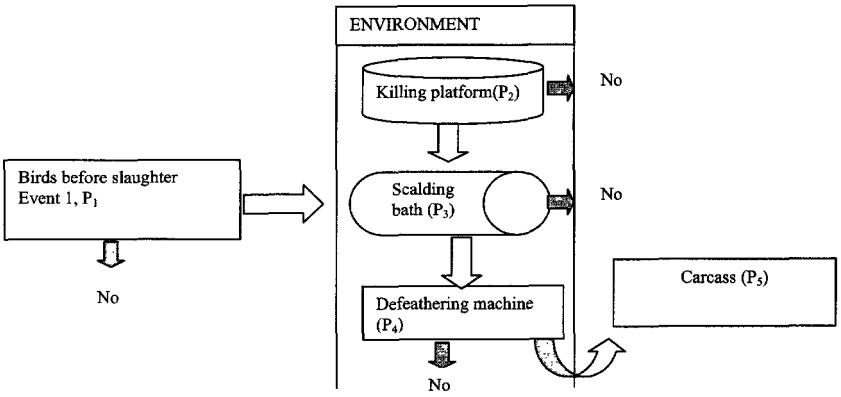

Figure 1. Conceptual framework for scenario pathway model for the risk assessment of *C. jejuni* in live-poultry market

$$P_{end-state} = \prod_{i=1}^{k} P_i$$

$P_{end-state}$ is the final probability outcome, and P_i represents an estimate of the probability at each event (E_i).

$$f(x) = \frac{2(x-a)}{(b-a)(c-a)} \quad \cdots \quad for\ a \leq x \geq b$$

a = minimum, b most-probable, and c = maximum values

RECOMBINANT MAREK'S DISEASE VIRUS-DERIVED CELL LINES AND THE STUDY OF VIRUS REACTIVATION FROM LATENCY

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We have recently described the construction of recombinant Marek's disease viruses and the establishment of cell lines using these viruses (5). Currently, we have established two cell lines which contain versions of the green fluorescent protein (GFP). One cell line, MDCC-UA04, contains a GFP (S65T) cassette regulated by the cytomegalovirus immediate-early promoter (CMVie) inserted at the nonessential US2 locus (2). Like the SV40 promoter driving the expression of lacZ in other recombinant-MDV-derived cell lines (5), we have found that the CMVie promoter-driven expression of GFP is also regulated in a manner similar to MDV lytic genes during latency. Consequently, the induction of fluorescence is a sensitive measure of early events in virus reactivation from latency. Currently, we are studying the surface antigens and associated signal transduction pathways involved in eliciting MDV reactivation from latency.

We now report that GFP expression is induced in UA04 cells not only by iododeoxyuridine (IudR) and bromodeoxyuridine (BrdU), classic inducers of herpesvirus reactivation (3), but that GFP expression is induced in UA04 cells by phorbol ester (TPA), chicken interferon-II (IFN-_-like), and cholera toxin (CT). We have found that TPA-induction is insensitive to inhibitors of: DNA polymerase (Ara-C), protein kinase C (Calphostin C, H7, H89), and ornithine decarboxylase (DFMO). We have also found that the induction of GFP expression by IFN-II can be blocked by pre-treatment of the cells with chicken interferon-I (IFN-a-like). These results support the conclusion of others (6), that MDV latency may be regulated in vivo by IFN-I. IFN-I-treatment did not block induction by TPA, however, suggesting that induction of MDV genome expression in transformed cells may be regulated via several distinct pathways.

We have also established another recombinant-MDV-derived cell line, MDCC-UA20, containing a soluble-modified GFP (smGFP) expression cassette (1). This cassette is also regulated by the CMVie promoter, but is inserted at the site of deletion of the recently-discovered MDV viral interleukin 8 homolog (vIL-8) (4). This vIL-8 gene is encoded within the repeats flanking the unique long region (TRL and IRL) of the genome, hence the smGFP cassette is present in two copies per genome. These cell lines promise to provide insight into the regulation of MDV latency and reactivation.

REFERENCES

1. Davis, S. J., and R. D. Vierstra. Soluble, highly fluorescent variants of green fluorescent protein (GFP) for use in higher plants. Plant Mol. Biol. 36:521-528. 1998.

2. Dienglewicz, R. L., and M. S. Parcells. Establishment of a lymphoblastoid cell line using the mutant MDV containing a green fluorescent protein expression cassette. Acta Virol. 43:106-112. 1999.

3. Dunn, K., and K. Nazerian. Induction of Marek's disease virus antigens by IdUrd in a

chicken lymphoblastoid cell line. Pathol. Biol. (Paris) 25:143-145. 1977.

4. Liu, J.-L., S.-F. Lin, L. Xia, P. Brunovskis, D. Li, I. Davidson, L. F. Lee, and H.-J. Kung. MEQ and v-IL8: cellular genes in disguise? Acta Virol. 43:94-101. 1999.

5. Parcells, M. S., R. L. Dienglewicz, A. S. Anderson, and R. W. Morgan. Recombinant Marek's disease virus (MDV)-derived lymphoblastoid cell lines: regulation of a marker gene within the context of the MDV genome. J. Virol. 73:1362-1373. 1999.

6. Volpini, L. M., B. W. Calnek, B. Sneath, M. J. Sekellick, and P. I. Marcus.
Interferon modulation of Marek's disease virus genome expression in chicken cell lines. Avian Dis. 40:78-87. 1996.

RE-EMERGENCE OF SOME INFECTIOUS DISEASES IN LAYER FLOCKS KEPT UNDER ALTERNATIVE REARING SYSTEMS

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Epidemic diseases, including a wide range of viral, bacterial and parasitic diseases, are of great importance in modem methods of keeping poultry. Successful vaccination programs and management routines have reduced the risk of outbreaks of these diseases in modern egg production. European table egg production is being moved towards non-cage systems by legislation. By year 2012 all traditional cages (non-enriched) will be banned. In Germany, currently, about 90% of commercial layers are kept in cages and several producers have started to produce table eggs in so called alternative rearing systems such as free range and/or perchery systems. In such rearing systems increased health problems and reemergence of some disease conditions were observed.

AVIPOXVIRUS INFECTION

In two layer flocks from different origins and kept on two different farms with free range rearing systems, clinical signs of fowl pox were observed 8 week after placement of the birds on the farms. Birds exhibited nodular lesions on combs, wattles, eyelids and at the corner of the mouth. In addition, diphtheritic lesions on mucous membrane of mouth and pharynx were detected. The signs were accompanied by a drop in egg production and increased mortality. The mortality rates were 5.5% in farm 1 and 16% in fan 2, within 8 weeks after the onset of clinical signs. Examination of tissue from the cutaneous nodules and mucous membrane lesions with electron microscopy (EM) revealed numerous typical pox virion particles. From both farms poxvirus was isolated on the chorioallantoic membrane (CAM) of 11-day-old SPF - embryonated chicken eggs. The isolated virus was identified using the agar gel precipitation test, EM and histopathological examination of CAM. Currently, further identification using monoclonal antibodies is in progress. Most of the poultry industry in Germany is not vaccinated against fowl pox, since the disease has not been observed for many years.

ERYSIPELAS AND HISTOMONIASIS

In the last 25 years no Erysipelas cases were reported in laying hens in Germany. In 1998 on a farm with 43000 laying hens kept in a free range system an increase of mortality accompanied with a drop in egg production was observed at 34 weeks of age. The birds were purchased as 18-week-old pullets from another farm. The birds showed depression, ruffled feathers, somnolence and diarrhoea. Gross lesions consisted of generalized congestion of internal organs, haemorrhage in pericardial fat, in heart muscle and in abdominal fat as well as salpingitis, peritonitis, and enteritis. The liver and spleen were enlarged and showed multiple necrotic foci. Bacteriological examinations of internal organs resulted in isolation of *Erysipelothrix rhusiopathiae* of serotype 1. In addition *Escherichia coli* and *Pasteurella multocida* could be isolated from the ovary and peritoneum. In spite of treatment with penicillin, the mortality reached 35.8% at the end of production period. On the same farm one year later (1999) in a new restocked flock, an outbreak

of erysipelas was observed at 34 weeks of age. The birds were purchased as 17-week-old pullets from another farm and vaccinated at 17 weeks of age against *E. rhusiopathiae* using inactivated vaccine contain serotype 1 and 2.

Four weeks later the same flock again showed increased daily mortality. At autopsy spherical necrotic foci in the liver were detected. No gross lesions in the caeca were noted. Histopathological examination of sections from the liver and caeca stained with hematoxylin and eosin (HE) and periodic acid-shiff stain (PSA) revealed the presence of *Histomonas*. The last available report on Histomonas among pullets in Germany was from 1984 in a flock kept on floor. Currently, in Germany no drugs are allowed to be used for treatment of histomoniasis in food producing animals. The total mortality due to erysipelas and histomoniasis at the end of the laying period reached 50%.

CONCLUSION

The new alternative rearing systems are accompanied by re-emergence of some disease conditions. Infectious diseases represent an important risk in poultry and the consequences of these diseases are an animal welfare concern. More efforts are needed to adapt new prophylactic measures and different approaches to hygiene.

RENAL COCCIDIOSIS IN A COMMERCIAL FLOCK OF GEESE

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The first report of renal coccidiosis in geese was in 1890 in France. The organism was first named *Coccidium truncatum*, but later renamed *Eimeria truncata*. This report describes an endemic of renal coccidiosis in two commercial flocks of geese. Five-to-seven-week-old geese from two commercial flocks were submitted to the laboratory with a history of increased mortality, depression, weakness and decreased weight gain. Expected total mortality at the time of submission was 3 - 4%, but the flocks were experiencing 6 - 10% total mortality. Birds are sold live to a customer who either custom processes them or sells them at a live market.

Necropsy revealed severely enlarged, pale kidneys with severe perirenal hemorrhaging in some birds and severe mottling of the kidneys in other birds. Histologic examination of these kidneys revealed severe interstitial inflammation with infiltration of lymphocytes and heterophils. There were numerous coccidial oocysts in various stages of development, in bundles and scattered throughout the tubules. There were multinucleated giant cells surrounding some of the bundles of oocysts. The organisms were not speciated. In some birds, there was severe thickening of the air sacs with small nodules scattered throughout. On histology, areas of severe fibrinoheterophilic inflammation with centers of eosinophilic cellular debris surrounded by a zone of multinucleated giant cells were found in the air sacs. Within these areas were numerous bacteria and occasional fungal hyphae.

Minimal information is available on the treatment of renal coccidiosis in geese. After consideration of therapeutic agents, it was decided to use amprolium in the drinking water at a rate of 1 gallon of amprolium per 800 gallons of water for 5 days. Follow-up submissions of dead birds were examined to determine response to treatment. Immediately following treatment, there was a decrease in mortality, but submitted dead birds still had renal coccidiosis. One week after treatment, weekly mortality was down to 0.5 - 2.3%/week, with improvement in overall flock appearance. Nonetheless, some of the submitted dead birds still had renal coccidiosis. Two weeks after treatment, weekly mortality was 0.2 - 0.4%/week and the flock appeared normal. However, dead birds still had renal coccidiosis. In 10-week-old geese, one goose had very pale kidneys, while one had grossly normal kidneys. However, a wet smear of both kidneys revealed mild to moderate numbers of coccidial oocysts present. In 12-week-old geese, the kidneys appeared grossly normal, but there were rare coccidia present.

The source of the coccidia is unknown. The geese were grown on a ranch where turkeys were previously grown. The ranch had been vacant for 3 years prior to placement of the geese. These were two of 9 flocks that were presently being grown. It appears that the treatment was beneficial. However, birds that were examined post-treatment appeared to still be infected with coccidia. Nonetheless, mortality decreased significantly and birds appeared normal. There were no reported problems with any of the processed birds.

ROLE OF UTAH STATE UNIVERSITY EXTENSION IN ENVIRONMENTALLY IMPROVING TURKEY HEALTH

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Confined rearing of turkeys is a relatively new practice in Utah. Optimal environmental conditions for raising turkeys in total confinement there have not been clearly documented. To begin establishing ventilation guidelines for the local turkey industry, a two-year study was carried out by Utah State University Cooperative Extension. Funding for the project ($43,514) was obtained through industry, state, and university sources. The objectives of the study were to 1) acquire and quantify baseline environmental quality of totally confined turkey production units in Utah, and 2) apply that information in a prospective manner to improve production efficiency. The first phase was devoted mainly to data acquisition. Twelve volunteer growers were used in the study. A total of 14 brooder and 25 growout buildings belonging to these growers were surveyed at various times of the year. Data collected consisted of ambient temperature, relative humidity and levels of O 2, CO, CO 2, and NH 3. The second phase of the project consisted of establishing benchmark total confinement environmental guidelines by correlating the collected data from phase one with best performance observations. The purpose of establishing benchmarks was to provide the growers with measured and quantified guidelines for confined turkey rearing under Utah conditions. These benchmarks were then used to evaluate the economic benefit to producers raising turkeys at various levels of production and performance.

Results from the brooder buildings showed an oxygen level (in relation to monitoring equipment zeroed to 20.9% O 2 immediately outside the building to be measured) ranging from 19.6% to 20.9% with an average of 20.5%. Carbon monoxide ranged from 0 to 97 ppm, averaging 9 to 15 ppm. Carbon dioxide ranged from 300 to 3425 ppm and averaged 1700 ppm. Brooder NH 3 level generally was found to be 0 to 3 ppm, but occasionally reached levels as high as 15 to 20 ppm in under-ventilated buildings housing turkeys 4 to 5 weeks of age. Relative humidity varied from 15% to 55%, depending on age of flock and ventilation status of the brooder. In growout facilities oxygen levels varied from 20.3% to 20.9%. Carbon monoxide remained below 3 ppm in most growouts. Ammonia levels ranged from 0 and 63 ppm. Levels above 15 ppm were common in growout buildings without exhaust fans. Relative humidity varied from 27% to above 90%. Temperature varied from 40 degree F to 77 degree F.

Early in the study we realized ambient temperature, both in the brooder and growout, varied considerably from grower to grower as well as from flock to flock. Mortality and feed efficiency also varied according to the temperature at which the turkeys were raised. Because of these findings, we felt it important to include environmental temperature guidelines in the project.

After comparing mortality and general flock performance observations, environmental management benchmarks were established (Table 1). These guidelines were then taken to the field to help growers improve management. We worked with various types of growers from small well-managed ranches to larger units with excellent facilities but having potential for better management of those facilities. "Large" vs. "small" and "well-managed" vs. "potentially well-managed" units were classified relative to Utah turkey industry standards.

Table 1. Established environmental benchmarks.

An example of economic yield derived from the field application of these guidelines is illustrated in Table 2. Four documented cases representing Utah grower types from comparatively small to large, and from comparatively well-managed to potentially well-managed are shown. "Net improvement" represents additional income generated from application of the benchmarks compared to previous normal flock management practices. Net improvement also takes into consideration additional production costs incurred, such as increased natural gas use, equipment purchases, and any other incidental expenditures needed to comply with benchmark guidelines.

Table 2. Net economic improvement of four turkey growers using project-generated benchmarks compared to previous management techniques.

Economic improvement resulted from 1) an avenge 5% reduction in mortality from early starvers, poult enteritis, spontaneous cardiomyopathy, airsacculitis, and enteritis; 2) 12% average increase in feed efficiency; and 3) increased market weight at an equivalent age at processing. Growers who participated in the study found that by following recommended ventilation guidelines, consistent temperature regulation -- particularly temperature control in the growout -- improved flock performance.

The long-term benefits of the project, however, far outweighed the short-term economic improvement presented in Table 2. Marketing flocks with excellent feed conversion and consistency in weight became the norm rather than the exception. Some participating growers found they could better utilize existing buildings for future year round production. Others also realized that the return for investment in raising turkeys indoors made it economically feasible to construct new facilities.

This study serves as an example of how a modest investment of time and funds by Utah State University Cooperative Extension produced benchmark environmental management guidelines that yielded a profitable return to the turkey growers of Utah.

Table 1. Established environmental benchmarks.

Factor Measured	Brooder Level	Growout Level
O_2 (%)	>20.3	>20.6
CO (ppm)	<10.0	< 3.0
CO_2 (ppm)	<1500	No Data
NH_3 (ppm)	<7.0	<15.0
Relative Humidity (%)	15 to 30	45 to 70
Temperature (^oF)	85[a], 99[b], 115[c]	65 to 70

[a]At edge of circle, [b]at rim of hover, [c]centered directly under hover

Table 2. Net economic improvement of four turkey growers using project-generated benchmarks compared to previous management techniques.

Comparative Production Size	Status	Net Improvement ($) (1 year production cycle)
Small	Well-managed	12,000
Small - medium	Well-managed	15,000
Medium	Potentially well-managed	45,000
Large	Potentially well-managed	40,000

[a]Data available for one flock only

SALINOMYCIN SENSITIVITY ANALYSIS OF COCCIDIAL SPECIES OBTAINED FROM COCCIVAC B OR ANTICOCCIDIAL TREATED POULTRY GROW-OUT FACILITIES

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Mixed coccidial (*Eimeria*) species obtained from pooled litter samples collected from 7 geographically different commercial poultry grow-out facilities (5 farms/facility) treated with repeated immunizations with Coccivac BTM live oocyst vaccine (American Scientific Laboratories, Inc., Millsboro, DE) or various anticoccidial shuttle programs were tested for sensitivity to 60 ppm salinomycin in battery cage experiments. Day old male broiler birds were placed on either unmedicated or medicated diets and at 10 days of age were challenged with a pretitrated dosage of a mixed coccidia inocula obtained from one of the pooled litter samples. Two separate experiments were run comparing the results seen with the Coccivac B litter oocyst inocula and oocyst inocula from litter of 3 facilities on anticoccidial treatment. Parameters of weight gain, feed conversion and intestinal lesion scores were measured at 6 days post-challenge and compared between non-medicated unchallenged (NMUC), non-medicated challenged (NMC), medicated unchallenged (MUC), and medicated challenged (MC) groups. In the first experiment, little difference was seen between Coccivac B oocyst inocula and inocula from 2 of the anticoccidial medicated facilities (designated facilities 1 and 2), and all of these inocula were significantly more sensitive to 60 ppm salinomycin than oocyst inocula from the third facility (designated facility 3). The MC groups from the Coccivac B and facilities 1 and 2 were significantly higher in average weight and numerically lower in feed conversions than their respective NMC control groups and the MC group from facility 3. Intestinal lesion scores of all 4 oocyst inocula MC groups were significantly lower for the mid-intestine and ceca compared to NMC groups, but more variation was seen with the upper-intestinal lesions with facility 3 inocula having a significantly higher lesion score than the NMC group and facility 1 inocula showing no significant difference between the MC and NMC group. In the second experiment, all 3 inocula from anticoccidial medicated facilities (designated 4, 5 and 6) were less sensitive to salinomycin than the Coccivac B inocula. The MC Coccivac B group was significantly higher in average weight gain than the MC groups for facilities 4, 5 and 6, and numerically lower in feed conversion than facilities 5 and 6. Lesion scores for the Coccivac B inocula were significantly lower for upper- and mid-intestine and ceca compared to the NMC controls, while a wide range of variability was seen with lesion scores for facilities 4-6. Facility 3 MC group showed reduction in only upper intestinal lesions, facility 4 MC groups showed reduction in just cecal lesions and facility 6 MC group showed reduction in both mid-intestinal and cecal lesions but not the upper-intestinal lesions. All MC groups from both experiments were significantly lower in average weight gain, numerically higher in feed conversions and significantly higher in lesion scores than the NMUC and MUC controls. The results indicated that repeated immunizations with Coccivac B would increase sensitivity to 60 ppm salinomycin medication in broiler grow-out facilities.

(A full length article will be published in *Avian Diseases*.)

SALT DEFICIENCY IN BROILERS AND LAYERS

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Diagnosticians are well aware of problems associated with excess dietary salt. However, salt deficiency is rarely diagnosed. The symptoms that have been reported in young birds include poor growth, impaired feed utilization, softening of bones, disturbances in the fluid and electrolyte balances, nervous signs and mortality, and in layers a decrease in egg production, egg size, loss of weight and cannibalism (1). Similar symptoms may also be observed with many other diseases. Salt requirements of poultry have been well documented twenty to forty years ago. Sodium and chlorine requirements are between 1200 and 2000 p.p.m. for most species of poultry with higher requirements listed for young birds(2). Sodium chloride contains 39.3% sodium and 60.7% chloride and, if added at 0.2 to 0.5%, meets the nutritional sodium and chloride needs in poultry diets. Recent studies by Murakami et al. (3, 4) have confirmed that salt requirements of today's fast growing broilers are still within this range. With this report we would like to draw attention of diagnosticians as well as feed mill operators to the fact that salt deficiency will cause not only significant production problems but also a differential diagnostic challenge. The first case involved hundreds of small flocks throughout northern New England and eastern New York over a 5-month period. Subsequently salt deficiency occurred in two commercial layer operations. Different feed manufacturers were involved.

CASE REPORTS

Clinical observations. Salt deficiency in small flocks affected broilers, pullets, ducks, geese, pheasants, quail, turkeys, guinea fowl and cygnets. Nervous signs (incoordination, falling on the sides, erratic movements), refusing to eat, listlessness, poor growth, poor feathering and mortality were observed after 2 weeks of age with the exception of ducklings which showed clinical signs within the first week. While mortality of greater than 50% was reported from 65% of 58 surveyed flocks in Maine, poor growth and not mortality was the primary concern in several well-managed broiler flocks. In several flocks mortality became a concern after 4 weeks of age and after placing broilers on a normal salt diet. In layer flocks, a drop of egg production and increased cannibalism were observed.

Epidemiology. A case control study was undertaken in New Hampshire and Vermont. Owners of 38 affected and 23 unaffected flocks participated in the study. Statistically significant associations were found with feeding a particular brand of commercial poultry feed (odds ratio = 29.2, 95% confidence interval = 3.17-675.8. p = 0001) and indoor housing (odds ratio =4.0, 95% confidence interval = 1.1 14.87, p = 0.03). Poultry fed the implicated brand exclusively were more likely to be affected than those that were fed other brands in addition to the implicated brand (odd ratio = 5.6, 95% confidence interval = 1.l-30.3, p = 0.03). Other management factors,

sources of poultry and poultry raising experience (median 10 years) were not associated with the illness.

Pathology. Uncomplicated cases exhibited stunted growth, immature feathering, pliable bones, atrophic bursa, spleen and thymus, dilated flaccid hearts, proventricular ulceration, pale kidneys. Histopathologically nephrocalcinosis, ventriculitis and proventriculitis, atrophy of lymphoid tissue, cardiac myodegeneration, and dense chromatin in the Purkinje cells of the cerebellum were observed. Broiler chickens that died after the age of 4 weeks and that had been placed on an adequate sodium diet usually showed lesions of right ventricular failure, which included congested lungs, dilated hearts, hydropericardium and ascites and swollen, fibrotic livers.

Feed analysis. Thirteen implicated feeds from different locations and from broiler starter and finisher, pullet grower, game bird, chick starter and layer mash, crumbles or pellets were analyzed and had an avenge sodium content of 240 p.p.m. (SD 104.1 p.p.m.). The mean chloride content of 7 feed samples was 655.6 p.p.m. (SD 265.8 p.p.m.). In comparison, 6 normal feeds had a sodium content of 1593.5 p.p.m. (SD 283.7 p.p.m.).

Differential diagnosis. Obvious lesions of concurrent diseases were present in a few flocks and included coccidiosis, bacterial infections, bronchitis/pneumonia, infectious bursal disease or leukosis. Some implicated feed samples were analyzed for pesticides, heavy metals, mycotoxins and excess ionophores with negative results except for one layer feed sample which contained 0.8 p.p.m. deoxynivalenol. Two livers from affected birds were analyzed for abnormal mineral levels, heavy metals, aromatic chlorinated compounds and 20 pesticides. No abnormal results were obtained. The atrophy of the lymphoid organs suggested the presence of an immunosuppressive agent. Avian leukosis S-virus was suggested as a possible cause. AL J-virus infection of meat-type birds has recently been described (5) in the US and reported to cause severe stunting in broilers (6). Eighteen plasma samples from affected flocks were tested for reticuloendotheliosis virus (REV) and AL J-virus by Dr Fadly (USDA-ARS, Avian Disease and Oncology Laboratory, East Lansing, MI). No REV was isolated. Non-group A or E ALV was isolated from 7 of 18 samples and confirmed by PCR as AL S-virus.

EXPERIMENTAL SODIUM DEFICIENCY

Experimental designs. To clarify what role a sodium (salt) deficient diet had in the mortality/stunting syndrome we decided to conduct a feeding trial. Seventy two day-old male Avian Farms broiler chickens were wing-banded and divided into 9 groups of 8 chicks. The chicks were raised in cages equipped with trough feeders and drinkers. Feed and water was provided ad lib throughout the trial. The three diets used consisted of a sodium deficient commercial chick starter diet (diet 1), the same diet with added salt (diet 2), and a commercial broiler starter diet from another company (diet 3) (Table 1).

Table 1. Analysis of the three experimental diets.

Three groups were randomly assigned to each diet treatment. Diet treatment 1 and 2 were fed for 4 weeks. These treatment groups were switched to diet 3 during weeks 5 and 6. Diet treatment 3 received diet 3 from day one to day 42, the end of the experiment. The feed fed to each group was weighed in and uneaten feed was weighed out at the end of each week.

Each chick was weighed and the shank length and width were measured on arrival, at the beginning of each week and at the end of the trial. Two chickens were removed from each group at 4 weeks and the bursa, the liver, heart and left lung lobe were weighed and the organ body weight ratios were determined. Blood was taken from each chicken. At the end of the experiment the same tissues were weighed and blood was taken. Serum from each chicken was sent to Avian Farms for S-virus PCR testing. Blood chemistry was done at Tufts University veterinary diagnostic laboratory. In all, 32 criteria were compared between the treatment groups. Lung, liver and kidney tissue was fixed in buffered formalin from all chickens that were euthanized at 4 and 6 weeks.

Results. All chicks but one tested negative for J-virus. There were no significant differences in any criteria between diet 2 and 3 treatment groups with the exception of a higher cumulative feed conversion in diet treatment group 2. Weight, shank length, shank width were significantly reduced and the cumulative feed conversion was significantly increased throughout the trial in diet treatment I groups (Table 2).

Table 2. Mean body weight, feed consumption and cumulative feed conversion of broilers on either low (diet 1) or normal sodium diets (diets 2, 3).

The bursa/body weight ratio was reduced at 4 weeks but this ratio recovered completely to normal levels by 6 weeks compared to treatment groups 2 and 3. The heart/body weight ratio was increased at 4 weeks and the lung/body weight ratio was increased at 6 weeks in diet treatment group 1. The sodium level was not affected but the potassium level was slightly lower at 4 weeks, which increased the sodium/potassium ratio. No significant differences were noted in the blood chemistry at 6 weeks with the exception of slightly lower total protein, albumin, glucose, cholesterol and AST levels.

The overall mortality was 13% during this experiment with no significant differences between the treatments. The causes of death included drowning (1), staphylococcus infection (2), nephrosis (2), slipped tendon (2), undetermined (2) and ascites (1). At six weeks 47% (7 chickens) of the chickens in diet treatment I had signs of right ventricular failure (ascites, enlarged heart, hydropericardium, congested lungs, swollen and fibrotic liver and/or swollen kidneys). These lesions were also observed in 2 chickens in diet treatment 2 but not in diet treatment 3.

Conclusion. Severe stunting as a result of reduced feed consumption and increased feed conversion ratio as observed in the field was reproduced experimentally with a sodium deficient diet. The bursa/body weight ratio was significantly reduced. Feed consumption, feed conversion and body weight increased after the change to a normal sodium diet but did not reach the same level as the groups on normal sodium level from day one. The bursa depletion was completely reversed. Blood chemistry data at 6 weeks did not vary significantly between treatments. There was no difference in the mortality during the first 4 weeks between the treatment groups. However, when broilers were switched from a salt deficient diet to a high energy normal sodium diet more chicks developed ascites and right ventricular failure. The high mortality observed in many field flocks may be explained by immunosuppression, environmental stress factors and in a few cases other complicating diseases. To what extent AL S-virus in some flocks may have contributed to poor growth and mortality is unknown.

CONCLUSIONS

The diagnostician should include salt deficiency in the differential diagnosis when high morbidity associated with poor growth, anorexia, poor feathering, lymphoid tissue atrophy and nervous signs in young poultry and a drop of egg production in layers are observed. Due to the absence of typical pathognostic gross and histological lesions a diagnosis will not be possible without concurrent feed analysis. Sodium and chloride deficiencies are easily preventable by continuous and vigilant feed quality control in the feed mills. Poultry diets should contain between 0.15 to 0.2% sodium or 0.35 - 0.5% salt.

REFERENCES

1. Austic, R. E., and M. L. Scott. Nutritional Diseases. In: Diseases of Poultry. 10th ed. B. W. Calnek, H.J. Barnes, C.W. Beard, L.R. McDougald, and Y.M. Saif, eds. Iowa State University Press, Ames, IA. pp. 63-64. 1997.

2. Anonymous. Nutrient Requirements of Poultry. 9th Revised Edition. National Academy of Sciences, Washington, D.C. 1994.

3. Murakami, A. E., S. E. Watkins, E. A. Saleh, J. A. England, P. W. Waldroup, and D. A. Dickey. Effect of level and source of sodium on performance of male broilers at 56 days. J. Appl. Poultry Res. 6:128-136. 1997.

4. Murakami, A. E., S. E. Watkins, E. A. Saleh, J. A. England, and P. W. Waldroup. Estimation of the sodium and chloride requirements for the young broiler chick. J. Appl. Poultry Res. 6:155-162. 1997.

5. Fadly, A. M., and E. J. Smith, Isolation and some characteristics of a subgroup J-like avian leukosis virus associated with myeloid leukosis in meat-type chickens in the United States. Avian Dis 43:391-400. 1999.

6. Stedman, N. L., and T. P. Brown. Body weight suppression in broilers naturally infected with avian leukosis virus subgroup J. Avian Dis. 43:604-610. 1999.

Table 1. Analysis of the three experimental diets.

Ingredient	Analysis (% of dry matter)		
	Diet 1	Diet 2	Diet 3
Crude protein	20.9	20.6	23.6
Fibre	6.2	6.8	6.5
Calcium	0.79	0.76	0.96
Phosphorus	0.68	0.68	0.66
Potassium	1.1	1.1	0.84
Sodium	0.027	0.19	0.16

Table 2. Mean body weight, feed consumption and cumulative feed conversion of broilers on either low (diet 1) or normal sodium diets (diets 2, 3).

Week	Mean Body Weight (gm)			Feed consumption (gms/bird/week)			Cumulative Feed Conversion		
	Diet			Diet			Diet		
	1	2	3	1	2	3	1	2	3
1	95	139	126	115	166	109	2.5	1.8	1.4
2	145	280	269	117	279	236	2.5	2.0	1.5
3	195	616	586	196	514	404	2.9	1.8	1.5
4	232	904	950	197	692	674	3.4	2.0	1.7
		Diet			Diet			Diet	
	3	3	3	3	3	3	3.	3	3
5	526	1417	1430	381	861	903	2.5	2	1.9
6	892	1949	1831	486	882	767	2.2	2	1.8

SEROTYPING OF *HAEMPHILUS PARAGALLINARUM* ISOLATES FROM MEXICO BY THE KUME HEMAGGLUTININ SCHEME

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Serogroups A, B and C were identified among *Haemophilus paragallinarum* isolates from Mexico as previously reported (2). In the present paper, a total of 42 isolates of *Haemophilus paragallinarum* were serotyped by the Kume hemagglutinin scheme as described (1, 3). Results obtained were 11 isolates being A-1 serovar, 7 isolates being A-2 serovar, and 14 isolates being C-2 serovar. A total of 10 isolates showed cross-reactions between serovars A-2 and B-1. A detailed study of these isolates is needed.

REFERENCES

1. Blackall, P.J., L.E. Eaves, and D.G. Rogers. Serotyping of Haemophilus paragallinarum by the Kume hemagglutinin scheme-proposal of a new serovar and altered nomenclature. J. Clin. Microbiol. 28:1185-1187. 1990.

2. Fernandez, R. P., G.A. Garcia, G.P. Ochoa, and V.E. Soriano. Biotyping of Haemophilus paragallinarum from avian coryza cases in Mexico. Proc. 48th West. Poult. Dis. Conf., Vancouver, British Columbia, Canada. pp. 110-111. 1999.

3. Kume K, A. Sawata, T. Nakai, and M. Matsumoto. Serological classification of Haemophilus paragallinarum with a hemagglutinin system. J. Clin. Microbiol. 17:958-964. 1983.

SIGNIFICANCE OF CLOSTRIDIUM SORDELLII ISOLATED FROM THE INTERNAL NAVEL OF CHICKS

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The pathologic investigation of young chicks is frequently one of the more challenging cases seen in the diagnostic laboratory. Laboratory examination can be useful as an impartial documentation of obvious abnormalities such as omphalitis, septicemia or aspergilliosis. Less obvious abnormalities such as weak chicks, starveouts or overall lack of chick quality are highly subjective evaluations. These subjective evaluations are frequently dependent on history of the breeder flock, hatchery conditions and/or brooder management. These three aspects of the history can often be at odds with each other or just unknown.

A series of cases of newborn chicks with rather unique lesions illustrate how many facets of the history are critical to this diagnostic problem. The initial submission consisted of day-old chicks in which a high number of dead-on-arrivals (DOA's) were noted at placement. Gross lesions were striking and consisted of severe subcutaneous edema over the ventral abdomen and extending down the medial surface of the thigh. The yolk sacs were also severely edematous but did not appear inflamed. Externally, the navels were closed and appeared normal. Internally, the navels consisted of a firm irregular nodule about two millimeters in diameter. Many of the chicks had variable degrees of hemorrhages on the liver from petechial to ecchymotic and covering the entire liver. These same lesions were also observed in the field. Histopathologic examination demonstrated significant lesions primarily in the liver and navel. The liver had extensive hepatic vacuolation that is normal for this age of bird. The hemorrhage observed grossly was actually extensive pooling of blood in the sinusoidal spaces. The external surface of the navel had a normal appearance. The internal nodule was a mass of fibrin, hemorrhage, necrotic debris and most sections had large Clostridia-like bacterial rods. No significant bacteria were cultured (aerobically) from either the yolk sacs or livers. Due to the extensive subcutaneous edema and the possibility of exudative diathesis, vitamin E analysis was performed on the livers. A level of 237 ppm was obtained.

Additional investigation into high numbers of DOA's at placement on different ranches and involving different strains of broilers narrowed the focus to one hatchery. Laboratory investigation centered around the Clostridia-like rods and the internal navel lesion. Anaerobic cultures were initially attempted from the yolk sac and resulted in no bacterial growth, aerobically or anerobically. This initial attempt was from chicks directly pulled from the hatchers. Anaerobic cultures were subsequently taken from the internal nodule (Table 1). *Clostridium sordellii* was recovered most frequently and in the largest amount (quantitated as rare, small, moderate or large). *Clostridium perfringens* and a *Clostridium* sp. were also isolated.

Table 1. Clostridial isolations from the internal navel.

The most frequent aerobic bacteria cultured from the navel were *Enterococcus faecalis* and *E. coli*. *Staphylococcus aureus, Klebsiella pneumonia*, *Enterococcus* sp. and *Salmonella*

livingstone were also recovered. Navel cultures taken from birds 2 days of age or greater were much more likely to have multiple bacterial species present. These older birds were also much more likely to have aerobic bacteria isolated from internal organs.

A histologic comparison was made from normal and affected navel samples collected from chicks from the suspect hatchery in addition to navel samples collected from two additional ("normal") hatcheries. The histologic appearance of the navels from the two "normal" hatcheries appeared to be normal healing of the navel. The external surface was intact with fibrosis, small focal areas of hemorrhage, and necrotic debris and slight heterophilic inflammation present on the internal surface. No bacteria were observed in any of the sections (5 sections/hatchery). Normal navel Sections from the problem hatchery also showed normal healing of the navel but a few sections had bacterial cocci present. Affected navel sections from the problem hatchery were somewhat variable in appearance but reflected what was seen in the initial case. The main exception was the visualization of different types of bacteria (i.e. not just Clostridial-like bacteria).

To further investigate the significance of *Clostridium sordellii*, a crude inoculation of newborn chicks was performed. The first inoculation was performed on newly hatched chicks pulled from the hatchers after normal dry-down time. These chicks were inoculated with 0.05 ml of a 48-hour broth culture. The chicks appeared normal at 12 hours and 36 hours post-inoculation. Histology of the navels was normal and showed the presence of a few bacterial rods in an occasional section. The second inoculation attempt involved pulling chicks from the hatchers at about 50% hatch when the chicks were still wet. A cotton swab was dipped into a 24-hour broth culture of *C. sordellii* and painted over the external navel. Forty-eight hours later, a variable sized nodule was present on the internal surface of the navel. *C. sordellii* was observed and cultured from the internal navel, but no significant abnormalities were present histologically.

The reproduction of a clinical problem is the heart of Koch's postulates. Unfortunately, chick quality encompasses numerous constantly changing variables and is a critical control point between breeder, hatchery and brooder management. The possibility exists that certain unknown variables may have to be present, along with *Clostridium sordellii*, whereby the gross lesions of the initial case were produced. When a problem in chick quality exists, a detailed field and laboratory investigation is necessary to eliminate or substantiate many of the variables that go into good chick quality.

Table 1. Clostridial isolations from the internal navel.

	Submissions	Cultures
Clostridium sordellii	3/5	12/24
Clostridium perfringens	1/5	3/24
Clostridium sp.	2/5	3/24

SUMMARY OF STUDIES ASSESSING THE EFFECT OF VIRGINIAMYCIN ON SALMONELLA SHEDDING

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Foods of avian origin, table eggs and meat, are widely produced throughout the world and remain a primary source of nutrition for humans. Despite evidence that poultry meat in the U.S. today is safer than anytime in the past, consumer concerns about microbial contamination of foods have become of paramount importance. The media and public reports of food borne illnesses in the 1980s and 1990s have been largely responsible for raising the awareness of this issue and, unfortunately, these same sources have failed to fully educate the public about the entire "picture". As a result, the poultry industry continues to face increasing consumer pressure to guarantee food safety and meet export requirements.

A primary food borne pathogen of concern has been and continues to be Salmonella. There have been numerous products marketed, concepts offered, and management practices implemented to ultimately decrease the numbers of this organism on the final product. Certainly, there are scientifically documented strategies, such as properly administered competitive exclusion products, which have repeatedly proven to be successful in helping to accomplish the goal of reduced Salmonella colonization of the chicken intestinal tract. However, there are also many products or strategies with claims of efficacy in this area which are not well documented. The problem is to determine how much of the available information is reliable enough to make a rational decision that can truly affect the area of concern.

The issue of a growth promotant's effect on *Salmonella* shedding is such an example. Although the topic has been raised at various times over the last few decades, there has been a "renewed" interest on microflora management in poultry in order to minimize *Salmonella* contamination. Trials by various companies have been conducted to demonstrate a particular product's "advantages" on the bird's microflora, but are the results reproducible? How does one make a decision when a company shows data suggesting one thing, and a competitor has information stating another? In actuality, evaluation of data from individual trials can be misleading. A more reliable method would be to examine a larger body of data to detect whether trends exist. Although the world of biology provides plenty of surprises when conducting individual studies, one should appreciate the power of multiple assessments over time in getting closer to the truth.

Does virginiamycin (VM) in the diets of animals affect the persistence of *Salmonella* in the intestines/fecal material? Table 1 summarizes fourteen studies where virginiamycin and other feed additive antibiotics were evaluated for their effect on *Salmonella* persistence following challenge. Most of the studies were conducted in chickens and have been used to support various submissions for the product's approval. In the majority of the trials, VM had no effect upon *Salmonella* shedding. In some instances, the Salmonella levels actually decreased with virginiamycin exposure, and in two situations, temporary increases were recorded. It appears from the overall results of this group of experiments that VM does not increase *Salmonella* excretion in animals. There are a few more recent and unpublished examples of poultry studies with similar findings, adding further credibility to this conclusion.

These findings can be in part explained by the mode of action of virginiamycin. It has been documented that VM as well as the other growth promotants exert an influence on various components of the intestinal flora. VM's activity is mainly confined to gram-positive bacteria, especially *Lactobacillus*. Lactic acid produced by this bacterial genus is an important component of the competitive exclusion (CE) properties of the intestinal tract, but VM does not eliminate this organism. It simply decreases the Lactobacilli numbers to levels which optimize nutrient utilization by the bird without causing loss of the CE property. In addition, VM has minimal effect in the unique environment of the cecum, where *Salmonella* and other gram-negative bacteria tend to colonize and predominate, respectively. What this means is that VM has minimal effect on volatile fatty acid (WA) production in the ceca, an important component in the fight to prevent colonization of *Salmonella* in this organ.

Table 1. Summary of *Salmonella* studies involving virginiamycin.

In conclusion, industry personnel must make many decisions affecting performance, cost effectiveness, and food safety. Unfortunately, these decisions are often made using limited information or simply to make a change without knowing whether it will really have an impact. In the latter instance, should an improvement occur, an errant association may be drawn. Ideally, any decision should be based on solid scientific evidence with an eye towards critical analysis of the data presented to us. In addition, it is vital to know that every growth promotant has a mode of action specific for that compound, and conclusions drawn from one should not be transferred to others. This decision making process is often not easy nor totally accurate, but should be based on science when possible.

Table 1. Summary of Salmonella studies involving virginiamycin.

Level of drug (ppm)	Salmonella serotype	Salmonella shedding	Length of Trial (days)	Author
CHICKENS				
10	typhimurium	0	109	H. Williams-Smith, J. of Hygiene 75:293-301
100	typhimurium	0	109	H. Williams-Smith, J. of Hygiene, 1975.
20	typhimurium	0	35	W. Gunther Leuchtenberger, 1981.
20	typhimurium	+	42	W. Gunther Leuchtenberger, 1981.
30	typhimurium	0	42	W. Gunther Leuchtenberger, 1981.
20	typhimurium	+	49	W. Gunther Leuchtenberger, 1981.
20	typhimurium	0	42	W. Gunther Leuchtenberger, 1981.
10	typhimurium	0	56	Gustafson,R. Zbl.Vet.Med.B29:119-128. 1981.
25	typhimurium	0	56	Abou-Youssef,M.H. Poult.Sci.62:30-37. 1982.
20	typhimurium	0	7	Bailey, J.S., Avian Dis. 32:324-329. 1988.
20	typhimurium	0	7	Humbert, F., Avian Pathol. 20:577-584. 1991.
TURKEYS				
20	hadar	0	84	Smith, H., Vet. Rec. 107:289. 1989.
SWINE				
55	typhimurium	0	31	Jones, F. T., J. Anim. Sci. 49:128-133. 1979.
55	typhimurium	0	59	Abou-Youssef, M.H. J. Anim. Sci.49:128-133. 1979.

* + indicates a temporary, albeit not significant, increase in Salmonella excretion.

SURVEILLANCE ON ANTIMICROBIAL RESISTANCE IN ESCHERICHIA COLI AND SALMONELLA ISOLATES FROM POULTRY BETWEEN 1993 AND SEPTEMBER 1999

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The present investigation was carried out in the State of Baden-Wurttemberg between 1993 and September 1999 to detect the development of antimicrobial resistance of *Escherichia coli* and *Salmonella* isolated from poultry to neomycin, tetracycline, enrofloxacin as well as furazolidone and chloramphenicol. The use of last two antibiotics has not been allowed in the European Union (EU) since 1994 and 1995 respectively. After 1998 bacteria were not tested for resistance to furazolidone and chloramphenicol. In total 2120 E. coli and 568 *Salmonella* isolates were tested. All isolates were obtained from turkey, layer and broiler farms attended by the Poultry Health Service, Stuttgart, and isolated for diagnosis or surveillance in the Chemical and Veterinary Laboratory, Stuttgart. The antimicrobial sensitivity test was done by a diffusion technique using Diagnostic Sensitivity Agar (DST-agar) (Oxoid, Wesel) and discs containing antimicrobials.

In the present survey the resistance of *E. coli* strains to individual antibiotics differed markedly from the resistance of *Salmonella* strains, The results obtained by testing *E. coli* revealed that there have been some gradual decreases in resistance to neomycin, furazolidone and chloramphenicol. Resistance to tetracycline remained all the time at a high level. The resistance to enrofloxacin showed a slight fluctuation. Most of the *Salmonella* isolates were sensitive to enrofloxacin. while there has been a significant increase in the resistance to tetracycline. The resistance to neomycin, furazolidone and chloramphenicol fluctuated or remained at the same level. Although the use of furazolidone and chloramphenicol in the European Union has been banned since 1994 and 1995 respectively, some resistant *E. coli* and *Salmonella* strains were found.

In conclusion, the results of this study show that the resistance of *E. coli* and *Salmonella* strains to tetracycline is at a high level and tends to increase. Consequently, the use of tetracycline should be only applied for therapeutic purposes after an accurate diagnosis and after testing of resistance in vitro. Furthermore tetracycline should be used at accurate doses and for sufficient duration.

THE 1999 AVIAN INFLUENZA (H7N1) EPIDEMIC IN ITALY

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INTRODUCTION

Italy has experienced both highly pathogenic avian influenza (HPAI) and low pathogenic avian influenza (LPAI) throughout the years. Evidence of HPAI was reported in 1935-1937 by Petek (13), with no other out breaks being reported up to 1997, when a virulent H5N2 virus was isolated in north-eastern Italy by Capua et al. (4). A different situation has been reported for viruses of low pathogenicity, and viruses of various subtypes have been isolated throughout the years from poultry, especially turkeys, and feral waterfowl (7, 10, 13, 11). Similar isolations have been reported in most countries producing poultry and have often been linked with the introduction of viruses from migratory waterfowl (1). Mutation of LPAI to HPAI has never been reported in Italy.

From the 29th of March to the end of October, 1999, one hundred-sixty-three outbreaks of LPAI were diagnosed in the Veneto and Lombardia regions which are located in the northern part of Italy. The outbreaks affected several avian species including turkeys, chicken breeders, broilers, and layer, and guinea fowl breeders and meat birds. The highest number of outbreaks was diagnosed in turkeys, 121 in broilers and 6 in breeders. Nineteen outbreaks were reported in broiler breeders and only 3 in chicken broilers. A total of 12 outbreaks were diagnosed in layers, one in guinea-fowl breeders and one in meat-type guinea fowl. On the 17th of December, 1999, highly pathogenic avian influenza was diagnosed in a meat turkey flock and subsequently it spread to other turkey flocks (breeders and meat-type) and to commercial layer flocks. The clinical, post-mortem, histopathological and virological findings differed substantially between the LPAI and the HPAI outbreaks and are reported below.

MATERIALS AND METHODS

Serological and virological investigations were performed in accordance with the guidelines indicated in EU directive 92/40/EEC (CEC, 1992). The haemagglutinin (H) and neuraminidase (N) subtypes of influenza A isolates were determined using polyclonal chicken antisera as described by Alexander and Spackman (2). All isolates were tested for virulence by intravenous pathogenicity index (IVPI) tests in six-week-old SPF chickens. Furthermore, nucleic acid from the viruses isolated was subjected to nucleotide sequencing in the region of the genome coding for the cleavage site of the haemagglutinin molecule (17, 18), by the EU Reference Laboratory for Newcastle disease and avian influenza, at Weybridge, UK. During the LPAI epidemic,

serology was mainly performed with the haemagglutination inhibition test using homologous antigen, since turkeys were vaccinated with inactivated multivalent vaccines containing H6 and H9 antigens which interfered with the AGP test.

RESULTS

Clinical and post-mortem findings (LPAI). In turkeys reared for meat the severity of the clinical and post-mortem disease varied considerably, with mortality ranging from 5% to 97% depending on the age of the affected birds. The clinical signs were dominated by respiratory distress which started with rales and snicking and then developed into severe dyspnea, associated with swelling of the infraorbital sinuses and conjunctivitis. This condition was always accompanied by complete loss of appetite, a febrile condition, ruffled feathers and depression. In some cases, an involvement of the pancreas which appeared haemorrhagic and hardened was observed and histologically, pancreatitis with severe, extensive necrosis of acinar cells was the main finding. Pancreatic lobes exhibited strong irregular eosinophilic staining caused by acinar necrosis and the most severe necrotic foci were lined by a thin rim of inflammatory cell debris. Interstitial oedema was also present, associated with fibrinous peritonitis affecting both pancreas and intestine. No other relevant lesions were detected elsewhere.

In older birds the clinical signs regressed with recovery in most of the affected birds, while in younger birds, up to 40 days of age, the clinical signs evolved into a more severe respiratory problem, which in some cases resulted in air-sac rupture with development of a subcutaneous emphysema, and was associated with mortality rates ranging from 40 to 97%. The most striking post-mortem lesion that was present in birds from a large majority of the affected flocks was the presence of fibrinous clots in the sinuses and trachea, which appeared in most cases to have caused death by suffocation. The trachea and lungs appeared to be congested and in some cases haemorrhagic. Petechial haemorrhages were also present in some cases on the epicardium.

A milder form of the same clinical condition was also observed in turkey breeders that consistently exhibited rales, coughing and swelling of the infraorbital sinuses and a febrile condition associated with loss of appetite. Egg production dropped from 30% to 80% during the acute phase, but partially recovered to subnormal levels within three weeks from the onset of the disease. Egg quality also decreased with misshapen, fragile and whitish eggs being produced during the drop in production. Mortality rates ranged from 5 to 20%, while morbidity rates reached 100%. The post-mortem findings were of affected respiratory and reproductive tracts with congestion of lung and trachea, sinusitis and conjunctivitis and often the so-called "egg-yolk peritonitis" was observed. A milder condition was seen in affected guinea fowl breeders, with conjunctivitis being a severe and consistent clinical sign.

Only a limited number of broiler and broiler breeder flocks was affected. In broiler breeders, an initial loss of appetite was followed by a drop in egg production of 5 - 20%. During this phase cyanosis of the combs and wattles could be seen. All the flock appeared to be affected and mortality ranged from 3 - 8%. Similar to the turkey breeders, misshapen eggs were also produced in considerable quantity. Pathological findings were restricted to the ovary and oviduct with colliquation of ovarian follicles, associated with catarrhal or fibrinous peritonitis. The only other lesion which appeared to be consistent was congestion of lungs and trachea, which in some cases appeared as pulmonary oedema.

In broiler chickens, H7N1 infections were not apparent in some flocks and in others characterised by anorexia and mild respiratory signs with mortality rates which were generally

low in the order of 2 - 3%. In one case mortality reached 20%. The post-mortem lesions were limited to the lungs and trachea which appeared congested with associated catarrhal tracheitis.

Outbreaks in commercial layers were similar to those observed in broiler breeders; initial signs were of loss of appetite and depression, followed by drops in egg production which ranged from 3 to 10%, and in some cases egg production losses reached 30%. Recovery to pre-disease levels occurred in only a few cases, while egg production in most flocks remained 2 - 3 points below expected levels. Clinical signs were usually present in about 20% of the birds, but mortality never exceeded 5%. Gross lesions mainly involved the reproductive organs and abdominal cavity: the ovary and oviduct appeared oedematous, and on opening the oviduct it contained a catarrhal exudate and fibrin clots, often this was associated with fibrinous and egg-yolk peritonitis. Lungs and tracheas at times appeared congested.

Guinea fowl broilers exhibited respiratory signs similar to those observed in meat turkeys accompanied by nervous signs with opistotonus, torticollis and paralysis of the wings with mortality reaching 30%.

Clinical and post-mortem findings (HPAI). In turkeys (breeders and meat birds), the only clinical signs which could be observed were anorexia, severe depression and nervous symptoms such as torticollis, opistotonus and incoordination. Mortality reached 100% in three days. No post-mortem lesions were observed apart from congestion of internal organs. In layers sudden mortality of 10% daily was followed by eradication of the affected flock.

Virology and serology. Virus isolation attempts yielded haemagglutinating agents on first passage, often accompanied by early embryo mortality (within 48 hours). Viruses were characterised serologically and all influenza isolates were of the H7N1 subtype. The IVPI test performed on a selected number of early (LPAI) isolates gave a result of 0.0. The deduced amino acid sequence of the region coding for the cleavage site of the haemagglutinin molecule for the early isolates was . . .PEIPKGR*GLF. . . while for some other isolates obtained subsequently it was. . . .PEVKGR*GLF. . .both sequences are typical of low pathogenic viruses. On the contrary the results of the IVPI test performed on the HPAI isolates was 3.0, and the deduced amino acid sequence of the region coding for the cleavage site of the haemagglutinin molecule was . . .PEIPKGSRVRR*GLF. . .which exhibits multiple basic amino acids indicative of high virulence, not present in the LPAI strains.

DISCUSSION

Data collected in the H7N1 1999 Italian epidemic, confirm the clinical and pathological findings reported in other LPAI outbreaks (5). In particular, due to the non pathognomonic clinical signs and lesions present in adult birds, the possibility of initially misdiagnosing the clinical condition can lead to a delay in identifying the agent, thus resulting in a considerable spread of infection in a relatively short period of time. LPAI can be a devastating disease in turkey poults, causing up to 97% mortality in affected flocks. The capability of the virus to spread and infect many flocks apparently virtually simultaneously, may cause great economic losses to the poultry industry, forcing some companies out of business. During the Italian 1999 influenza epidemic a HPAI virus emerged in turkeys. The mutation of LPAI to HPAI has been proved in experimentally infected chickens, (16) and has similarly occurred in the field in chickens (8). Moreover, the possibility of humans being infected with LPAI (9) and HPAI (4, 14, 15) places these viruses in a different context compared to the one they held when Directive 92/40/EC was drafted. Considering these two points, it would seem advisable that an eradication

policy should be implemented before the HPAI virus emerges. However, eradicating a significant number of infected flocks is only feasible if there is financial support available. With current legislation this appears to be inapplicable for LPAI. An alternative to an eradication policy is a vaccination strategy, which reduces but does not prevent the virus from replicating (5) and is therefore useless from a biological point of view, and furthermore, would have dramatic implications on intra and extra community trade within the current legislation.

A reconsideration of European Union legislation could possibly be a solution to a number of problems which have emerged in the Italian 1999 H7N1 epidemic and in other recent epidemics in which it appeared that LPAI outbreaks caused by H5 and H7 subtypes must be controlled in order to avoid heavy economic losses to the poultry industry in Europe.

REFERENCES

1. Alexander, D.J. The epidemiology and control of avian influenza and Newcastle disease - review article. J. Comp. Pathol. 112:105-126. 1995.

2. Alexander D.J., and D. Spackman. Characterization of influenza A viruses isolated from turkeys in England during March-May (1979). Avian Pathol. 10:281-293. 1981.

3. Capua I., S. Marangon, L. Selli, D.J. Alexander, D.E. Swayne, M. Dalla Pozza, E Parenti, and F.M. Cancellotti. Outbreaks of highly pathogenic avian influenza (H5N2) in Italy during October (1997) to January (1998). Avian Pathol. 28:455-460. 1999.

4. Claas C.J., A.D.M. Osterhaus, R. Beek, J. De Jong, G.F. Rimmelzwaan, D.A. Senne, S. Krauss, K.F. Shortridge, and R.J. Webster. Human influenza A H5N1 virus related to a highly pathogenic avian influenza virus. Lancet 351:472-477. 1998.

5. CEC (1992): Council Directive 92/40/EEC of 19 May 1992 introducing Community measures
for the control of avian influenza. Official Journal of the European Commission, L167, 1-15. 1992.

6. Easterday B.C., V.S. Hinshaw, and D.A. Halvorson. Influenza. In: Diseases of Poultry 10th ed. B.W Calnek, H.J. Barnes, C.W. Beard, L.R. McDougald and Y.M. Said, eds. Iowa State University Press. Ames, IA. pp. 583-605. 1997.

7. Franciosi C., P.N. D'Aprile, D.J. Alexander, and M. Petek. Influenza A virus infections in commercial turkeys in North East Italy. Avian Pathol. 10:303-331. 1981.

8. Kawaoka Y., W.J. Bean, and R.G. Webster. Molecular characterisation of the A/ Chicken/ Pennsylvania/83 (H5N2) influenza viruses Proc. Second International Symposium on Avian
Influenza. D.E. Swayne and R.D. Slemons. eds. Georgia Center for Continuing Education, University of Georgia, Athens, Georgia. pp197-206. 1986.

9. Kurtz J., R.J. Manvell, and J. Banks. Avian influenza virus isolated from a woman with

conjunctivitis. Lancet 348:901-902. 1996.

10. Meulemans G. Status of avian influenza in Western Europe. Proc. Second International Symposium on Avian Influenza. D.E. Swayne and R.D. Slemons, eds. Georgia. Center for Continuing Education, University of Georgia, Athens Georgia. pp. 77-83. 1987.

11. Papparella V., A. Fioretti, and L.F. Menna. The epidemiological situation of avian influenza in Italy from (1990) to (1993) in feral bird populations and in birds in quarantine. Proc. Joint First Annual Meeting of the National Newcastle Disease and Avian Influenza Laboratories of Countries of the European Communities, Brussels. pp 19-21. 1994.

12. Papparella, V., A. Fioretti, and L.F. Menna. The epidemiological situation of avian influenza in Italy (1994). Proc. Joint Second Annual Meetings of the National Newcastle Disease and Avian Influenza Laboratories of Countries of the European Union, Brussels. pp 14-15. 1995.

13. Petek, M. Current situation in Italy. Proc. First International Symposium on Avian Influenza. Carter Composition Corporation, Richmond, USA. pp 31-34. 1982.

14. Suarez, D.L., M.L. Perdue, N. Cox, T. Rowe, C. Bender, J. Huang, and D.E. Swayne. Comparison of highly virulent H5N1 influenza A viruses isolated from humans and chickens from
Hong Kong. J. Virol. 72:6678-6688. 1998.

15. Subbarao, K., A. Klimov, J. Katz, H. Regnery, W. Lim, H. Hall, M. Perdue, D. Swayne, C. Bender, J. Huang, M. Hemphill, T. Rowe, M. Shaw, X. Xu, K. Kukuda, and N. Cox. Characterization of an avian Influenza A (H5N1) virus isolated from a child with a fatal respiratory illness. Science 279:393-396. 1998.

16. Swayne D.E., J.R. Beck, M. Garcia, M.L. Perdue, and M. Brugh. Pathogenicity shifts in experimental Avian Influenza virus infections in chickens. Proc. Fourth International Symposium on Avian Influenza. D.E. Swayne and R.D. Slemons, eds. Georgia Center for Continuing
Education, University of Georgia, Athens Georgia. pp.171-181. 1998.

17. Wood G.W., J. Banks, J.W. McCauley, and D.J. Alexander. Deduced amino acid sequences of the haemagglutinin of H5N1 avian influenza virus isolates from an outbreak in turkeys in Norfolk, England. Arch. Virol. 134:185-194. 1994.

18. Wood G.W., J. Banks, L.H. Brown, I. Strong, and D.J. Alexander. The nucleotide sequence of the HA1 of the haemagglutinin of an H1 avian influenza isolate from turkeys in Germany
provides additional evidence suggesting recent transmission from pigs. Avian Pathol. 26:347-355.
1997.

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THE EFFECT OF T CELL DEPLETION ON VACCINE PROTECTION AGAINST INFECTIOUS BURSAL DISEASE

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Infectious bursal disease (IBD) is an economic problem for the chicken industry despite vigorous vaccination strategies. Current IBD vaccines are selected based on their ability to induce humoral immunity. Despite widely practiced vaccination, IBD outbreaks continue to occur in commercial chickens with associated economic loss. Recently developed experimental recombinant vaccines also fail to provide adequate protection against IBDV-induced bursal lesions although almost all induce circulating anti-IBDV antibodies. An adequate T cell response seems to be necessary for the development of protective immunity against most viruses (3,6,7). The significance of the cellular immune response in protection against IBDV is largely unknown. The purpose of our study was to investigate the role of T cells in IBD. We hypothesized that the depletion of functional circulating T cells will lead to insufficient protection of chickens vaccinated with inactivated IBDV.

In two repeat experiments, specific-pathogenfree chickens were depleted of functional circulating T cells by thymectomy (1,5) and cyclosporin A (CsA) treatment, 100 mg/kg body weight given intramuscularly (4). This treatment resulted in reduction of circulating T cell numbers by 50-60 % in comparison to controls while the B cell numbers were not affected. Further, the proliferative response of T cells to a T cell-mitogen was reduced by more than 90 % in comparison to that of controls. The T cell-compromised chickens as well as control chickens with intact T cell functions were vaccinated with formaldehyde-inactivated IBDV. At 10 days post-vaccination, the anti-IBDV antibody levels were determined. Birds that were depleted of functional T cells developed significantly lower virus-neutralizing antibody titers than the T cell-competent birds (log2 9 versus log2 11, $P < 0.05$). Eighty percent of the vaccinated controls in comparison to 49 % of the T cell-depleted birds developed detectable anti-IBDV antibodies. This observation indicated that functional T cells were necessary for optimum antibody development.

Thirteen days post vaccination, vaccinated chickens as well as unvaccinated controls were challenged intraocularly with IBDV-IM (2). Only 8 % of the vaccinated T cell-intact chickens developed mild pathological and histopathological bursa lesions. Vaccinated birds compromised in their T cell functions had a mortality rate of 23 % at 5 days PI and a total IBDV incidence of 88 %. The protection against challenge correlated with the level of circulation antibodies. Birds with detectable neutralizing anti-IBDV antibody titers of log2 11 or higher were protected against IBDV-induced pathological and histopathological lesions. Only 20 % of the T cell-depleted birds had antibody titers above log2 11 and were protected against IBDV-induced bursa lesions in comparison to 90 % protection in controls. Birds with lower circulating antibody levels developed lesions such as atrophy, necrosis and gelatination of the bursa of Fabricius.

From this study we concluded that T cells were critical in chickens for developing a protective immune response against an inactivated IBDV-vaccine. Chickens with compromised T cell function had poor humoral immune responses. Although some of the T cell-compromised birds developed detectable antibody levels, the titers were in average lower in comparison to those in control-vaccinated chickens. Our study indicated that antibody titers may have to reach a

certain threshold to protect chickens sufficiently against IBDV. T cell help may be needed to reach these antibody levels.

REFERENCES

1. Cihak, J., H. W. L. Ziegler-Heitbrock, H. Stein, and U. Loesch. Effect of perinatal anti-TCR2 treatment and thymectomy on serum immunoglobulin levels in chicken. J. Vet. Med. 38:28-34. 1991.

2. Kim, I.-J., M. Gagic, and J. M. Sharma. Recovery of antibody-producing ability and lymphocyte repopulation of bursal follicles in chickens exposed to infectious bursal disease virus.
Avian Dis. 43:401-413. 1999.

3. Nash, A. A., and P. Cambouropoulos. The immune response to herpes simplex virus. Semin. Virol. 4:181-186. 1993.

4. Nowak, J. S., D. Kai, R. Peck, and R. M. Franklin. The effects of cyclosporin A on the chicken immune system. Eur. J. Immunol. 12:867-876. 1982.

5. Sharma, J. M., R. L. Witter, and H. G. Purchase. Absence of age resistance in neonatally thymectomized chickens as evidence for cell-mediated immune surveillance in Marek's disease. Nature
253:477-479. 1975.

6. Zinkernagel, R. M. Immunology taught by viruses. Science 271:173-178. 1996.

7. Zuckermann, F. A., R. Husmann, R. Schwartz, J. Brandt, E. Mateu de Antonio, and S. Martin. Inter-leukin-12 enhances the virus-specific interferon gamma response of pigs to an inactivated pseudorabies virus vaccine. Vet. Immunol. Immunopathol. 63:57-67. 1998.

THE EFFICACY OF A COMPETITIVE EXCLUSION FLORA (AVIGUARD™) AGAINST EXPERIMENTAL *SALMONELLA* CHALLENGE IN TURKEY POULTS

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INTRODUCTION

Since its first description in 1973 by Nurmi and Rantala (7) and Rantala and Nurmi (10), the principle of competitive exclusion (CE), which is also known as the Nurmi concept (8), has been widely accepted by poultry scientists, veterinarians and poultry producers as a method of educing or inhibiting the intestinal colonisation of newly hatched poultry with Salmonella organisms. The current size of the worldwide CE market is approximately 450 million doses per year. The efficacy of CE has been shown in turkeys (1, 3, 5, 11, 12, 13). Aviguard™ is a lyophilised fermentation preparation of the indigenous caecal flora of healthy chickens. The trials presented in this paper were conducted to assess the efficacy of the undefined CE product Aviguard against experimental *Salmonella* challenge in turkey poults.

MATERIALS AND METHODS

Two trials were conducted according to the Recommended Assay of Mead et al. (6), which is commonly used to assess the efficacy of CE products in chickens.

Trial No. 1. Day-old turkey stags were kept in cardboard boxes on coarse wood shavings at ambient temperature. Sterile feed and sterile water were provided ad libitum. The birds were orally dosed with three different batches of Aviguard on day of hatch. Four groups of 12 birds each were used per batch. One day later, two of the groups were orally challenged with approximately 10 4, the other two groups with approximately 10 3 *Salmonella kedougou* cells. The Aviguard treated birds were compared to two challenge control groups, which remained untreated, but were challenged with either 10 3 or 10 4 *S. kedougou* organisms. Seven days after challenge, the stags were necropsied and the level of caecal Salmonella colonisation was recorded. Based on the colonisation levels of the individual birds, the infection factor (IF) and log 10 reduction (LR) were calculated for each of the groups. The formulas used for the calculations are given below: [See formula at end of manuscript.]

Trial No. 2. This trial was conducted under the same environmental conditions and according to the same basic protocol as Trial No. 1. However, this time, day-old turkey stags and day-old male broiler chicks were treated with the same Aviguard batch in order to compare the efficacy of the chicken derived CE flora Aviguard against caecal *S. kedougou* colonisation in the two avian species. Two groups of 12 poults each and two groups of 12 chicks each were orally dosed with 1/10 of an Aviguard dose. The same number of turkeys and chicks was treated with one single dose and the tenfold dose of the same batch. Twelve stags and chicks each served as

challenge control groups. All the birds were challenged with approximately 5 x 10 3 *S. kedougou*.

RESULTS

Trial No. 1. The challenge control groups showed a mean *Salmonella* count (IF) of 8.1 and 8.4 for the 10 3 and 10 4 challenge, respectively. The mean IF of all treated turkeys, which had been challenged with 10 4 *S. kedougou* was 3.3 compared to a mean IF of 2.1 for the birds challenged with 10 3 *S. kedougou*. The resulting mean log reductions were 5.1 and 6.6 following a challenge with 10 4 and 10 3 *S. kedougou*, respectively. All treatments led to a highly significant reduction of the caecal *Salmonella* count.

Trial No. 2. The IFs of the challenge control groups were 7.8 and 8.4 for the turkeys and chickens, respectively. Treatment with 1/10 of a dose reduced the caecal *Salmonella* count by 2.1 and 0.7, treatment with the normal dose by 6.3 and 6.6 and treatment with the tenfold dose by 6.8 and 8.1 log 10 in turkeys and chickens, respectively. Treatment with the normal dose and the tenfold dose induced a highly significant reduction of the caecal *Salmonella* colonisation.
Significant differences between the normal and the tenfold dose could not be found indicating that a tenfold overdose of Aviguard does not boost efficacy.

DISCUSSION

Reciprocal protection of the chicken and turkey against intestinal *Salmonella* infection by a chicken derived CE flora was reported by various authors (3, 4, 11, 12, 13). These findings could be confirmed by the significant log reductions obtained in turkeys by treatment with the product Aviguard. Although chicken derived CE floras are generally considered not as efficacious in turkeys as in chickens (3, 4, 11, 12), the results of Trial No. 2 indicate, that under certain conditions, the administration of a normal dose of Aviguard can induce equal protection against intestinal *Salmonella* colonisation in day-old chicks and turkey poults. Weinack et al. (13) treated day-old chickens and turkeys with homologous CE floras and challenged them with increasing doses of *Salmonella*. They demonstrated that, more in turkeys than in chickens, overwhelming challenge doses hamper the protection afforded by CE. Optimum CE protection was obtained for challenge doses of ,</=4 log 10 and </=3 log 10 in chickens and turkeys, respectively. The better response to CE of the turkeys challenged with the lower dose in Trial No. 1 supports the observations of Weinack et al. (13). The previously reported good efficacy of the product Aviguard in turkeys (2, 9) was confirmed by the results presented in this paper.

REFERENCES

1. Barnum, D. A., W. Anderson, W. Reid, E. Davis, and W. R. Mitchell. The application of Competitive Exclusion in the prevention of Salmonella colonization in turkeys. Proc. 8th International Symposium of the World Association of Veterinary Food Hygienists: pp. 88-93. 1981.

2. Cameron, D. M., J. N. Carter, P. Mansell, and V. A. Redgrave. Floor-pen efficacy study with Aviguard against Salmonella typhimurium DT 104 colonization in turkeys. Proc. Salmonella and salmonellosis, Ploufragran, France: pp. 481-485. 1997.

3. Hollister, A. G., D. E. Corrier, D. J. Nisbet, R. C. Beier, and J. R. DeLoach. Comparison of effects of chicken caecal micro-organisms maintained in continuous flow culture and provision of dietary lactose on cecal colonization by Salmonella typhimurium in turkey poults and broiler chicks. Poult. Sci. 73:640-647. 1994.

4. Impey, C. S., G. C. Mead, and S. M. George. Evaluation of treatment with defined and undefined mixtures of gut micro-organisms for preventing Salmonella colonisation in chicks and turkey poults. Food Microbiol. 1:143-147. 1984.

5. Lloyd, A. B., R. B. Cumming, and R. D. Kent. Prevention of Salmonella infection in poultry by pretreatment of chickens and poults with intestinal extracts. Austr. Vet. J. 53:82-87. 1977.

6. Mead, G. C., P. A. Barrow, M. H. Hinton, F. Humbert, C. S. Impey, C. Lahallec, R. W. A. W. Mulder, S. Stavric, and N. J. Stern. Recommended assay for treatment of chicks to prevent Salmonella colonisation by 'competitive exclusion'. J. Fd. Prot: 52:500-502. 1989.

7. Nurmi, E. V., and M. Rantala. New aspects of Salmonella infection in broiler production. Nature 241:10-211. 1973.

8. Pivnick, H., and E. V. Nurmi. The Nurmi Concept and its role in the control of Salmonella in poultry. In: Developments in Food Microbiology, 1st ed. R. Davies, ed. Applied Science Publishers Ltd., Barking, UK. pp. 41-70. 1982.

9. Primm, N. D., K. Vance, L. Wykle, and C. L. Hofacre. Application of normal avian gut flora by prolonged aerosolization onto turkey hatching eggs naturally exposed to Salmonella. Avian Dis. 41:455-460. 1997.

10. Rantala, M., and E. V. Nurmi. Prevention of growth of Salmonella infantis in chicks by the flora of the alimentary tract of chickens. Brit. Poultry Sci. 14:627-630. 1973.

11. Schneitz, C. and L. Nuotio. Efficacy of different microbial preparations for controlling Salmonella colonisation in chicks and turkey poults by competitive exclusion. Brit. Poult. Sci. 33:207-211. 1992.

12. Snoeyenbos, G. H., O. M. Weinack, and C. F. Smyser. Protecting young chicks and poults from salmonellae by oral administration of "normal" gut microflora. Avian Dis. 22:273-287. 1978.

13. Weinack, O. M., G. H. Snoeyenbos, C. F. Smyser, and A. S. Soerjadi. Reciprocal Competitive Exclusion of Salmonella and Escherichia coli by native intestinal microflora of the chicken and turkey. Avian Dis. 26:585-595. 1982.

$$IF = \frac{\log_{10}(\text{bird 1}) + \ldots + \log_{10}(\text{bird 12})}{12}$$

$$LR = IF_{\text{challenge controls}} - IF_{\text{test group}}$$

POSTERS

THE PATHOPHYSIOLOGY OF STUNTING SYNDROME DISEASE OF TURKEYS: PRO-INFLAMMATORY CYTOKINES AND INTESTINAL EPITHELIUM

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Stunting syndrome agent (SSA) is an enteric virus that causes stunting syndrome (SS) in turkeys resulting in diarrhea and weight loss. Using an embryo model we previously reported that the immune cells are involved in the pathophysiology of the intestinal disease. We found that the intestinal fluid secretion in immune deficient turkey embryos was less than in the control embryos following SSA infection. The present study was initiated to determine the involvement of the pro-inflammatory cytokines during SSA infection. The presence of pro-inflammatory cytokines including interleukine-1 (IL-l), IL-8, tumor necrosis factor-alpha (TNF- alpha) and monocyte-macrophage inflammatory protein- alpha (MIP- alpha) were evaluated in the intestinal epithelial cells (IEC) of embryos by RT-PCR. There was an increase in the level of IL-8 mRNA from 48 to 96 h post-inoculation. The maximum level of mRNA for MIP- alpha was observed at 96-h post-inoculation. The TNF- alpha mRNA was detected in IEC only from infected embryos. These observations suggest that the viral infected intestinal epithelial cells produced pro-inflammatory cytokines that may be involved in the pathophysiology of SS.

TRANSMISSION AND PATHOLOGY OF AVIAN LEUKOSIS VIRUS SUBGROUP J (ALV-J)

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Avian Leukosis Viruses (ALV) are members of the Retroviridae family. These viruses are noted for producing a variety of transmissible benign and malignant neoplasms. ALVs are divided into 6 subgroups (A, B, C, D, E and J) based on their host range, interference patterns and viral envelope antigens. ALV-J is the most recently described subgroup with its origin attributed to recombination occurring between an exogenous ALV and endogenous ALV gene sequences within the chicken genome. The study objective was to evaluate direct and indirect transmission of ALV-J and any early pathology induced. The study was conducted in a biosecurity level (BSL) 2 containment facility. Direct transmission was accomplished by mixing infected chickens (ALV p27 group specific antigen positive) with uninfected chickens in cage batteries at 0, 7, 14, 21 and 28 days post-hatch. Following exposure, the chickens were monitored weekly by collecting blood samples for p27 ELISA (Enzyme Linked Immunosorbent Assay), PCR (Polymerase Chain Reaction), blood cell differentials and serum for antibody testing. The study was terminated at 8 weeks post-hatch and the chickens were weighed, bled and necropsied. Tissues were taken for histopathological evaluation. Indirect exposure was accomplished by placing infected and uninfected chickens in the same room. Samples were collected as described above. Results indicate that virus transmission was accomplished by both exposure methods. No consistent gross and microscopic lesions were found which correlated with ALV early infection.

USE OF LOW DOSES OF CLENBUTEROL TO REDUCE INCIDENCE OF ASCITES SYNDROME IN BROILERS

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Various beta-adrenergic agonists have been shown to be capable of improving weight gain when added to the feed of poultry (1,4, 10, 11). One of the key features observed when beta adrenergic agonists are added to the feed is a reduction in the proportion of fat in tissue (2, 6), an increase in nitrogen accretion, and a reduction in the saturated fatty acid concentration within the fat associated with muscular fibers (7).

It has been suggested that the ascites syndrome is a multifactorial disease with a common triggering factor: a reduced cardiovascular reserve (9, 14). It has also been shown that beta -adrenergic agonists can increase cardiac performance and regulate the number and bioavailability of beta -receptors in target tissue (3). Based on the hypothesis that a continuous stimulation of beta -adrenergic receptors, using clenbuterol as beta -adrenergic agonist, could improve the cardiovascular sufficiency of the birds and reduce the incidence or severity of the ascites syndrome, a trial using low doses of clenbuterol-medicated feed throughout the complete fattening cycle, was carried out.

MATERIALS AND METHODS

Thirty two thousand one-thy-old Arbor-Acres broiler chicks from a commercial hatchery were randomly divided and housed in 8 groups (4 replicates per group) of 4,000 birds each, under controlled standard conditions. The temperature of the poultry houses with natural light (13 h) was maintained at 34 degree C initially, and then reduced by 3 degree C/wk until it reached 21 degree C, at which temperature, the room was maintained for the rest of the feeding period. Humidity was held at 65%, and bird density at the end of the trial was 27 kg/m 2. Birds were fed throughout the production cycle as per the recommendations of Cuca et at (5) (Table 1).

Table 1. Composition of basal diets.

The diet fed from 0 to 3 weeks of age contained 22% CP and 3,000 kcal ME/kg, and the second diet, fed from 3 to 7 weeks of age, contained 20% CP and 3,020 kcal ME/kg. In this trial, diets were supplemented with 0.25 ppm of clenbuterol (using a clenbuterol premix 0.6%, containing: clenbuterol hydrochloride and calcium carbonate as excipient) in the experimental groups throughout the first 4 weeks of the production cycle. Chicks were allowed to have free access to both diets up until week 4 and then slight restriction was implemented. They also had free access to water. The vaccination program included vaccines against infectious bursal disease (three times), Newcastle disease and infectious bronchitis (two times each).

The following variables were assessed: weight gain, feed conversion rate, mortality rate, abdominal fat body weight ratio, and ventricular index, measured according to the following formula VI RV/LV; where VI = ventricular index; RV = right ventricle; and LV = left ventricle. Data were processed using an analysis of variance for weight gain and conversion rate and for

ascites mortality, abdominal fat body weight ratio, and ventricular index. Man-Whitney "U" tests were utilized (12).

RESULTS AND DISCUSSION

Table 2 shows the mean and standard deviation increments in body weight, and conversion rate values at days 21 and 52. Table 3 summarizes data for mortality due to the ascites syndrome and the abdominal fat:body weight ratio. Table 4 summarizes values for cardiac weight, weight of the right and left ventricles, and ventricular index.

Table 2. Mean +/ SD of body weight (BW) and conversion rate (CR) in the control and clenbuterol-treated groups on days 21 and 52.

Table 3. Mean and standard deviation data: for mortality due weight ratio at 52 d in the control and clenbuterol-treated group.

Table 4. Mean values in grams of total cardiac weight, weights of the right and left ventricle, and ventricular index.

There was a statistically significant increase in the weight of the right ventricle in the clenbutero-treated birds, relative to that of the untreated control group ($P< 0.05$). A larger ventricular index ($P< 0.08$) was obtained for the clenbuterol-treated chicks.

There were no significant differences in body weight ($P> 0.1$) or ventricular index ($P> 0.1$). However mortality due to the ascites syndrome and abdominal fat; body weight radio differed significantly ($P< 0.05$, and $P < 0.01$, respectively). Also, a significant negative correlation was found between ventricular index and cardiac weight with mortality rate ($r = 0.98$).

In contrast with other studies, the experimental model used in this trial failed to show a growth-promotion effect with clenbuterol. This lack of effect may be due to the well-balanced diet used in this trial, and to the comparatively low concentrations of clenbuterol added to the diet. Muramatsu et al. (11) found a similar lack of effect when near to optimal nutritional and husbandry conditions were observed. As expected, the use of clenbuterol as a feed additive significantly reduced the abdominal fat body weight ratio (13).

Results show a significant reduction in mortality due to ascites syndrome. This study is first in mentioning the effects of clenbuterol in decreasing mortality due to ascites syndrome. Greater values for the ventricular and cardiac indexes obtained in the clenbuterol-treated birds, may have contributed to the reduced mortality due to ascites syndrome. A more balanced distribution of intraventricular pressures and volumes may have been achieved, which, in turn, allowed the birds to withstand the cardiovascular changes ascribed to the ascites syndrome (14). However, this speculation requires confirmation.

These results could benefit the poultry industry if adequate withdrawal times are established and enforced or if other means of adrenergic stimulation prove equally effective.

REFERENCES

1. Alpizar, S. O., R. F. Perez-gill, g. E. Avila, S. V. V. aaes, C. C. Lopez, and C.

L. Ocampo. Efecto de un agonista beta -adrenergico en la alimentacion de pollos de engorda. Vet. Mex. 24:37-41. 1993.

2. Bobowiec, R., P. Radymska, and K. Wawryziniak. Effect of adrenergic agonist clenbuterol on plasma lipoprotein fractions, fatty acid content and muscular fibre types in chicks. Med. Vet. 50:457-460. 1994.

3. Brian, B. H., and R J Lefkowitz. Catecolaminas y Drogas Sympaticomimeticas. In; Las Bases farmacologicas de la terapeutica. 6th ed. Editorial Panamericana, Mexico City, Mexico. p.223. 1991.

4. Buyse, J., E. Decuypere, G. Hygheabaert, and M. Herremans. The effect of clenbuterol supplementation on growth performance and on plasma hormone and metabolite levels of broilers. Poult. Sci. 70:993-1002. 1991.

5. Cuca, G. M., G. C. Avila, and M. A. Pr6. Alimentacion de las aves. 8th ed. Universidad Autonoma de Chapingo. Mexico, 1996.

6. Foglia, T. A., A. L. Cartwright, R. J. Gyurik, and J. G. Philips. Fatty acid turnover rates in the adipose tissue of the growing chicken (Gallus domesticus). Lipids 29:497-502. 1994.

7. Hamano, Y., S. Kobayashi, and Y. Terashima. Effect of beta adrenergic turnover in broiler chicks fed either low or adequate protein diet. Anim. Sci. Technol. 65:1105-1110. 1994.

8. Macri, A., F. Vefre, C. Pieramati, L. Achene, and A. Ioppolo, Effect of clenbuterol on broiler chickens under intake restriction. Italian J. Sci. 6 255-262. 1993.

9. Machorro, V. E., and L. H. Paasch. Evaluacion del efecto de hipertension pulmonar, en la presentacion del sindrome ascitico en Mexico. Vet. Mex. 12:2.4. 1981.

10. Malucelli, A., F. Ellendorff, and H. H. D. Meyer. Tissue distribution and residues of clenbuterol, salbutamol, and terbutaline in tissues of treated broiler chickens. J. Anim. Sci. 72:1555-1560. 1994.

11. Muramatsu, T., M. Kakita, Y. Aoyagi, and J. Okamura. Research note; beta -adrenergic agonist effects on liver and breast muscle protein syntesis in female chicks. Poult. Sci. 70:1630-1632. 1991.

12. Siegel, L. Estadistica no parametrica. 1st, ed. Trillas, Mexico City, Mexico. P86. 1980.

13. Takahashi, K., K. Akiba, and M. Horiguchi, Effects of a beta-adrenergic agonist (clenbuterol) on performance, carcase composition, hepatic microsomal mixed function oxidase and antibody production in female broilers treated with or without corticosterone. Br. Poult Sci. 34:167-175. 1993.

14. Wideman, R. F., and Y. K. Kirby. Electrocardiographic evaluation of broilers during the onset of pulmonary hypertension initiated by unilateral pulmonary artery occlusion. Poult. Sci. 75:407-416. 1996.

Table 1. Composition of basal diets.

Ingredients and Composition	Starter	Finisher
Corn yellow	74.36	60.32
Soybean meal	27.65	32.28
Oil	3.61	3.01
Calcium phosphate	2.02	1.78
Limestone	1.30	1.20
Salt	0.35	0.35
DL-methionine	0.22	0.17
Vitamin mix[1]	0.25	0.25
Mineral mix[2]	0.10	0.10
Bacitracin	0.05	0.05
Ethoxyquin	0.04	0.04
Coccidiostat	0.05	0.05
Pigment	0.4	0.4

Calculated composition: Crude protein, 22 and 20; ME, kcal/kg 3000 and 3020; TSAA, 0.90 and 0.80; Methionine, 0.55 and 0.48; Lysine, 1.20 and 1.02; Phosphorus, nonphytate, 0.50 and 0.45; Calcium, 1.00 and 0.90.

[1]Contents per kilogram of diet: vitamin A. 5000 I.U., cholecalciferol, 1,100 IU; tocopheryl acetate, 11 IU; menadione, 1.1 mg; thiamine- HCL, 2.2 mg; riboflavin, 4.4 mg; pyridoxine HCL, 2.2 mg;; cyanocobalamin, 0.66 meq; niacin, 44 mg; Ca pantothenate, 12 mg; choline chloride, 220 mg; folic acid, 0.55 mg; D-biotin, 0.11 mg.

[2]Provided in milligrams per kilogram of diet: Mn,60;Zn,50;Fe,30;Cu,5.0;I,1.2;Co,0.2;Se,0.1.

Table 2. Mean ± SD of body weight (BW) and conversion rate (CR) in the control and clenbuterol-treated groups on days 21 and 52.

Group	21 d		52 d	
	BW	CR	BW	CR
Control	578 ± 56.6	1.64 ± 0.9	2,380 ± 215	2.57 ± 1.1
Treated	561 ± 58.8	1.67 ± 0.8	2,437 ± 221	2.22 ± 1.2

Table 3. Mean and standard deviation data: for mortality due to ascites syndrome and for abdominal fat:body weight ratio at 52 d in the control and clenbuterol-treated group.

Group	Mortality (%)	Abdominal fat: body weight ratio
Control	5.6 ± 1.3 *	23.58 ± 6.4 (1.37 %)
Treated	1.8 ± 0.7	21.47 ± 5.2 (1.15%)

* $P < 0.05$

Table 4. Mean values in grams of total cardiac weight, weights of the right and left ventricle, and ventricular index.

Group	Cardiac Weight	Weight of The right Ventricle	Weight of the left ventricle	Ventricular Index
Control	7.8	1.83*	6.2	0.30
Clenbuterol treated	8.82	2.33*	6.6	0.34

*$P < 0.05$

VACCINATION OF CHICKENS AND TURKEYS WITH A COMBINATION VACCINE

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Combination vaccines represent an important trend of vaccinology in recent years with obvious advantages. In modem poultry production facilities, multiple administration of different monovalent vaccines are costly if not impractical. A combination vaccine is a stable single vaccine preparation containing more than one type of standardized antigen which is ready for administration by injection or by other means. A new combination vaccine (Phlombo^{TM}), composed of adjuvanted bacterial antigens from avian origin killed *Pasteurella multocida* (4 serotypes), *Hemophilus paragallinarum* (2 serotypes), *Escherichia coli* 078 and *Salmonella typhimurium*, was developed and evaluated for its intended use in chickens and turkeys. Safety and laboratory scale efficacy studies on chickens and turkeys indicate that the vaccine is safe and efficacious when injected subcutaneously in commercial birds. Enzyme linked immunosorbent assay (ELISA), using sera from the vaccinated birds demonstrated significant increase of specific serum antibodies compared to the non-vaccinated controls. Challenge studies were performed after the 2nd dose of vaccine in these target species of birds using the homologous virulent strains of *P. multocida, H. paragallinarum* or *E. coli*. The morbidity and mortality data following the challenge doses indicated significant or remarkable protection of the vaccinated birds compared to their control cohorts.

WEST NILE FEVER - A REEMERGING ZOONOSIS

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In late August - October, 1999, an outbreak of viral encephalitis occurred concurrently in wild birds, notably crows, zoo birds, horses and humans in the states of New York, New Jersey and Connecticut. In two recent publications from workers at the CDC and their collaborators, and from a Connecticut group of arbovirologists findings have been documented. One describes isolates of West Nile Virus (WNV) from two species of mosquitoes, brains of crows and a Cooper's hawk. The CDC group have published partial sequence data of WNV isolates from several crows, a sandhill crane, a Chilean flamingo from the Bronx zoo, and from two human cases. All these isolates yielded an E gene nucleotide sequence that was extremely homologous to a WNV isolate from a goose in Israel in 1998 and to a Romanian isolate from 1996. In this communication the appearance in 1999 of WNV for the first time in North America will be evaluated within the context of what is known about the geographical distribution of arthropodborne viruses (arboviruses) in the world, and about the distribution and zoonotic properties of WNV in more detail.

The most important veterinary arboviruses belong to the Togaviridae family and are sub-divided into the Alphavirus and Flavivirus genera. Both genera have avian enzootic hosts that are migratory birds with unique flight patterns. Alphaviruses are also known as the New World viruses and are associated with nervous diseases, mainly of horses, but occasionally of game birds and domestic fowl. The major examples are the Eastern, Western and Venezuelan equine encephalitides that occur in North, Central and South America. These are zoonotic diseases by contrast to another North American virus, Highlands J, that causes an egg drop syndrome in turkey breeders and sometimes, equine encephalitis. Zoonotic alphaviruses identified in the Eastern Hemisphere include Sindbis, initially isolated in Egypt, but now also isolated from the other three continents, and Ockelbo virus isolated in Sweden only. Wild bird reservoirs of both viruses have been identified.

With the exceptions of St Louis encephalitis virus and more recently of West Nile virus that have appeared in North America, all the flaviviruses are Old World viruses. The principal veterinary pathogen in this genus is Japanese encephalitis (JE) that has epidemic and endemic patterns in temperate and tropical regions of Asia respectively. JE was recently described in Australia for the first time. The second most important veterinary flavivirus is considered to be WNV that was first isolated in 1937 from a Ugandan woman with a mild fever. Since then WNV has been isolated on numerous occasions over the last 50 years from countries throughout Africa and Eurasia where it is considered endemic. The epidemic form of West Nile fever (WNF) has quite a sporadic history. Until 1996-97 when more than 500 human cases were diagnosed in Bucharest, Romania, the disease was almost unknown as an epidemic in Europe. The only significant outbreak was in France during 1962-65 when 10 severe human cases were recorded. More recently in 1997, 5 human cases were reported in Southern Moravia, Czech Republic. In Africa, hundreds of human cases were reported in epidemics that raged from the early 1950s through 1974 when a major epidemic hit South Africa involving 3,000 persons. More recently 8 patients died out of a total of 50 cases of WNF in Algeria. In Romania, the case-fatality was about 10%.

In recent years the involvement of horses in WNF epizootics has become more pronounced. In the early 1950s several deaths were reported in Egypt and an infectivity trial was conducted with a local isolate. During the French outbreak in 1962, 50 cases of equine encephalomyelitis were recorded. More recently in 1998 in Northern Italy, 6 out of 14 WNV-affected horses either died or were euthanized, while in the same year 42 out of 94 affected horses died in Morocco. In the New York epidemic in 1999, 8 of 18 febrile horses died on Long Island.

Reports of natural WNF in birds were almost nil until last year when considerable mortality was observed among American crows in the New York area. In the 1950s an extensive series of infectivity experiments was performed in Egypt on pigeons, chickens, gulls, ducks and crows. Some species died of encephalitis and in others the virus persisted for extended periods. The involvement of the crow was a remarkable feature of these studies. Many observations are documented on the role of migrating birds in the transmission of arboviruses between countries and continents. Serosurveys carried out in European, Eurasian and African countries yielded the presence of HI and virus-neutralizing antibodies in a wide range of trapped birds. Ornithologists in the Middle East have counted hundreds of thousands of white storks as they fly over the region to and from Europe and Africa each year. The route covers Israel, the Nile River and Delta, and the Sinai Peninsula. In 1998, large numbers of these birds were grounded because of inclement weather conditions in Southern Israel and some were bled and others that did not survive were examined for WNV in the brain. These isolates were characterized and PCR E gene products were sequenced by Dr Vincent Deubel at the Pasteur Institute, Paris (manuscript in preparation). We consider that the stork has a major role in the transport of WNV in the region and its nesting habits in Europe during the spring and summer make it a strong candidate for seeding European countries during its annual flyover.