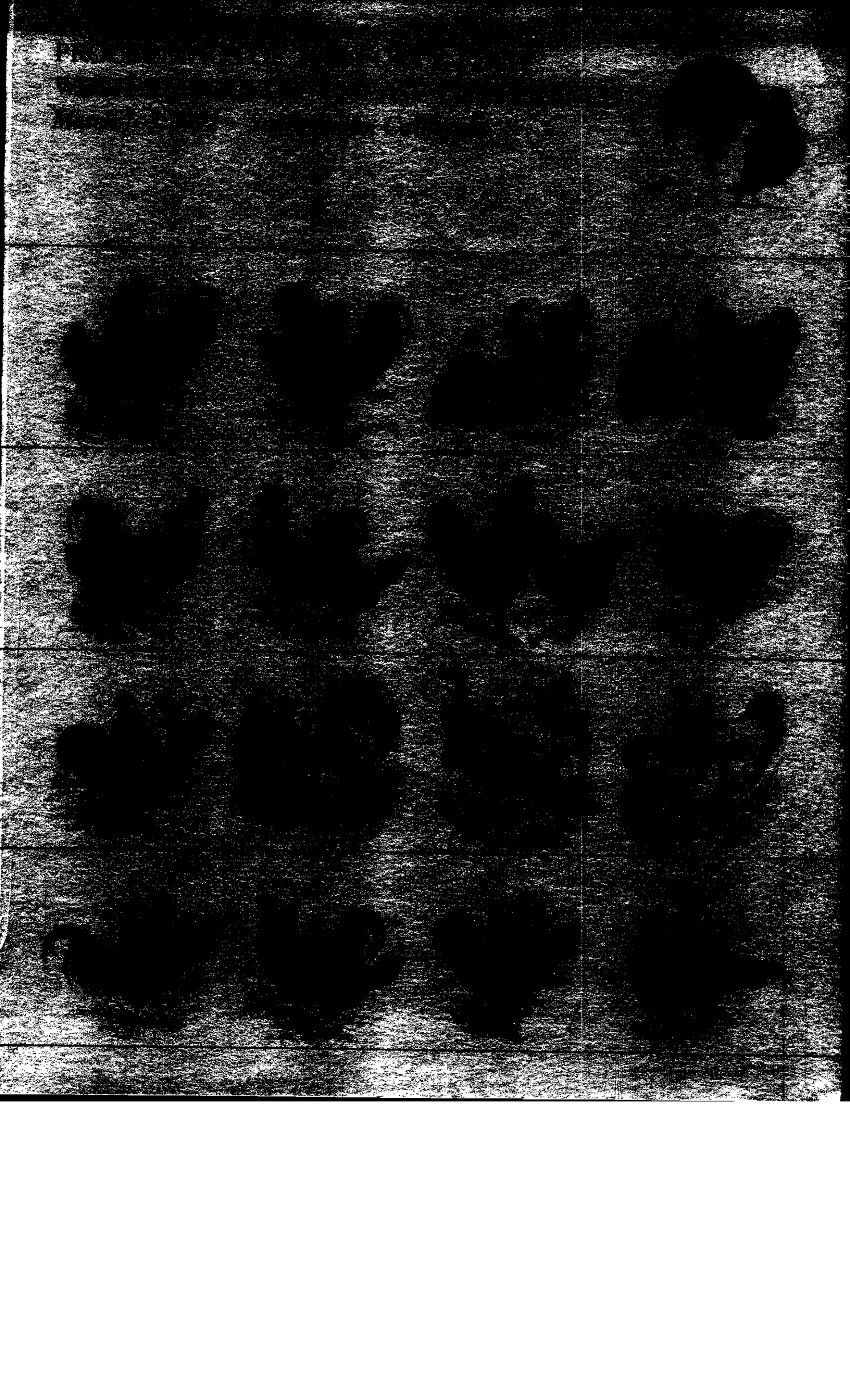
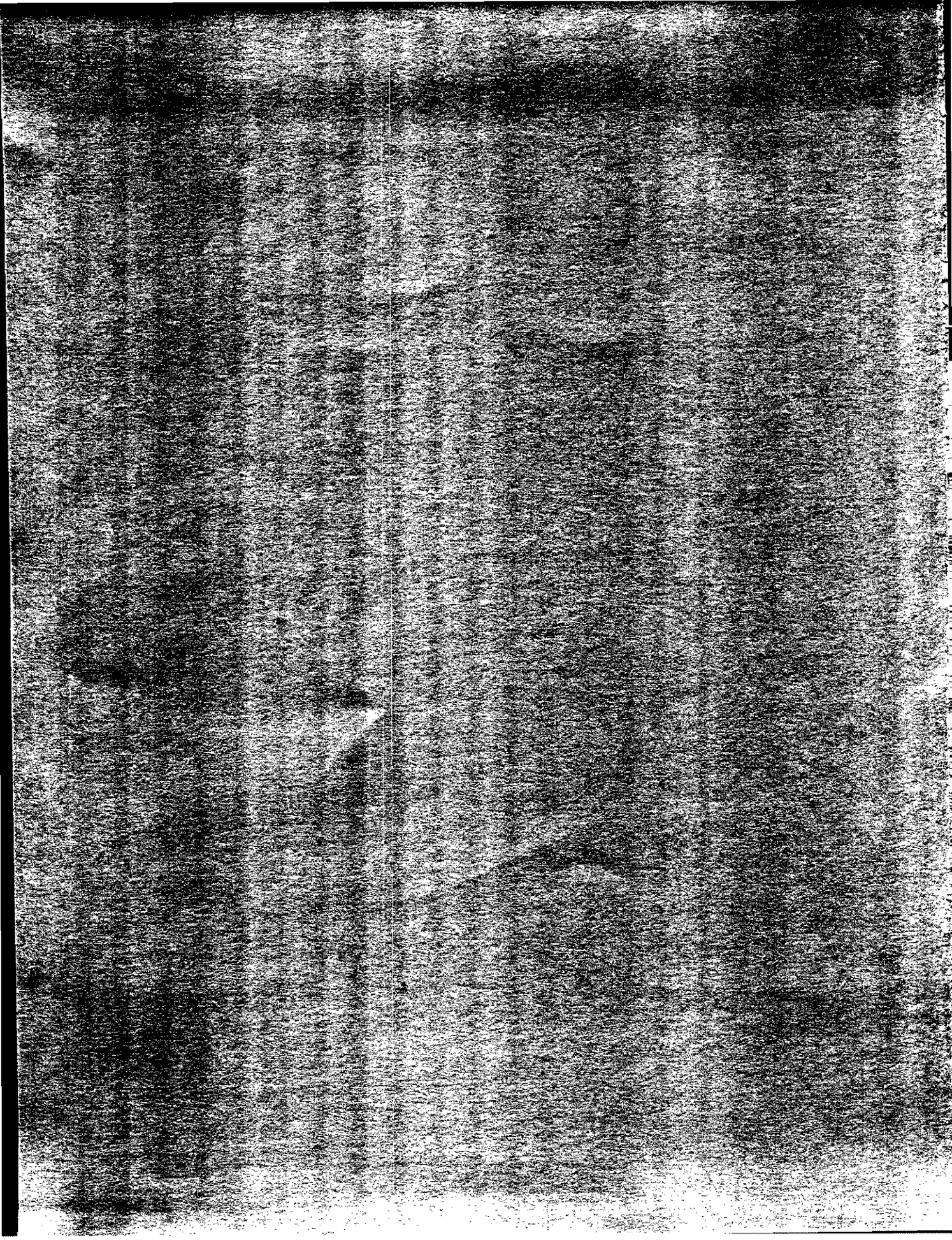
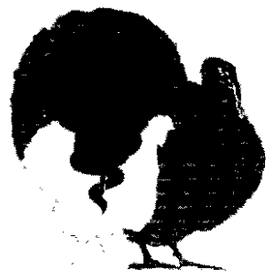




PROCEEDINGS OF THE FORTY-FIRST WESTERN POULTRY DISEASE CONFERENCE

March 1–3, 1992 · Sacramento, California



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Dr. Richard P. Chin
President
California Veterinary Diagnostic Laboratory System
University of California
2789 S. Orange Avenue
Fresno, CA 93725

Dr. Rocky J. Terry
Program Chair
P.O. Box 1858
Glen Rose, TX 76043

Dr. A Singh Dhillon
Program Chair-elect
Washington State University
Puyallup Research and Extension Center
Department of Microbiology and Pathology
Puyallup, WA 98371

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Provo, UT 84602

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The proceedings of the 41st Western Poultry Disease Conference are presented as a service and a source of information to those attending the conference and to others who wish to gain some insight as to the discussion and information presented. As has been stated in the past, these proceedings are not considered or regarded as an "official publication" but simply as a source of information.

Copies of proceedings are available from: A.S. Rosenwald
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Price: $12.50 postpaid (AAAP members: $10.00) for USA.
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SPECIAL ACKNOWLEDGMENT

The Western Poultry Disease Conference (WPDC) is pleased to acknowledge substantial contributions to its Speaker's Fund. These contributions provide support for outstanding scientists whose participation might otherwise not be possible. Over thirty organizations and companies have given substantial financial support, and many companies and organizations, including some that also contribute to the Speaker's Fund, send speakers at no expense to the conference. We thank all these.

We especially thank the American Association of Avian Pathologists for its generous program enrichment grant that helps bring speakers from other regions. WPDC also sends "thanks" to the Asociacion Nacional de Especialistas en Ciencias Avicolas de Mexico (ANECA) for support from the joint ANECA-WPDC Conference of 1991 that was passed on to the 1992 Conference and for jointly defraying expenses of several of our speakers. WPDC is most grateful to Pfizer Animal Health for sponsoring and hosting the exciting welcoming reception, the "Pfizer Express."

Our distinguished patrons, donors, and sustaining members are listed on the following pages. We greatly appreciate their generosity.

Many have provided special services that contribute to the continued success of this conference. We express special thanks to those in charge of local arrangements at Sacramento; members of the translation crew (M. Salem and V. Mireles), who make a major contribution in providing the excellent English/Spanish translation and other bilingual assistance; the program chair; and all others who contribute to the program and host committees.

We express our gratitude to all authors who submitted manuscripts. We give thanks and acknowledgment to Brigham Young University, Department of Microbiology, who handled the publication of the proceedings. We especially thank Angela Adair for typing and formatting the manuscripts and Jennifer Harrison for coordinating the computer desktop publishing of the proceedings. We also thank Bruce Patrick (Graphic Communications, Brigham Young University) for the cover design and interior layout.

WPDC SPECIAL RECOGNITION AWARDS

At this year's conference, the WPDC is pleased to present its Special Recognition Award to the following persons for their many contributions and for their many years of support and service to this conference. The 1992 Awardees are:

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MINUTES OF THE 1991 40TH WPDC BUSINESS MEETING

President Jeanne M. Smith called the meeting to order at 12:45 p.m. on April 26, 1991 at the Acapulco Plaza Hotel, Acalpulco. The minutes of the 39th WPDC business meeting as printed in the 40th proceedings were read and approved.

President Smith reported that the Capitol Plaza Holiday Inn, Sacramento had been reserved for the 41st conference to be held on March 1-3, 1992. The hotel is close to many of the attractions in downtown Sacramento such as the Capitol, "Old Sacramento," shopping etc. The hotel is devoting essentially all of its meeting rooms to WPDC so the conference will have sufficient space for all of its activities.

The president indicated that Dr. Singh Dhillon was nominated by the executive committee to serve as program chair-elect for 1993, and asked for additional nominations from the floor. There were no nominations from the floor; Dr. Dhillon was elected by voice call. Dr. Dhillon agreed to serve as program chair for 1992 in the event that Dr. Rocky Terry was unable to serve because of a change in his employment and place of residence. An alternate program chair-elect for 1993 was elected in the event that Dr. Dhillon became program chair for 1992. Drs. Ken Lam and George Cooper were nominated from the floor. Dr. George Cooper was elected by voice vote.

A question regarding the need to change the by-laws arose since the group had elected two program chair-elect for 1993. It was pointed out by Dr. Rosenwald that the group does not have any by-laws except in 1984 a constitution was adopted that indicated that the name of the organization shall be "The Western Poultry Disease Conference" and the purpose of the organization was to hold meetings annually in the Western region; an ad hoc committee had been appointed to develop the by-laws further but the committee has remained inactive. After further discussion it was clarified that Dr. Cooper's election would become void in the event that Dr. Dhillon remained as program chair-elect for 1993.

The president announced the following officers for 1992:

President:	Dr. Richard P. Chin
Program Chair:	Dr. Rocky Terry
Secretary:	Dr. Richard Yamamoto
Treasurer:	Dr. Arnold S. Rosenwald
Program Chair-elect, 1993:	Dr. Singh Dhillon

The President announced that the officer not mentioned above was Dr. Marcus Jensen who had served as Proceedings Editor for the past 5 years. Dr. Jensen had informed the president that Scholarly Publications at BYU was no longer able to produce the proceedings which means that the conference will have to pay for this service. Dr. Jensen is willing to continue the duties as Proceedings Editor but asked if there were others who would be willing to take on this responsibility. There were no volunteers. The discussion centered around ways to reduce the time commitment and cost to prepare the proceedings with the likely necessity of down-grading its quality. Dr. Jensen would be able to do the work more easily if, for example, papers came in as 1-page abstracts or camera-ready form. There have been problems with software programs that did not work (25%) and much editing, and re-writing of lengthy papers having elaborate tables and figures. It was suggested that papers of one page or less in length could be submitted as hard copies without the use of computer disks. Laboratories that submit several papers such as the CVDLS might consider combining their papers on a disk or two in a camera-ready form. Dr. Jim Case indicated the possibility of the use of publication capabilities of the CVDLS to maintain the high quality of the proceedings. He volunteered to work with Dr. Jensen to write guidelines to increase the probability of being able to automatically get either hard copy or disk-based information into the computer so that they may be formated the same. Dr. Jensen indicated that the real problem was in having people conform to guidelines and systems. After further discussion, the membership voted and approved limiting of paper length to a maximum of 3 pages (no tables or figures). It was again emphasized that full-length papers published in a proceedings such as WPDC would most likely be rejected when submitted to a refereed journal.

The membership approved two proposals submitted by the Executive Committee:

1. **Poster Award.** The program chair will appoint a committee to select the best poster.
2. **Service Award.** One given annually. *Criteria:* Recipient must show significant contribution to avian medicine in the Western Region in areas of teaching, research, extension or conference activities. Nominations will be accepted from the general membership and final selection will be done by the Executive Committee.

Dr. A. S. Rosenwald, Treasurer, reported that a complete accounting was not available because expenses were being shared between WPDC and ANECA. A balance of approximately $4,000 was estimated at the end of this joint conference.

Dr. Rosenwald also mentioned some problem areas in running the day-to-day operations of the WPDC. He was hopeful that some day we would have a Secretary/Treasurers'office to conduct the daily business of WPDC. Consideration should also be given to combining the positions of secretary and treasurer for smoother operation of the organization. Dr. Yamamoto mentioned that since WPDC is a self-sustaining organization, funds should be available for secretarial help ($1,000 to $2,000). With this type of assistance from the WPDC, more benefits from his EPM Department could follow. Currently, the conference has access to the EPM Department computers. Dr. Rosenwald indicated his work-study student assistance is costing the WPDC about $1,200 to $1,500. Drs. Rosenwald and Yamamoto will attempt to coordinate their activities for a smoother operation for the coming year.

The joint 40th WPDC-16th ANECA meeting was dedicated to Dr. A.S. Rosenwald for his tireless effort to maintain close ties with our colleagues from the South. The WPDC membership again congratulated Dr. Rosenwald for this honor.

President Smith turned the gavel of the conference over to the new president, Dr. Richard P. Chin, who presented the "President's Plaque" to Dr. Smith. Dr. Chin moved to adjourn the meeting which was seconded and passed.

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Note: Both the oral and poster presentations of the 41st WPDC are listed below in alphabetical arrangement by presenting author. Authors and titles of all papers scheduled for presentation at the conference are listed.

PROCEEDINGS OF THE FORTY-FIRST WESTERN POULTRY DISEASE CONFERENCE

SEVERE OUTBREAKS OF INFECTIOUS BURSAL DISEASE IN MOROCCO

K. Bouzoubaa, T. Jaouzi, A. Amara, M. El Houadfi, F. Kichou, M. Mouahid, and J.G. Bell

Institute of Agronomy and Veterinary Medicine
Hassan II, Avian Diseases Department
BP 6202 Rabat-Institute, Morocco

Infectious Bursal Disease (IBD) or Gumboro disease had been recorded in Morocco since the late 1970s[1]. However, the incidence was very low and no vaccination was used. In 1989, subclinical IBD cases were reported and some farms started vaccinating their flocks with available commercial vaccines[2]. In the summer of 1991, IBD appeared with unusually high mortalities, and within 3 months, the disease covered all parts of the country. Then speculations arose about the origin of the disease.

In this paper, we are reporting cases of IBD that were diagnosed at the Avian Diseases Department at the Institute of Agronomy and Veterinary Medicine Hassan II, Rabat, Morocco as part of a study to elucidate the nature of these severe IBD outbreaks.

MATERIALS AND METHODS

Field Cases. Between May and December 1991, 60 cases from outbreaks of IBD were presented to the Institute. Histories of IBD, vaccine (type, schedule), age at onset of the disease, symptoms, and mortalities were recorded.

Specimens. Bursae having the characteristics gross lesions of IBD were collected from necropsied birds in some flocks suffering from severe IBD. They were used for virus isolation and for histopathology.

Virus Isolation. Aliquots of bursae from broilers, pullet layers, or breeders were homogenized with equivalent amounts of phosphate-buffered saline. Supernatant from the resulting homogenate was used for virus isolation. Ten-day-old chicken embryos from parent flocks unexposed to IBD virus were inoculated by the chorioallantoic membrane route[3].

Histological Examination. Enlarged and hemorrhagic bursae from birds were collected. They were fixed in 10% formalin and section (3-5um thick) were cut and stained with hematoxylin and eosine for studying histopathological changes.

RESULTS

Epidemiological Data. Data collected from a total of 60 outbreaks of IBD are presented in Table 1. Thirty-nine outbreaks were observed in broilers, 18 in pullet layers, and 2 in breeders. One case was reported in a native breed. Table 2 presents the evolution of IBD in broilers and pullet layers since May 1991.

The Natural Disease. The clinical manifestation of the disease appeared suddenly in all chicken types. The initial signs were depression and watery-whitish diarrhea, followed by ruffled feathers, trembling, severe prostration, and death. The duration of the disease varied from 5-10 days with an average of 1 week. The mortality rate varied from 0.8 to 90% for pullets, from 0.9 to 73% (in 1 broiler farm) in broilers and from 6 to 10% in breeders as shown in Table 1.

The main gross lesions in all chicken types were enlarged and severely hemorrhagic bursa, hemorrhages of variable degrees in the muscles of the thigh and breast, petechia in kidneys and sometimes hemorrhages in the proventriculitis and intestines. In some broiler flocks, IBD was associated with cecal coccidiosis. The thymus was often congested. The liver presented grayish streaks. In some cases, caseous material was found on the bursa. On histopathological examination, typical lesions of IBD such as intrafolicular edema mixed with such phagocytic cells as heterophils were observed. Most cases were confirmed by histopathology.

Virus Isolation. The causative virus was isolated from broilers, from pullet layers and from breeders. Typical lesions in the inoculated embryos were observed.

DISCUSSION

During the summer and autumn of 1991, several outbreaks of IBD appeared on several farms in Morocco. During these outbreaks, unusual mortalities were observed especially in pullet layers. This study reports 60 such outbreaks and shows the great incidence of IBD in Morocco. The disease was severe between 35-50 days in pullets and between 28-40 days in broilers as reported by others-[4,5,6]. The virus has been isolated; however, it needs to be characterized. There is an urgent need to study vaccination programs using available vaccine and including challenge with the locally isolated virus. Finally, comprehen-

sive epidemiological studies need to be conducted to fully characterize the disease in Morocco.

REFERENCES

1. Bouzoubaa, K., M. Mouahid, M. El Houadfi, A. Amara, J.G. Bell et T. Jaouzi. Les dominantes pathologiques en aviculture au Maroc: Etude retrospective 1977-1987. Maghreb Veterinaire. 4:15-19. 1989.

2. Flissi, H. Contribution a I etude de la bursite infectieuse au Maroc. These Veterinaire, I.A.V. Hassa II, Rabat, Morocco. 1991. pp 105.

3. Winterfield, R.W. Infectious Bursal Disease. In: Isolation and Identification of Avian Pathogens. American Association of Avian Pathologists. Hitchner, Domermuth, Purchase and Williams eds. 2nd ed. Texas. pp. 79-80. 1982.

4. Winterfield, R.W. and S.B. Hitchner, Gumboro disease. Poultry Digest. 23:206-207. 1964.

5. El Batrawi, A.M. Studies on severe outbreaks of Infectious Bursal Disease in Egypt. I. The natural and experimental disease. Proceedings of 2nd Scientific Meeting. Egypt. vet. poult. assoc. 12-14 March, Cairo. pp. 239-252. 1990.

6. Van Den Berg, M. Gouze and Meuleumans. Acute Infectious Bursal Disease: Isolation and characterization of a highly virulent strain. Avian Path. 20;133-143. 1991.

7. Boualem, M. Maladie de Gumboro au Maroc. Etude epidemiologique dans la region de Rabat-Sale-Temara et dans les couvoirs Nationaux. These veterinaire I.A.V. Hassan II, Rabat, Morocco. 1978. pp 80.

Table 1. Chicken type, age at onset of the disease and mortality rate among investigated outbreaks.

Chicken type	No. of outbreaks	Age at onset of the disease (days)			Mortality rate (%)		
		Minimum	Maximum	Average	Minimum	Maximum	Average
Layers	18	22	70	37.5	0.8	90	29
Broilers	39	11	40	25.3	0.9	73	12.6
Breeders	2	28	35	31.5	6	10	8.0
Native breeds	1	--	--	35	--	--	50

Table 2. Evolution of the outbreaks in broilers and pullet layers in 1991.

Month (1991)	No. of outbreaks		Average Mortality (%)	
	Broilers	Pullets	Broilers	Pullets
May	2	-	2	--
June	5	1	18.15	60
July	11	3	12.6	20
August	5	4	10.3	19
September	4	3	18.5	33.3
October	5	3	14.5	34.1
November	4	-	4	--
December	3	4	3	31.0
Total	39	18	12.6	29.0

SUDDEN DROP IN EGG PRODUCTION ASSOCIATED WITH VITAMIN A TOXICITY IN CHICKENS

H. Kinde[A], H.L. Shivaprasad[B], F.D. Galey[C], G. Cutler[D], and D. Hamar[E]

[A]California Veterinary Diagnostic Laboratory System, San Bernardino Branch,
105 West Central Avenue, San Bernardino, California 92412
[B]California Veterinary Diagnostic Laboratory System, Fresno Branch,
2789 South Orange Avenue, Fresno, California 93725
[C]Davis Central Reference Lab, P.O. Box 1770, Davis, California 95617
[D]8450 Happy Camp Road, Moorpark, California 93020
[E]Veterinary Diagnostic Laboratory, Colorado State University,
300 West Drake #E.100, Fort Collins, Colorado 80523

A severe drop in egg production (60-80%) was noted in a flock of 38-week-old layers given a new batch of feed. The clinical signs observed included anorexia, depression, profound photophobia, and closed eyelids (stuck shut). Water consumption was normal. Mortality was negligible. At necropsy, regression of the ovaries and friable livers were noted. The suspected feed was fed to 14, 30-week-old layers. Six other birds were fed normal feed. Clinical signs were similar to those in the field. Necropsy examination was done at 1, 3 and 4 week intervals during the trial period. Loss of body weight and regression of the ovaries were noted. Histological findings included conjunctivitis, parathyroid hyperplasia and decreased mineralized osteoid seams of the long bones. No clinicopathological abnormalities were found in the control birds. Analysis of the feed revealed vitamin A levels of 1800 mg/kg. Vitamin A levels in sera, livers, and egg yolk of birds exposed to the suspected feed were also in excess amount. Not surprisingly, vitamin A was not detected in the egg whites (albumin) of these birds.

ETHOXYQUIN TOXICOSIS IN BROILER CHICKS

Veronica Yin-Ming Leong and Tom P. Brown

Poultry Disease Research Center
University of Georgia, Athens, Georgia 30602

Ethoxyquin (1,2-dihydro-6-ethoxy-2,2,4-trimethylquinoline) is an antioxidant used commonly in the feed industry. It protects feedstuff against oxidation and prevents peroxide formation, rancidity, and degradation of fat-soluble vitamins and pigments. A commercial broiler operation had a sudden increase of mortality in 4 different flocks at 2 different locations. Daily mortality of 0.4-1.0% of 2-week-old broilers was attributed to contamination of starter feeds with approximately 6,500 mg/kg ethoxyquin. All affected houses and some unaffected houses had red dust on the interior ceilings, walls, and equipment. Gross necropsy findings in chicks consisted of pale, swollen kidneys with excessive urates in ureters, kidneys and joints, as well as dark brown-green, swollen livers. Histologically, multifocal proximal tubular necrosis, dilated sinusoids, bilary hyperplasia, and brown pigment in livers, kidneys, and vessel lumens were found. The clinical disease and lesions were reproduced experimentally with 12,500 mg/kg ethoxyquin.

COCKLEBURS (XANTHIUM SPP.) ARE NOT TOXIC TO BROILER CHICKS

Mark A. Goodwin[A], Edward T. Mallinson[B], John Brown[C], E. Craig Player[A], Kenneth S. Latimer[D], N. Dale[E], Wayne V. Shaff[B], and T.G. Dickson[A]

[A]Georgia Poultry Laboratory, P.O. Box 20 Oakwood Road
Oakwood, Georgia 30566
[B]University of Maryland, College Park, Maryland 20740
[C]Department of Medical Microbiology
[D]Department of Veterinary Pathology
College of Veterinary Medicine
[E]Extension Poultry Science, University of Georgia,
Athens, Georgia 30602

Cockleburs are toxic for several mammalian species including cattle, sheep, pigs, and rats. Spiking mortality syndrome (SMS) is an illness of young broiler chicks, characterized—at least in part—by 1) a sudden rise in mortality rate, 2) a variety of signs that include one or more of the following: depression, huddling, uneasiness, sternal or lateral recumbency, extensor rigidity, terminal convulsions, "blindness." Gross lesions may include all or none of the following: pale or dark viscera, mottled dark or pale liver, or wide growth plates. The etiology of SMS is not known.

SMS in chickens resembles cocklebur toxicity in cattle, sheep, and pigs. The purpose of the present study was to determine if cockleburs would be toxic when fed to broiler chicks.

Ingestion of 25% w/w cocklebur (CB) or 25% rice hull (RH) diets resulted in highly significant failure to properly-gain body weight. Failure of CB-fed chicks to gain body weight could have been due to low calorie intake alone.

Chicks receiving the three diets otherwise did not develop clinical signs of illness or gross or microscopic lesions. Cocklebur toxicity is a differential for the diagnosis of failure of broiler chicks to properly-gain body weight, but is not the (a) cause of SMS.

GANGRENOUS DERMATITIS, "BLUE WING," IN DELMARVA BROILERS

Mariano Salem, C. Pope, J. Rosenberger, S.S. Cloud, and E.M. Odor

Animal Science Department
University of Delaware

During the period of May 14 through July 18, 1991, five cases of gangrenous dermatitis/Blue wing disease were submitted to the Poultry Diagnostic Laboratory at the University of Delaware, Georgetown, Delaware. Cases involved two different integrated poultry companies. The total number of affected flocks in Company "A" were nine, with total mortality ranging form 5.32% to 12.78% at seven weeks of age. In Company "B," the total number of flocks was six with total mortality ranging from 7% to 22% at seven weeks. In all cases, broilers submitted were between 2 and 3 weeks of age and were progeny of breeders from 28 to 36 weeks of age (second to tenth week of production). Breeders involved (from both operations) were in production in North Carolina.

Post mortem findings included extensive areas of gangrenous dermatitis in wing, breast, neck, back, and foot. Some muscle hemorrhages were seen. There was paleness of liver and spleen and also hypoplasia of thymus and bursae. The bone marrow showed white-yellow decoloration. Some livers presented a pale screen pattern.

Of birds sampled, 52% had packed cell volume values of <27 and these low readings ranged form 11 to 25.

Subcutaneous cultures of areas affected yielded *Staphylococcus aureous* but not *Clostridium s.p.* Chicken anemia virus (CAV) was isolated from an homogenate of buffy coats (blood) of liver and spleen samples from affected birds in all of the cases.

Histological findings included: atrophy of the bone marrow, cortical lymphocyte loss, and rare large round eosinophilic inclusion bodies within enlarged nuclei of reticular thymic cells. Inclusion bodies, though described in experimental CAV challenge, have not been reported in field cases. Other lesions include: follicular lymphocyte loss with minimal cystic epithelial changes with and without medullary reticuloendothelial hyperplasia and ellipsoid hyperplasia with rare hyalin degeneration on spleen sections.

In Company "A," one affected farm was treated with erythromycin via drinking water. In Company "B," tetracycline also in the water was used. Both treatments were reported to be ineffective.

PATHOGENIC, PHENOTYPIC, AND GENOTYPIC CHARACTERISTICS OF AN ATYPICAL STRAIN OF PASTEURELLA GALLINARUM

R. Droual[A], R.L. Walker[B], H.L. Shivaprasad[A], J. Jeffrey[A], C.U. Meteyer[A], R.P. Chin[A], and D.P. Shapiro[C]

California Veterinary Diagnostic Laboratory System
University of California, Davis
[A]Fresno Branch, 2789 S. Orange Ave., Fresno, California 93725
[B]Davis Central Laboratory, P.O. Box 1770, Davis, California 95617
[C]Zacky Farms, 315 North H. Street, Fresno, California 93701

Pathogenicity of a strain of *Pasteurella gallinarum* isolated in Fresno County, California was compared to the type strain (ATCC 13361). Broiler chickens were inoculated intranasally with 10^7 colony forming units (CFU), and intramuscularly with 10^5 CFU, of each strain. Chickens inoculated intranasally and intramuscularly with the type strain, and intranasally with the Fresno strain, did not develop lesions and did not differ significantly in weight gain from sham-inoculated controls 10 days post-inoculation. In contrast, chickens inoculated intramuscularly with 10^5 CFU of the Fresno strain of *P. gallinarum* developed severe myositis at the inoculation site, pericarditis, perihepatitis, airsacculitis and synovitis. *P. gallinarum* was reisolated from these lesions. Phenotypic characteristics of type and Fresno strains of *P. gallinarum* were identical except in reactions to ONPG broth and ability to ferment xylose. Protein banding patterns for the 2 strains were identical except for a single band difference in the 35 kDa region. Restriction endonuclease analyses confirmed differences between the two strains. Plasmid analysis revealed that the type strain had 2 plasmids while no plasmids were detected in the Fresno strain. This finding suggests that pathogenicity in the Fresno strain is chromosomally mediated.

HEMATIC VALUES IN FLOCKS AFFECTED WITH INCLUSION BODY HEPATITIS

M. Tamayo[A], J.J. Gomez[B], and R. Senas[B]

[A]Intervet Mexico, S.A. de C.V.
[B]Departamento de Produccion Animal: Aves, UNAM, Mexico, D.F.

Inclusion body hepatitis is known in Mexico as hydropericardium and hepatitis syndrome. It's a viral disease of fowl which takes a severe course.

This disease was reported in Mexico in 1973 by Antillon and Lucio but it has had a devastating effect on the broiler industry in the past few years (1989-1991). Chick-

ens become affected at age 3-7 weeks and the clinical signs observed were ruffling of feathers, dehydration and paleness.

The aim of this study was to observed blood changes in birds affected naturally with inclusion body hepatitis. The study was carried out on 60 chickens from 4 flocks in order to determine the number of erythrocytes and total protein.

The study revealed that the incidence of anemia was (35% [21/60]) and the concentration of total protein varied from 35 to 42 g/l.

On the basis of these results, it can be stated that chickens infected naturally with the virus of inclusion body hepatitis showed serious blood disturbances.

More studies need to be carried out in order to establish if the anemia is due only to the dysfunction of the liver or if there is another agent involved.

THE DIAGNOSTIC USE OF IMMUNOPEROXIDASE STAINING OF AVIAN MYCOPLASMA

B.R. Charlton[A], R.P. Chin[B] and R. Walker[C]

UC Davis, California Veterinary Diagnostic Laboratory System,
Turlock[A]/Fresno[B]/Davis[C], 1550 North Soderquist Avenue,
P.O. Box 1522, Turlock, CA 95381

Immunostaining involves the detection of specific antigens by the corresponding specific antibody which is coupled to a reporter system. Immunofluorescent staining has been used for several decades. It involves an antibody labelled to a fluorescent group, but requires the use of a fluorescent microscope. Recently, immunoperoxidase (IP) techniques have been developed utilizing enzymes coupled to antibodies. The enzyme converts a colorless substrate to an insoluble colored product at the position of the enzyme and can be visualized with an ordinary light microscope. There are a variety of advantages and disadvantages for each procedure.

Two different applications of IP staining of avian mycoplasmas are currently being evaluated at CVDLS. The first application is the identification of isolated avian mycoplasma colonies. The second application is enzyme immunohistochemical staining on formalin-fixed paraffin-embedded tissues for *Mycoplasma* species. Both procedures utilize specific primary rabbit antisera to the four pathogenic avian mycoplasma species. Identification of mycoplasma colonies on agar plates utilizes an indirect IP procedure[1]. This procedure involves the placement of filter discs saturated with specific mycoplasma antisera directly on agar with mycoplasma colonies. Following incubations, the discs are removed and replaced with filter discs saturated with horseradish peroxidase conjugated goat antirabbit IgG. After the second incubation, the discs are removed and the plate is developed. Mycoplasma colonies are examined for dark purple staining by viewing under a dissection scope. The advantages of this procedure over the indirect fluorescent antibody (IFA) procedure are: (1) a fluorescent microscope is not needed, (2) antisera can be diluted to a greater degree, and (3) is technically simple to perform and to read. One hundred thirty-four mycoplasma cultures have been identified utilizing both the indirect FA and the indirect IP procedure. Complete agreement between the two procedures occurred with 32 *Mycoplasma gallisepticum* (MG) cultures, 25 *M. meleagridis* (MM) cultures, 23 *M. synoviae* (MS) cultures, and 49 cultures which did not react with MG, MS, MM or *M. iowae* (MI) antisera. The IP procedure identified 1 case each of MG, MS, and MM which the FA procedure failed to identify. The FA procedure identified one case of MG and MM and 3 cases of MS which the IP procedure failed to identify. The specific primary antisera used in the IP procedure were at a 1:800 dilution compared to a 1:400 dilution (MG, MM, and MI) or a 1:100 dilution (MS) for the FA procedure.

Immunohistopathology performed on formalin-fixed paraffin-embedded tissues utilized an avidin-biotin kit supplied by Vector Laboratories (Burlingame, California) and the procedure of Haines and Chelack[2]. Primary antibodies to the various *Mycoplasma* sp. were added at a dilution of 1:1000. The mycoplasma organisms were most easily observed on the surface of the epithelial mucosa of the sinuses and trachea. The organisms were not observed to date within the lung tissue or air sac epithelium. Positive results were not adversely affected by formalin fixation times of up to 3 weeks. Although positive results were obtained from tissues in which either MG or MS were recovered, the procedure was not particularly sensitive. Usefulness of the procedure may be limited to situations where secondary bacterial growth obstruct the culturing of *Mycoplasma* sp.

REFERENCES

1. Imada, Y. et al. 1987. Rapid identification of Mycoplasmas by indirect immunoperoxidase test using small square filter paper. J. Clin. Microbiol. 25:17-21.

2. Haines, D. M. and B. J. Chelack. 1991. Technical considerations for developing enzyme immunohistochemical staining procedures on formalin-fixed paraffin-embedded tissues for diagnostic pathology. J. Vet. Diagn Invest 3:101-112.

ISOLATION IN EMBRYONATING CHICKEN EGGS OF ROTAVIRUSES FROM TURKEY POULTS

P.R. Woolcock[A], A.E. Castro[B], J. Chinsangaram[C], D. Larkin[B], H.L. Shivaprasad[A], R. Chin[A], R. Droual[A], J. Jeffrey[A], and C.U. Meteyer[A]

California Veterinary Diagnostic Laboratory System,
University of California, Davis
[A]Fresno Branch, 2789 S. Orange Avenue, Fresno, California 93725
[B]Davis Central Reference Laboratory, P.O. Box 1770, Davis, California 95617
[C]Department of Pathology, School of Veterinary Medicine,
University of California, Davis, California 95616

Rotaviruses are one of the causal agents implicated in the poult enteritis syndrome. They are known primary enteropathogens in food-producing animals[12] and are often associated with outbreaks of diarrhea. Rotaviral-caused enteritis in poults has been reported from the United Kingdom, United States, and France[7].

Rotaviruses are classified as a genus within the Reoviridae on the basis of their characteristic morphology and a genome containing 11 segments of dsRNA within a double shelled capsid. To date, seven distinct groups of rotaviruses have been identified from human, mammalian, and avian species[13]. These groupings are based on serological and electropherotyping analyses and consist of group A rotaviruses, which have a common group antigen that distinguishes them from the non-group A (nonA) rotaviruses, which include groups: B, C, D, E, F, and G. Only groups A, D, F, and G have been associated with avian species[13]. Good agreement between molecular characterization of avian rotaviruses and serological results has been reported[10]. The first isolations of rotaviruses from turkeys and chickens were made in primary chick kidney or chick embryo liver cell cultures[6,8,9]. Subsequently, virus isolations have been reported in continuous cell lines[5,16] and lymphoblastoid cell lines[15]. Serial propagation of rotaviruses in cell culture usually requires trypsin treatment of the virus inoculum. Most isolates are noncytopathic on primary isolation and require several passages in cell culture before a cytopathic effect is seen. Isolation of avian rotaviruses in the yolk sac of embryonating chicken eggs was first reported from lovebirds[3]. This was the only report of a yolk sac isolation of a rotavirus until Castro, et al.[2] reported the isolation of embryo-lethal rotaviruses from Californian turkey poults by direct inoculation into the yolk sac of embryonating chicken eggs.

Rotaviral isolates from poultry flocks with episodes of diarrhea have been serologically placed into groups A, D, F, and G[1]. Approximately 31% of the rotaviruses detected in diarrheic turkey flocks were electropherotyped as group D[4]. In another electropherotyping survey, group D was detected most frequently in 3- to 4-week-old poults[11]. In chickens, rotaviruses in group F were reported as most prevalent in birds 9- to 16-days-of-age and group G in birds of 43- to 50-days-of-age[13,14]. These findings indicate the variability of serotyping associated with avian rotaviruses and the need to identify the appropriate group types occurring at different stages of the enteric syndrome in affected birds.

The economic significance of rotaviral enteritis to the poultry industry has yet to be defined but by analogy with the situation in mammals, it is considered to be significant[7]. In Californian turkey flocks, poult enteritis is one of the most frequently diagnosed syndromes. Analyses of submissions of turkey accessions to the California Veterinary Diagnostic Laboratory System (CVDLS) for 1990 and 1990 (January to October) show that poult enteritis cases constituted 13.4% and 13.9%, respectively.

This methodology of isolation and preliminary characterization of rotaviruses from clinical accessions from poults will be described. By ELISA, these isolates have been shown to belong to both group A and non-group A rotaviruses. Results on further characterization of these

isolates by electropherotyping to determine group type and their relationship to the clinical problem will be presented.

The direct isolation of group A and non-group A rotaviruses in specific pathogen-free embryonating chicken eggs represents a significant technical advance, particularly since strains of non-A rotaviruses have been detected.

REFERENCES

1. Bridger, J.C. Novel rotaviruses in animals and man. In: Novel diarrhoea viruses. Ciba foundation symposium 128. John Wiley & Sons Ltd., Chichester, UK. 1987.

2. Castro, A.E., J. Moore, S. Hammami, R.B. Manalac, and R.P. Chin. Direct isolation of rotaviruses from turkeys in embryonating chicken eggs. Veterinary Record: in press.

3. Gough, R.E., M.S. Collins, G.W. Wood, and S.A. Lister. Isolation of a chicken embryo-lethal rotavirus from a lovebird (Agapornis species). Veterinary Record 122:363-364. 1988.

4. Kang, S.Y., K.V. Nagaraja, and J.A. Newman. Physical, chemical and serological characterization of avian rotaviruses. Avian Dis. 32:195-203. 1988.

5. Kang, S.Y., K.V. Nagaraja, and J.A. Newman. Primary isolation and identification of avian rotaviruses from turkeys exhibiting signs of clinical enteritis in a continuous MA104 cell line. Avian Dis. 30:494-499. 1986.

6. McNulty, M.S., G.M. Allan, D. Todd, J.B. McFerran, E.R. McKillop, D.S. Collins, and R.M. McCracken. Isolation of rotaviruses from turkeys and chickens: Demonstration of distinct serotypes and RNA electropherotypes. Avian Path. 9:363-375. 1980.

7. McNulty, M.S. Rotavirus Infections, in: B.W. Calnek, with H.J. Barnes, C.W. Beard, W.M. Reid, and H.W. Yoder, Jr. (Eds) "Diseases of Poultry" 9th Edn, pp. 628-635. (Ames, Iowa State University Press). 1991.

8. McNulty, M.S., G.M. Allan, D. Todd, and J.B. McFerran. Isolation and cell culture propagation of rotaviruses from turkeys and chickens. Archives of Virology 61:13-21. 1979.

9. McNulty, M.S., G.M. Allan, D. Todd, J.B. McFerran, and R.M. McCracken. Isolation from chickens of a rotavirus lacking the rotavirus group antigen. Journal of General Virology 55:405-413. 1981.

10. Pedley, S., J.C. Bridger, D. Chasey, and M.A. McCrae. Definition of two new groups of atypical rotaviruses. Journal of General Virology 67:131-137. 1986.

11. Reynolds, D.L., Y.M. Saif, and K.W. Theil. A survey of enteric viruses of turkey poults. Avian Dis. 31:89-98. 1987.

12. Saif, L.J. Introduction, in: L.J. Saif and K.W. Theil (Eds) "Viral Diarrheas of Man and Animals," p. 3. (Boca Raton, FL, CRC Press, Inc). 1990.

13. Saif, L.J. Nongroup A rotaviruses, in: L.J. Saif and K.W. Theil (Eds) "Viral Diarrheas of Man and Animals," pp. 73-95. (Boca Raton, FL, CRC Press, Inc). 1990.

14. Saif, L.J. Comparative aspects of enteric viral infections, in: L.J. Saif and K.W. Theil (Eds) "Viral Diarrheas of Man and Animals," pp. 10-31. (Boca Raton, FL, CRC Press, Inc). 1990.

15. Schat, K.A., and T.J. Myers. Cultivation of avian rotaviruses in chicken lymphocytes and lymphoblastoid cell lines. 1987.

16. Theil, K.W., D.L. Reynolds, and Y.M. Saif. Isolation and serial propagation of turkey rotaviruses in a fetal rhesus monkey kidney (MA104) cell line. Avian Dis. 30:93-104. 1986.

STUDIES ON INCLUSION BODY HEPATITIS WITH REFERENCE TO ITS EPIDEMIOLOGY AND DIAGNOSIS IN INDIA

S.N. Sharma, M.S. Oberoi, and B. Singh

Department of Veterinary Bacteriology and Virology,
College of Veterinary Science,
Punjab Agricultural University,
Ludhiana - 141 004, India

In the course of present study the diagnosis of inclusion body hepatitis (IBH) was confirmed on the basis of presence of intranuclear inclusion bodies in the hepatocytes of birds submitted for diagnosis. The affected liver tissue from such confirmed outbreaks were processed for isolation of avian adenovirus and bacteria in chicken embryo liver cell culture and various bacteriological media respectively. Simultaneously, the occurrence of concurrent diseases and detailed information about age, housing system, vaccination, atmospheric temperature, and relative

humidity was recorded. Feed samples from nine outbreaks were analyzed for the presence of mycotoxins. Starting from October 1990 to September 1991, 13 outbreaks were diagnosed as IBH. The disease occurred in broiler chicks of 2 - 5 weeks of age which were reared on a deep litter system and were vaccinated only with Newcastle disease F strain vaccine at one day of age. The outbreaks were more frequently recorded during the months of October and November when the atmospheric temperature varied from 11.5 to 30.7°C and relative humidity was 63 to 65%. Out of 13 outbreaks avian adenovirus could be isolated from 12 outbreaks in cell culture. The avian adenovirus isolates were identified on the basis of their reaction in counterimmunoelectrophoresis with standard type 1 antiserum. The concurrent diseases diagnosed at the postmortem examination were mycotoxicosis, infectious coryza, chronic respiratory disease and coccidiosis. Mycotoxins in feed could be detected in 7 out of 9 feed samples tested in the range of 200 to 400 ppb. The mortality in the outbreaks ranged from 3.3 to 20%. The mortality in mycotoxin associated outbreaks ranged from 7 to 20% as compared to mycotoxin negative cases (3.3 to 10%). The various bacterial organisms isolated were *E. coli* (3), *Salmonella gallinarum* (3), *Pasteurella multocida* (3), *Salmonella spp.*, *Proteus vulgaris* and *Staphylococcus aureus* (1 each).

AN UNUSUAL HIGH MORTALITY IN YOUNG TURKEYS

A. Singh Dhillon

Washington State University-Puyallup, Research and Extension Center,
Puyallup, Washington 98371

A flock of 22,312 5-week-old turkey poults suffered a severe respiratory disease problem. Low mortality was seen during the fifth and sixth week of brooding. Mortality increased in the seventh week when the flock was moved to grow-out houses. Highest mortality was seen in the eighth week of growing. The weekly mortality in both toms and hens from week one to week thirteen was 293, 111, 42, 91, 70, 133, 345, 2635, 1534, 256, 141, 188, and 156, respectively. Total losses were 6,001 turkeys from a flock of 22,312 resulting in a 26.9% total mortality.

Necropsy Examination of Five-week-old Turkeys. Clinical examination of the live birds revealed coughing, sneezing, and rubbing of the faces on the shoulders. Gross postmortem alterations were of catarrhal tracheitis. Lungs were slightly congested in some birds. The air sacs were normal. No significant alterations were present in the livers, spleens, or kidneys.

An *E. coli* was isolated in high numbers from 6 tracheas and 2 lung specimens. A *Pseudomonas sp.* was isolated in moderate numbers, from 2 tracheas. No bacterial pathogens were isolated from 1 lung specimen. *Bordetella avium* was not isolated from any of the trachea or lung specimens. Results of *Salmonella* isolation were negative.

Serology. Ten serum samples were tested for Newcastle using ELISA serology. The results were negative. All samples were negative for *Mycoplasma gallisepticum* antibodies.

Virology. The tissues of trachea and lung were inoculated in Specific Pathogen Free embryos. No embryonic mortality was present. Preformed chicken embryo liver (CEL) monolayers were inoculated with trachea and lung specimens. Two passages were made in CEL monolayers. No cytopathologic effects were present. The virus isolation attempts were negative using both procedures.

Diagnosis. Pulmonary congestion and catarrhal tracheitis of possible viral etiology complicated by *E. coli*.

Necropsy Examination of 8-week-old Turkeys. Eight turkeys were selected from this flock for postmortem examination. Extremely high daily mortality was reported. The flock was not medicated with any of the antibiotics. Clinical examination revealed lethargy and some CNS disorder. The birds had marked loss of balance. Gross postmortem alterations were of severe pericarditis with large quantities of fibrinous exudate present in the heart sac. The fibrinous material was also present on the surface of the liver, but in lesser amounts. Other changes in the livers were of hepatomegaly in 4 out of 8 specimens. Multiple white foci were present in the liver of 3 birds. The air sac membranes in most of the birds were thickened with the presence of froth. The spleens were hemorrhagic, mottled, and enlarged in all birds. The bursae of Fabricius were flaccid and pale in color. The brains appeared to be pale. The lungs were frothy and consolidated in 3 birds; the lungs of 2 birds had focal pale areas.

The results of bacteriologic evaluation were as follows:

Bird 1	Liver	No bacterial growth
	Heart	1+ *Staph*; 1 CFU *E. coli*
Bird 2	Liver	No growth
	Heart	6 CFU *E. coli*
Bird 3	Liver	No bacterial growth
	Heart	3 CFU *E. coli*
Bird 4	Liver	No bacterial growth
	Lung	3+ *E. coli*
	Heart	No bacterial pathogens isolated
Bird 5	Liver	10 CFU *E. coli*
	Lung	1+ *Staph*; 2+ *E. coli*
	Heart	1+ *E. coli*

To summarize, an *E. coli* was isolated in low numbers from the livers of 1 out of 5 birds. An *E. coli* was isolated in low numbers from the hearts of 1 out of 5 birds. High numbers of *E. coli* were isolated from 1 lung specimen. Moderate numbers of *E. coli* were isolated from another lung specimen. A *Pseudomonas sp.* was isolated in high numbers from 3 tracheas and in moderate numbers from 1 trachea. The isolation of *E. coli* in such low numbers was not considered significant from a flock with high mortality and lesions of severe septicemia. The flock was medicated with chlortetracycline at a level of 400 grams per ton of feed for 4 weeks. The mortality dropped shortly after medication.

Histopathology. Histopathologic alterations were of sever fibrinous pericarditis, hepatitis, splenitis, encephalitis, airsacculitis, and pneumonia with heterophilic cell infiltrate. Special staining revealed the presence of elementary bodies of chlamydia in the tissues of lungs.

Serology. Ten samples tested using ELISA serology were negative for Newcastle antibodies. The specimens were also negative for MG, MS, and avian influenza.

Virology. The tissues collected from these birds were inoculated in Specific Pathogen Free embryos. Embryonic mortality and stunting were present. Tissues were also inoculated on preformed liver cell monolayers. Cytopathologic effects were seen. The virus isolated hemagglutinated avian red blood cells.

Supportive Diagnosis. Eighty-two serum samples were collected by the USDA and shipped to NVSL, Ames, Iowa. Those samples were found to be positive for *Paramyxovirus-1* (PMV-1) antibodies. The samples were found to be negative for PMV-2 and PMV-3 antibodies. Only 3 out of 82 samples were positive for chlamydial antibodies using complement fixation test. *Chlamydia psittaci* was not isolated from several tissues submitted.

SUMMARY

A flock of 22,312 turkeys suffered a severe respiratory outbreak at 5 weeks of age with lesions of catarrhal tracheitis, pulmonary congestion, and low mortality. Mortality increased from 6 to 9 weeks of age. Clinical signs observed at eight weeks were of lethargy and loss of balance, indicating a central nervous system disorder.

Gross pathologic alterations were of sever fibrinous pericarditis, hepatitis, splenitis, airsacculitis, and pneumonia with the presence of froth and consolidation. Histopathologic alterations were of pericarditis, hepatitis, splenitis, airsacculitis, pneumonia, and encephalitis. The cellular infiltrate was heterophilic. Special staining revealed elementary bodies of *Chlamydia psittaci*. Antibodies to paramyxomirus-1 were present in the serum samples of the turkeys. *Chlamydia psittaci* antibodies were present in a few serum samples. *Chlamydia psittaci* could not be isolated; however, a Newcastle virus with mild pathogenicity was isolated.

It is likely that the high mortality was associated to ornithosis and respiratory stress due to PMV-1 infection that predisposed the flock.

FACIAL CELLULITIS ASSOCIATED WITH FOWL CHOLERA IN COMMERCIAL TURKEYS

J. Jeffrey[A], H. Shivaprasad[A], C. Cardona[B], B. Charlton[B], H. Medina[C]

California Veterinary Diagnostic Laboratory System
University of California, Davis
[A]CVDLS-Fresno Branch, 2789 S. Orange Ave., Fresno, California 93725
[B]CVDLS-Turlock Branch, P.O. Box 1522, Turlock, California 95381
[C]Foster Farms, 1125 N. Golden State, Turlock, California 95381

Commercial turkeys from four ranches were presented to the Fresno and Turlock branch laboratories with extremely swollen heads between July 1989 and August 1991. *Pasteurella multocida* was isolated in pure culture from the skin in all cases. *P. multocida* isolates were: untypeable, serotype 1, and serotype 3 x 4. In at least two of the reported cases, birds submitted for necropsy with skin lesions had no lung or air sac involvement while those birds which had lung and air sac lesions did not have lesions in the skin. In one case, serotype 1 was isolated from the liver and lungs while the skin isolate was untypeable. Histologically the facial cellulitis was described as suppurative necrosis with occasional thrombosed vessels, fibrin deposition and scattered bacterial nests or extensive pyogranulomatous inflammation of the deep dermis with perivasculitis. Flocks ranged in age from 13 to 18 weeks and included both toms and hens. Concurrent disease processes identified in these cases were: *E. coli* airsacculitis (1), positive Newcastle disease virus serology (1), and seroconversion to adenovirus (1). Two of the four ranches had been vaccinated at 8 weeks of age for Newcastle disease, the other two ranches received no vaccinations.

PYOGRANULOMATOUS TYPHLITIS AND HEPATITIS OF MARKET TURKEYS

T.Y. Morishita[A] and A.A. Bickford[B]

[A]Department of Veterinary Pathology, School of Veterinary Medicine,
University of California, Davis, California 95616
[B]California Veterinary Diagnostic Laboratory System,
Turlock Branch Laboratory, P.O. Box P, Turlock, California 95381

There are a few diseases that affect mainly the cecum without involving the rest of the intestinal tract. The formation of cecal cores has been reported in only a few diseases including salmonellosis, coccidiosis, and histomoniasis. A flock of 7-week-old market turkeys demonstrated cecal core formation with subsequent cecal rupture. Histopathological examination revealed pyogranulomatous lesions in the liver and cecum of affected birds. No causal agent was determined. Because of the unknown etiology of these lesions, an epidemiological study was also performed to determine the cause and initiating factors or lesions leading to cecal core formation. This report describes the case history and pathology of three successive flocks that demonstrated these lesions and the subsequent epidemiological study that performed.

POSTMORTEM MONITORING OF FLOCK MORTALITY
1. ANIMAL WELFARE ASPECTS

D.A. McMartin

Veterinary Medicine Extension
University of California, Davis, California 95616

The United States is currently experiencing an upsurge of societal interest in the welfare (sometimes called the well-being) of animals utilized by humans, including those raised primarily for food. This may be due to several factors, including almost total separation of urban dwellers from everyday familiarity with agricultural animals, which therefore have become "exotic," and consequently of enhanced interest as living organisms.

Societal information on the ways in which agricultural animals are treated by their owners and handlers comes mostly via the mass media and as such is often inaccurate. One photograph in a national magazine can imply that many animals are badly treated, and television can do the same with a 15 second clip, or a 10 minute epic. In the last few years, veal, beef, poultry, dairy, salesyards, and horses have received media attention, either deliberately or unfortuitously. This is possible because in human/agricultural animal interactions, activities go on which to societal eyes indicate a lack of owner concern for animals. A majority of people surveyed in 1991 wanted more legislation **ensure** that all animals are well looked after. It must be emphasized however, that in many instances, e.g., animal behavior, we do not know the impractical or totally uneconomical. On the other hand, animal agriculture must actively seek programs by which it can promote its care and concern for animals to society.

Intensive poultry production systems have been the subject of numerous criticisms[2]. As mentioned before for animals in general, data is often limited or unavailable either to totally defend existing poultry management practices or to define better economically viable alternatives.

To compare the merits of different practices and systems for animal welfare, it has been suggested[1,2,3] that longevity of animals' lives may be an indicator of levels of well-being under different management systems, the assumption being that long life can be equated with optimal well-being. Such data will take many years to gather, even for animals such as poultry with a short generation time.

I suggest that poultry industries look at the converse of that proposal, namely, examine the birds which live the shortest. Producers should consider ways in which to derive information on what is now called "routine" or "normal" flock mortality. Dr. Bickford is going to discuss tangible scientific, practical benefits which could be obtained from such investigations. In addition, societal benefits less tangible, but in some ways possible of equally long-term significance, could be created by demonstrating poultry industries' concern for the dead birds, and utilizing knowledge gained from their death to enhance the well-being of the living.

REFERENCES

1. Hurnik, J.F. Animal welfare: ethical aspects and practical considerations. Poult. Sci. 69:1827-1834. 1990.

2. Hurnik, J.F. and H. Lehman. Ethnics and farm animal welfare. J. Agric. Ethics 1:305-310. 1988.

3. California Poultry Letter, Editor—R.A. Ernst, Avian Science, University of California, Davis, CA 95616. October 1988.

NECROPSY MONITORING OF ROUTINE MORTALITY OR CULLING LOSSES IN POULTRY FLOCKS

A. A. Bickford[A] and D. A. McMartin[B]

[A]California Veterinary Diagnostic Laboratory System
Turlock Branch, P.O. Box 1522, Turlock, California 95381
[B]University of California, Davis
Cooperative Extension, 1383 Surge III, Davis, California 95616

INTRODUCTION

In all intensive animal production systems some level of loss to death and culling is accepted as a biological norm and rule of thumb standards have been established for tolerable levels in various species. For commercial poultry such losses have been estimated to be as follows:

- 12% for table-egg production flocks (4% as pullets, 8% as layers) through 60 weeks of age
- 8% of meat turkeys through 16+ weeks of age
- 4% of broiler chickens through 45 days of age

A distinction is usually made between routine mortality and death losses attributed to specific flock-wide diseases based on the assumption that the latter would clearly exceed the established norm. Among the problems accounting for "normal" mortality are anomalies (cysts, skeletal, deformities, etc.), miscellaneous tumors (other than Marek's or leukosis), low pecking order status interfering with ability to eat and drink, inability to adapt to management changes (beak-trimming, change from open waterers to cup or nipple watering systems, etc.), cannibalism and other types of trauma, cloacal or oviductal eversion and prolapse, alimentary impactions, and a variety of other individual bird problems. Contrary to commonly held perceptions it is not always the "poor-doers" or misfits that succumb in large, dense populations. The super performers are also subject to unique stresses sometimes leading to premature death losses. Causes of death among meat birds with the most rapid weight gains include sudden death ("heart attack") syndrome, leg weakness and deformities and aortic rupture in turkeys whereas, in the best performing laying hens, death may be due to caged layer fatigue (adult rickets), fatty liver syndrome or late-breaking Marek's disease.

The range of conditions accounting for mortality in poultry flocks is so diverse that it is impossible to estimate or predict and specific conditions may vary widely from ranch to ranch. To be sure that "normal" mortality truly represents uncontrollable death loss, it is necessary to subject a reasonable sample of dead and cull birds to a thorough necropsy on a regular basis so that a baseline of causative conditions can be established site by site. Once established, this baseline should allow rapid identification of diseases presenting a threat to the flock.

PROPOSED MONITORING PROGRAM

It is suggested that freshly dead birds be collected from throughout each house (pen, range) and refrigerated (not frozen) if they must be held before submission. There should be some effort to randomize birds and no bird, no matter how obvious the problem, should be excluded from the selection pool. Up to 10 birds should be submitted on a regular schedule (weekly during growout, biweekly or monthly for adults).

CVDLS laboratories will perform a complete necropsy on each bird and report gross findings within 24 hours. Since decomposition of some degree is likely, no follow-up studies will be done on dead birds. However, if a disease of potential flock significance is detected the submitter will be notified and urged to select an appropriate sample of birds for regular diagnostic evaluation. When specific flocks are on a scheduled dead-bird necropsy program it should be possible to establish a standing submission form for each flock, adding only updating information with each submission. The fee for the dead-bird monitoring necropsy will be $20.00.

POTENTIAL BENEFITS OF MORTALITY MONITORING

In this era of concern for animal welfare, an effort to determine the cause(s) of spontaneous mortality is clear evidence of a producers commitment to assessment of the general welfare of his flocks and it may reveal specific stressors and environmental or management problems. Mortality monitoring will at the very least establish a knowledge base of the range of diseases or conditions responsible for death losses. In some instances the program will reveal potential flock problems in advance of a

flock-wide crisis. The program could be of special benefit in evaluating the effectiveness of control programs designed to curtail specific causes of mortality. Ultimately, mortality monitoring should allow the producer to establish specific targets for acceptable mortality rates and eliminate the need for accepting rule of thumb guidelines.

SUSPECTED BUDGERIGAR FLEDGLING DISEASE IN A LOVEBIRD — A CASE REPORT

Donald L. Singletary

Department of Veterinary Science, Animal Diagnostic Laboratory
The Pennsylvania State University, University Park, PA 16802

INTRODUCTION

Budgerigar fledgling disease (BFD) is an acute, highly fatal viral disease of fledglings less than a month of age[1,3]. Affected fledglings 2 to 4 weeks of age die within 1 to 2 days of showing symptoms. In budgerigar aviaries mortality ranges from 25% to 100%[1,3]. In large psittacines the disease occurs in birds 4 to 12 weeks of age, particularly in hand fed birds[7]. Originally reported in budgerigar aviaries, the disease is now reported in a large variety of other psittacines[6]. The causative agent is a Papovavirus[1,2]. The following report describes a suspected case of BFD ion a fledgling lovebird.

CASE REPORT

On September 19, 1991 a single dead 2½ week old lovebird was submitted to the Animal Diagnostic Laboratory, Pennsylvania State University for diagnosis. The submission was from a mixed colony of 25 pet birds which included 4 pairs of lovebirds. The lovebird pairs had five fledglings. Three of the five died within a ten day period. Blood was noticed on the first dead bird upon removal from the nest. The owner suspected that it had been picked up by the adults. The second dead bird was submitted for examination. The third died after efforts to keep it alive failed. The only other symptoms the owner reported were anorexia and poor growth. The fledglings were being hand fed.

At necropsy the crop was distended with food and a trace of ascites was noted in the thoraco-abdominal cavity. No other gross lesions were observed in any of the internal organs.

Since neonatal crop stasis is a common cause of death in hand fed birds[5] and because of the general lack of gross lesions and the presence of a full crop, a preliminary diagnosis of neonatal crop stasis was made.

Swabs of the cloaca, crop, liver and heart blood were submitted for routine aerobic bacteriologic culture, and a variety of tissues (crop, lung, intestine, pancreas, brain, spleen, liver, heart) were submitted in phosphate buffered formalin for histopathology.

Bacterial cultures from the crop were negative for *E. coli* and yeast. Cloacal swabs were negative for *E. coli* and yielded only a few yeast. Liver cultures produced no growth. Alpha streptococcus was isolated from the cloaca, crop and heart blood.

The most significant histopathologic changes were seen in the liver, spleen and kidney. The other tissues had no specific disease lesions. The liver had a severe multifocal to coalescing acute hepatocellular necrosis. A severe acute multifocal interstitial nephritis was evident. The spleen contained scattered pale basophilic intranuclear inclusion bodies and a mild diffuse necrosis.

A presumptive diagnosis of budgerigar fledgling disease was made based on the histopathologic findings and clinical signs.

DISCUSSION

It is a challenge for a poultry diagnostic laboratory which routinely sees chickens and turkeys to diagnose the occasional submission of a pet bird. This case provided such a challenge.

In this case, only one bird was submitted with limited history. The symptoms reported were general and nonspecific. A large number of symptoms have been reported for BFD. these included distended abdomens with reddening of the skin, poor weight gain, prolonged crop emptying, regurgitation, depression, glassy eyes, anorexia, diarrhea, dehydration, excessive bleeding when a feather is pulled or when the bird is injected, and abnormal feather development[1,3,6].

The typical gross lesions of BFD in budgerigars include hydropericardium, ascites, enlarged liver and heart, pinpoint white necrotic foci in the liver, heart and kidneys, and petechial hemorrhages in the liver and kidneys[1,3].

However, in the submitted bird none of these "classical" lesions were seen, other than minimal ascites.

Intranuclear inclusion bodies are a characteristic finding in BFD. They have been described as being clear, opaque or basophilic[1,3]. Inclusion bodies may be found in many tissues including kidney, spleen, heart, brain, bone marrow, feather follicles, skin and crop[1,3]. The kidney is the preferred organ in which inclusions are found in budgerigars, while the spleen is the best site in larger psittacines[7]. This bird had basophilic inclusions in the spleen suggestive of those of BFD virus.

Virology and serology, while not attempted in our case, can also be used in an effort to make a diagnosis. Budgerigar embryo fibroblasts must be used for the initial isolation of the causative virus[7]. A fluorescent antibody virus neutralization test is also available to detect BFD antibody[7].

Controlling an outbreak of BFD in an aviary is difficult. There is no effective treatment, and no vaccine is currently available (P.D. Lukert, pers. comm.). Nevertheless detailed procedures to re-establish a breeding facility following an outbreak have been reported[4]. Two procedures can be used. One is to completely depopulate the infected birds, C & D and repopulate with BFD free breeders. The other is to use the BFD infected breeders but follow specific management procedures.

REFERENCES

1. Bernier, G., Morin, M., Marsolais, G.: A generalized inclusion body disease in the budgerigar (*Melopsittacus undulatus*) caused by a Papovavirus-like agent. Avian Dis. 25:1083-1092, 1981.

2. Bozeman, L.H., Davis, R.B., Gaudry, D., Lukert, P.D., Fletcher, O.J., Dykstra, M.J.: Characterization of a Papovavirus isolated from fledgling budgerigars. Avian Dis. 25:972-980, 1981.

3. Davis, R.B., Bozeman, L.H., Gaudry, D., Fletcher, O.J., Lukert, P.D., Dykstra, M.J.: A viral disease of fledgling budgerigars. Avian Dis. 25:179-183, 1981.

4. Davis, R.B., Bozeman, L.H., Gaudry, D., Fletcher, O.J., Lukert, P.D., Dykstra, M.J.: An update on budgerigar fledgling disease (BFD). Proceeding of the 33rd Western Poultry Disease conference, Davis, CA pp 96-98, 1984.

5. Flammer, K. Pediatric Medicine. In: Clinical Avian Medicine and Surger. G. Harrison and L. Harrison, eds., W.B. Saunders Co., Philadelphia. pp. 647, 1986.

6. Gerlack, H. Viral diseases. In: Clinical Avian Medicine and Surger. G. Harrison and L. Harrison, eds. W.B. Saunders Co., Philadelphia. pp. 426-428, 1986.

7. Lukert, Phil D. Budgerigar Fledgling Disease. In: A laboratory manual for the isolation and identification of avian pathogens, 3rd ed. H.G. Purchase, L.H. Arp, C.H. Domermuth, and J.E. Pearson eds. American Association of Avian Pathologists, Kennett Square, PA. pp. 106-107, 1989.

DISEASE PROBLEMS ASSOCIATED WITH A UTAH GAMEBIRD FARM

Fred Dustan Clark[A] and Ray Olsen[B]

[A]Utah State University Provo Lab, 2031 South State, Provo, UT 84606
[B]Utah Division of Wildlife Resources, 1000 North Main, Springville, UT 84663

The Utah Division of Wildlife Resources (Salt Lake City, Utah) maintains a gamebird farm in Springville, Utah, for raising chukars and Hungarian partridges. This farm has facilities for breeding, hatching, and rearing chukars which are to be released into the wild. A review of the diagnostic records of the Utah State University Diagnostic Laboratory at Provo, Utah for the 10 year period 1982-1991 revealed numerous submissions from the gamebird farm at Springville, Utah. The diseases and conditions diagnosed in the chukars will be presented.

SPIROCHETOSIS IN A BACKYARD FLOCK OF CHICKENS

G.L. Cooper

California Veterinary Diagnostic Laboratory System
Turlock Branch, P.O. Box 1522, Turlock, California 95617

An outbreak of Spirochetosis was seen in a backyard flock of game chickens in the Central Valley of California. Symptoms consisted of depression, pale combs, and loose greenish droppings. Mortality was high in affected birds who ranged in age from 8-12 months. Gross lesions found in 2 birds submitted for necropsy included anemia, green mucoid enteritis, and slightly enlarged and mottled spleens. Large numbers of spiral shaped bacterial organisms were seen in the plasma of Giemsa-stained blood smears from both birds.

External parasites were not observed on either bird, but the owner recalled seeing "sticktight fleas" and "blue bugs" on some birds. *Argas persicus* is an important vector, and the principle reservoir or *Borrelia anserina*, the causative agent of fowl spirochetosis. This soft-bodied tick can occasionally be found living in the cracks and crevices of poultry houses. In this outbreak it is believed that infected ticks may have been introduced onto the ranch in used wooded poultry crates purchased by the grower before the onset of the disease outbreak.

AVIAN TUBERCULOSIS IN PHEASANTS

B.A. Singbeil[A] and A.A. Bickford[B]

[A]Veterinary Medical Teaching Hospital, School of Veterinary Medicine
Davis, California 95616
[B]Turlock Branch Laboratory, P.O. Box 1522, Turlock California 95381

Five of five pheasants (*Phasianus colchicus*), presented due to death associated with lethargy and respiratory difficulty over a two-day period were observed to have lesions consistent with avian tuberculosis. Tuberculous lesions were present in the liver, spleen, and intestine of all birds. These pheasants, presented at 10 months of age, were from a group of 200 breeding birds with a history of fowl cholera and capillariasis with acid-fast bacilli present in intestinal tissue at five months of age. Microscopic evaluation of lesions revealed granulomas that were primarily of epithelioid origin with acid-fast bacilli present. *Mycobacterium avium* was isolated and identified by DNA-probe analysis. The culture has been forwarded for serotyping.

SUMMARY OF DIAGNOSTIC FINDINGS IN OSTRICHES SUBMITTED TO CALIFORNIA VETERINARY DIAGNOSTIC LABORATORY SYSTEM–FRESNO

H.L. Shivaprasad[A], J.S. Jeffrey[A], A.E. Castro[B], R. Droual[A], R.P. Chin[A], C.U. Meteyer[A], R.L. Walker[B], B. Reynolds[B], D.D. Larkin[B], G. DeBaere-Tuter[B], and P.R. Woolcock[A]

California Veterinary Diagnostic Laboratory System,
University of California, Davis
[A]Fresno Branch Laboratory, 2789 South Orange Avenue,
Fresno, California 93725
[B]Davis Central Reference Laboratory, P.O. Box 1770,
Davis, California 95617

Files on ostrich cases submitted to CVDLS, Fresno between May 1990 and December 1991 were reviewed. Data were summarized for various parameters: clinical history, gross lesions, histopathology, bacteriology, virology, toxicology, and parasitology. Most of the birds submitted to the laboratory were 4 weeks or less with a history of diarrhea, weakness, loss of weight, and flock mortality ranging from 2 to 90%. Most of these birds had watery contents in the cecum and colon and some birds had fibrinonecrotic mucosa of the cecum and colon. Some of these birds had mild to moderate lymphoid necrosis and depletion in the bursa of Fabricius. Except for isolation of *Clostridium perfringens* from the intestine of a few birds, no significant bacteria were isolated nor were any parasites observed. Myxovirus-like particles were observed by electron microscopy from the intestine and its contents of many of these birds. Also, astroviruses were directly identified from the intestine of 2 birds by electron microscopy. Paramyxovirus type 1 and paramyxovirus type 2 were isolated and identified by serology from the intestine of a few birds. The significance of these viruses in relation to clinical signs, lesions, and mortality in ostriches is unknown and awaits further study.

Yolk sac infection in chicks due to *E. coli*, *Pseudomonas* sp., and other bacteria was common. Granulomatous ventriculitis due to a mucoraceous zygomycetes was seen in several cases. Pneumonia due to *Aspergillus* sp. and bacteria was also seen in many birds. These and other interesting diagnostic findings in individual ostriches or in groups of ostriches will be discussed.

PRESENT STATE OF HAEMOPHILUS CORYZA

P.J. Blackall

Queensland Department of Primary Industries, Animal Research Institute,
665 Fairfield Road, Yeerongpilly, 4105 Australia

INTRODUCTION

The Western Poultry Disease Conference has had a long and close association with the disease we know as infectious coryza. Many of the past Conferences have featured papers from such notable coryza workers as Drs. Bell, Rosenwald, Yamamoto and Matsumoto. It is a privilege to present a paper on this topic at a Conference that can be regarded as one of the "homes" of infectious coryza. This paper will be presented as a review of our latest knowledge on the disease. Particular emphasis will be placed on the knowledge that has been gained since the publication of the chapters on infectious coryza in latest editions of *Diseases of Poultry*[24] and *A Laboratory Manual for the Isolation and Identification of Avian Pathogens*[12].

CLINICAL DISEASE

Yamamoto[24] provides an excellent review of the pathology of infectious coryza as well as much useful infor-

mation on the distribution of the disease, the economic losses associated with the disease and so on. Although the disease is now accepted as being only significant in layers, a recent report from California notes that the disease can still be a problem in meat chickens[17].

CLASSIFICATION AND IDENTIFICATION

The avian haemophili are gram-negative organisms that have been isolated from birds and which have a demonstrated *in vitro* growth factor requirement. The factors that can be required by haemophili for *in vitro* growth are haemin (X factor) and/or nicotinamide adenine dinucleotide (NAD; V factor).

While early reports described the causative agent of infectious coryza as *Haemophilus gallinarum* an X and V factor dependent organism, the only haemophili that have been recovered from poultry since the 1960s have been shown to require V factor only. It has recently been claimed that the early descriptions of X and V factor dependent avian haemophili were incorrect due to deficiencies in the testing media available in the 1930s to 1960s[11]. Thus, it is possible that *H. gallinarum* never existed!

Currently, it is widely accepted that at least two species of avian haemophili exist. Both species require V factor only. One species is known as *H. paragallinarum* and is regarded as the causative agent of infectious coryza. The second species is known as *H. avium* and is regarded as non-pathogenic. Detailed studies have recently suggested that *H. avium* is in fact a grouping of at least three species with the new species being *P. avium, P. volantium* and *Pasteurella* spp. A. Blackall and Yamamoto[12] provide a review of this recent work plus identification tables. It is worth noting that not all isolates of *H. avium* can be assigned to the new *Pasteurella* spp.

VIRULENCE MECHANISMS AND PROTECTIVE ANTIGENS

Neither the protective antigens of *H. paragallinarum* nor those factors associated with the virulence of *H. paragallinarum* have been convincingly identified. Yamamoto[24] covers much of our knowledge in this area.

Convincing evidence of the role of hemagglutination (HA) antigens in protection has recently emerged. Takagi and colleagues have shown that chickens treated with monoclonal antibodies against the HA antigen of *H. paragallinarum* serovar A showed no clinical signs on challenge[22]. As well, the monoclonal antibodies reduced the amount of the challenge organism present in the sinuses of the treated chickens compared with untreated controls[22].

There have been several suggestions that not all isolates of *H. paragallinarum* are equally virulent. In particular, it has been suggested that at least some isolates of Page serovar B are non-pathogenic for chickens. However, in a recent study using some of the strains described by others as non-pathogenic, we found that all four strains of Page serovar B we examined were pathogenic, causing the typical macroscopic lesions of infectious coryza[23]. We did find that one strain (0222) did not cause clinical signs but this strain did cause macroscopic lesions in the sinus.

SEROTYPING SCHEMES

There are now three schemes available for serotyping *H. paragallinarum*—the Page scheme[20], the Kume scheme[19] and the Hinz scheme[18]. The latter scheme has not been widely used. The following sections provide the latest information on the Page and Kume schemes.

The Page Scheme. This is the oldest scheme for serotyping *H. paragallinarum*. The scheme is traditionally performed using an agglutination test and recognises three serovars (A, B and C). Some reports have cast doubt on the validity of Page's serovar B, suggesting that the members of this serovar are really only variants of serovars A or C. In a recent collaborative study, we have conclusively demonstrated that Page serovar B is a true serovar[23]. A major drawback of the Page scheme is the high incidence of either autoagglutinating or non-agglutinating strains—up to 38% in one of our studies. Recently, we have been able to show that all strains of *H. paragallinarum*, regardless of their Page serovar, have hemagglutination (HA) activity, provided glutaraldehyde-fixed chicken red blood cells are used. This HA activity can be used as the basis for a hemagglutination (HI) test using the rabbit antisera for serovars A, B and C. We have shown that the use of the HI methodology in the Page scheme resulted in the correct serotyping of 70/72 isolates of *H. paragallinarum*[3].

In collaboration with colleagues in Japan, we have also been evaluating the use of monoclonal antibodies to assign *H. paragallinarum* isolates to a Page serovar. We have recently shown that a panel of four monoclonal antibodies can be used in either a HI test or a dot-blot test to successfully assign field isolates to Page serovars[14]. The use of the four monoclonal antibodies gave better results (76/76 correctly serotyped) than a earlier study from our laboratory in which we had only two monoclonal antibodies available (81/97 correctly serotyped)[10].

The Kume Scheme. The Kume serotyping scheme is based on the detection of hemagglutinins in bacterial cells that are first treated with potassium thiocyanate and then sonicated. The HA activity is detected using glutaraldehyde fixed chicken red blood cells. The Kume scheme recognises three serogroups (I, II, and III) which can be further subsplit into a total of seven serovars using absorbed antisera. It has recently been shown that Kume serogroups I, II, and III correspond to Page serovars A, C and B respectively[6]. Recently, the Kume scheme has been

extensively used in my laboratory resulting in the recognition of two new serovars[6]. Due to the proliferation of new serovars, we have suggested a new terminology for the Kume scheme. We now refer to Kume serogroups A, B and C (previously Kume serogroups I, III, and II). The serovars within the serogroups are identified numerically. Currently the recognised Kume serovars are serovars A-1, A-2, A-3, A-4, B-1, C-1, C-2, C-3 and C-4[6]. The new terminology emphasises that the Page serovars and the Kume serogroups are equivalent.

The Best Serotyping Scheme? It is obvious that no one particular serotyping scheme will suit every laboratory. The Page scheme, when used with a HI test as described in our studies[3], would appear to be the simplest option available for most laboratories. It is important that when using the HI test in the Page scheme that the HA antigen is produced in way similar to that described in our study. This particular production method avoids the need for any treatment of the antigen for the majority of isolates. The fact that the Page serovars also correspond to immunovars is another powerful inducement to the use of the Page scheme.

The use of the monoclonal antibodies is restricted to those laboratories with direct access to these reagents as these antibodies are not yet commercially available. The Kume scheme gives the greatest subtyping but is a technically demanding test that may not suit many laboratories.

BIOTYPING

Biotyping of bacteria can be regarded as the recognition of subgroups of isolates of the same genus and species. This technique has proven to be a powerful tool in studying the epidemiology of infectious disease outbreaks. However, there have been few attempts to establish biovars within the species *H. paragallinarum*. The only subdivisions that have been reported are the serological schemes described in the previous sections.

This relative shortage of methods for subdividing *H. paragallinarum* has, we believe, inhibited epidemiological studies on infectious coryza. For this reason, our laboratory has been attempting to develop methods for biotyping *H. paragallinarum*. We have concentrated on the use of three methods: the Kume serotyping scheme, carbohydrate fermentation patterns and antimicrobial drug resistance patterns. Full details of the methods and their applicability are given in our publication[5]. The important point to emerge from the application of these biotyping techniques is that subdivisions within *H. paragallinarum* are possible. By combining all three methods, we were able to subdivide a collection of 92 isolates of *H. paragallinarum* into 19 subgroups[5].

FINGER-PRINTING OF HAEMOPHILUS PARAGALLINARUM BASED ON BIOTECHNOLOGY\MOLECULAR BIOLOGY

The recent advances in the areas of molecular biology and biotechnology have opened new possibilities for the creation of subdivisions within a bacterial species. Rather than traditional biotyping, it is now possible to apply techniques that are commonly regarded as generating "finger-prints" of bacteria. My laboratory has been evaluating the use of some of these new "finger-printing" techniques with *H. paragallinarum*.

We have found that plasmids are not common in *H. paragallinarum*; amongst over 100 isolates we have examined to date only one was found to possess a plasmid[14]. This would indicate that plasmid profiles are not likely to be useful. Protein profiles have been shown to have some capability of subdividing *H. paragallinarum*. Both whole-cell and outer membrane protein profiles have been shown to fall into two major banding patterns[9,13]. As only two subdivisions have been recognised, it is unlikely that protein profiles will be widely useful as a finger-printing technique. The use of computerised image analysis may allow finer subdivisions to be recognised.

Restriction endonuclease analysis (REA) of chromosomal DNA has proven to be a very useful tool for subdividing isolates of *H. paragallinarum*. When we examined a collection of 15 *H. paragallinarum* collected from outside Australia, we found that all 15 isolates possessed unique REA profiles[4]. Thus, REA appears to be a powerful tool for establishing whether isolates of *H. paragallinarum* are related or not. The use of computerised image analysis may increase the power of REA to subtype *H. paragallinarum*.

APPLICATION OF BIOTYPING AND FINGER-PRINTING TO THE EPIDEMIOLOGY OF INFECTIOUS CORYZA

The application of the biotyping and finger-printing techniques mentioned above to the epidemiology of 16 cases of infectious coryza has recently been described[8]. Antimicrobial biotyping and REA profile typing proved most useful. The other techniques (Kume serotyping, biochemical biotyping, plasmid profiles and protein profiles) gave either limited or no subdivision amongst the isolates. The combined results of the laboratory study indicated that, rather than six unrelated outbreaks as suggested by the field data, the 16 cases represented three pairs of related outbreaks. This study represents the first application of sensitive biotyping and finger-printing techniques to outbreaks of infectious coryza. Two important points emerged from the study. It was shown that farms

can be chronically infected with a single strain of *H. paragallinarum* that re-emerges at intervals. As well, the first detailed evidence that replacement stock are a major source of infectious coryza was obtained.

INACTIVATED INFECTIOUS CORYZA VACCINES

Much of the definitive work on the safety and efficacy of vaccines against infectious coryza was performed in the 1970s. Yamamoto[24] provides an excellent review of this material. There has been some recent activity on infectious coryza vaccines, mainly evaluating the efficacy and safety of a range of different adjuvant systems.

A number of studies have confirmed that aluminum hydroxide gel is an effective adjuvant system[24]. Several studies have reported the occurrence of minor reactions at the vaccination site, although the reactions are generally considered mild. There has been some disagreement over the efficacy of vaccines containing mineral oil type emulsions. Some workers have reported that water-in-oil type emulsions are effective[15,16]. In studies performed at my laboratory, we have found water-in-oil emulsions to be both poorly efficacious and the cause of major adverse side reactions[21].

A double emulsion adjuvant represents an alternative method of combining the antigen phase and the adjuvant phase. A double emulsion is created by re-emulsifying a simple water-in-oil emulsion in an outer water phase. In contrast, in a simple water-in-oil vaccine the antigen is dispersed in water droplets distributed through out the oil phase. In our initial evaluation of double emulsions[2], we found that these vaccines to be safe but capable of only giving very poor levels of protection. Our recent work with double emulsion systems has given far more encouraging results. We have collaborated with a commercial manufacturer experienced in the production of double emulsion type vaccines. Double emulsion vaccines produced by this manufacturer (Australian Poultry Limited) have proven to be very effective, giving high levels of protection[7]. As well, these double emulsion vaccines cause very little in the way of adverse reactions at the site of inoculation (breast muscle). These results indicate that the double emulsion system, provided it is formulated correctly, represents both a safe and effective adjuvant system for infectious coryza vaccines.

FUTURE

We have gained much useful knowledge on infectious coryza since the pioneering days of workers such as Schalm and Beach at the University of California, Davis. The future promises even more exciting adventures. The application of the new techniques of molecular biology and other reagents such as monoclonal antibodies open new areas for research. It is possible that by the end of the 1990s we will have available rapid and specific diagnostic tools. Such tests will rapidly and specifically detect antibody in chicken sera or the presence of antigen in sinus washings. As well, we may have both live and inactivated vaccines. These new generation vaccines could be based on strains that have been manipulated/engineered so that they possess a range of cross-protecting antigens but none of the antigens associated with adverse side reactions.

REFERENCES

1. Blackall, P. J. Antimicrobial drug resistance and the occurrence of plasmids in Haemophilus paragallinarum. Avian Dis. 32:742-747. 1988.
2. Blackall, P. J. Further comparison of adjuvants for an inactivated infectious coryza vaccine. Avian Dis. 32:831-835. 1988.
3. Blackall, P. J., L. E. Eaves, and G. Aus. Serotyping of Haemophilus paragallinarum by the Page scheme: comparison of the use of agglutination and hemagglutination-inhibition tests. Avian Dis. 34:643-645. 1990.
4. Blackall, P. J., L. E. Eaves, and C. J. Morrow. Comparison of Haemophilus paragallinarum isolates by restriction endonuclease analysis of chromosomal DNA. Vet. Microbiol. 27:39-47. 1991.
5. Blackall, P. J., L. E. Eaves, and D. G. Rogers. Biotyping of Haemophilus paragallinarum by hemagglutination serotyping, and carbohydrate fermentation, and antimicrobial drug resistance patterns. Avian Dis. 33:491-496. 1989.
6. Blackall, P. J., L. E. Eaves, and D. G. Rogers. Proposal of a new serovar and altered nomenclature for Haemophilus paragallinarum in the Kume hemagglutinin scheme. J. Clin. Microbiol. 28:1185-1187. 1990.
7. Blackall, P. J., L. E. Eaves, D. G. Rogers, and G. Firth. An evaluation of inactivated infectious coryza vaccines containing a double-emulsion adjuvant system. Avian Dis. 36:in press. 1992.
8. Blackall, P. J., C. J. Morrow, A. McInnes, L. E. Eaves, and D. G. Rogers. Epidemiologic studies on infectious coryza outbreaks in northern New South Wales, Australia, using serotyping, biotyping, and chromosomal DNA restriction endonuclease analysis. Avian Dis. 34:267-276. 1990.
9. Blackall, P. J., D. G. Rogers, and R. Yamamoto. Outer-membrane proteins of Haemophilus paragallinarum. Avian Dis. 34:871-877. 1990.
10. Blackall, P. J., T. Yamaguchi, Y. Iritani, and D. G. Rogers. Evaluation of two *monoclonal antibodies* for serotyping Haemophilus paragallinarum. Avian Dis. 34:861-864. 1990.
11. Blackall, P. J., and R. Yamamoto. "Haemophilus gallinarum" - a re-examination. J. Gen. Microbiol. 135:469-474. 1989.

12. Blackall, P. J., and R. Yamamoto. Infectious Coryza. In: A Laboratory Manual for the Isolation and Identification of Avian Pathogens, 3rd ed. H. G. Purchase, L. H. Arp, C. H. Domermuth, and J. E. Pearson, eds. American Association of Avian Pathologists, Philadelphia, Pennslyvania. pp. 27-31. 1989.

13. Blackall, P. J., and R. Yamamoto. Whole-cell protein profiles of Haemophilus paragallinarum as detected by polyacrylamide gel electrophoresis. Avian Dis. 33:168-173. 1989.

14. Blackall, P. J., Y.-Z. Zheng, T. Yamaguchi, Y. Iritani, and D. G. Rogers. Evaluation of a panel of monoclonal antibodies in the subtyping of Haemophilus paragallinarum. Avian Dis. 35:(in press). 1991.

15. Coetzee, L., G. S. Strydom, and E. J. Rogers. The value of oil-adjuvant vaccines in the control of Haemophilus paragallinarum infection (infectious coryza) in egg producing birds in South Africa. Develop. Biol. Standard. 51:169-180. 1982.

16. Davis, R. B., R. B. Rimler, and R. B. Shotts Jr. Efficacy studies on Haemophilus gallinarum bacterin preparations. Am. J. Vet. Res. 37:219-222. 1976.

17. Droual, R., A. A. Bickford, B. R. Charlton, G. L. Cooper, and S. E. Channing. Infectious coryza in meat chickens in the San Joaquin Valley of California. Avian Dis. 34:1009-10016. 1990.

18. Hinz, K.-H. Heat-stable antigenic determinants of Haemophilus paragallinarum. Zbl. Vet. Med. B 27:668-676. 1980.

19. 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.

20. Page, L. A. Haemophilus infections in chickens. 1. Characteristics of 12 Haemophilus isolates recovered from diseased chickens. Am. J. Vet. Res. 23:85-95. 1962.

21. Reid, G. G., and P. J. Blackall. Comparison of adjuvants for an inactivated infectious coryza vaccine. Avian Dis. 31:59-63. 1987.

22. Takagi, M., N. Hirayama, H. Makie, and S. Ohta. Production, characterization and protective effect of monoclonal antibodies to Haemophilus paragallinarum serotype A. Vet. Microbiol. 27:327-338. 1991.

23. Yamaguchi, T., P. J. Blackall, S. Takigami, Y. Iritani, and Y. Hayashi. Pathogenicity and serovar-specific hemagglutinating antigens of Haemophilus paragallinarum serovar B strains. Avian Dis. 34:964-968. 1990.

24. Yamamoto, R. Infectious coryza. 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, Iowa. pp. 186-195. 1991.

PRELIMINARY DEVELOPMENT OF AN ELISA ASSAY FOR INFECTIOUS CORYZA (HEMOPHILUS PARAGALLINARUM INFECTION) IN CHICKENS

N. Raie[A], S. Hietala[B], J. Barton[A], and D. Read[A]

California Veterinary Diagnostic Laboratory System
School of Veterinary Medicine, University of California-Davis
[A]San Bernardino Branch Laboratory, P.O. Box 5579,
San Bernardino, California 92412
[B]Davis Laboratory, P.O. Box 1770, Davis, California 95617

Infectious coryza is an acute respiratory disease of chickens caused by the bacterium *Hemophilus paragallinarum*. Though infectious coryza is associated with a low mortality, the economic impact on egg production in infected flocks is significant and warrants disease control measures. Inactivated vaccines and controlled exposure to live antigens are currently recommended as the most effective measures to reduce production losses due to infectious coryza. This study reports on the development of an ELISA for use in the economic management of infectious coryza. Preliminary results suggest the assay can be used to monitor the serologic response to *H. paragallinarum* in vaccinated or exposed birds. The ELISA was optimized to detect exposure and to assess protection in vaccinated and challenged birds. Field and laboratory trials were conducted to monitor the serologic response of birds to live challenge with *H. paragallinarum* in vaccinated and non-vaccinated flocks.

SEROLOGIC RESPONSE TO COMMERCIAL INFECTIOUS CORYZA BACTERINS: PRELIMINARY RESULTS AS MEASURED BY A NEWLY DEVELOPED ELISA

J. Barton[A], N. Raie[A], W. McKeen[B], M. Hammarlund[C], G. Cutler[D], D. Read[A], A. Bickford[E], and S. Hietala[F]

California Veterinary Diagnostic Laboratory System
School of Veterinary Medicine, University of California-Davis
[A]San Bernardino Branch Laboratory, P.O. Box 5579, San Bernardino, California 92412
[B]University of California, Cooperative Extension
777 East Rialto Avenue, San Bernardino, California 92415
[C]P.O. Box 7698, Riverside, California 92513
[D]P.O. Box 1042, Moorpark, California 93020
[E]Turlock Branch Laboratory, P.O. Box 1522, Turlock, California 95381
[F]Davis Laboratory, P.O. Box 1770, Davis, California 95617

Infectious coryza is an ongoing cause of depressed production on multiple-age egg farms caused by the bacterium, *Hemophilus paragallinarum*. It has been recognized as a problem in California since as early as 1933[2,3]. Active infection may cause 10-40% decrease in egg production[5]. Current practice within the industry is to achieve controlled infection through spray or drinking water exposure to virulent *H. paragallinarum* prior to onset of egg production. This is done both with and without previous immunization. Co-infection with *Mycoplasma gallisepticum* results in a chronic manifestation of infectious coryza[1,2,4]. The "F strain" of *M. gallisepticum* is commonly utilized in southern California to reduce losses from chronic respiratory disease; however, its role as a co-pathogen with *H. paragallinarum* is unknown. The economic impact of infectious coryza on egg production is significant and warrants disease control measures.

Under field conditions, chickens were vaccinated against infectious coryza. All vaccinated birds, as well as unvaccinated controls, were exposed to a virulent isolate of *H. paragallinarum* utilized on the ranch for "controlled exposure." Under laboratory conditions, chickens were similarly vaccinated. One-half of this group was infected with "Modesto" strain of *H. paragallinarum*. In both the field and laboratory trials, sera were collected at intervals and stored. Serum antibody levels to *H. paragallinarum* were measured by an Enzyme Linked Immunosorbent Assay (ELISA) under development at the San Bernardino Branch of the California Veterinary Diagnostic Laboratory System. Results of serologic response as measured by this newly developed ELISA will be presented.

REFERENCES

1. Adler, H.E., and R. Yamamoto. Studies on Chronic Coryza (Nelson) in the Domestic Fowl. Cornell Vet. 46:337-343. (1956)

2. Beach, J.R. and O.W. Schalm. Studies of the Clinical Manifestations and Transmissibility of Infectious Coryza of Chickens. Poultry Sci. 15:466-472. (1936)

3. Page, L.A. Haemophilus infections in chickens. III. Factors in intraflock transmission of infectious coryza and its chemical and antibiotic therapeusis. Avian Dis. 6:211-225. (1962)

4. Page, L.A., A.S. Rosenwald, and F.C. Price. Haemophilus infections in chickens. IV. Results of laboratory and field trials of formalinized bacterins for the prevention of disease caused by *Haemophilus gallinarum*. Avian Dis. 19:318-322. (1963)

5. Yamamoto, R. Infectious Coryza in Diseases of Poultry-9th Edition, Edited by B.W. Calnek et.al. pp. 186-195. (1991)

CLINICAL EFFICACY OF DANOFLOXACIN IN THE THERAPY OF E. COLI AIRSACCULITIS IN BROILERS

J. Tyler Holck[A], Frank Sterner, and William S. Swafford[A]

Pfizer, Animal Health, 1107 South 291 Highway
Lee's Summit, Missouri 64081

Danofloxacin, a new fluoroquinolone antibacterial, was evaluated in the therapy of a naturally occurring outbreak of *Escherichia coli* respiratory disease in a commercial broiler flock in Delaware. Approximately 4 weeks after placement, a severe outbreak of colibacillosis originated in a broiler flock of 27,282 birds. Simultaneously, a mild respiratory disease outbreak began in a nearly identical house containing 27,200 birds of identical age and origin at the same production site. When the birds were approximately 5 weeks of age, the flock experiencing the most severe disease received danofloxacin at 5 mg/kg/day for 3 days while the second flock was medicated with oxytetracycline at 422 mg/l of drinking water for 5 days. Birds from both flocks were slaughtered at approximately 7 weeks of age. In the danofloxacin-medicated flock, mortality was reduced during the medication and post-treatment observation periods ($P \leq 0.05$) compared to the level present on the pre-treatment day. Lower treatment-to-slaughter mortality and fewer condemnations at slaughter, especially those attributed to airsacculitis and septicemia, were recorded for the flock medicated with danofloxacin. The minimum inhibitory concentration (MIC) of danofloxacin against *E. coli* isolates ranged from 0.015 to 0.125 µg/ml and was >64 µg/ml for oxytetracycline.

INTRODUCTION

Danofloxacin is a potent, third-generation fluoroquinolone which has bactericidal activity against a broad spectrum of bacteria and mycoplasmas of veterinary importance, including *E. coli*[1]. The use of danofloxacin as a respiratory therapeutic agent in broilers is supported by its superior pharmacokinetic profile, which demonstrates respiratory tissue concentrations exceeding plasma levels by a factor of 4[2]. The superior potency and pharmacokinetics of danofloxacin suggest that it should be efficacious in the treatment of naturally occurring *E. coli* respiratory disease.

This study was conducted to assess the safety and efficacy of 5 mg/kg/day of danofloxacin given for 3 days in the drinking water for the treatment and control of *E. coli* respiratory disease (airsacculitis/septicemia) of broiler chickens under conditions of commercial production and exposure to natural disease.

MATERIALS AND METHODS

Two flocks of approximately 27,000 broiler chicks each were used to evaluate the safety and efficacy of danofloxacin for treatment and control of *E. coli* respiratory disease. The trial was conducted at a commercial broiler production facility in Delaware. The chicks were vaccinated against Newcastle's disease (NDV), Marek's disease (MD), and infectious bronchitis virus (IB) at the hatchery.

The chicks were housed in a conventional production facility with approximately 0.06 m^2 floor space per animal and were given *ad libitum* access to water and feed. The basal ration consisted of a corn/soy base with anticoccidial and growth promotant added. The basal ration contained no antibacterials at the time the test medications were administered.

Procedure. Two broiler houses on the same farm were monitored during the growout for clinical signs of *E. coli* respiratory disease with concurrent rising flock mortality. Medication was initiated when the daily mortality was 0.1% (> 1 bird/1000) or greater, a minimum of 5% of the birds exhibited clinical signs of respiratory disease, and gross post-mortem examination of a representative number of birds confirmed the presence of *E. coli* respiratory disease. The first flock was medicated for three days with danofloxacin at 5 mg/kg/day in the drinking water using a proportioner system. The second flock was medicated with oxytetracycline, also administered through a proportioner, at the rate of 422 mg/l for 5 days.

Birds were observed for mortalities, clinical disease, and specific adverse reactions to medication. Up to a maximum of 40 dead birds per treatment group per day were randomly selected for post-mortem examination. Lesions of airsacculitis, pericarditis, and perihepatitis were scored according to the method of Goren[3] and recorded. In addition, swabs were taken from the airsac or liver and examined bacteriologically for the presence of *E. coli*. The susceptibility of a representative sample of recovered isolates to the test drugs was determined by the Sensititre® broth microdilution method. At the end of the growing period, birds were sent for processing and inspection. The total liveweight, number of birds condemned and the reasons for condemnations were recorded for each house.

Data Analysis. Daily mortality was determined and recorded the day after it occurred. To assess the effect of treatment on percent total daily mortality, a general linear model (GLM) procedure was used. The model for percent daily mortality (analyzed as the arcsin of the square root of the percent daily mortality) partitioned the total variation into treatment groups, periods, and interaction between treatment group and period. Periods evaluated were as follows:

- Background (DOT -8 to -2)
- One day pretreatment (DOT -1)
- Treatment (DOT 0 to 2 or 0 to 4 depending on treatment)
- Five days post-treatment (DOT 3 to 7 or DOT 5 to 9 depending on treatment)

Planned comparisons among periods within a treatment group and treatment groups within a period were tested using the interaction least squares means.

RESULTS

Clinical, Pathological and Microbiological Observations. Clinical observations of snicking, depression, and mortality consistent with respiratory disease attributable to *E. coli* were observed in birds from both houses at approximately 4 weeks of age, with therapy initiated 7 days later. The severity of the outbreak was more intense in the danofloxacin-treated flock, with moderate to severe airsacculitis, pericarditis, and perihepatitis present at post-mortem examination of birds which died during the 24-hour period **prior** to medication. Culturing of these birds revealed pathological evidence of *E. coli* respiratory disease in 73% of the birds examined in the danofloxacin flock and in 20% of the birds in the oxytetracycline flock.

The clinical appearance of danofloxacin-medicated birds improved during the post-treatment observation period and snicking was reduced. Treatment with oxytetracycline was less effective as evidenced by increasing clinical signs of respiratory disease during post-treatment observation periods.

Both flocks experienced a rise in mortality following the post-treatment observation period. Adenovirus was isolated from a pooled trachea sample of oxytetracycline-treated birds submitted for diagnostic evaluation at the end of the growing period.

Mortality. The severity of disease outbreak prior to medication was significantly greater ($P \leq 0.05$) in the danofloxacin-treated flock as evidenced by the background and pretreatment mortality. The recorded mortality during the 7-day period prior to the disease outbreak and during the 24 hours prior to medication was 0.304% and 0.411% respectively for the danofloxacin and 0.091% and 0.061% respectively for the oxytetracycline-medicated flock.

Medication with danofloxacin reduced average daily mortality to 0.275% during the medication period and to 0.185% during the 5-day post-treatment observation period. The latter was significantly ($P \leq 0.05$) less than that recorded during the pretreatment period. The mortality in the flock treated with oxytetracycline remained stable during the treatment period; however, the flock experienced an increase in mortality to 0.202% during the 5 days post-treatment, significantly ($P \leq 0.05$) higher than the treatment period for that flock.

Flock Productivity. Birds were slaughtered at approximately 7 weeks of age; the total mortality and liveweight at slaughter reflected the extreme contrast in severity of the disease between the 2 flocks, with 11.3% mortality and lower liveweights recorded for the danofloxacin flock compared to 7.7% mortality recorded for the oxytetracycline flock. However, danofloxacin therapy did reduce the treatment-to-slaughter mortality compared to oxytetracycline, with 2.81% and 3.49% mortality recorded for the respective flocks following the initiation of therapy. Additionally, the flock medicated with danofloxacin had 0.95 less total percent condemnations at slaughter, including 0.39 due to airsacculitis and 0.48 due to septicemia, when compared to the flock medicated with oxytetracycline.

MIC Values for Isolates of *E. coli*. The MIC against 58 *E. coli* isolates collected during post-mortem examinations ranged from 0.015 to 0.125 µg/ml for danofloxacin and was >64 µg/ml for oxytetracycline.

Safety Observations. No adverse reactions to the administration of danofloxacin were noted.

REFERENCES

1. McGuirk, P.R., M.R. Jefson, T.R. Shryock, and T.K. Schaaf. The synthesis and antibacterial activity of danofloxacin: a new quinolone for veterinary medicine. Twenty-ninth Interscience Conference on Antimicrobial Agents and Chemotherapy, Houston, Texas. September 1989.

2. Frame, G.M., D.D. Mann, and M.J. Lynch. Pharmacokinetics of the quinolone antibiotic danofloxacin (CP-76, 136) in cattle, swine, and poultry. Twenty-ninth Interscience Conference on Antimicrobial Agents and Chemotherapy, Houston, Texas. September 1989.

3. Goren, E. Observations on experimental infection of chicks with E. coli. Avian Pathology, 7:213-224. 1978.

TOXOIDS AND ESCHERICHIA COLI BACTERINS IN TURKEYS: IMMUNITY AND PROTECTION

Elie K. Barbour, James B. Johnson, Peter E. Poss,
Marshall K. Brinton, and Brian J. Zeug

Department of Research and Development,
Brinton Laboratories, Inc., Willmar, Minnesota

The immunity and protection against colisepticemia in turkeys vaccinated at 5 weeks of age with either the whole cells of *Escherichia coli*, whole cells of *E. coli* plus their toxoids, and toxoids alone were evaluated.

The immune responses by all vaccinates were significantly higher ($P < 0.05$), 3 weeks post vaccination, compared to unvaccinated birds. The magnitude of immune responses 3 weeks post vaccination was significantly higher in birds administered the toxoid alone compared to the other vaccines.

A preliminary controlled challenge 3 weeks post vaccination was performed. A 6 hr-culture of 4 strains of *E. coli* was given in the left thoracic air sac in 1 ml of tryptic soy broth per bird. Birds given the toxoid alone had higher protection against colisepticemia than birds given the other *E. coli* bacterins. An expanded controlled protection experiment showed a significant reduction in mortalities ($P < 0.05$) in birds given toxoids compared to the unvaccinated birds.

Field application of the whole cell bacterin, whole cell plus toxoid, and toxoid alone in 5000 bird-flocks was done. Between 7-10 weeks of age the mortalities due to colisepticemia were 1.32%, 0.96%, and 1.5% respectively. The unvaccinated flock resulted in 12.3% mortality during the same observed period ($P < 0.05$).

PASSIVE IMMUNE PROTECTION: A REEVALUATION

Michael W. Naveh and Eliora Z. Ron

Department of Molecular Microbiology and Biotechnology
Tel-Aviv University, Tel-Aviv, Israel

Passive immune protection—usually by horse antibodies—has been extensively practiced in human medicine, but its use has been reduced with the development of effective vaccines. At present, the use of passive immunization is expanding due to new purification techniques and the availability of monoclonal antibodies.

In poultry, intensive production started at the time that active immunization by vaccination has already become the method of choice. Actually, in a way, the industrialized production became possible largely due to the availability of such vaccines.

Today, active immunization is still the method of choice for prevention of severe diseases, using attenuated-live vaccines, inactivated vaccines or a combination of both (Newcastle, ILT, Marek's, IB, etc.). However, in many circumstances vaccination does not solve the problem:

a. Pathogens for which vaccines do not provide satisfactory protection (bacterial vaccines against *Pasteurella, E. coli, Salmonella*).
b. Pathogens that constitute a problem at an early age, when vaccination is not very effective (such as IBDV).
c. "Unexpected pathogens"—disease outbreaks by pathogens that are not frequent in the area.
d. Vaccines that produce severe, disease-like reactions (using "hot strains" such as Komarov, live TRT etc.).
e. Vaccines containing several immunologically-distinct strains, that may interfere with each other (multistrain *Pasteurella*, IBDV).

For most of these problems passive immune protection can provide an effective solution.

In preparing antibodies from poultry for passive immunization there is the unique advantage of using the eggs instead of serum. The egg yolk of hyper-immunized hens is a rich and inexpensive source of specific polyclonal antibodies (IgY - immunoglobulins of yolk origin). It is interesting to note that the fact that chicken eggs are a convenient source of antibodies was published as early as 1893[1] and it was not less than 80 years later that publications appeared[2,3,4] demonstrating the existence of specific antibodies and suggesting possible applications.

Specific antibodies appear in the yolk about three weeks after immunization, reach a plateau four weeks later, and remain high for at least two months. Following vaccination, it is possible to obtain from each egg a total amount of about 60 mg of immunoglobulins, which contain about 2 mg of specific antibodies. It is interesting to note that the antibody content of one egg is equivalent to that of 1 liter of horse serum!

One of the problems that can be solved by using passive immune protection is avian colisepticemia. This disease is responsible for heavy economical losses worldwide. The use of drugs is often not effective, due to the fact that the bacteria rapidly develop resistance to the available antibacterial agents. The problem is intensified when the *E. coli* infection is superimposed on a primary condition—viral or environmental—which is not affected by the drugs at all. Immunization against colisepticemia also presents a problem because of the large number of pathogenic *E. coli* strains which can cause the disease, and do not cross-react immunologically.

Antibody preparations protective against colisepticemia can be produced in two ways:

1. Immunizing each hen with one *E. coli* strain and combining the IgY preparation from several hens, each immunized with a different strain, thus covering all the strains that constitute a problem in the specific region.
2. Immunizing with a polyvalent vaccine (containing a mixture of relevant strains, or an antigen shared by the whole group.)

An example of the effectiveness of IgY antibodies is presented in Table 1. In addition to colisepticemia, passive immune protection was obtained against a number of viral diseases.

REFERENCES

1. Klemperer, F. 1893. Arch. exptl. Pathol. und Pharmakol. 31, 356.
2. Rose, M.E. et al. 1974. European Journal Immunology 4, 521.
3. Bar-Joseph, M. and M. Malkinson. 1980. J. Virol. Methods 1, 179.
4. Piela, Th.H. et al. 1984. Avian Dis. 28, 877.

Table 1. Immune protection against colisepticemia induced by intraperitoneal injection of virulent bacteria.

Bacteria	Treatment	Survivors/ total	% survivors	p
E. coli 078	none	0/18	0	
	anti 078	20/21	95	<0.01
E. coli 02	none	0/9	0	
	anti 02	8/9	89	<0.01

VIRAL ENTERIC INFECTIONS IN TURKEY FLOCKS IN ISRAEL

Y. Weisman[A], R. Meir[A], M. Malkinson[A], D.L. Reynolds[B]

[A]Kimron Veterinary Institute, Bet Dagan, Israel
[B]Veterinary Medical Research Institute, College of Veterinary Medicine, Iowa State University, Ames, Iowa

Over the past two years (1990-1991), the number of cases of viral enteritis (VE) in young turkey flocks in Israel has increased dramatically. Together with turkey rhinotracheitis (TRT), these two conditions are most prevalent in the 1 to 6 week age group.

Morbidity, due to VE has reached 100% in severe outbreaks, while up to 40% mortality is not uncommonly encountered in the field. The severity of the losses depends to a large extent on intercurrent diseases such as TRT and *Escherichia coli*. In a few instances, acute

coccidiosis was diagnosed despite treatment with efficacious anti-coccidial drugs. The following symptoms were encountered in VE: diarrhea, nervousness, litter eating, stunting, lack of uniformity, and abnormal feathering. Skeletal abnormalities and uneven growth were prevalent in flocks throughout their lifetime. On necropsy, the intestines were filled with fluid, the caeca were dilated and filled with gases. The gut wall in many cases was very thin (gut thinness) and in some cases gizzard erosions were present, findings previously recorded only by Sell[2].

A total of 125 flocks of turkey poults have been monitored; intestines were removed from freshly dead or killed birds and double-stranded RNA viruses were identified from intestinal contents by genome electropherotyping techniques[1]. In some flocks, samples were taken more than once, including when the flock had recovered from VE. Of the 160 gut preparations that were examined, 42% were negative, 36% contained group D rotavirus, 10% group A rotavirus, 3.1% both group A and group D rotavirus, and 7.5% were positive for rotavirus but unresolvable as to group. Some of the negative samples were from flocks that had recovered from VE but were identified as having had either group D rotavirus or group A rotavirus infections. In the most severe outbreaks of VE, groups D rotavirus was detected. Only in 5 outbreaks were group A rotavirus considered to be the causal agent; however, both virus detection and seroconversion were demonstrated in these flocks. Seventy-eight percent of those samples from which group D rotavirus was detected and 69% of those samples from which group A rotavirus was detected were from flocks less than 4 weeks old.

The enzyme-linked immunosorbant assay (ELISA) was used to detect serologic antibody to group A rotavirus. The ELISA used employed group A rotavirus grown in MA-104 cells as the antigen for coating the plates. All the turkey breeding flocks, and most of the meat flocks, that were monitored were shown to be serologically positive. No relationships between group A rotavirus antibody positive status and VE were observed, with the exception of the 5 outbreaks mentioned above.

REFERENCES

1. Reynolds D.L. and B.S. Pomeroy. Enteric viruses. In: A Laboratory manual for the isolation and identification of avian pathogens. Ed. H.G. Purchase and H. Chop, C.H. Domermuth, J.E. Pearson, Kendall Hunt Publishing Company, Iowa pp. 128-134, 1989.

2. Sell J.L., personal communication.

PRODUCTION AND APPLICATION OF MONOCLONAL ANTIBODIES AGAINST AVIAN REOVIRUSES

Nahla D. El-Mahdy, A.S. Dhillon, and William C. Davis

Washington State University-Puyallup
Research and Extension Center, Department of Microbiology & Pathology
Puyallup, Washington 98371

Reovirus infection is a world-wide poultry problem in growing chickens and turkeys[3,4,6]. It has been responsible for significant avian health problems among which leg problems (viral arthritis) and reduced weight gain (malabsorption syndrome) are the most important manifestations. The economic losses are due to low hatchability and high mortality, poor body weight gains, excessive culls, and condemnation during processing in broilers.

Avian reoviruses are members of the Reoviridae family which have a double-stranded, segmented RNA genome, non-enveloped with icosahedral symmetry, and double-capsid structure.

Antigenic differences among reoviruses have been mainly studied by virus neutralization tests using polyclonal antibodies[7,2,8] but these studies have been inadequate in determining the serological differences in various strains of reoviruses. An approach that holds promise is the use of monoclonal antibodies directed against virus surface antigens that delineate strain differences and thus serve as a tool for identification and classification of reovirus isolates.

Monoclonal antibodies (MAbs) were first describes in 1975 by Kohler and Milstein[5] who produced MAbs by fusion of spleen cells from virus-immunized mice with murine myeloma cells. The hybridoma cells secrete MAbs that are homogenous preparations of antibodies of defined specificity. MAbs of various specificities can be produced and selected *in vitro* using appropriate screening assays such as ELISA. MAbs can be used in all applications where conventional polyclonal antibodies have been used. In addition, the purity and specific nature of MAbs

have made them valuable in immunodiagnosis for rapid and correct identification of serotypes.

BALB/c mice were hyperimmunized with plaque purified Reovirus S-1133. The spleen cells were fused with murine myeloma cells[1]. A suspension ELISA test using whole cells as an antigen was developed to screen the hybridoma supernatants. Fourteen hybridomas that secreted Reo S-1133 specific-antibodies were cloned and isotyped. Immunodiffusion tests revealed that 8 MAbs produced IgM and 6 produced IgG1. The details of other properties and their applications will be discussed at the meeting.

REFERENCES

1. Davis, William C. Enhancement of myloma-B-cell hybridoma outgrowth in primary cultures with B cell mitogens. Periodicum Biologorum. 90:367-374. 1988.

2. Giambrone, J.J. and W. Solano. Serologic comparison of avian reovirus isolates using virus neutralization and an enzyme-linked immunosorbent assay. Avian Dis. 32:678-680. 1988.

3. Kawamura, H., F. Shimizu, M. Maeda, and H. Tsubahara. Avian Reovirus: its properties and serological classification. National Inst. of Animal Health Quarterly. 5:115-124. 1965.

4. Kibenge, F.S.B. and G.E. Wilcox. Tenosynovitis in chickens. Veterinary Bulletin, Weybridge. 53:431-443.

5. Kohler, G and C. Milstein. Continuous cultures of fused cells secreting antibody of predefined specificity. Nature 256:495-497. 1975.

6. Robertson, M.D., G.E. Wilcox, and F.S.B. Kibenge. Prevalence of reoviruses in commercial chickens. Aust. Vet. Jour. 61:319-322. 1984.

7. Rosenberger, J.K. Characterization of reoviruses associated with runting syndrome in chickens. Proc. Int. Union Immunol. Soc., Sydney, Aust. 66:141-152. 1983.

8. Wood, G.W., R.A.J. Nicholas, C.N. Hebert, and D.H. Thornton. Serological comparisons of avian reoviruses. Jour. Comp. Pathol. 91:29-38. 1980.

THE ROLE OF CELL MEDIATED IMMUNITY IN MEDIATING RESISTANCE IN CHICKS TO CRYPTOSPORIDIUM BAILEYI INFECTION

Josh Hatkin

Poultry Science Department,
Auburn University, Auburn, Alabama 36849

The role of cell mediated immunity (CMI) in mediating resistance to infection with *Cryptosporidium baileyi* was studied. This was accomplished by a reduction of the birds CMI system with cyclosporin A (CsA). Chicks received either a single challenge dose of 2 X 10^6 *C. baileyi* oocysts at 28 days of age or a priming dose of 2.3 x 10^5 *C. baileyi* oocysts at 7 days of age followed by the challenge dose. All birds were necropsied 14 days following the challenge dose and selected tissues were examined for developmental stages of *C. baileyi*. CsA treated chicks that received 2 doses of *C. baileyi* were smaller and had more severe gross and microscopic lesions than non-CsA treated controls. CsA treated chicks that received only the challenge dose had greater numbers of developing *C. baileyi*, and greater gross and microscopic lesions than non-CsA treated controls. Depressed CMI function was demonstrated in CsA treated birds by the delayed wattle reaction, when surface sterilized, sonicated *C. baileyi* were used as antigen. Humoral immunity was not altered in CsA treated birds. Humoral immunity was evaluated with an enzyme labeled immunoassay, which detected specific anti-*C. baileyi* antibody, and with a hemagglutination assay, which detected natural antibody against rabbit erythrocytes.

THE DETERMINATION OF HEMORRHAGIC ENTERITIS VIRUS ANTIBODY IN TURKEYS BY ELISA

Martin Blankfard[A], Saira Malik[A]
and Jan V. J. M. van den Hurk[B]

[A]Kirkegaard and Perry Laboratories
2 Cessna Court, Gaithersburg MD 20879
[B]Veterinary Infectious Disease Organization,
124 Veterinary Road, Saskatoon, Saskatchewan, Canada S7N 0W0

Hemorrhagic enteritis (HE) is an acute disease in turkeys caused by hemorrhagic enteritis virus (HEV) and characterized by enteritis, splenomegaly, hemorrhages and death[1]. Hemorrhagic enteritis is widespread among turkey flocks and causes significant economic losses to turkey growers[1]. Serologic studies indicate that most adult turkeys flocks have been infected with HEV[2]. Presently no commercially available ELISA exists for the detection of HEV antibody.

A new indirect ELISA for the detection of HEV antibody in turkey serum has been developed and utilizes an affinity purified HEV hexon protein antigen[3]. Serum panels, consisting of monospecific sera to a variety of infectious disease agents (both chicken and turkey) were used to determine test specificity. Results indicated excellent specificity to HEV sera and no cross reactivity to heterologous sera. In addition, antisera to avian adenoviruses groups 1, 3 and 5 also demonstrated no cross reactivity when assayed by the HEV ELISA. Sensitivity studies reveal that the HEV ELISA is at least 16 times more sensitive than the conventional agar-gel percipitin test. Commercial turkey flocks vaccinated with spleen derived HEV vaccines exhibit high (4,000 to 8,000) and uniform (CV= 20% to 50%) HEV ELISA antibody titers. Turkey flocks vaccinated with tissue culture HEV vaccine exhibited low (500 to 1000) and non-uniform (CV= 100% to 200%) antibody titers.

These data indicate that the HEV ELISA demonstrates excellent specificity, sensitivity and reproducibility.

REFERENCES

1. Domermuth, C. H., R. T. DuBose, W. B. Gross and V. J. Yates. Avian Adenovirus group II. The hemorrhagic enteritis-marble spleen disease viruses. 49th N.E. Conf. Avian Disease. Proc. 1977

2. Domermuth, C. H. and W. B. Gross. Hemorrhagic enteritis and related diseases. In: Diseases of Poultry 9th edition, Ames Iowa University Press, 1991; 22:567-572.

3. van den Hurk, J. V. and S. van Drunen Littel-van den Hurk. Characterization of Group II avian adenoviruses with a panel of monoclonal antibodies. Can. J. Vet. Res. 1988; 52:458-467.

EFFECTS OF NUTRITION ON BROILER HEALTH AND LEG STRUCTURE

Phillip Hargis

Texas A&M University Overton, Texas

Leg problems are and have been the topic of discussion in many poultry companies across the United States. From time to time most companies have leg problems in their birds. It is not that leg disorders are problems that cannot be prevented. Leg problems are challenges that can be confronted and successfully remedied. Nutrition of the bird plays a major part in the cause and prevention of leg problems in broilers and turkeys as well as all other poultry. Disease conditions will cause leg structural problems. However, the majority of the leg problems in birds today are based on nutritional aspects of the feeds.

Along with leg structure problems come bird health problems related to the fact that birds have trouble walking to feed and water. Discomfort in their body convinces

the bird that it is better to just sit and survive instead of eating, drinking and growing. This restriction on daily nutrients places a stress on the bird's total system opening the door for disease conditions that adversely affect the bird. Reduced resistance to disease can be directly related to the nutritional intake of the bird.

QUALITY CONTROL IN FEED INGREDIENTS

There are several areas of nutritional management that can be linked to bird health and leg structure. One important area is quality control of the feed ingredients. Quality control is most often thought to be the purchase of the best possible quality of a particular ingredient. Quality control also means deals more with eliminating problems within and between feed ingredient shipments. Quality control deals more with purchasing more consistency within feed ingredients. Purchasing good or poor quality ingredients is not the most important issue. Purchasing the same degree of quality each time is most important. If the particular quality level of an ingredient is known, that information can be reflected in the ingredient matrix of the feed formulation program. Understanding what is being used and knowing what the next load consist of is the key to any nutritionist. An ingredient poor in quality does not mean it cannot be used to the company's advantage. Consistently poor quality is the key. Good and poor ingredient quality does not mean detrimental nutrition problems like mycotoxins. Things like protein, calcium, phosphorus and calorie level are the nutrients that must be monitored. If these nutrients are significantly different with each load of soybean meal, then the feed made today will be different from the feed made last week. Birds cannot be expected to grow the same today each day under these conditions.

Two additional areas of importance are the fat and animal protein sources. Fat is added to feeds for its calorie content. Calories increase growth rate and feed efficiency. When the fat source fluctuates in calorie content per pound due to moisture the birds get more calories than expected today and less than expected tomorrow. This influence on growth puts pressure on bone development. Even growth patterns are important. If fat is used that is going or is already rancid, the destructive power of the fat on other essential nutrients such as vitamins can cause bird health and structural problems.

Animal protein is also very important. Animal protein sources are used because they offer a tremendous amount of nutrients for each pound of ingredient used. Protein, *calcium* and phosphorus are critical nutrients that must be consistent. Calcium and phosphorus from animal protein sources may be the sole source of phosphorus in the diet. Limestone may be used to supplement calcium. However, no dicalcium phosphate or deflourinated phosphate is used to meet the phosphorus requirement. Fluctuations in calcium and phosphorus intake from day to day cause extreme pressure on the structural development of the bird. The proper ratio of calcium to phosphorus must be maintained in order for the bird to develop desired bone characteristics. The cheaper nutrient calcium is often in excess in most ingredients supplying minerals. Calcium is often in excess in the average diet. The bird will use the calcium in some manner. That use includes making bone structures that will not survive the production stage of today's bird.

PHOSPHORUS AND CALCIUM

There have been many studies done and articles written on the importance of phosphorus and calcium in the structural development of birds. Studies have been done on the importance of addition trace minerals, including manganese and zinc, in the proper development of bone. Additional studies have been conducted on the proper ratio in broiler feeds. Zeolite nutrition has been studied regarding their influence on leg and structural disorders. There is a vast reserve of information dealing with the mentioned topics as well as others such as tibia dyschondroplasia. Most of the research has been done with available phosphorus. The non-phytin phosphorus portion in plant protein sources has been considered to be 30-33% of the total plant phosphorus. This is considered available phosphorus to the bird. Under today's farming practices and the genetic potential of poultry this three percent variance may not be sufficient to produce consistently sound structural development. Inorganic phosphorus is more important when considering a feed formulation. Sources of inorganic phosphorus such as dicalcium phosphate are more consistent and more dependable sources of phosphorus. Even within these sources there can be problems.

Some deflourinated phosphate sources have been found to be as low as 80% available to poultry. With most sources of dicalcium and deflourinated phosphate being from 95 to 100% available these lower availabilities can cause problems. Animal protein sources are good sources of inorganic phosphorus. Consistent quality in these ingredients and constant monitoring of the calcium and phosphorus levels will allow for a good daily intake of nutrients essential for proper bone development.

Feeds formulated with all of the phosphorus coming from animal protein sources do not have the assurance of a constant phosphorus intake. A key point to remember is that either calcium or phosphorus in excess causes the other to precipitate in the intestine of the chicken. As feeds are formulated, swings in actual mineral content can cause an intestinal absorption problem. If this occurs for only one day in the life of the broiler or turkey, that may be the stress that the bone structure cannot overcome

when the bird begins to put on tremendous amounts of muscle right before market.

The calcium/phosphorus ratio gets a lot of attention when discussing leg problems in poultry. The proper ratio has been well documented. However, other things play an important role in structural and resulting health problems in poultry. Manganese nutrition is a key player in leg disorders. If our ingredients provide the finished feed with an actual calcium level much higher than the calculated calcium level birds can experience leg problems. This can happen due to the inexpensive price of calcium and its use by ingredient suppliers within various ingredients. Combine this with the fact that the maximum level of calcium can easily be the decision of the feed formulation program and problems can be seen. The problem may be that the temporary rise in calcium with correct phosphorus levels may disrupt the metabolism of manganese leading to increased leg problems.

It is important to remember that the cation/anion ratio may be more important than the calcium/phosphorus ratio in poultry feeds. Dietary cations calcium and magnesium along with dietary anions phosphorus, chloride and sulfur must be considered when studying feeds related to structural problems in poultry. The addition of zeolites to a finished feed tends to increase percent bone ash which leads to fewer birds exhibiting severe bone disorders. However, if percent bone ash is at optimum levels before the addition of zeolites no beneficial effects can be expected. Borderline nutritional feeding programs may see good results, whereas, feeding programs with a higher plane of nutrition may see no effects of zeolite addition. There are no simple answers in this area. However, the solutions may not be as drastic as believed. Little things in small packages can make significant differences.

VITAMIN NUTRITION

Information has been around for years regarding the importance of certain vitamins in the formation of bone and the utilization of certain nutrients critical to proper structural development. It has been known that a niacin deficiency causes certain leg abnormalities. With tryptophan sparing niacin and excess arginine, leucine and glycine increasing the niacin requirement, the feed formula must account for many things every day.

Most vitamin premixes are purchased in large amounts with specifications changed infrequently. Ingredients are constantly changing within the feed mill. Too many times the vitamin premix is formulated at the minimum bird requirement due to the cost savings incurred. As ingredients in the feed mill change the vitamin requirement by the bird may change significantly. Leg problems may occur even when no nutritional changes are made to existing feeds.

Niacin is very stable against oxidation and heat. High occurrence of leg problems in the summer months due to vitamin storage conditions and finished feed storage conditions in on-farm feed bins cannot be blamed on a niacin deficiency. However, in broiler and turkey feeds that switch from corn to milo and the companion ingredient corn gluten meal is used the niacin requirement for the bird increases. This is due to the amount of leucine that corn gluten meal brings to the formulation. Most vitamin premixes are not reformulated based on ingredient changes in the feed mill. If the bare minimum in vitamin supplementation is practiced ingredient changes can adversely affect the growth patterns of the bird's skeleton.

Beyond nutrition there is evidence that within breed and strain of turkeys and broilers, leg abnormalities may result from defects in intestinal metabolism of such vitamins as D3. Although research indicates that the dietary amount of vitamin D3 may not alter the rate of bone metabolism, if the nutrient is not being digested properly leg problems can occur in diets with adequate supplementation of the vitamin.

Pyridoxine nutrition is another area that may become critical if the feed formulation puts the right ingredients into the right combination and the supplemental vitamin level is not altered. With pyridoxine important as a coenzyme component in the tryptophan conversion to niacin and the biosynthesis of coenzyme A, a change in feed formulation eliminating animal protein sources may put pressure on the pyridoxine requirement if the vitamin premix is not updated.

As grain sources change, biotin supplementation becomes a critical issue. Milo utilization can challenge the biotin requirement of the bird resulting in improper bone structure. Vitamin A additions in extreme excess of the bird's requirement can interfere with vitamin D3 utilization due to the two vitamins competing for the same absorption sites within the gut. Sufficient amounts of supplemental D3 must be present to prevent the additional vitamin A from indirectly reducing bone ash resulting in increased leg problems.

Many leg disorders in progeny can be traced to the breeder hens. In both turkeys and broilers, the breeders must be phased fed the vitamin premix to insure that adequate daily vitamin intake is maintained during periods of reduced feed intake. Carry-over of vitamins increases as breeder birds age. This has been shown to be especially important in turkey hens. Young turkey hens need more vitamin supplementation compared to older birds to ensure that the progeny are quality offspring. Not altering the vitamin nutrition due to low feed intake or age of the breeder flock can result in offspring that have the tendency for leg weakness even before they hatch.

TRADITIONAL CAUSES OF LEG PROBLEMS

There are traditional nutrition aspects that directly affect proper bone formation and thus the incidence of leg disorders. Mycotoxins, such as aflatoxin B1, are known to interfere with the absorption of dietary fat in the gut of the bird. With fat soluble vitamin requirements existing for all poultry, it seems likely that intestinal absorption of these vitamins is hindered by these mycotoxins. Mycotoxins are in every quality control program. However, no discussion of leg weaknesses in poultry would be complete without mentioning the adverse effects of the presence of mycotoxins.

The use of antioxidants in vitamin premixes is another traditional area that must be considered when working with leg problems. The preservation of those vitamins that affect the bone formation process within the bird must be maintained through the use of the proper antioxidant. There are too many vitamin premixes today that do not use an antioxidant or use the wrong one. The results are field problems including leg disorders that coincide with the age of the vitamin premix or with the manner and conditions in which the vitamin premix was stored. Proper stabilization of fat and animal protein sources is important. Rarely is that a problem today. However, nutritional disorders arise every now and then from rancid fat or poultry meal. Consistent mixing practices by the feed mill will always reduce the incidence of leg problems in poultry. If the turkey grower feed is mixed the same way every day, the birds will grow in a manner that is conducive to good bone structure. Seemingly insignificant variations in limestone and phosphate additions to a batch of feed will contribute directly to leg problems in poultry.

A personal account best describes how the mixing of feed can affect the health of birds. A federal prison system asked for help in streamlining their feed mill operations. One area that had an immediate impact on feed price was the changing of ingredients from 100 pound sacks to bulk supplies. Although feed prices for the same formula were lower, the occurrence of leg problems in the holiday turkey flock took a dramatic turn for the worse. The problem was that the individuals could not read well. Past batch cards indicated ingredient amounts by number of sacks. Revisions in feed mill operations included a set of scales that required batch cards with target scale weights. The men mixing the feeds had problems getting the ingredients mixed right. When ingredients were again purchased in sack form, the turkey leg problems disappeared. Feed mill management is very important.

TOMORROW'S GENETICS

The birds being fed today are not the same birds fed last year and will not be the same birds fed next year. Genetic changes create nutritional challenges that must be confronted daily. Feeds formulated last year may not work in the same manner today. Vitamin nutrition of years past may not supply adequate amounts for today's poultry.

Broilers and turkeys are growing faster in less time. Genetics has reduced the percent skeleton in birds while increasing the muscle mass. Larger birds are walking around on slimmer legs. Small nutritional problems can cause large defects in birds. Birds do not have the large margins of safety that were standards several years ago. Seemingly small stresses of yesterday are magnified today.

Birds are being asked to do a lot during their lifetime. The nutritional aspect of bird management must remain one step ahead of the bird's genetic potential. If it does not, many things including leg problems will be the rule instead of the exception.

CONCLUSION

This discussion of the affects of nutrition on bird health and leg structure has not uncovered anything new. Hopefully, it has pointed out that some of the contributing problem areas are very minute. These nutritional interactions are not new to the field of poultry nutrition. Business is so fast today that many times the little things get by us. Today, little things will insure that our broilers and turkeys have sound bones capable of withstanding our current management challenges.

REFERENCES

1. Ballard, Rebecca, and Hardy M. Edwards, Jr., 1988. Effects of dietary zeolite and vitamin A on tibial dyschondroplasia in chickens. Poultry Sci. 67:113-119.

2. Bar, Arie, Jacob Rosenberg, Riki Perlman, and Shmuel Hurwitz, 1987. Field rickets in turkeys: relationship to vitamin D. Poultry Sci. 66:68-72.

3. Cook, M.E., P. H. Patterson, and M. L. Sunde, 1984. Leg deformities: inability to increase severity by increasing body weight of chicks and poults. Poultry Sci. 63:620-627.

4. Dekich, Mark A., 1985. Leg problems in broilers: a clinical view. Pages 1-3 in: Proceedings Southeastern Poultry and Egg Association's Poultry Health Seminar, Atlanta, Georgia.

5. Ferket, Peter R., 1990. Nutrition and leg problems in turkey toms. Pages 74-85 in: Proceedings Arkansas Nutrition Conference, North Little Rock, Arkansas.

6. Ferket, P. R., and J. L. Sell, 1989. Effect of severity of early protein restriction on large turkey toms. 1. Performance characteristics and leg weakness. Poultry Sci. 68:676-686.

7. Halley, J. T., T. S. Nelson, L. K. Kirby, and Z. B. Johnson, 1987. Effect of altering dietary mineral balance

on growth, leg abnormalities, and blood base excess in broiler chicks. Poultry Sci. 66:1684-1692.

8. Hester, Patricia Y., K. K. Krueger, and Mark Jackson, 1990. The effect of restrictive and compensatory growth on the incidence of leg abnormalities and performance of commercial male turkeys. Poultry Sci. 69:1731-1742.

9. Hulan, H.W., G. DeGroote, G. Fontaine, G. DeMunter, K. B. McRae, and F. G. Proudfoot, 1985. The effect of different totals and ratios of dietary calcium and phosphorus on the performance and incidence of leg abnormalities of male and female broiler chickens. Poultry Sci. 64:1157-1169.

10. Jensen, L. S., D. L. Fleteher, M. S. Lilburn, and Y. Akiba, 1983. Growth depression in broiler chicks fed high vitamin A levels. Nutr. Rep. Int. 28:171-179.

11. Lofton, J. T., and J. H. Soares, Jr., 1986. The effects of vitamin D3 on leg abnormalities in broilers. Poultry Sci. 65:749-756.

12. McNaughton, James L., 1988. Niacin's role in poultry nutrition. Poultry Digest, October, pp. 476-478.

13. Nelson, T. S., G. C. Harris, L. K. Kirby, and Z. B. Johnson, 1990. Effect of calcium and phosphorus on the incidence of leg abnormalities in growing broilers. Poultry Sci. 69:1496-1502.

14. Nestor, Karl E., Wayne L. Bacon, Gerald B. Havenstein, Y. Mohamed Saif, and Philip A. Renner, 1988. Carcass traits of turkeys from lines selected for increased growth rate or increased shank width. Poultry Sci. 67:1660-1667.

15. Pierson, F. W., and P. Y. Hester, 1982. Factors influencing leg abnormalities - a review. World's Poult. Sci. J. 38:5-17.

16. Potter, L. M., 1988. Bioavailability of phosphorus from various phosphates based on body weight and toe ash measurements. Poultry Sci. 67:96-102.

17. Riddell, C., 1985. Anatomy of skeletal disorders in broiler chickens. Pages 1-3 in: Southeastern Poultry and Egg Association's Poultry Health Seminar, Atlanta, Georgia.

18. Ruiz, N., and R. H. Harms, 1988. Niacin requirement of turkey poults fed a corn-soybean meal diet from 1 to 21 days of age. Poultry Sci. 67:760-765.

19. Ruiz, N., R. H. Harms, and S. B. Linda, 1990. Niacin requirement of broiler chickens fed a corn-soybean meal diet from 1 to 21 days of age. Poultry Sci. 69:433-439.

20. Smith, O. B., and E. Kabaija, 1985. Effect of high dietary calcium and wide calcium-phosphorus ratios in broiler diets. Poultry Sci. 64:1713-1720.

21. Soares, J. H., Jr., and J. T. Lofton, 1986. Recent studies on vitamin D and skeletal problems in broilers. Pages 1-7 in: Proceedings Maryland Nutrition Conference.

22. Summers, J. D., Huile Shen, S. Leeson, and R. J. Julian, 1984. Influence of vitamin deficiency and level of dietary protein on the incidence of leg problems in broiler chicks. Poultry Sci. 63:1115-1121.

EFFECTS OF NUTRITION ON TURKEY HEALTH AND LEG STRUCTURE

H. John Kuhl, Jr.

2060 N. Palm Ave.
Rialto, California 92376

It is beyond the time constraints and most likely the author's capability to do complete justice to all of the areas for which this time applies. I will take the liberty of limiting my presentation to practical, everyday occurrences of nutritional involvement on turkey health and leg structure.

This talk will be divided into three areas; development of nutrient requirements, ingredient variability, and mill errors and environmental effects upon feed and feed intake.

Nutrient requirements must first be based on the ultimate use of the turkey. If it is grown for meat production, then at what age and weight is it expected to go to market? If it is grown for breeding purposes, then what lighting weight do we desire? The number of feeds chosen for a given growth period depends upon the feed mill finished feed storage and our own preference. The larger the number of feeds available, the less drastic are the nutrient intake changes for the turkey when feeds are changed. Table 1 depicts nutrient levels which I use for one strain of turkey grown for meat production. Turkey breeding companies all provide nutrient recommendations for their individual strains. You will not that all of these recommendations exceed the National Research Council

(NRC) levels for calcium and phosphorus during the first six to eight weeks of life (Table 2). Recommended vitamin levels are also much higher than NRC (Table 3). Feed cost makes up about 70% of the cost of turkey production and, believe me, nutritionists are reminded of this fact often. So why are these recommendations so much higher in level and subjected to enteritis (bacterial, viral, and coccidiosis) which increases the rate of passage of feed through the intestine and reduces the amount of time spent at absorption sites in the small intestine. Turkeys are fast-growing creatures. Even the slightest decrease in intake or absorption at young ages can affect the structure and shape of leg bones.

I have included (Table 4) recommendations for turkey breeder hen for your information. We must remember that much of the poult's vitamin needs are drawn from the yolk over the first week of life. For this reason, leg weakness during the first week which can not be explained by low calcium or phosphorus in the starter feed may be due to low Vitamin D3 fortification of the hen breeder diet.

Ingredient variability can ruin the best formulated, best managed feeding program. All feed ingredients are by-products of food stuffs for humans. Inherent in these products is variation in nutrient content. The trick in feed formulation is to use ingredients at levels which reduce the risk of the most variable nutrients. Meat and bone meal is a by-product which rightly is pointed to as a feed ingredient with great variability. However, if only one supplier of meat and bone meal is used, then the variability is greatly reduced. Table 5 lists some maximum usage levels of ingredients for use in turkey feeds and also possible reasons for these limits.

Over the years, I have had the "opportunity" to observe leg problems in turkeys. In cases in which I was convinced the probable cause was feed related, I would list the order of cause as (1) salt or sodium (too much or too little), (2) phosphorus (not enough), (3) vitamin D3 (not enough or not metabolized), (4) contaminants (aflatoxin and mycotoxin) and (5) trace minerals (not enough zinc or manganese). The number one cause, salt or sodium, can be quickly and chcaply determined in feed ingredients and drinking water. Sodium or salt content of drinking water needs to be factored into the formulation of feeds. A recommendation to reduce sodium levels of feeds below .17% should be based on a recent water analysis with sodium in excess of 200 ppm. Effects of low sodium intake on bone structure are shown in Table 6.

The environment, temperature primarily, affects feed intake and stability of nutrients. Both feed intake and nutrient stability can adversely affect bone structure. Feed intake can be compensated with nutrient changes in the formulas. Nutrient stability must be compensated with feed management. Mold inhibitors are cheap insurance and will reduce the risk of mold toxins during feed storage. Feed should never be kept in feed tanks for periods longer than two weeks. Peroxides are generated in near-logarithmic fashion during feed storage and even the most effective antioxidants are overcome in time. Vitamin E is behind. Prompt turnover of feed in tanks and feeds help insure that the nutrient levels planned to be in the feed will be present for the poults to consume.

One last point concerning treatment or prevention of leg disorders in poults. Vitamin stress packs should contain relatively low levels of Vitamin A. Fat soluble vitamins compete for absorption sites in the intestine and these sites are easily swamped with high levels of Vitamin A.

Table 1. Suggested nutrient specifications.

NUTRIENT	PRESTART	STARTER	GROWER 1	GROWER 2	FINISH 1	FINISH 2	FINISH 3
M.E. (KCAL/LB)	1320.000	1360.000	1420.000	1480.000	1530.000	1580.000	1580.000
ARGININE %	1.790	1.680	1.550	1.410	1.120	0.930	0.840
LYSINE %	1.750	1.630	1.520	1.380	1.104	0.910	0.820
METHIONINE %	0.620	0.560	0.520	0.480	0.400	0.350	0.300
METH & CYSTINE %	1.100	1.040	0.980	0.870	0.750	0.640	0.550
THREONINE %	1.050	1.000	0.920	0.830	0.710	0.620	0.530
TRYPTOPHANE %	0.285	0.270	0.250	0.235	0.190	0.150	0.150
CALCIUM %	1.400	1.350	1.250	1.200	1.100	0.950	0.900
PHOSPHORUS-AV %	0.820	0.770	0.710	0.660	0.600	0.500	0.460
SODIUM %	0.180	0.180	0.180	0.180	0.180	0.180	0.180
AGES TO FEED	1 - 20	21 - 41	42 - 62	63 - 83	84 - 104	105 - 125	126 +

- Fat additions will vary depending on grain selection. The finisher feeds will require from 6.5% to 9% added fat for these energy levels.
- The available phosphorus levels are based on the premise that 100% of the phosphorus in animal protein sources and inorganic phosphates is available to the turkey. Pitman-Moore's (1990) feed analysis chart shows that meat and bone meal has 88% available phosphorus. If we consider the difference between 100% and 88% available phosphorus from meat and bone meat at 4.4% total phosphorus and usage at 6% of the diet then we would see a difference of .03% in available phosphorus simply due to the interpretation of how much phosphorus is available. Inorganic sources have been shown to vary from 100% down to 80% available phosphorus.

Table 2. A comparison of NRC (1984) energy, calcium and available phosphorus with suggested levels for male turkeys.

AGE (WEEKS)	ENERGY (KCAL/KG)	CALCIUM (%)	PHOSPHORUS AV.%
NRC 0-4	2800	1.2	0.6
NRC 4-8	2900	1.0	0.5
NRC 8-12	3000	0.85	0.42
NRC 12-16	3100	0.75	0.38
NRC 16-20	3200	0.65	0.32
NRC 20+	3300	0.55	0.28
KUHL 0-3	2900	1.4	0.82
KUHL 3-6	3000	1.35	0.77
KUHL 6-9	3125	1.25	0.71
KUHL 9-12	3250	1.2	0.66
KUHL 12-15	3365	1.1	0.6
KUHL 15-18	3475	0.95	0.5
KUHL 18+	3475	.9	0.46
BUT 0-4	2800	1.31	0.73
BUT 4-8	2900	1.27	0.73
BUT 8-12	3000	1.19	0.69
BUT 12-16	3100	1.11	0.65
BUT 16-19	3200	1.07	0.60

NRC=National Research Council (1984)
KUHL=Authors' Suggested Levels
BUT=British United Turkeys

Table 3. A comparison of vitamin levels in turkey feeds (0-8 weeks).

Units per ton of Complete Feed

VITAMIN	UNITS (1)	NRC (1984)	ROCHE (2)	% NRC
A	MIU	3.63	11.4	3.1
D3	MICU	0.82	3.2	3.9
E	TIU	10.9	58.0	5.3
B12	MG	2.72	41.5	15.2
RIBOFLAVIN	G	3.27	10.6	3.2
NIACIN	G	63.5	136.0	2.1
d-PANTO ACID	G	9.98	26.3	2.3
CHOLINE	G	1724.0	2292.0	1.3
MENADIONE	G	0.91	1.0	1.1
FOLIC ACID	G	0.91	2.6	2.8
THIAMINE	G	1.82	5.8	3.2
PYRIDOXINE	G	4.08	8.9	2.2
d-BIOTIN	MG	181.0	447.0	2.5

1) MIU= Million International Units
MICU=Million International Chick Units
TIU=Thousand International Units
G=Grams
MG=Milligrams
2) Vitamin nutrition of poultry, Hoffman LaRoche

Table 4. Suggested vitamin addition levels for turkey breeder hens.

Units per Ton of Feed		
VITAMIN		UNITS (1)
A	10.0	MIU
D3	4.0	MICU
E	45.0	TIU
MENADIONE (K3)	2.25	G
B12	25.0	MG
NIACIN	80.0	G
RIBOFLAVIN	7.5	G
D-PANTOTHENIC ACID	20.0	G
FOLIC ACID	2.5	G
THIAMINE	2.8	G
PYRIDOXINE	7.5	G
BIOTIN	425.0	MG
CHOLINE	550.0	G

1) MIU=Million International Units
MICU=Million International Chick Units
TIU=Thousand International Units
G=Grams
MG=Milligrams

Table 5. Suggested maximum usage levels for various feed ingredients.

INGREDIENT	MAXIMUM LEVEL	REASON TO LIMIT USAGE
MEAT AND BONE MEAL	6%	CALCIUM, PHOSPHORUS AND SALT
BAKERY BY-PRODUCT	7%	SALT
POULTRY BY-PRODUCT	5%	PROTEIN, PHOSPHORUS AND PEROXIDES
RAPESEED MEAL	7%	ERUCIC ACID AND GLUCOSINOLATE
CASSAVA (TAPIOCA)	6%	LINAMARIN (A GLUCOSIDE)
TRITICALE	15%	PROTEIN VARIATIONS
FAT (POULTS < 2 WEEKS)	2.5%	LIPASE SECRETION OF POULT

FEEDING BIRDS FOR OPTIMUM IMMUNE COMPETENCE

Kirk C. Klasing and Nora A. Halquist

Department of Avian Sciences
University of California, Davis 95616

Interactions between nutrition and infectious disease can take two forms. First, nutrient requirements may be altered by the presence of clinical or even non-clinical disease in the flock. Second, nutritional status may affect immunocompetence of the bird and consequently its resistance to infectious agents[1]. We do not have scientific results detailing the exact interaction between the 40 or more known nutrients and the dozens of infectious agents currently causing problems in the field. However, research on interactions between major nutrient categories and the immune response permits some generalizations. In an attempt to address the ways by which nutrition can affect immunocompetence in a systematic fashion, we have proposed the following mechanisms:

1. Impact of Nutrition on Substrate Supply. It is well documented that a chronic, severe nutrient deficiency impairs immunocompetence as a result of insufficient availability of that nutrient for its structural or metabolic function in the cells of the immune system. Since poultry are rarely subjected to chronic nutrient deficiencies, this large literature base is not particularly relevant. More importantly, do birds have a greater requirement for optimal immunocompetence than for maximal growth, feed efficiency, component yield or reproductive capacity? Although the literature base pertaining to this question is not extensive, it is apparent that for most nutrients, levels which optimize growth are also adequate for optimal immunocompetence. Exceptions may include vitamins A and E[2] and methionine[3]. Given the capacity of the stimulated immune system to mobilize large quantities of nutrients from other tissues, via leukocytic cytokines[4], it is not surprising that the immune system is relatively resistant to marginal nutrient deficiencies. Additionally, the binding affinities of transport proteins on the cell membranes of leukocytes suggest that the immune system has a high priority for circulating nutrients and is able to compete favorably with many other tissues when nutrient levels are low.

2. Nutrients as Immunoregulatory Agents. Dietary manipulations of some nutrients result in immunoregulatory consequences due to the participation of the nutrient or its products in cellular communication. Probably the best example of this is the role of nonessential dietary polyunsaturated fatty acids in modifying the gener-

ation of eicosenoid second messengers during an immune response. Dietary n-3 and n-6 fatty acids have a profound impact on the fatty acid composition of the membranes of leukocytes and consequently the amounts of prostaglandins and leukotrienes released in response to extracellular signals. These second messengers are responsible for positive and negative modulation of immune responses. For example, dietary n-3 fatty acids enhance the antibody response of pullets to SRBC vaccinations but suppress rates of lymphocyte mitogenesis after mitogen stimulation[5].

3. Influence of Nutrition on Hormonal Milieu. The immune system is not an autonomous system but is influenced by other physiological systems. For example, hormones such as insulin, glucagon, thyroxin, growth hormone and glucocorticoids quantitatevly effect most immune responses. Diet composition and meal frequency are potent regulators of the circulating concentrations of these hormones. Poultry are often subjected to acute nutritional manipulations due to management decisions or problems in the feed delivery system. It is becoming apparent that severe, acute changes in nutrition are more debilitating to the immune system than most marginal but chronic nutritional problems. Short periods of starvation (24 hr) surprisingly enhance both cellular and humoral immunity[6]. Over consumption of feed has the opposite effect, impairing immunoglobulin production and delayed type hypersensitivity.

4. Impact of Nutrients on the Pathology Due to an Immune Response. When the immune system responds against invading pathogens, it produces a wide variety of noxious agents including proteolytic and other hydrolytic enzymes, reactive oxygen intermediates, and reactive nitrogen derivatives which destroy bacteria, parasites or infected cells. These defensive agents can injure normal host cells and result in various pathologies. Several nutrients can modulate the degree of pathology induced by an immune response. For example, antioxidants such as vitamins E and A, and xanthophils, protect host cells from the damaging effects of superoxides and limit pathology. The quantity of these antioxidants in tissues is directly affected by dietary levels.

The quantity of nutrients in the feed as well as the pattern of feeding can impact immunocompetence of poultry. Optimizing immune responsiveness of birds is a safe and economical means of improving flock performance.

REFERENCES

1. Cook, M.E. Nutrition and the Immune Response of the Domestic Fowl. Crit. Rev. Poult. Biol. 3:167-190, 1991.

2. Nockels, C. F. The role of vitamins in modulating disease resistance. Vet. Clin. N. Am. Food An. Pract. 4:531, 1988.

3. Tsiagbe, V.K., Cook, M.E., Harper, A.E. & Sunde, M.L. Enhanced immune response in broiler chicks fed methionine-supplemented diets. Poult. Sci. 66:1147-1154, 1987.

4. Klasing, K.C. Nutritional aspects of leukocytic cytokines. J. Nutr. 118: 1-11, 1988.

5. Fritsche, K.L., Cassity, N.A. & Huang, S. Effect of dietary fat source on antibody production and lymphocyte proliferation in chickens. Poult. Sci. 70:611-617, 1991.

6. Klasing, K.C. Influence of acute starvation or acute excess intake on immunocompetence of broiler chicks. J. Poult. Sci. 67:626-634, 1988.

ABNORMAL CARDIOVASCULAR RESPONSE TO EXERCISE IN HEAVY TURKEY AND RELEVANCE TO SUDDEN DEATH SYNDROME

M. Boulianne and D.B. Hunter

Department of Pathology, University of Guelph
Guelph, Ontario, N1G 2W1, Canada

For the past decade, turkey producers have recognized an increase in the number of apparently healthy turkeys that die suddenly for unexplained reasons, with lesions of generalized passive congestion. The cause of this mortality is unknown and the term Sudden Death Syndrome (SDS) has been used to describe the condition. Field observations have related occurrence of SDS to increased activity in the flock, fighting, a stressful event, such as moving the birds form one barn to another, tilling the litter, etc. We decided to use exercise stress testing to assess the cardiovascular system, to identify potential pathophysiological risk factors not apparent at rest, nor at necropsy. Preliminary studies have revealed that following exercise, heavy turkeys developed hyperthermia, severe lactic acidosis, and decreased arterial blood pressure[3].

The drop in blood pressure contradicts previous publications on exercise physiology in avian species[1,2,4,5].

The purpose of this study was to measure blood pressure, heart rate, cardiac output, total peripheral resistance, stroke volume, stroke index, and lactate, during rest and treadmill exercise in an attempt to define the cardiovascular capabilities of the male and female heavy turkeys.

RESULTS

Mean, systolic and diastolic arterial blood pressures decreased significantly in both sexes during exercise. Heart rate and cardiac output increased significantly. Total peripheral resistance showed a significant fall in both sexes. Exercise had no significant effect on stroke volume and stroke index. Due to technical problems, the number of samples collected for lactate analysis was insufficient. Nonetheless, clinical observations showed that lactate values increased up to 6 times the resting values.

DISCUSSION

It is difficult to compare our results with previous literature because of the different exercise regimes used. The turkeys of the present study were submitted to ten minutes of light exercise with a treadmill speed of 0.15m/sec. However, their response was similar to that of birds submitted to severe exercise and some turkeys showed signs of exhaustion. The marked increase in lactate reveals that they were functioning anaerobically.

During exercise there is usually a marked vasodilation in muscles, which is followed by skin vasodilation. Total peripheral resistance falls and cardiac output rises to maintain blood pressure. Whether an insufficient increase in cardiac output or too great a fall in total peripheral resistance was responsible the drop in blood pressure was not determined in this study. Nevertheless, the cardiovascular system was unable to meet the metabolic needs generated by the exercise. In view of these results we conclude that exercise of the domestic heavy turkey may create hemodynamic instability leading to sudden death.

REFERENCES

1. Baudinette, R. V., A. L. Tonkin, J. Orbach, R. S. Seymour, and J. F. Wheldrake. Cardiovascular function during treadmill exercise in the turkey. Comp. Biochem. Physiol. 72A:327-332. 1982.

2. Bech, C., and S. Nomoto. Cardiovascular changes associated with treadmill running in the pekin duck. J. Exp. Biol. 97:345-358. 1982.

3. Boulianne, M., and D.B. Hunter. Measurements of cardiovascular and blood parameters during exercise in turkeys and relevance to the Sudden Death Syndrome, Addendum. In: Proceedings of the 40th North Central Avian Disease Conference, Y.M. Saif, ed. Ohio Agriculture Research and Development Center, Wooster, Ohio. pp. 127-128. 1989.

4. Grubb, B. R. Cardiac output and stroke volume in exercising ducks and pigeons. J. Appl. Physiol. 53:207-211. 1982.

5. Kiley, J. P., W. D. Kuhlmann, and M. R. Fedde. Respiratory and cardiovascular responses to exercise in the duck. J. Appl. Physiol. 47(4):827-833. 1979.

SKELETAL MYOPATHY ASSOCIATED WITH EXPERIMENTAL DOSING OF TURKEYS WITH MONENSIN

C.J. Cardona[A], A.A. Bickford[A], and F.D. Galey[B]

[A]California Veterinary Diagnostic Laboratory System
Turlock Branch, P.O. Box 1522, Turlock, California 95381
[B]California Veterinary Diagnostic Laboratory System
P.O. Box 1770, Davis, California 95617

Monensin is a sodium selective carboxylic ionophore widely used in chickens and turkeys as a coccidiostat. Since 1987, it has been approved for use in turkeys at a level of 54 to 90 grams/ton of complete feed.

Monensin and other ionophores will act on all cells of the body, but skeletal and cardiac muscle are the tissues in which toxicoses are manifest. At low dose levels of monensin calcium influx and a related release of catecholamines leads to increased myocyte contractility. At high levels of monensin, there is an uncoupling of oxidative phosphorylation and subsequent energy depletion in the cell leading to mitochondrial swelling. These changes lead to contraction and eventual myofiber death.

A rear limb skeletal myopathy associated with therapeutic levels of monensin has been described in commercial turkeys. The present report describes an attempt to determine the effect of monensin on turkey skeletal muscle under field conditions.

QUANTITATING PATHOLOGIC CHANGES IN BROILERS

Bruce Stewart-Brown

Solvay Animal Health, Inc., 1201 Northland Drive
Mendota Heights, Minnesota 55120

Evaluation of pathologic changes in the tissues of broilers has always been useful as an aid in diagnosis of a disease. It has also been used to a limited degree in making quantitative judgments on the significance of specific lesion-causing viruses.

In 1991, tissues from broiler chickens throughout the U.S. were taken at ages ranging from 1 to 56 days. Tissues collected included bursa, thymus, trachea, and Harderian gland. Lesions of each tissue were quantitated using a computerized image analysis system. Serology for infectious bursal disease virus (IBDV), Newcastle disease virus, infectious bronchitis virus, *Mycoplasma gallisepticum*, and *Mycoplasma synoviae* was also done on these same flocks.

Bursae were analyzed for lymphocyte depletion. Depletion was most significant starting at 21 days and older. Depletion often, but not always, coincided with seroconversion to IBDV.

The thymus evaluation involved a judgment on cortical thickness as well as macrophage infiltration. Thymus lesions were most prevalent between 28 and 42 days of age.

The tracheas of the chickens were quantitatively judged by the cellular infiltration in the area just under the mucosal surface. Cellular infiltration was highest at 14 days and again at 35 to 49 days.

Harderian glands were analyzed for concentration of plasma cells. This method has been difficult but preliminary results will be discussed.

The genetic potential of today's chicken is not often attained. Quantitating changes that occur during the production cycle can help identify areas of opportunity. Once identified, the possible solutions can then be optimally judged.

THE INCIDENCE OF ANEMIA AND POLYCYTHEMIA IN GEORGIA BROILERS DURING 1988 AND 1989

Mark A. Goodwin[A], James F. Davis[A], John Brown[B], and T. G. Dickson[A]

[A]Georgia Poultry Laboratory, P.O. Box 20,
Oakwood Road, Oakwood, Georgia 30566
[C]Department of Medical Microbiology College of Veterinary Medicine
University of Georgia Athens, Georgia 30602

We have established the incidence of anemia and polycythemia in packed cell volume- (PCV) tested clinically-ill Georgia broilers that were submitted to the Georgia Poultry Laboratory during 1988 and 1989. During this period more than 66% (324/488 = 66.4%) of PCV-tested broiler chicks were anemic. In contrast, less than 2% (8/488 = 1.6%) of PCV-tested chicks were polycythemic. The incidence of anemia was significantly ($P < 0.001$) higher than expected (2.5%) in 7 (56.9%), 14 (83.9%), 21 (74.7%), 28 (58.7%), and 35 (57.9%) day old broilers. The incidence of polycythemia was significantly ($P < 0.001$) higher than expected (2.5%) in 35 (21.1%) day old

broilers but was not significantly different from the expected rate in 7 (0%), 14 (1.8%), 21 (0.3%), and 28 (4.3%) day old chicks.

These rates of anemia are much higher than we would have hypothesized. This causes us to believe that either 1) An etiology(ies) for anemia is present in epidemic proportions in Georgia broilers, or 2) The standard method for establishing references intervals for anemia in animals does not apply to broiler chicks. No matter what the rate, the causes for anemia and polycythemia in broiler chickens should be determined.

AN OUTBREAK OF BOTULISM IN A WELL-DEFINED WATERFOWL POPULATION IN WEST-CENTRAL GEORGIA

Paul C. Smith and Herron Baxley

Department of Pathobiology, College of Veterinary Medicine,
Auburn University, Alabama 86849

Outbreaks of botulism in waterfowl populations are uncommon in the Southeastern United States. A sudden increase in mortality in ducks in a high density water fowl population in a public park in Columbus, Georgia resulted in an outcry from local residents. Local veterinarians made an erroneous, presumptive clinical diagnosis that added to the confusion and concern of local citizens. Three of four blood samples taken from dead or clinically affected birds were positive in the mouse protection test for *Clostridium botulinum*, toxin type C. The importance of a laboratory diagnosis, the explanation of the etiology, role of environmental conditions and possible courses of action available to reduce the incidence and reoccurrence will be emphasized.

CAUSE AND CONTROL OF A PERACUTE FORM OF INCLUSION BODY HEPATITIS

T.M. Grimes

Ingham-Tegel Poultry Company
P.O. Box 120 Camden, Australia 2570

A disease identical pathologically to inclusion body hepatitis (IBH), but not associated with infectious bursal disease (IBD) virus or chicken anaemia agent (CAA), sporadically caused up to 40% mortality in 7-14-day-old meat chicken flocks in Australia, commencing in the early 1980s. Research and in-house company studies indicated that egg transmission of a fowl adenovirus (FAV) with a specific genetic makeup was the likely cause and that vaccination of breeding flocks with this FAV prevents this peracute form of IBH.

INTRODUCTION

IBH is described in scientific literature[5] as a disease of 3-7-week-old chickens with sudden mortality of up to 10% over a 5-day period in an affected flock. Autopsy shows lesions including hemorrhages, enlarged mottled liver and atrophied bursa of Fabricius, intranuclear inclusion bodies in hepatocytes, and the isolation of a Group 1 FAV of any 12 serotypes from the liver. Concurrent infection with immunosuppressing agents, such as IBD virus or CAA, has been considered to be a necessary part of the pathogenesis of IBH.

However, in the early 1980s, a disease identical clinically and pathologically to IBH occurred sporadically in meat chicken flocks or various companies in all Australian states but mortalities up to 40% occurred at 7-21 days of age[1,3,4]. FAV was isolated form the livers of chicks that died of this peracute form of IBH. Affected broiler flocks often appeared to be derived from the one breeder flock.

Studies were initiated in 1985 at Australian research institutes including the veterinary laboratories of the New

South Wales and Victorian Governments and the CSIRO Melbourne. Field investigations and epidemiological follow-up were undertaken by the technical staff of Ingham-Tegel poultry company.

AETIOLOGY

A Group 1 FAV of serotype 8 and a subtype of a Group E genome[2] is proposed as the cause of peracute IBH in Australia for the following reasons:

1. All FAV isolated from 52 peracute IBH outbreaks from 1985-89 and subsequently characterized were classified as above. FAV from classical IBH outbreaks in older birds or from normal chickens were of a different genomic makeup, even though they were serotype 8 FAV (E. Arzey pers. com., 2).
2. Seroconversion in breeder flocks, as determined by virus neutralization of a serotype 8 FAV, occurred concurrently with peracute IBH outbreaks in progeny flocks.
3. A disease similar to peracute IBH has been reproduced by exposing young chicks by natural routes of infection to FAV with these characteristics[1,2]. Sera from convalescent chicks were negative for antibodies against IBD virus, CAA and reticuloendotheliosis virus.

TRANSMISSION

It is proposed that young chicks become infected by either vertical transmission from their parents via embryonated eggs or by horizontal transmission from their parents via embryonated eggs or by horizontal transmission from the chicks' environment whether that be hatchery, delivery truck, or broiler farm. Evidence for vertical transmission includes:

1. FAV are known to be vertically transmitted[5].
2. Disease occurrence at 7-14 days suggests either vertical transmission or infection of chicks from their environment in the first few days of life. Since all Ingham-Tegel meat chicken flocks are placed on an "all out, all in" basis and entire sheds are depopulated and disinfected before placement, the likelihood of infection from the shed environment has been minimized.
3. Experience within the Ingham-Tegel company that the majority of sheds of progeny from a single meat breeder flock can be affected with peracute IBH for a 3-6 week period, irrespective of which geographical areas (including different states) they are placed. Progeny placed from other breeder flocks into the same geographical locations were often not affected.
4. Seroconversion of breeder flocks to serotype 8 FAV has been correlated with peracute IBH occurrence in progeny in a number of outbreaks in Ingham-Tegel chicken flocks.

Evidence for horizontal transmission includes:

1. Chicks placed from parent flocks, other than the one apparently producing peracute IBH outbreaks at that time, occasionally are affected with peracute IBH.
2. Experimental transmission studies[1] indicate that young chicks in contact with infected chicks can die of peracute IBH.

INVOLVEMENT OF IBD VIRUS

IBD virus is not considered to be involved in peracute IBH because:

1. IBD virus is not vertically transmitted and it is unlikely to infect up to 40% of chicks by 7-14 days of age in fully cleaned out and disinfected chicken sheds.
2. Maternal antibody levels to IBD virus in some affected broiler flocks would have been relatively high since parent flocks in some cases were vaccinated with both live and killed IBD virus vaccines and the peracute IBH outbreaks often occurred shortly after the onset of lay.
3. Serological testing of affected broiler flocks at slaughtering age revealed that some were negative to IBD virus antibody.
4. IBD virus antigen could not be detected by ELISA testing conducted by the CSIRO Melbourne in the atrophied bursa of Fabricius from some chicks that died of peracute IBH.
5. Peracute IBH has been reproduced with FAV that have been plaque-purified 3 times in chicken embryo kidney cell cultures produced form SPF eggs and the absence of IBD virus confirmed by serological testing of surviving chicks.

INVOLVEMENT OF CAA

CAA is not considered to be necessary for peracute IBH to occur beeause:

1. Peracute IBH has been reproduced with FAV that have been plaque-purified 3 times in chicken embryo kidney cell cultures produced from SPF eggs and the absence of CAA confirmed by serological testing of surviving chicks.
2. Clinical signs of runting and paleness, low blood-packed cell volumes, autopsy lesions of pale bone

marrow and thymic atrophy, and secondary infections with *E. coli*, salmonella and aspergillus are common features of CAA disease but not peracute IBH.

3. Breeder flocks which produced numerous progeny flocks that were affected with peracute IBH were confirmed seropositive for CAA at 15-17 weeks of age by indirect fluorescent antibody testing undertaken in Germany. In some cases, seroconversion was a result of vaccination at 9-10 weeks of age with a living virus CAA vaccine now used routinely in breeder flocks of some Australian poultry companies. Seroconversion to CAA prior to lay is considered to prevent egg transmission of CAA and the development of CAA disease in young chicks[6].

VACCINATION

Field experience within the Ingham-Tegel company with peracute IBH outbreaks in the last 5 years indicates that outbreaks are usually associated with specific breeder flocks, that outbreaks abate within a 3-6 week period, that at the onset of outbreaks, the breeder flock involved is negative for serum neutralizing antibody to serotype 8 FAV, and that outbreaks cease following seroconversion.

Similar epidemiology occurs with avian encephalomyelitis and CAA disease, both of which can be prevented by vaccinating breeder flocks in rearing with non-attenuated, live virus vaccines.

A serotype 8, hypervirulent, subtype Group E genomic FAV isolated in SPF chicken embryo liver cell cultures from the livers of 9-day-old meat chickens with peracute IBH was purified by limit-dilution technique, seedstocks produced in SPF cell cultures, and an FAV vaccine using a titre of 10^4 $TCID_{50}$/dose was produced. Replacement breeders vaccinated *per os* or by drinking water administration in mid-rearing were bled at 16 weeks of age and tested for serum neutralizing antibodies against type 8 FAV to validate the vaccine titre and administration methods.

FAV vaccination of Ingham-Tegel breeder flocks has now been undertaken for 2 years commencing in areas where peracute outbreaks have been most prevalent in the past. Field studies indicate that the vaccine is safe, breeding flocks are serologically positive to type 8 FAV prior to onset of lay and peracute IBH outbreaks have not occurred in progeny of flocks immunized in this way.

REFERENCES

1. Barr, D., and P. Scott. Proc. 2nd Pacific Poultry Health Conference. Post-Grad. Committee in Vet. Sci., Univ. of Sydney Proc., 112:323-326. 1988.
2. Erny, K., D. Barr, and K. Fahey. Avian Pathol. 20:597-606. 1991.
3. Reece, R., D. Barr, D. Grix, W. Forsyth, R. Condron, and M. Hindmarsh. Aust. Vet. J. 63:201-202. 1986.
4. Reece, R., D. Barr, and D. Grix. Aust. Vet. J. 64:365-367. 1987.
5. McFerran, J. 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 Univ. Press, Ames, pp. 552-563. 1991.
6. Vielitz, E., and H. Landgraf. Avian Pathol. 17:113-120. 1988.

PACKED CELL VOLUME REFERENCE INTERVALS TO AID IN THE DIAGNOSIS OF ANEMIA AND POLYCYTHEMIA IN BROILER CHICKENS

John Brown[A], Mark A. Goodwin[B], and James F. Davis[B]

[A]Department of Medical Microbiology,
College of Veterinary Medicine, University of Georgia,
Athens, Georgia 30602
[B]Georgia Poultry Laboratory, P.O. Box 20, Oakwood Road,
Oakwood, Georgia 30566

Packed cell volume (PCV) reference intervals that define cut-offs for use in diagnosing anemia and polycythemia in young broiler chickens never have been established. Blood samples were collected from over one hundred 3- to 50-day old clinically-healthy conventionally-reared broiler chicks. PCVs were determined and analyzed.

PCVs regressed significantly ($\underline{P} < 0.025$) on age. This means that neonatal physiologic anemia occurs in broiler chicks. The definition for anemia varies with broiler

chick age. Anemia is a PCV $\leq$ 26%, 29%, 33%, 32%, 33%, 33%, 31, or 28% for broiler chicks 3, 7, 14, 21, 28, 35, 42, and 49 days old, respectively. Polycythemia is a PCV $\geq$ 42%, 43%, 41%, 40%, 42%, 42%, 41%, or 40% for broiler chicks 3, 14, 21, 28, 35, 42, and 49 days old, respectively. Results from our study provide guidelines that will allow diagnosticians and researchers to detect anemia and polycythemia in young broiler chicks of various ages.

HISTOLOGICAL, IMMUNOHISTOCHEMICAL, AND ULTRASTRUCTURAL FINDINGS IN A THYMIC TUMOR IN A MATURE CHICKEN

S. Mark Hall[A] and Dr. Derek Mosier[B]

[A]USDA, FSIS, Western Laboratory, 620 Central Ave.,
Alameda, California 94501
[B]Department of Pathology, College of Veterinary Medicine,
Kansas State University, Manhattan, Kansas 66506

INTRODUCTION

Thymomas are generally benign tumors that originate from thymic tissue; they are composed of lymphoid and epithelial tissue elements[1]. In the chicken, the thymus is composed of elongated to multi-lobulated lobes that extend linearly from the anterior cervical to the anterior thoracic region. There are typically fourteen lobes, seven on each side of the neck along the jugular veins[3]. The lobes are encapsulated and each lobe (0.2cm - 0.4 cm in 10-12-week-old broilers) is roughly divided into outer cortical (lymphoid) and inner medullary (epithelial, reticular, and myoid) regions[3,5]. Case reports of thymomas in the chicken are limited, but thymomas are generally described as firm, large, grayish-pink, elongated masses occupying the jugular groove anywhere in the cervical region or base of the neck[1]. Thymomas are not associated with the avian leukosis complex or Marek's disease and usually occur in the absence of other lymphoid neoplasia.

GROSS FINDINGS

At slaughter, a mature chicken (approximately 18 months old) presented with a subcutaneous mass approximately 1x2x8 cm. The mass extended bilaterally from midway down the neck to the crop. The mass was gray-white in color and had a lobular or "cauliflower-like" appearance. There were areas of hemorrhage and necrosis present within the neoplastic mass. No other gross abnormalities were observed.

HISTOLOGICAL FINDINGS

Sections of the neoplastic mass were collected in a 10% buffered formalin solution. Routine paraffin embedded tissues were sectioned at 6um and examined by hematoxylin and eosin (H&E), periodic acid Schiff (PAS), Gomori methenamine silver (GMS), phosphotungstic acid-hematoxylin (PTAH), Ayoub-Shklar (AS), Mayers mucicarmine (MC), and Masson trichrome (MT) stains. The neoplastic mass was composed of dense sheets and occasional cords of cells arranged into large lobules by wide bands of dense connective tissue and further subdivided into small circular lobules or whorls and rowing cords by thin bands of fine connective tissue. Scattered throughout the mass were small circular aggregates (100-300um) of flattened cells lining cleared central spaces. In general, the cells of the mass had a small to moderate amount of pale reticular to foamy eosinophilic (occasionally fibrillar) cytoplasm. They had round to slightly oval, eccentric nuclei with marginated chromatin and often a central dark basophilic nucleolus. About 20% of the cells had angular to spindle shaped nuclei. Atypical mitotic figures were 1-2 per high powered field. A moderate number of small lymphocytes were present diffusely and in focal aggregates throughout the mass. About 50% of the neoplastic tissue consisted of patchy necrotic foci and numerous variably sized foci of hemorrhage were present. Cells with globular eosinophilic cytoplasm stained by MT and globular blue cytoplasm by PTAH were present in the normal thymus and neoplastic tissues. Both the neoplasia

and normal thymus contained a few MC positive cells. GMS demonstrated a fine reticular fibrous pattern of the connective tissue throughout the neoplasia. Conclusive staining for keratin was not observed in the neoplasia or in the normal thymus by AS.

ULTRASTRUCTURAL FINDINGS

Formalin fixed tissue from the neoplastic mass and control (normal) chicken thymus were transferred to 2.5% glutaraldehyde and washed in cacodylate buffer, and fixed in 4% osmium tetroxide. The tissues were dehydrated to 100% ethanol, placed in a mixture of 50/50 propylene oxide and polybed resin, desiccated, and placed in 100% polybed resin. Thin sections were placed on copper grids, stained with uranyl acetate, and examined (transmission electron microscopy). The mass contained cells that had features and characteristics most consistent with epithelial cells; some thymic lymphocytes[2], and presumed myoid cells were observed[5]. The majority of the cells had round to oval eccentric nuclei with prominent marginated chromatin and a central large nucleolus. Most of the cells had voluminous cytoplasm containing numerous dilated mitochondria and myelin figures. Apparent intracytoplasmic canaliculi were observed in a few cells. Some cells (myoid) contained abundant variably sized electron dens globules, and often adjoined cells containing fibrillar material within their cytoplasm.

IMMUNOHISTOCHEMISTRY

Assays were performed on formalin fixed paraffin embedded normal chicken thymus and the neoplastic mass. The assays used monoclonal or polyclonal antibodies against vimentin, desmin, high and low molecular weight cytokeratins, and lysozyme. Biotinylated secondary antibodies followed by avidin-peroxidase complex were used. The chromogen was 4-chloro-1-naphthol and the sections were counter stained with hematoxylin. Results of immunohistochemical stains were equivocal, but areas of positive staining for lysozyme were present in both the normal thymic medulla and the neoplasia.

CONCLUSIONS

The neoplastic mass had gross, histological, and ultrastructural features that were consistent with a thymoma. Special histochemical stains and immunochemistry provided some supporting evidence to confirm the diagnosis[4]; but in general, supporting evidence was equivocal or relatively non-specific in verification of the origin or histogenesis of the mass.

REFERENCES

1. Campbell, J.G. Tumors of the Fowl. J.B. Lippincott Co., Philadelphia. 215-17. 1969.
2. Clawson, C.C., M.D. Copper, and R.A. Good. Lymphocyte fine structure in the bursa of Fabricius, the thymus, and the germinal centers. Lab. Invest. 16:407-421, 1967.
3. Hodges, R.D. The Histology of the Fowl. Academic Press, London. 213-21. 1974.
4. Kuo, T-t., J-P. Chang, F-J. Lin, W-C. Wu, and C-H. Chang. Thymic carcinomas: histopathological varieties and immunohistochemical study. Am.J. Surg. Pathol. 14:24-34. 1990.
5. Seifert, R., and B. Christ. On the differentiation and origin or myoid cells in the avian thymus. Anat. Embryol. 181:287-298. 1990.

THE IMPORTANCE OF LIVE PRIMING AND ANTIGENIC DIFFERENT VACCINE STRAINS IN PROTECTION AGAINST INFECTIOUS BRONCHITIS

M. Voss, E. Vielitz and C. Conrad

Lohmann Tierzucht GmbH, Veterinary Laboratory
Abschnede 64, 2190 Cuxhaven Germany

INTRODUCTION

Protection against infectious bronchitis virus (IBV) is still a problem due to the occurrence of new serotypes. Most of the breeder flocks in Germany are vaccinated with the classical Massachusetts type vaccines including live vaccinations with H 120 and H 52 and killed M 41-vaccines. Some veterinarians include also the Dutch variant strains D-274 and D-1466 into their vaccination program. Also, vaccination during production with live

H 120 or H 52 was shown to be safe and to improve the immunity, which was demonstrated several times by improvement of egg production and egg quality.

But the occurrence of several known or new isolates/serotypes complicates the vaccinal protection against IBV. Generally it is a problem to include these new IBV strains specially into live vaccines because of the long lasting licensing procedure for new vaccines. Therefore we performed, within the last 3 years, different trials to get answers for the following questions:

1) Is the use of different serotypes in killed vaccines effective to protect birds which are primed only with live vaccines of the Massachusetts serotype?
2) Are there IBV isolates other than the classical Massachusetts strain H 120 and H 52 which can produce broader crossprotection against heterologous field infections?

MATERIAL AND METHODS

For the serological control during these challenge trials we used the ELISA (IDEXX) and the Micro-Serumneutralization-Test in chicken embryo kidney cells.

Oculonasal vaccinations were done by application of one drop of vaccine each to eye and nose and 0.2 ml intratracheally. Drinking water vaccination was done under field condition with an addition of 0.2% skin milk powder.

Challenge infections were carried out intratracheally with $10^{3.5}EID_{50}$/bird using the pathogenic strains M41 and D-274, in the first trial also IBV-229/75 and IBV-10. The evaluation of the vaccinal protection after challenge was based on clinical symptoms, ciliostasis, and reisolation of the challenge virus.

CHALLENGE TRIALS AND RESULTS

In regard to the first question mentioned above, we included in our studies two German IBV isolates, IBV-10 and IBV-229/75. IBV-10 was isolated in 1962 from birds with respiratory symptoms, IBV-229/75 in 1975 from broiler breeders with respiratory symptoms and drop in egg production. The choice for these strains was made due to the detection of relatively high levels of neutralizing antibodies in several tested breeder flocks. But it was not clear if these antibody titers were induced by field infections with these strains or due to crossreactivity to other strains. Cross neutralization tests, performed by Jane Cook, indicated no significant cross reactions between the strains IBV-10 and 229/75 and any of the tested antisera against European IBV strains.

In the first trial, therefore, we compared the strains IBV-10 and 229/75 with the vaccine strain H 120 to test the protection against homologous and heterologous challenge; SPF birds (Valo, Lohmann Cuxhaven) were vaccinated 2 times with the live strains H 120, IBV-10 or 229/75 respectively by oculonasal infection. Three weeks (for H 120, 2 weeks) after the second vaccination, groups of birds were challenged with the homologous virus and also with the heterologous strains M 41 and D-274. The results are shown in Table 1. The results demonstrate good protection in regard to the homologous challenge for the strains 299/75 and IBV-10; better than the results for the H 120 vaccinates. The M 41 challenge showed the best results, as expected, in the H 120 vaccinated birds, followed by the 229/75 and than the IBV-10 group.

In the second trial the question was, if a homologous live-priming is necessary to induce sufficient protection after vaccination with inactivated vaccines. Each 40 SPF birds (Valo) were vaccinated oculonasally with H 120 or D-274 live vaccines respectively. Twenty birds an than again half of these birds were challenged after 4 weeks with M 41 or D-274. The results are summarized in Table 2. The best results in regard to the 3 challenge criteria are in those groups where the challenge virus was homologous to the live-priming. This is especially true for D-274. If we look for the individual serology of some of the birds (which were used for reisolation) we get interesting results (see Table 3).

Revaccination with inactivated vaccines heterologous to the live-priming induced no significant increase in neutralizing antibody levels against the serotype used in the inactivated vaccine. But there is a good correlation between neutralizing antibody titer and protection against challenge, not between ELISA and protection. Birds with high ELISA titers are fully susceptible if challenge was done with an IBV strain heterologous to the live-priming. The results show also the great variation of ELISA titers within a group of birds.

Back to the introduction of new serotypes into vaccines, we performed a third trial with an experimental inactivated M 41+229/75 vaccine. Each 20 SPF birds were vaccinated oculonasally with live H 120 or 229/75 respectively. After 6 weeks they were revaccinated with M 41+229/75 inactivated vaccine and after another 4 weeks challenged with M 41 or D-274 respectively. The results are shown in Table 4. Both vaccinated groups challenged with M 41 were well protected with and without live H 120 priming. But the groups with D-274 challenge were less protected in regard to ciliostasis and virus reisolation. Nevertheless the birds with 229/75 live-priming showed only very low clinical symptoms. Together with the serological results, this indicates that after a live 229/75 vaccination, a killed combination of M 41 plus 229/75 will booster the IBV 229/75 immunity as well as the Massachusetts immunity. The live H 120 priming did not show this clear of a picture.

For our last trial (trial 4) an experimental killed IB/ND/IBD-vaccine was produced which contained the

IBV strains M 41, IBV-10 and 229/75. Fifty LSL chicks were placed in our rearing farm and vaccinated at an age of 3 and 8 weeks with live H 120 vaccine via the drinking water. At an age of 18 weeks, the birds were transferred to our isolation cabins and half of them were vaccinated again with H 120; the others with a combination of live IBV-10 and 229/75 vaccine by oculonasal application. Five weeks after this third live vaccination, 19 birds of each group were revaccinated with the experimental IB(3)/ND/IBD-vaccine and challenged 3 weeks later with M 41 or D-274. Six birds of each group didn't receive this killed vaccine. The challenge results of this trial are shown in Tables 5 and 6.

Birds vaccinated with the live IBV-10+229/75 vaccine showed high ELISA and Micro-SN titers at point of revaccination with the killed vaccines, whereas the H 120 vaccinates showed, in most of the groups, ELISA titers less than 1000. After revaccination with the killed vaccine, all groups showed very high ELISA titers of more than 13,000. These groups which were vaccinated with the live IBV-10+229/75 vaccine showed high neutralizing antibody titers against the Massachusetts strains as well as the strains IBV-10, 229/75 and D-274. Also against D-1466, there was some crossreactivity. In regard to the challenge criteria, best results were obtained in the oil vaccinated groups challenged with M 41, but the IBV-10+229/75 primed birds also showed good protection against the D-274 challenge. Compared with the non oil vaccinated groups, the application of the trivalent inactivated IB-vaccine improved the protection against both challenge viruses.

CONCLUSION

From these trials we have come to the following conclusions:

1) IBV-10 and IBV-229/75 are German IBV field isolates which are not related to other European and North African strains.
2) These strains provide broader cross protection against heterologous challenge than the Massachusetts strains H 120 and H 52.
3) Best protection is affected by homologous live and inactivated vaccines.
4) Vaccination with killed vaccines—prepared with strains of less crossreactivity (e.g. D-274)—without live-priming with these strains, will not give a sufficient immunity.
5) The application of killed vaccines prepared with different strains of broader crossreactivity will provide better protection without live-priming than a program using live vaccines only.
6) Isolate IBV-229/75 appears to be a good priming virus.
7) Polyvalent inactivated vaccines given after live-priming with IBV-229/75+IBV-10 improves the protective level considerably, which may be sufficient to resist field challenge in Germany.

Table 1. Serological and challenge results of trial 1.

Vaccine Virus	Challenge Virus	Serology at challenge ELISA	H120	M41	D274 hom.*		Challenge results clinic	cilostasis	reisol.
H 120	M 41	775	5,4	≤2,4	≤0,6	-	0,2	1/5	2/5
	D-274	1669	6,3	≤3,1	≤1,2	-	1,6	5/5	2/5
229/75	229/75	2045	4,0	4,1	6,3	9,3	0,0	0/5	0/5
	M 41	1729	3,7	4,9	6,1	8,5	0,2	1/5	3/5
	D-274	1941	4,0	4,4	6,3	9,0	0,2	0/5	2/5
IBV-10	IBV-10	1650	≤2,7	≤2,7	≤2,6	9,9	0,35	0/5	0/5
	M 41	1410	≤3,4	≤2,6	3,6	10,1	0,6	4/5	2/5
	D-274	1871	≤3,5	≤3,2	2,9	10,0	0,15	0/5	2/5

*homologous neutralization titers

Table 2. Challenge results of trial 2.

1 Vaccination (Live)	2. vaccination (killed)	Challenge Virus	Challenge results clinic	cilostasis	reisolation
H 120	M 41	M 41	0,33	1/4	0/4
		D-274	0,88	0/4	4/4
H 120	D-274	M 41	0,56	0/4	1/4
		D-274	0,38	0/4	2/4
D-274	M 41	M 41	1,36	2/4	4/4
		D-274	0,11	0/4	0/4
D-274	D-274	M 41	0,89	2/4	2/4
		D-274	0,00	0/4	0/4

Table 3. Comparison of IB titers at challenge and protection (trial 2).

Vaccination live	killed	Chall. virus	Micro-SN (log 2) ELISA	H120	M 41	D-274	Challenge result clinic	reisolation
H 120	M 41	M 41	5063	10	9	4	0	-
			6223	11	14	≤3	0	-
H 120	M 41	D-274	12544	13	11	≤3	0	+
			8892	13	11	≤3	2	+
H 120	D-274	M 41	4034	9	10	≤4	1	-
			454	≤3	6	≤4	1	+
H 120	D-274	D-274	9788	9	10	5	1	-
			7973	10	10	≤4	1	+
D-274	M 41	M 41	6223	≤4	5	6	3	+
			9049	5	5	9	3	+
D-274	M 41	D-274	7911	5	6	8	1	-
			148	≤4	6	6	0	-
D-274	D-274	M 41	7440	5	6	9	1	-
			82	≤3	≤3	6	3	+
D-274	D-274	D-274	137	≤3	≤3	7	0	-
			4357	4	6	10	0	-

Table 4. Serological and challenge results of trial 3.

1.Vaccin. (live)	2.Vaccin. (killed)	Chall. Virus	Serology (Micro-SN log 2) ELISA	H120	M41	D274	229/75	Challenge results clinic	ciliostasis	reisolat.
H 120	M41+229	M 41	a) 615	5,8	2,8	0,2	1,6	0/4	0/4	0/4
			b) 7638	9,8	9,9	4,5	6,2			
		D-274	a) 448	5,7	2,1	0,6	1,2	1,2	2/4	3/4
			b)10376	10,6	11,3	5,1	6,5			
229/75	M41+229	M 41	a) 581	5,0	2,0	1,1	9,0	0,3	0/4	1/4
			b) 6942	9,3	9,8	5,5	9,7			
		D-274	a) 1262	6,4	3,2	3,8	9,8	0,3	1/4	3/4
			b) 7441	9,1	8,3	5,1	9,8			
	Controls	M 41						2,7	2/3	3/3
		D-274						1,0	1/3	3/3

a) serology at revaccination with killed vaccine
b) serology at challenge

Table 5. Serological results of trial 4.

3.vaccin. (live)	4.vaccin. (killed)	Chall. Virus	time[1]	ELISA Titer	Micro-SN (log 2) H120	M 41	D-274	D-1466	IBV-10	229/75
H 120	IB-3[2]	M 41	a)	651	5,1	3,3	≤2,0	n.d.[3]	≤2,0	≤2,2
			b)	690	7,4	6,2	≤3,0	≤1,8	3,0	3,4
			c)	13161	11,3	11,6	4,6	3,4	6,9	7,0
229+10	IB-3	M 41	a)	651	5,1	3,3	≤2,0	n.d.	≤2,0	≤2,2
			b)	4200	9,4	8,1	6,3	2,0	9,2	7,6
			c)	13573	10,2	10,1	7,1	3,0	9,6	8,6
H 120	IB-3	D-274	a)	651	5,1	3,3	≤2,0	n.d.	≤2,0	≤2,2
			b)	734	6,6	5,5	≤3,2	≤2,8	2,6	3,1
			c)	14061	10,3	10,4	6,3	3,6	6,4	8,1
229+10	IB-3	D-274	a)	651	5,1	3,3	≤2,0	n.d.	≤2,0	≤2,2
			b)	5525	9,9	9,2	6,0	2,1	9,0	7,6
			c)	14436	10,4	9,9	7,1	3,9	9,4	8,5

1) Serology (mean titer) at a) 3. vaccination (live); b) 4. vaccination (killed); and c) challenge
2) killed experimental IB (M 41+229/75+IBV-10)/ND/IBD-vaccine
3) n.d.=not done

Table 6. Challenge results of trial 4.

3.vaccin. (live)	4.vaccin. (killed)	challenge Virus	No. birds	challenge results clinic	ciliostasis	reisolation
H 120	IB-3*	M 41	8	0,88	0/3	0/3
H 120	no	M 41	2	0,5	n.d.**	n.d.
229+10	IB-3	M 41	8	0,38	0/3	0/3
229+10	no	M 41	3	1,67	n.d.	n.d.
H 120	IB-3	D-274	10	1,3	1/3	3/3
H 120	no	D-274	2	2,5	n.d.	n.d.
229+10	IB-3	D-274	8	0,88	0/3	0/3
229+10	no	D-274	3	2,0	n.d.	n.d.
controls		M 41	3	3,0	3/3	3/3
		D-274	3	3,0	3/3	3/3

* IB-3=killed experimental IB (M 41+229/75+IBV-10/ND/IBD-vaccine
** n.d.=not done

PRELIMINARY INVESTIGATION ON DIFFERENT TURKEY RHINOTRACHEITIS (TRT) VIRUS ISOLATES FROM DIFFERENT COUNTRIES

H.M. Hafez

State Veterinary Laboratory, 7000 Stuttgart 1, Germany

INTRODUCTION

Turkey rhinotracheitis (TRT) is a highly contagious respiratory disease of turkeys and has been reported in many countries worldwide[2,6,8,12,14,15]. In the USA there has not been any evidence of the disease in turkey flocks.

As a causative agent, a pneumovirus has been isolated and identified[3,4,10,16].

Studies using electron microscopy, virus neutralization test (VN), indirect immunofluorescence, and enzyme linked immunosorbent assay (ELISA) suggest close morphological and antigenic relationship between isolates from different countries[1,7,10]. However, testing of serum samples originating from different countries in ELISA tests using different TRT isolates revealed that some serum samples obtained from France reacted positive only with a homologous antigen. On the other hand, sera originating from England and Germany reacted positive with both homologous and heterologous antigens and at higher levels with the heterologous antigen[9]. Similar discrepancies were also observed by testing chicken sera for TRT antibodies in two different laboratories using ELISA tests prepared with different isolates. In these tests, more positive flocks were detected using French isolates[5].

The purpose of this work is to compare 4 TRT viruses isolated in different laboratories of different countries.

MATERIALS AND METHODS

Viruses. The following viruses, isolated from turkey flocks showing respiratory manifestations, were used in this study:

1) **BUT1= 8544**[15] obtained from British United Turkeys Ltd. (BUT), Chester, England. The initial isolation was on turkey embryo tracheal organ culture which was subsequently adapted in chicken embryo fibroblast and cloned. The number of passages in cell culture before use in this laboratory is unknown. In this laboratory, the virus strain was passaged 3 times the CER- cell line[13] before being used.
2) **CVO3**, obtained from Rhone Merieux Laboratories, Lyon, France, after isolation and 28 passages in monkey kidney cell culture (VERO). In this laboratory, the virus strain was passaged once in VERO cells.
3) **STG 761/88**[10] isolated in this laboratory (Germany). The initial isolation and 16 passages were in the CER cell line .
4) **STG 854/88**, was also isolated in the same manner as STG 761/88 and passaged 11 times in CER cells (Hafez,1989 unpublished date).

The infected cultures were incubated at 37°C for 5-6 days until extensive cytopathic effect (CPE) characterized by syncytium formation was observed. They were then frozen and thawed once and clarified by low speed centrifugation and stored at -70°C prior to used. Isolates showed similar titers; approximately 4.5-5.5 $\log_{10}$/ml.

Physical and chemical treatment. Thermostability, chloroform sensitivity and the effect of 5-iodo-2'-deoxy-uridine (IUDR) were performed in CER and VERO cells[10].

Haemagglutination tests. Chicken, turkey, guinea pig, rabbit, mice, bovine and sheep red blood cells at a concentration of 1.0% in NaCl buffer were used. The incubations were carried out at 4C for 30 and 90 minutes.

Antisera. Antisera against BUT and CVO3 strains were obtained from Dr.Baxter-Jones, BUT, Chester, England and Dr. Le Gros, Rhone Merieux, Laboratories, Lyon, France respectively.

Antiserum against STG 671/88 was prepared in 4-week-old turkey poults free from TRT antibodies. The birds were inoculated intranasally and intraocularly with 1 ml of culture medium containing 10^5 $TCID_{50}$/ml. After 26 days the birds were boostered by intravenous and intranasal routes, and exsanguinated one week later.

Antiserum against STG 854/88 was prepared in 4-week-old SPF chickens. The birds were inoculated intramuscularly and intraoccularly with 10^5 $TCID_{50}$/ml/bird. A second inoculation followed 2 weeks later and the birds were bled after anothor week.

Virus neutralization tests (VN). The micro-beta VN tests (constant virus diluted serum) were performed in microtiter plates using 100 $TCID_{50}$ of each virus. Neutralization titres were expressed as base 2 logarithms of the reciprocal of the highest dilution of serum that completely neutralized the virus[10].

Purification of the Viruses. Cell debris was removed by centrifugation at 3000 g for 20 minutes. Centrifugation at 30,000 g for 60 minutes followed and the obtained pellet was resuspended in a small amount of deionized water before being placed on a 30% sucrose cushion. After centrifugation at 50,000 g for one hour the obtained pellet was resuspended in a 0.01M Tris-HCl buffer (pH 7.0) and used for sodium dodecyl sulphate (SDS) polyacrylamide gel electrophoresis. Non infected control culture was treated in the same way.

SDS-poly acrylamide gel electrophoresis (SDS-PAGE). The SDS-PAGE on virus preparations were carried out according to the method described[11] using 11% acrylamide gels. The polypeptides were detected by staining the gel with 0.2% Coomassie brilliant blue R.

RESULTS

Exposure of all viruses to 56°C or 60°C resulted in complete loss of infectivity after 30 minutes. The loss of infectivity after chloroform treatment indicates that all isolates are enveloped. On the other hand, IUDR treatment did not adversely affect the infectivity and proved that all viruses contain RNA.

The isolates were not able to haemagglutinate erythrocytes from any of the species so far examined after 30 or 90 minutes.

Virus neutralization tests revealed marked two way cross reactions with BUT1=8544, STG 761/88 and STG 854/88. The CVO 3 isolate showed only partial reaction (Table 1).

The SDS-PAGE profiles of 3 TRT viruses (1 from England and 2 from Germany) and the molecular weights of viral polypeptides were very similar. On the other hand, the CVO 3 strain showed some variation in SDS-PAGE (Figure 1). Some polypeptides of different molecular weights such as 52, 50, 39, 37 and 28 kDa are visible only in the BUT1=8544 , STG 761 and STG 854 strain profiles; they could not be detected in the profile of the CVO 3.

DISCUSSION

Although many reports on TRT clinical observations and serological surveys in many countries have been published, however there have been relatively few reports to date on virus isolation.

Studies using TRT virus isolates from different laboratories in England showed morphological and antigenic similarity[1,16]. Also, results obtained by comparison of isolates obtained from different laboratories and countries revealed close morphological and antigenic relationship[7,10]. In the present investigations results indicate that the tested isolates shared similar physio-chemical properties and are antigenically related. However, the relationships between the BUT1=8544 and the two German STG strains were closer than to the strain obtained from France (CVO3). Results of SDS-PAGE analysis also revealed that the German and English isolates have similar polypeptide profiles, while the CVO3 strain showed some variation. Similar results have been reported also by Gough and Collins[7]. Their SDS-PAGE polypeptides analysis of 3 TRT isolates- CC220 and 14/1 (from England) and 1556 (from France) - showed that the 1556 strain (from France) has a similar but not identical profile. Further investigations using molecular biology techniques are in progress.

ACKNOWLEDGEMENTS

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REFERENCES

1. Baxter-Jones, C., J. K. A. Cook, J. A. Frazier, M. Grant, R.C. Jones, and G.P. Wilding. Closer relationship between TRT virus isolates. Vet. Rec. 121:562. 1987.

2. Buys, S.B. and J.H. Du Preez. A preliminary report on the isolation of a virus causing sinusitis in turkeys in South Africa and attempts to attenuate the virus. Turkeys, June, pp 36 and 46. 1980.

3. Buys, S. B., J.H. Du Preez, and H.J. Els. The isolation and attenuation of virus causing rhinotracheitis in turkeys in South Africa. Onderstepoort J. Vet. Res. 56:87-98. 1989.

4. Collins, M. S., R. E. Gough, S. A. Lister, N. Chettle, and R. Eddy. Further characterisation of a virus associated with turkey rhinotracheitis. Vet. Rec. 119:606. 1986.

5. El Houadfi, M., A. Hamam, J. Vanmarcke, and J.K.A. Cook. Swollen head syndrome in broiler chickens in Morocco. Proc. 40th West. Poult. Dis. Conf. Acapulco, Mexico. April 24-27, pp. 126-127. 1991.

6. Giraud, P., G. Bennejean, M. Guittet, and D. Toquin. Turkey Rhinotracheitis (TRT) in France: Isolation and characteristics of a new infectious agent. Meeting of the working group 7-8; "Turkey and water fowl". Eur. Fed. WPSA, Lyon, France. Aug. 20-22. 1986.

7. Gough, R. E., and M. S. Collins. Antigenic relationships of three turkey rhinotracheitis viruses. Avian path. 18:227-238. 1989.

8. Hafez, H.M.. Rhinotracheitis der Puten. Schriftreihe der Dtsch. Vet. Ges. - Fachgr. "Geflügelkrankheiten" 34. u. 35. Fachgespräch, Hannover.pp.107-120. 1987.

9. Hafez, H. M.. Turkey rhinotracheitis: Review and experience in Germany. Wien. Tierärztl. Mschr. 78:95-200. 1991.

10. Hafez, H.M., and F. Weiland. Isolierung des Virus der Rhinotracheitis der Puten (TRT). Tierärztl. Umsch. 45:103-111, 1990.

11. Laemmli, U.K.. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature, London. 227:680-685. 1970.

12. Pollan, B., H.M. Hafez,and L.Vasicek. Rhinotracheitis bei Puten in Österreich. Wien. Tierärztl. Mschr.(in Press), 1992.

13. Smith, A. L., G. H. Tignor, and K. Mifune. Isolation and assay of rabies serogroup viruses in CER cells. Intervirology. 8:92-99. 1977.

14. Weisman. Y., C. Strengel, R. Blumenkranz, Y. Segal.Turkey Rhinotracheitis (TRT) in turkey flocks in Israel. Virus isolation and serological response. Proc. 37th West. Poult. Dis. Conf. Feb. 1988. Davis, California. pp. 67-69. 1988.

15. Wilding, G. P., C. Baxter-Jones, and M. Grant. Ciliostatic agent found in rhinotracheitis. Vet. Rec. 118:735. 1986.

16. Wyeth, P. J., R. E. Gough, N. Chettle, and R. Eddy. Preliminary observations on a virus associated with turkey rhinotracheitis. Vet. Rec. 119:139. 1986.

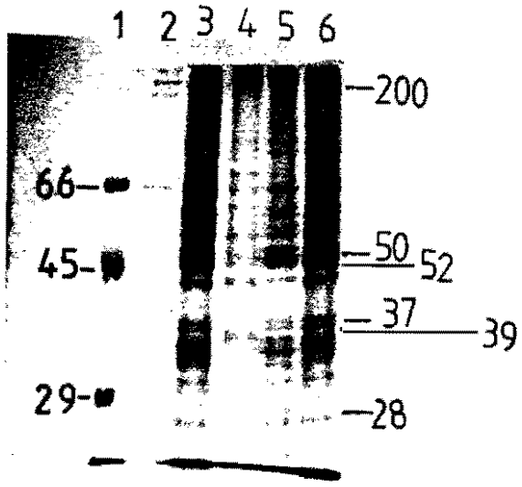

Figure 1. SDS-polyacrylamide gel electrophoresis of the 4 TRT virus isolates.

1) Bovine serum albumin
2) Tissue culture preparation
3) BUT1 = 8544
4) CVO 3
5) STG 761/88
6) STG 854/88

Table 1. Comparison of virus neutralising antibody titres.

TRT	Antisera			
isolates	BUT=8544	CVO 3	STG 761	STG 854
BUT=8544	10.3	4.5	8.3	8.3
CVO 3	5.0	**8.0**	6.0	7.0
STG 761/88	9.0	4.5	**10.0**	10.3
STG 854/88	9.3	n.d.*	8.0	**10.3**

* not done

APPLICATIONS OF MONOCLONAL ANTIBODIES IN THE DIAGNOSIS OF IBV INFECTION

Kemal Karaca, Syed Naqi and Benjamin Lucio

Department of Avian and Aquatic Animal Medicine, College of Veterinary Medicine, Cornell University, Ithaca, NY 14853

Infectious bronchitis virus (IBV) infection may be diagnosed by demonstration of rising antibody titers in the serum, or preferably by virus isolation and identification. These methods have been considered less than ideal for a number of reasons but were regularly used because alternate methods were not available. Last year we reported development of monoclonal antibodies (Mabs) specific to IBV serotypes Arkansas, Connecticut, and Massachusetts which could be used for rapid diagnosis in ELISA and immunoperoxidase assay.

Recently, using Mab-based ELISA and immunoperoxidase techniques we have screened a number of standard laboratory strains belonging to homologous and heterologous IBV serotypes, and several field isolates. Suitability of chorioallantoic membrane versus allantoic cells from infected chicken embryos for use in the immunoperoxidase assay was assessed. Correlation of results of ELISA or the immunoperoxidase method with conventional virus neutralization procedure was established.

In a separate study, a blocking ELISA (B-ELISA) was used to determine whether a serotype-specific diagnosis of IBV infection can be established using sera from infected chickens. For this purpose, 3 groups of chickens were intranasally inoculated with either Ark-CA, Con-08, or 8-311 strain belonging to Ark, Conn, and Mass serotypes, respectively. Twenty-one days later, the 3 groups were exposed to an inoculum containing all 3 IBV strains. Chickens were bled weekly for 3 weeks after primary and secondary exposure, and individual serum samples were tested in a B-ELISA. Chicken sera specifically inhibited binding of Mabs to their homologous antigens. These results correlated well with those from a virus-neutralization test suggesting that B-ELISA has an excellent potential for use in the field diagnosis of IBV infection.

SUSCEPTIBILITY OF EMBRYOS OF SIX AVIAN SPECIES TO A VIRULENT STRAIN OF PIGEON PARAMYXOVIRUS (PPMV), TYPE 1

A.E. Castro[A], D.D. Larkin[A], M. Pool[A],
S.A. Bokhari[C], and B. Charlton[B]

[A]California Veterinary Diagnostic Laboratory System,
Davis Branch, P. O. Box 1770, Davis, CA 95617
[B]Turlock Branch, P. O. Box P, Turlock, CA 95381
[C]University of California Cooperative Extension
733 County Center III Court, Modesto, CA 95355

INTRODUCTION

The pigeon paramyxovirus (PPMV-1) is a variant strain of Newcastle Disease Virus (NDV)[4,7]. The PPMV-1 contains a single-stranded RNA, a hemagglutinin and neuraminidase protein which is used in its classification into the family Paramyxoviridae. However, the strain classification of PPMV-1 is based on its reaction with monoclonal antibody[1] and on the pathogenicity tests of the isolates based on their chicken embryo mortality after allantoic route inoculation[3,4]. This pathogenicity test in embryonating chicken eggs (ECE) or mean death time (MDT) has been one of the basic tests for the classification of paramyxovirus, Type 1 or NDV for its virulence as: high (embryo mortality of < 60 hours), moderate (deaths occurring between 60 to 90 hours), or low (deaths occurring > 90 hours) post inoculation[3,4]. There are other pathogenicity tests used for the classification of virulence of NDV which include the intracerebral pathogenicity index in day-old chicks (ICPI) and the intravenous pathogenicity index in 6-week-old chickens (IVPI)[3,4]. The pathogenicity of 6 PPMV-1 isolates have been determined using the ICPI and IVPI tests[2]. In this latter study, a decline in virulence for chickens after serial passage in birds was suggested to be due to selection pressures in the bird[2]. In another study of PPMV-1 isolates from outbreaks in Italy, the virus strains isolated were classified as mesogenic to lentogenic[6]. These strains had low pathogenicity for quail and were non-pathogenic for chickens[6].

BACKGROUND ON VIRUSES

Two accessions (K515 and D3515) were received from a squab farm located in California. These 2 submissions were a component of a further surveillance for NDV on the premises where more than 1000 pigeons had died.

An organ pool of D3515 was used to inoculate, via the allantoic route, into specific-pathogen-free ECE and it produced a hemagglutinin titer of 1:64 in the chorioallantoic fluids (CAFs). From a tissue pool of K515 a hemagglutinin with a titer of 1:128 was obtained after similar inoculation into ECE. The 2 virus isolates failed to react with antisera to PMV-2 (Yucaipa) or PMV-3 but a hemagglutination-inhibition (HI) titer of 1:32 was obtained for D3515 isolate and an HI of 1:64 for K 515 isolate. The 2 virus isolates were also examined by electron microscopy and typical paramyxovirus particles were found.

The 2 virus isolates were also serotyped as PPMV-1 by the National Veterinary Services Laboratory (NVSL) using monoclonal antibodies. The viruses were also further classified by NVSL as lentogenic based on their mean death time in ECE.

RESULTS

To ascertain the potential danger of the PPMV-1 isolate to neighboring game bird growers, a comparative study of the MDT was done in embryonating eggs of game turkeys, pheasants, chukars, pigeons and quail using

chicken embryos as the reference standard. A serial 10 fold titration was done on the isolate K515 in ECE to establish the minimal lethal dose for assessing the pathotype of the virus. The pathotype in chicken embryos was determined to be lentogenic based on a MDT of 114 hours.

Prior to the performance of the MDT studies in eggs of other avian species, a size measurement of 10 eggs for each species was made (Figure 1). This measurement was used to establish an equivalent inoculation volume for each egg type with the chicken inoculum as the reference standard (Table 1).

The findings of these evaluations established that the pathotype of the K515 isolate ranged from mesogenic to lentogenic for the avian species tested. The virulence classifications based on adjusted MDT (in hours) were: turkey (mesogenic), chicken (lentogenic), pheasant (mesogenic), chukar (mesogenic) and quail (lentogenic). The MDT for the pigeon embryos was 48 hours, which placed the PPMV-1 pathotype for this species in the mesogenic range.

DISCUSSION AND CONCLUSIONS

Of the embryonating eggs of the species tested, quail embryos were the most resistant and pigeon embryos the most susceptible. The MDTs were adjusted to reflect the differences in hatching time of eggs for each species. A variable that should be paramount in such studies is the ability to obtain eggs from the species all at the same day of embryonation. To overcome this problem, eggs were selected for testing from the layers when a predictable date could be ascertained with the exception of the pigeon eggs.

Whether this isolate of PPMV-1 retains its virulence after serial passage is yet unknown. The approaches used for the prevention, control and vaccination of the pigeons in this outbreak will be reviewed.

REFERENCES

1. Alexander, D. J. Antigenic and biological characterization of avian paramyxovirus type 1 isolates from pigeons—an international collaborative study. Avian Path. 14:365-376. 1985.

2. Alexander, D. J., and G. Parsons. Pathogenicity for chickens of avian paramyxovirus type 1 isolates obtained from pigeons in Great Britain during 1983-85. Avian Path. 15:487-493. 1986.

3. Alexander, D. J. Newcastle Disease. In: A laboratory manual for the isolation and identification of avian pathogens. H. G. Purchase, L. H. Arp, C. H. Domermuth and J. E. Pearson. eds. Amer. Assoc. of Avian Pathologists. Kendall/Hunt Publishing Co., Dubuque, Iowa, 3rd ed. pp. 114-120. 1989.

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 University Press, Ames, Iowa. 9th ed. pp. 496-519. 1991.

5. Barton, J. T., A. A. Bickford, and G. C. Cooper. Paramyxo-virus type 1 infections in racing pigeons in California. 1. Pathology, serology and clinical signs. Proc. of the Western Poultry Dis. Conf. 40:20. 1991.

6. Biancifiori, F., and A. Fioroni. An occurrence of Newcastle Disease in pigeons: virological and serological studies on the isolates. Comp. Immuno. Microbiol. Infect. Dis. 6:247-252. 1983.

7. Kaleta, E. F., D. J. Alexander, and P. H. Russell. The first isolation of the avian PMV-1 virus responsible for the current panzootic in pigeons. Avian Path. 14:553-557. 1985.

Table 1. Recommended egg inoculation volumes for virus suspensions based on the size of embryonating egg.

Species	Inoculation Volume (ml)*
Chicken	0.20
Turkey	0.20
Pheasant	0.15
Pigeon	0.10
Chukar	0.10
Quail	0.05

*Chicken is the standard reference

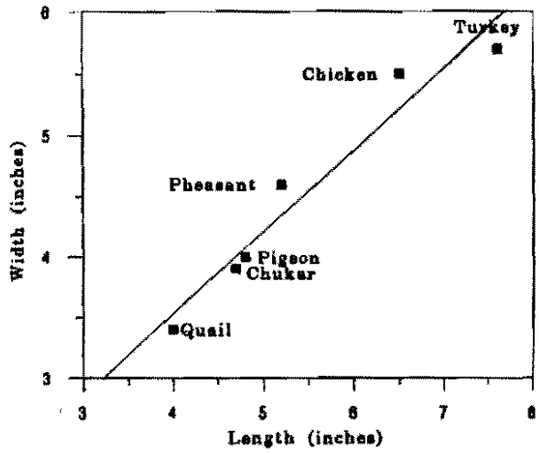

Figure 1. Comparative relationship of the size of the egg using chicken eggs as the standard of embryonating eggs (measured as Circumference of egg in inches) of different domestic species.

Slope of line (Y) - 5.4E-2 + 0.86X
correlation coefficient (R2) - 0.99

A SUBUNIT AVIAN INFLUENZA VACCINE

V. Sivanandan, S. Kodihalli, K.V. Nagaraja, and D.A. Halvorson

Department of Veterinary PathoBiology
College of Veterinary Medicine
University of Minnesota, St. Paul, Minnesota 55108

Avian influenza virus, belonging to the sub-type H5N2 (A/Ty/MN/1700/82), was propagated, purified, and treated with detergent N-octyl glucoside. Highly enriched viral nucleoprotein with residual hemagglutinin (NP-HA) was extracted in 1M NaCl solution and used in the subunit vaccine preparation. This preparation did not contain any detectable levels of matrix (M) and neuraminidase (NA) proteins and was free of infectious virus. The NP-HA subunit was incorporated into "immunostimulating complex" (ISCOM) and tested at different concentrations for its efficacy as vaccine in turkeys. The immune response of the turkeys vaccinated with HP-HA ISCOM was measured by ELISA. The vaccinated turkeys were partially protected to heterologous challenge. The NP-HA vaccine did not prevent virus infection but aided in the recovery from infection by limiting the virus multiplication in the lungs and trachea.

EXPERIENCES WITH SALMONELLA ENTERITIDIS (SE) BACTERIN

Kenneth H. Eskelund

Maine Biological Laboratories
Waterville, Maine 04901

Three serials of *Salmonella enteritidis* (SE) bacterin were tested. In Trial SEB-2, SPF chickens, 16 weeks of age were divided into groups of 10 birds each and vaccinated with 0.5 ml of one of the SE bacterin serials by intramuscular injection. Another 10 birds were held in the same pen as unvaccinated controls. At 3 weeks post-vaccination (PV), all groups were revaccinated as above with the same serials except 10 birds from the serial AD41 group. At 3 weeks post-revaccination (PRV), serum samples were collected. Serum plate tests and microtiter agglutination tests were conducted on all sera. At 3 weeks PRV, all birds were sent to Dr. Mike Opitz at the University of Maine for challenge. All birds were challenged by crop installation of a 8.6 Log 10 CFU of SE organisms per bird. Composite fecal samples were collected daily for 7 days post-challenge (PC). At 7 days PC, all birds were sacrificed and the intestines and organ pools (liver, spleen and kidney) were cultured for SE. All results are shown in Table 1. It can be seen that one vaccination stimulated antibody production (serial AD41). After revaccination, microagglutination titers were significantly increased. Infection of internal organs was reduced by 50% and 30% respectively in birds vaccinated twice with serials AC13 and AD41. SE was isolated from the gut of a high percent of all groups. Most important was the finding that fecal counts after challenge showed that fecal shedding of SE was reduced by 97.3% in once vaccinated birds and 98.0% to 99.3% in twice vaccinated birds, compared to the unvaccinated challenged controls. From this, we conclude that immunity from the SE bacterin has significantly reduced the colonization of the gut from the SE challenge.

It is difficult to conduct good efficacy studies with SE bacterin based on challenge results. Other factors, such as stress and natural resistance seem to be big factors in the pathogenicity and infectivity. In Maine Biological Laboratories (MBL) trials, all challenges have been conducted by Dr. Mike Opitz at the University of Maine. It has been most difficult to establish a challenge dose that will consistently invade the organs of a high percentage of the controls without overwhelming the vaccinates. In Trial SEB-2 (Table 1) the heavy challenge (8.6 Log 10 CFU per bird) overwhelmed the vaccinates. In Trial SEB-2 (Table 3) a lesser challenge (7.0 Log 10) was given and it only infected the organs of 16.6% of the controls. Although the vaccinates had less organ infection and cleared faster, the results are not significant because of lack of infection in the controls. In Trial SEB-9 (Table 2) the challenge dose of only 6.0 Log 10 CFU caused 50% or-

gan infection in the controls compared to only 10% and 11% in the vaccinates.

Although there was a significant decrease in SE infection of intestines and organs in Trial SEB-9, we do not believe that protection against organ infection is the most important function of this bacterin. We believe that the SE problem in chickens can best be controlled by preventing colonization and fecal shedding. This is shown in Field Trial SEB-102 (Table 4). In this trial a commercial egg complex consisting of 7 (90,000 bird) laying houses was depopulated after SE was diagnosed. The complex was cleaned and disinfected and approved for repopulation by the USDA Salmonella Enteritidis Task Force. Three pullet flocks being raised for housing in this complex were vaccinated with an autogenous SE bacterin produced by MBL. The bacterin contained high levels of antigens for phage types 8, 14B, and 23. One of the pullet growing houses used for rearing these flocks was shown to be environmentally positive to SE at the time the baby chicks were placed; however, the test results were not known until after placement. In early November 1990, the pullet houses were checked and all 3 houses were found to be SE positive. At approximately 6 weeks PV, these flocks were moved into 3 of the 7 layer houses in the complex. The other 4 houses were repopulated with birds from a different source and did not receive the SE bacterin. These birds had been tested and found to be SE negative prior to housing. In early January, 1991, flocks in two of the unvaccinated houses experienced a drop in production. On January 14, 1991, birds were submitted to a diagnostic laboratory and phage type 14B of SE was isolated from birds from both houses. This was the same phage type that had previously been isolated from this complex. On or about February 6, 1991, about 13 weeks after housing of the vaccinated birds, all houses were tested for SE contamination. Twenty-one drag swabs were used in each house and sent to Dr. Mike Opitz. Results are shown in Table 4.

This was a most dramatic result with these negative vaccinated houses surrounded by heavily SE contaminated houses even though there was a lot of traffic between the houses. In November 1991 the vaccinated houses were tested at about 47 weeks post-housing and all 3 houses were still SE negative, at or near the end of the laying cycle.

Field Trial SEB-103 was set up to evaluate the efficacy of an autogenous SE bacterin as an aid in the eradication of SE from an SE contaminated, continuously operating commercial egg laying complex. This complex consisted of 12 commercial egg cage houses each having a capacity of about 30,000 birds. The environment in these houses had been monitored for SE by the use of drag swabs. All houses, except No. 8, had tested SE positive. Phage types 13A, 14B, 23 and "not typeable" were isolated from this complex. Starting in August of 1991, after depopulation, all houses were thoroughly cleaned, washed and disinfected (some several times). Each house was swabbed and shown to be SE negative before repopulation with SE clean pullets. When these houses were tested at 6 weeks (House 3) and 4 weeks (House 6) post-housing, both flocks were environmentally positive to SE. These results confirm that even though a house is thoroughly washed and disinfected and shown to be SE negative by acceptable procedures, there are still living SE organisms present. When susceptible birds are placed in these houses, they pick up the SE organisms and multiply them rapidly to cause environmental contamination. Starting with House 11 on December 26, 1990, all pullets have been vaccinated at the time of housing with an autogenous SE bacterin produced by MBL and revaccinated 3 to 5 weeks later. Over the past year all vaccinated houses have been tested for environmental SE contamination by the use of drag swabs at various intervals after housing. All swabs have been tested at the University of Maine. Results of environmental testing are shown in Table 5. It will be noted that the two unvaccinated houses (3 and 6) became environmentally positive at 4 to 6 weeks post-housing. With few exceptions all drag swabs taken from all vaccinated houses have tested SE negative. Considering that these flocks were not vaccinated until the time that they were placed in the laying houses, it is evident that the SE bacterin has been very efficacious in preventing the colonization and shedding of SE in this previously contaminated complex. It is also interesting to note that *Salmonella* species other that SE are usually still isolated form the cleaned and disinfected houses even when SE is no longer isolated. If these salmonellae are still present, it is difficult to believe that all SE has been removed or inactivated. During the production cycle, these other salmonellae have been isolated from the swabs of most tests on all houses. These same swabs have been negative to SE. It is evident that the vaccinated birds have resisted infection with SE even though they are still susceptible to other *Salmonella* species.

MBL is also producing an autogenous SE bacterin for another company that has had a confirmed SE trace-back. This complex consists of 36 houses with a total capacity of 2,400,000 layers. They started an SE vaccination program in May 1991. Pullets are being vaccinated at about 14 weeks of age and revaccinated about 4 weeks later. After the SE positive spent hens are removed, each house is cleaned, washed and disinfected before placing the new SE vaccinated flock. As of November 22, 1991 they had monitored 13 vaccinated flocks between the ages of 25 and 40 weeks, using 20 drag swabs per house. All of these flocks were environmentally SE negative.

It has been difficult to get true information about the incidence of SE. When an egg producer learns that he has SE positive houses, he doesn't want to advertise it. Even if he vaccinates, he is reluctant to share results. We

really don't know how many SE positive flocks there are in the field. However, random testing of spent hens at a dressing plant in Georgia showed 17% SE positive flocks. One would expect the incidence of SE to be higher in the Northeast where most of the "trace-backs" have occurred. The problems with SE in both humans and chickens are becoming more widespread and more serious. The SE Task Force Status Report lists a total of 21 reported human outbreaks in the first 8-1/2 months of 1991. However, there were another 21 cases reported in the following 2 months. Within the past few months there have been first time "trace-backs" to commercial egg flocks in Michigan and Colorado. In late November, there were several new trace-backs to Pennsylvania flocks some for the second time. It is obvious that the present program of depopulation, cleaning, disinfection is not adequate. Our results indicate that the additional use of a good SE bacterin can make the difference. To summarize these results, MBL has produced and sold over 10 million doses of Autogenous Salmonella Enteritidis Bacterin since May of 1989. Sales (and actual use) could be broken down as follows: 1989: 105,000 doses; 1990: 1,129,000 doses; 1991: 9,000,000 doses. All of this bacterin has been used in large commercial egg production complexes. To my knowledge, as of November 27, 1991, all houses containing vaccinated birds are testing environmentally SE negative or have been tested SE negative at the end of the production cycle. Also, all replacement pullets being raised for these complexes were being vaccinated against SE.

The present USDA testing program starts with the testing of the environment (the chicken houses) by the use of drag swabs. This is the same in proposed future testing programs. The FDA has proposed that they take over the SE control program from USDA and only allow interstate shipment of eggs from flocks that have been certified to be SE clean. This would also be dependent on proving an SE negative environment. In all programs, the first thing to be tested is the environment. If the poultry house is tested SE negative by the use of drag swabs, the flock is considered negative and no further testing is done. Eggs can be marketed normally; therefore, it is most important that every effort be made to eliminate SE from the poultry houses. The autogenous SE bacterin has been shown to play an important role in cleaning up contaminated premises by reducing or eliminating fecal shedding. This must indicate that immunity from vaccination greatly inhibits the ability of the SE organisms to colonize the intestine. In our laboratory challenge trials, millions of organisms were placed directly into the crop, and compared to controls; only 1% or 2% were able to colonize the gut and be shed in the feces. Contrast this challenge with a well cleaned and disinfected commercial layer house. Many studies have concluded that it is practically impossible to eliminate SE from laying houses by acceptable "C&D" procedures. When susceptible birds are placed in these houses, the few SE organisms are rapidly multiplied. However, if the birds have enough immunity to resist infection with these few organisms, the house will remain environmentally negative by accepted routine monitoring procedures. Our field data on some 5 million vaccinated birds show that we are accomplishing this with our SE bacterin.

I fully realize that my approach to this problem is unusual. It is based on highly significant reduction in fecal shedding (gut colonization) and our data are based on testing of chicken houses rather than chickens. However, the only way to solve this human disease problem is by eradicating SE from the chicken houses. Whereas these salmonellae are ubiquitous, eradication will not be accomplished by usual eradication procedures alone. Our approach is innovative; however, it has been highly successful under practical (even adverse) conditions. There has also been some concern that birds in an environmentally negative house could still be SE infected and spread the organism to humans. It only stands to reason that if the SE can't colonize the gut (the first line of defense) the chances of passing through the gut wall, into the blood stream is negligible. From a practical point of view, if the house is environmentally negative the flock will be given a clean bill of health and the birds or the eggs will not be tested.

All of the SE bacterin that MBL has produced has been autogenous bacterin. This means that it is produced specifically for specific premises with an SE isolate or isolates that have been found to infect that specific farm (or complex). We have generally used 3 or 4 different SE phage types in each bacterin. This is done to provide the widest possible coverage. Our studies indicate that the use of a good SE bacterin can make the difference between success and failure in reestablishing environmentally SE negative poultry houses and SE negative flocks on previously infected complexes.

Table 1. Trial SEB-2.

SERIAL NO.	AC13	AD41	AD41	AD46	NONE
NO. OF VACCINATIONS	2	1	2	2	0
SERUM PLATE TEST (POS/TOT)	10/10	10/10	10/10	9/9	1/10
CULTURE RESULTS ORGANS - % POS	50	100	70	100	100
INTESTINES - % POS	90	90	90	100	100
MEAN FECAL COUNT PER GRAM X 1000	1.1	2.0	1.5	0.5	74.9
% REDUCED FECAL SHEDDING	98.5	97.3	98.0	99.3	0

Table 2. Trial SEB-9.

BACTERIN: SERIAL AF23 - PHAGE TYPES 8, 14B & 23
SERIAL AF30 - PHAGE TYPES 8, 13A, 23 & 28
VACCINATIONS: 11 & 14 WEEKS
CHALLENGE: 17 WEEKS - 10^9 CFU
NECROPSY: 10 DAYS POST-CHALLENGE

	MEAN FECAL COUNT X 100 SE/GRAM (DAYS PC)									
SERIAL	1	2	3	4	5	6	7	8	9	10
AF23	P*	P	10	63	0	10	0	10	0	2
AF30	P	P	0	0	0	0	2	20	0	0
Controls	P	P	3162	3162	2512	2512	25	20	40	25

*P - The lab was unable to quantitate fecal pools due to faulty media.

TREATMENT	SERIAL NUMBER AF23	AF30	CONTROLS
CULTURE RESULTS (10 DAYS PC)			
INTESTINES	20%	11%	83%
ORGANS	10%	11%	50%
SERUM PLATE TEST (POS/TOT)			
SALSBURY ANTIGEN	10/10	9/9	1/6
VINELAND ANTIGEN	10/10	9/9	0/6

Table 3. Trial SEB-10.

BACTERIN: : PHAGE TYPES 8, 13A, 14B, 23
VACCINATIONS : 10 AND 13 WEEKS
CHALLENGE : 16 WEEKS - 10^9 CFU
NECROPSY : 7 AND 14 DAYS POST-CHALLENGE

	MEAN FECAL COUNTS - SE PER GRAM (DAYS PC)						
TREATMENT	1	2	4	6	9	11	14
VACCINATES	36300	4	580	0	0	0	0
CONTROLS	1260	400	30900	13800	79400	18	0

TREATMENT	VACCINATES	CONTROLS
CULTURE RESULTS (% POS):		
INTESTINES: 7 DAYS PC	8.3	33.3
14 DAYS PC	0.0	0.0
ORGANS: 7 DAYS PC	8.3	16.6
14 DAYS PC	0.0	18.0
SERUM PLATE TEST (POS/TOTAL):		
SALSBURY ANTIGEN	23/23	2/23
VINELAND ANTIGEN	23/23	0/23

Table 4. Field trial SEB-102.

HOUSE NO.	VACCINATION STATUS	WEEKS HOUSED	CULTURE RESULTS (SWABS) POS/TOTAL	%POSITIVE
1	NO	9	20/21	95
2	YES*	13	1/21*	5
3	YES	13	0/21	0
4	YES	13	0/21	0
5	NO	9	20/21	95
6	NO	10	9/21	43
7	NO	10	19/21	90

* 5000 unvaccinated birds were used to complete the filling of this house

Table 5. Salmonella testing of drag swabs.

HOUSE NUMBER	FLOCK HISTORY			SALMONELLA TESTING OF DRAG SWABS		
Flock Number	Date Vaccinated	Date Revaccinated	Date Housed	Weeks Housed	Positive/Total	
					S.E.	Other
3 202/02	None	None	8-15-90	Empty 6 14 Sold	0/10 1/10 2/13 -	1/10 6/10 3/13 -
4 205/03	None	None	8-30-90	Empty 4 12 20 36 46 55 64 Sold	0/10 2/10 4/10 0/13 0/13 0/13 0/13 1/10 -	5/10 7/10 5/10 6/13 1/13 1/13 3/13 2/10 -
11 210/03	12-26-90	1-14-91	12-26-90	Empty 5 12 26 50	0/9 0/18 0/13 0/13 0/13	0/9 2/18 3/13 1/13 4/13
7 206/03	1-15-91	3-13-91	1-15-91	Empty 2 9 26 47	0/9 0/15 0/13 0/13 0/13	2/9 0/15 6/13 3/13 0/13
10 209/03	1-27-91	3-6-91	1-27-91	Empty 1/2 7 15 25	0/18 0/15 1/13 2/12 0/13	4/18 12/13 6/13 3/12 5/13
12 211/03	2-5-91	3-6-91	2-5-91	Empty 6 13 23	0/9 0/13 0/13 0/13	0/9 8/13 7/13 7/13
3 202/04	2-27-91	3-26-91	2-27-91	Empty 1 5 19 29	1/10 0/9 1/13 0/13 0/9	1/10 0/9 9/13 6/13 3/9
4 204/02	6-6-91	7-11-91	6-6-91	Empty 13 25	0/10 0/13 0/13	1/10 3/13 2/13
1 208/02	6-10-91	7-15-91	6-17-91	Empty 13 25	0/10 0/9 0/13	0/10 4/9 1/13
5 205/02	6-23-91 7-14-91 7-26-91	7-22-91 8-28-91 8-28-91	6-23-91 7-14-91 7-26-91	Empty 8-13 20-25	0/10 0/13 0/13	0/10 1/13 4/13
9 208/03	7-26-91	8-30-91	7-26-91	Empty 9 14 20	0/16 0/13 0/13 0/13	0/16 7/13 3/13 2/13

A POSSIBLE DNA PROBE FOR SALMONELLA ENTERITIDIS

Mazhar I. Khan

Department of Pathobiology,
University of Connecticut, Storrs, Connecticut 06269

Recently, *Salmonella* infections of humans have been associated with the use and consumption of commercially produced chicken eggs. This has created a major public health concern. During the past several years 10,253 humans have become ill from infections caused by *Salmonella enteritidis* (SE), serotype *enteritidis*, and 46 deaths have occurred. The resulting publicity has increased consumer concerns and resulted in a decline of egg consumption. The major problem contributing to the difficulty in investigation and control of SE involves the proper diagnosis and differentiating the SE isolates. Currently the diagnosis of SE in chickens include information obtained through serology using cross-reacting pullorum antigen or SE homologous antigen, as well as isolation and identification of the organism by grouping and phage typing. These tests are not rapid nor adequate to differentiate strains of SE.

We are developing a panel of SE-DNA probes in an effort to detect SE infection directly in field samples. Chromosomal DNA of the SE was digested with restriction enzymes and specific areas of the DNA sequences were identified by the cross DNA-hybridization technique, cloned into a plasmid vector and transformed in an *E. coli*. A recombinant clone specific for SE was identified by DNA hybridization techniques. When radioactively labelled and used as a probe, this recombinant clone has hybridized with SE isolates from human as well as avian sources; but did not hybridize with other types of *Salmonella* organisms.

THE INFLUENCE OF JMV-1 LYMPHOKINES ON AVIAN AND MAMMALIAN PATHOGENS

Martin Sevoian

Department of Veterinary & Animal Sciences,
Paige Laboratory, University of Massachusetts, Amherst MA 01003

The JMV-1 cell line has been adapted for growth in reduced serum (0.1%) or serum-free media to facilitate isolation and characterization of cell products in the cell culture supernatant. Cells have been adapted for growth in serum-free media by gradually reducing the amount of FCS and CS and substituting Ventrex Serum Replacement, a molecularly defined formulation containing insulin, transferrin, selenite, and testosterone (Ventrex Biotech Products, Portland, ME). Cell growth has been monitored daily by trypan blue exclusion counts. The serum-free, cell-free supernatant was tested in day-old-SPAFAS (specific-pathogen-free) for activity against a lethal challenge of JMV.

Because of its non-productive nature, JMV-1 does not contribute to the continued spread of MDV in the environment, thereby serving to reduce or eliminate reservoir hosts as a source of transmission. The cell-free culture supernatant from the JMV-1 cell line can be safely used as a non-infectious and non-oncogenic immunization against Marek's disease and most other infections. It has been demonstrated that chronic infections such as MDV, HVT, listeria, chlamydia, *Campylobacter* spp. *Salmonella* spp., etc. use up to 20% of the energy needs of the infected host (Dr. Harriet Robinson, personal communication). JMV-1 supernate has nothing infectious and no known contaminants. It can be lyophilized. It is important to determine what factors are present in the culture supernatant of JMV-1 cell line that are providing this protection in order to determine how they produce their protective effect and to determine if this (these) effect(s) can be enhanced.

The cell-free supernatant of JMV-1 is an economical, effective, antimicrobial, antiparasitic, anti-viral agent and immunomodulary. Two-dimensional reverses phase HPLC-SDS PAGE indicated that the protein constituents of the supernatant include several hundred distinct species

(Swadesh, unpublished, 1989). In order to produce a defined, reproducible antipathogenic product, the active agents must be identified and quantitated. Similarly, potentially immunogenic or pyrogenic species must be identified as such. In this way, reliable protocols of administration may be developed. Fractionation of a mixture could, of course, separate components whose interaction produces optimal antipathogenic response. Therefore, the development of rapid *in vitro* activity screen is needed to facilitate the identification of such interactions. We are now growing JMV-1 cell-line in serum-free media.

NEW APPROACHES TO STUDY CELL-MEDIATED IMMUNE RESPONSES TO MAREK'S DISEASE VIRUS

K.A. Schat,[A] W.D. Pratt,[A] R. Morgan,[B] and Z. Uni[A]

[A]Department of Avian and Aquatic Animal Medicine,
College of Veterinary Medicine, Cornell University,
Ithaca, New York 14853
[B]Department of Animal Science and Agricultural Biochemistry,
College of Agricultural Sciences, University of Delaware,
Newark, Delaware 19717

Cell-mediated immunity (CMI) is postulated to be an important part of the defense system against Marek's disease herpesvirus (MDV) infection. However, little or no progress has been reported on the understanding of the role of cytotoxic T cells (CTLs) and identification of MDV proteins recognized by these CTLs[3]. One of the major reasons for the lack of progress is the absence of chicken target cell lines expressing defined major histocompatibility complex class I (MHC-I) antigens. These cell lines are needed to present viral antigens in the context of MHC-I antigens. Syngeneic, but not allogeneic, CD8+ CTLs only recognize and lyse antigen-positive cells when the viral antigen is processed and expressed in the context of MHC-class I antigens. Previous work by two groups (reviewed in 3) had demonstrated the generation of syngeneic restricted, CD8+ CTLs after inoculation of chickens with reticuloendotheliosis virus (REV).

The other major reason for the lack of progress is the cell-associated nature of MDV. The demonstration of CTLs in *in vitro* assays such as chromium release assays (CRAs) requires that a majority of the target cells express the appropriate viral antigens. This is normally achieved by infecting MHC-I expressing cells with a high multiplicity of infection. Unfortunately, this is not possible with a cell-associated virus. Based on these impediments it is obvious that new approaches have to be used to study antiviral immune responses to MDV. Recently, molecular biology techniques have been used to define specific antigens recognized by virus-specific CTLs in mammalian herpesvirus infections. Transfection of murine cell lines with genes of cytomegalovirus resulted in the expression of immediate early (IE) proteins, which were recognized by CTLs from immunized syngeneic mice[1].

The development of a similar system for the identification of relevant genes for anti-MDV immune studies requires (1) appropriate target cell lines expressing MHC-I antigens, (2) transfection techniques allowing stable expression of genes and selection of stable transfected cells, and (3) demonstration of MDV-induced CTLs which can lyse transfected cells.

For the first requirement REV-transformed cell lines such as RECC-CU91 (CU91) were developed by infecting lymphoblastoid T cells with the transforming T-strain of REV. These cell lines were lysed by syngeneic CTLs obtained from REV-infected chickens. A plasmid (pNL1) containing the transposon Tn5 *neo*^{r} and the *lac*Z gene was constructed for the transfection studies. With this plasmid transient expression can be detected because of the *lac*Z gene, and selection for transfected cells and thus stable expression of the *neo*^{r} gene by the addition of G418 to the medium. REV-transformed cell lines were stably transfected using electroporation techniques[4].

Specific anti-MDV CMI responses were examined using the following approach. CU91 was latently infected with MDV and was renamed CU210. This cell line was subsequently transfected with pNL1 alone (CU212) or pNL1 and the *BamHI* A fragment of the MDV DNA library (CU211) (Table 1). Only the latter expressed the MDV-specific phosphoprotein with a molecular weight of 39 kilodalton (pp39). CU211, but not CU210 or CU212 were lysed by SB-1 induced CTLs[2] (Table 1). Studies using effector cells from birds inoculated with oncogenic

and attenuated MDV strain and the pp39 expressing CU211 are in progress.

REFERENCES

1. Koszinowski, V. H., G. M. Keil, H. Schwarz, J. Schickedanz, and M. J. Reddehase. A nonstructural polypeptide encoded by immediate early transcription unit 1 of murine cytomegalovirus is recognized by cytolytic T lymphocytes. J. Exp. Med. 166:289-294. 1987.

2. Pratt, W. D., R. Morgan, and K.A. Schat. Cell-mediated cytolysis of lymphoblastoid cells expressing Marek's disease virus-specific phosphoproteins. Submitted.

3. Schat, K. A. Importance of cell-mediated immunity in Marek's disease and other viral tumor diseases. Poult. Sci. 70:1165-1176. 1991.

4. Schat, K. A., W. D. Pratt, R. Morgan, D. Weinstock, and B. W. Calnek. Stable transfection of reticuloendotheliosis virus-transformed lymphoblastoid cell lines. Avian Dis. In press.

Table 1. Characterization of reticuloendotheliosis virus (REV)-transformed cell lines.

Cell line	REV transf	Latent MDV	Transfected with pNL1	Transfected with A-fragment	Expression of phosphoprotein	Lysis by SB-1 CTL
CU91	+	-	-	-	-	-
CU210	+	+	-	-	-	-
CU211	+	+	+	+	+	+
CU210	+	+	+	-	-	-

AVIAN HEALTH MONITORING—PREVENTIVE MEDICINE VIA COMPUTER DATA

R.W. Keirs[A], D.L. Magee[A], H.G. Purchase[A], R. Underwood[A], C.R. Boyle[B], and J. Freund[A]

[A]College of Veterinary Medicine,
[B]Mississippi Agricultural and Forestry Experiment Station,
Mississippi State, Mississippi 39762

Flock-health monitoring is important to optimize production efficiency, detect subclinical problems, acquire data to make valid comparisons of production practices and identify future directions of research. A flock-health monitoring system was initiated in 1982 with cooperation of the poultry industry in Mississippi. It has evolved over time so that it now consists of; (a) regular, representative live-bird sampling, necropsy and scoring of gross observations, (b) a computer program for data storage and summary, and (c) regular periodic preparation and distribution of reports to the industry. Of the 13 broiler-production complexes in Mississippi, 9 participate in the system. From each participating flock, representative samples are taken twice (2 weeks apart), from birds between the ages of 11 and 40 days. Five body systems receive particular attention; integumentary, skeletal, respiratory, hemolymphatic, and gastrointestinal. Fifteen observations are scored. The scores, weight, sex and origin of the birds are entered into a lap top computer in the necropsy room for later transfer to a microcomputer for storage and summarization.

Flock-health monitoring can be of great value to the industry and to regulatory agencies. It is used by the poultry industry to optimize its vaccination, parasite control (e.g. coccidiosis), management, and nutrition programs. For example, high gizzard scores alerted one company to a flock-health problem. Reducing the level of copper sulfate in the feed reduced the gizzard scores and eliminated the problem. Thus, flock-health monitoring programs emphasize health rather than overt disease. We believe it is the future for preventive veterinary medicine.

A complete text of this presentation has recently been published as "A new system for broiler flock-health monitoring" in Preventive Vet. Med., 11:95-103, 1991. Elsevier Sci. Publ., Amsterdam. Reprints are available from the authors.

ASSISTING FLEDGLING POULTRY ENTERPRISES IN LESS DEVELOPED COUNTRIES

D.V. Zander

Poultry Health Consultant,
18340 160th Ave NE,
Woodenville, Washington 98072

Many forms of assistance are available through various agencies from various countries, e.g. Heifer project, Agency for International Development (AID), Christian Veterinary Association, Volunteers in Overseas Cooperative Assistance (VOCA) agencies from Canada, Japan, and European countries. VOCA's emphasis is finding available specialists willing to go to less developed countries and assist the local cooperative requesting the assistance. The volunteers' expenses are paid but they receive no stipend. Volunteers are recruited from diverse specialties, e.g. poultry management, nutrition and disease control, dairy, beekeeping, marketing, pest and weed control, organization, finance, etc. The usual assignment is for 4 weeks.

My first assignment by VOCA (Nov. 1989) was to evaluate the diagnostic laboratory activities of a large poultry cooperative in Santa Cruz, Bolivia. The second assignment (Nov. 1991) was to assist a fledgling women's poultry cooperative in Uganda. The situation, needs, stage of development, language, physical assets, transportation, and communication were vastly different in the two countries. The farms in Uganda were in what I call "stage 1," i.e. very small flocks, few disease problems as yet, no sophistication such as slaughter plants or egg processing facilities, and no refrigeration. Knowledge of poultry and poultry husbandly was scant or lacking.

Farms of the Bolivian cooperative were in what I call "stage 2." The farms and flocks were relatively large, many supplied by large feed mills and hatcheries and, as a group, have their own diagnostic laboratory and veterinary staff. "Stage 2" is characterized by owners searching for any drug or vaccine that will cure the diseases that plague their large, multi-age farms.

One lay farm was reaching "stage 3"—management control and prevention of disease. "Stage 3" is characterized by an appreciation of the importance of biosecurity including proper isolation, planned farm layout, routine depopulation, quarantinable farm units, and layered cages.

Aspects of the VOCA assignments will be discussed and illustrated.

SPATIAL EPIDEMIOLOGY OF FOWL CHOLERA IN TURKEYS

Tim E. Carpenter

Department of Epidemiology & Preventive Medicine, School of Veterinary Medicine
University of California, Davis, California 95616

The epidemiology of fowl cholera in California turkeys has been studied extensively since 1985 by the California Turkey Project. The objective of the present study was to examine the spatial relationship of fowl cholera outbreaks in California turkey premises. The spatial distribution of outbreak premises was determined and compared to the distribution of non-outbreak (control) premises based on their respective means and standard dispersions. Outbreak patterns were also examined visually, both temporally and spatially, using a mapping program in an attempt to identify potential patterns of transmission among turkey premises. Finally, first and higher order nearest neighbor analyses were used to determine if distance to nearest or other neighbors affected the risk of a premises having an outbreak of fowl cholera. These findings were combined with previous risk factor and *DNA typing results in an* attempt to further unravel the complex questions involved in this disease transmission study.

STRATEGIC SEPARATION OF COMMERCIAL POULTRY PRODUCTION SITES IN CALIFORNIA I. INFECTIOUS DISEASE ASPECTS

D.A. McMartin

Veterinary Medicine Extension
University of California, Davis, California 95616

In the State of California, the San Joaquin Valley is a major location of large poultry industries—broilers, turkeys, and eggs. In the last few years, influences restricting industry development have emerged, affecting either proposed new facilities, or expansion or change of use of existing facilities. One source of objections has been other poultry industries, e.g., turkeys against caged layers, but sometimes also by owners of other animal facilities, e.g., horses. The main objections have been based on a perception of the threat of infectious disease. In the case of the turkey industry, this has been largely, although not totally, the threat of *Mycoplasma gallisepticum* (MG); in the case of horses, salmonellosis has figured prominently. A second major source of objections has been from urban origins, which will be dealt with by John Voris in paper II.

Animal industries and county planning and land use agencies have, therefore, been seeking guidance on appropriate separation of animal facilities from each other and from dwellings or institutions such as schools.

With regard to disease, factors which govern its spread include the biological characteristics of the organism, weather, topography, weight of infection, species involved, resistance of host, and existence of vectors. There is no equation showing that disease occurrence on poultry sites varies inversely with the distance between them, but expert opinion states that this is the case. "The closer the houses of one premises to those of another, the more likely is the spread of infection to healthy birds on an adjacent farm[1]."

Regarding maximal airborne travel, the viruses of hoof (foot) and mouth disease (FMD) and Aujeszky's disease have been shown on epidemiological evidence to have gone 60 km and 40 km (37 and 25 miles), respectively[2,3]. Comparing the properties of FMD with those of Newcastle disease virus (NDV), it has been postulated that NDV is theoretically capable of travelling "10s of kilometers[4]." NDV has been recovered from air samples 64 meters downwind from infected premises[5]. For turkey coryza, one mile seems to be commonly within its range in Utah. "If a flock breaks with coryza, most unvaccinated flocks downwind for at least one mile are at risk[6]." With regard to MG, although for many years long-range airborne transmission has not been regarded as a significant source of infection, there is some field evidence which indicates such transmission can occur. Again, in turkeys in Utah, "...MG transmission would definitely occur in repeatable and predictable patterns following prevailing winds... In at least one instance, transmission was known to travel at least 5 miles between flocks. More frequently, breaks would occur one-half to one mile apart[7]." Experimental evidence tending to support the possibility of occasional airborne spread of MG infection is found in work[8] reporting survival of MG in an artificial aerosol for up to 24 hours, and in a dehydrated state for more than 24 hours[9].

At a meeting in Visalia, CA, in December 1986, including personnel from poultry industries, California Department of Food and Agriculture, University of California, and county planning and land use agencies, I proposed a separation of poultry production sites by 2 miles, as a basis for discussion, taking into account both disease risk and urban pressure. This figure has been controversial, described at times as too much, and that 1 mile or even half a mile is adequate. On the other hand, some thinking in the turkey industry has gone beyond the 2-mile proposal, suggesting a distance of 5 miles between turkey facilities and multi-age caged layer operations.

REFERENCES

1. Zander, D.V., and E.T. Mallinson. In Diseases of Poultry, edited by Calnek, et al., Iowa State University Press, Ames, Iowa, 1991.

2. Hugh-Jones, M.E., and P.B. Wright. Studies on the 1967-8 foot and mouth disease epidemic. The relation of weather to spread of the disease. J. Hygiene (Cambridge) 68:253-271. 1970.

3. Christensen, L.S., et al. Evidence of long distance airborne transmission of Aujeszky's disease (pseudorablies) virus. Vet. Rec. 127:471-474. 1990.

4. Gloster. J. Factors influencing the airborne spread of Newcastle disease. Brit. Vet. J. 139:445-451. 1983.

5. Hugh-Jones, M.E., et al. The evidence for the airborne spread of Newcastle disease. J. Hygiene (Cambridge) 71:325-339. 1973.

6. David D. Frame, pers. comm., 1990.

7. David D. Frame, pers. comm., 1990, quoting Royal A. Bagley.

8. Beard, C.W. and David P. Anderson. Aerosol studies with avian mycoplasma. I. Survival in the air. Avian Dis., 9:54-59. 1967.

9. Whitear, K.G. Control of chicken mycoplasma infections in Australia. Published by the post-graduate committee in Veterinary Science, The University of Sydney, Australia and the International Union of Immunological Societies. Proceedings No. 66. Disease Prevention and Control in Poultry Production, pp. 251-261, August 1983.

STRATEGIC SEPARATION OF COMMERCIAL POULTRY PRODUCTION SITES IN CALIFORNIA II. NUISANCE COMPLAINT ASPECT

John C. Voris

University of California, Kearney Agricultural Center,
9240 S. Riverbend Avenue, Parlier, California 93648

INTRODUCTION

The rapid increase in the population of the Unites States and the expansion of the poultry industry to meet the increased demands has put the poultry industry and their neighbors on a collision course for land-use conflicts. The proper siting of poultry facilities resolves land-use conflicts before they arise. A cooperative program involving industry, the public, and representatives of local government for the development of siting and managemental guidelines can be beneficial to both industry and the public. Siting and managemental guidelines were developed in California that can be adapted to poultry-producing areas nationwide[6].

THE PROBLEM

The problem is the siting of facilities close to residences and the need to make the mitigation of nuisances a top priority by individual producers. The solution is to develop within the poultry industry an awareness of the importance of proper siting and nuisance management guidelines. Guidelines with industry and public input are welcomed by most local governing bodies, since it is easy to regulate an industry when the rules were developed by a coalition of that industry and potential neighbors. Once developed, by one governing body, these guidelines will be copied in adjacent areas with similar problems.

GUIDELINE COMPONENTS

Guidelines should include siting standards for separation from residences, sensitive uses, and other agriculture. Standards for a minimum set back from all property lines, ditches, canals and other waterways, and a standard for separation of poultry facilities from one another for biosecurity reasons would be considered. Management plans describing the operational practices necessary to control nuisances such as flies, feathers, dust, and odors should be discussed. Most finished ordinances will require that all new facilities will have to develop management plans to be filed with their building permit and approved by the health department[3].

NOTIFICATION CLAUSE

A notification clause is an important consideration for the public and the poultry producer. Many conflicts can be avoided by notifying potential new neighbors that a poultry facility is located in the neighborhood.

Most regions will have a notification requirement once a poultry facility permit is issued. A notice is sent to property owners within one-half mile of the proposed facility. This notice informs property owners that the county has issued the permit. This is an "after-the-fact" notice, and with no public hearing involved, will not impact the right of the new owner to build the facility. It does require disclosure to any potential buyer that a poultry facility exists within one-half mile.

WINDSHED DIAGRAMS

Windshed diagrams can be extracted from a wind rose developed for the area from locally available data. The California wind rose resulted from the wind velocity and direction data recorded at a local airport over a nine-year period[4]. This buffer zone, approximately the shape of an egg, is used to provide neighbors and communities with protection from the poultry facility[5].

CROP LIABILITY

Some crops are sensitive to dust and feathers[1,2]. In most areas, totally confined facilities are exempt from restrictions involving adjacent crops. Existing facilities are "grandfathered" in with the adoption of the ordinance, but may not expand unless they meet the guidelines. Neighbors are anxious about the potential of liability resulting from the drift of herbicides or pesticides onto a poultry facility. This concern includes the method of application and the possibility of poultry mortality from stampeding and piling.

POULTRY SEPARATION

In addition to biosecurity, the separation of poultry facilities reduces the opportunity for nuisance complaints. Large poultry facilities have the potential of producing substantial amounts of dust, odor, feathers, and flies. The two-mile restriction eliminates the possibility of having a concentration of poultry in any one area.

MANAGEMENT STANDARDS

In most regions, the health department will require the preparation of a management plan by the potential poultry producer for the control and management of nuisances. The grower/operator must provide a written management plan describing the management practices necessary to control feathers, flies, dust, and odor nuisances.

ADDITIONAL CONCERNS

An additional area often overlooked is the impact of numbers. The number of birds being placed on a facility is an important consideration in the guidelines. It is obvious that larger numbers are more likely to cause problems both from disease transmission and from the production of nuisances.

In the same way, the type of unit needs to be considered. The public views all poultry facilities as being the same. It does not differentiate between brooding, growing, and breeding. In each instance, the environmental impact is different.

Existing facilities not meeting all the guidelines that were "grandfathered" will not be allowed to expand. Some producers will feel their "rights" have been taken away. Neighbors frequently are adamant about not allowing any expansion. Preparation of guidelines with a section that allows existing facilities the "right" to expand a certain percentage will overcome this objection. A justification for this is the increase in size of at least 20 percent for today's turkey over the same bird 20 years ago. Many older facilities no longer have adequate square footage for the number of birds that they have traditionally grown.

In many regions, small towns and unincorporated areas are considered in a separate siting standard. Some suggest a mile radius from the "zone of influence" of the community. Others use the wind rose principal with a windshed of a mile. The orientation of the long axis is dependent upon the direction of the prevailing wind with the narrow end pointing in the direction of the prevailing wind. This siting standard further limits the amount of land available, but is preferable to the problems related to siting close to a growing community.

REFERENCES

1. Anonymous, 1982. Grape Pest Management. Division of Agricultural Sciences, University of California Pub.No. 4105:121-122.

2. Anonymous, 1990 (revised). USDA Handbook for Inspecting and Receiving Natural Condition Raisins. Feather Contamination, page 2.25.

3. Fresno County Zoning Ordinance, 1990. Section 868, Ordinance No. T-038-306, Public Works & Development Services Department, 2220 Tulare Street, Fresno, California.

4. Kreis, D.R., L.R. Shuyler, 1972. Beef Cattle Feedlot Site Selection for Environmental Protection. EPA-R2-72-129. U.S. Environmental Protection Agency, ADA, OK.

5. Kreis, D.R., 1978. Limiting the Environmental Impact of Animal Production Odors. Environment International Vol.1, pages 247-275 Pergamon Press Ltd. 1978. Printed in Great Britain.

6. Voris, J.C., 1992. Development of poultry guidelines to regulate interface with neighbors. J. of Appl. poultry research Vol.1, No.1 in press.

IMMUNE RESPONSE OF WHITE PEKIN DUCKS TO THE CU VACCINE ADMINISTERED BY WING WEB AND ORAL METHODS

S.M. Gergis[A], M.A. Shahata[B],
E.H. Rizkalla[C] and Suzan F. Gorgi[A]
[A]Veterinary Serum and Vaccine Research Institute,
Abbassia, Cairo, Egypt, P.O. Box 131
[B]Department of Poultry Diseases, Faculty of Veterinary Medicine,
Assiout University, Egypt
[C]Animal Health Research Institute, Dokki, Cairo, Egypt

White Pekin duck breeder candidates immunologically responded to exposure to the living attenuated Clemson University (CU) strain of *Pasteurella multocida* via both wing web (WW) and drinking water (DW) routes. High levels of gamma globulins, antibodies and protective immunity with no adverse reactions were induced by 2 vaccinations. Immunity was greatest 2 weeks after secondary vaccination by WW puncture, although immunity was evident by the second week after vaccination. The development of immunity after vaccinating twice by the DW route began 2 weeks after secondary exposure. A steady stepwise increase in immunity was observed among vaccinates till reaching a peak at 8 weeks after revaccination. Such ducks experienced significantly less mortality than unimmunized controls following homologous challenge.

INTRODUCTION

Economically, perhaps the most important enzootic bacterial disease that threatens duck breeding is avian cholera. Epornetics of the disease cause great losses for projects working in duck breeding. This is due to the fact that the ducks are highly susceptible to infection by *P. multocida* and also due to the rapidity of spread and the extra virulence of some *P. multocida* strains.

Autogenous bacterins prepared with formalin inactivated broth cultures have been reported to provide excellent control in the field[8], but Dougherty described only marginal efficacy in laboratory experiments[4]. At present, an oil emulsified bacterin is used in breeder ducks[10]. The frequent isolation of types 1 and 3 (Heddleston classification) *P. multocida* serotypes from White Pekin ducks in Egypt[7] and the successful application of the CU vaccine in chickens and turkeys[5] raised questions concerning the immunologic response of White Pekin ducks to this type of vaccine by DW and WW methods. The present study tried to answer these questions.

MATERIALS AND METHODS

Experimental birds. White Pekin ducks were obtained from governmental duck hatchery. They were raised in an isolated building until they reach 28 days of age and used in laboratory trials. Commercial duck feed without medication, drinking water and light were available continuously throughout the experiment.

Vaccine strain. Commercially produced lyophilized CU strain vaccine (Orachol, American Scientific Laboratories, Omaha Nebraska) used for vaccination of chickens and turkeys was reconstituted and used to vaccinate ducks via WW and DW.

Challenge strain. A virulent strain of *P. multocida* (P-1059) was provided by the National Animal Disease Center (NADC) in Ames, Iowa. 0.1 ml of 10 LD50 of this strain, previously estimated in ducks[7], was injected in the thigh of vaccinates as a challenge dose.

Oral vaccination. All principal groups of ducks consumed the recommended dosage of the CU vaccine in a mixture of skimmed milk and drinking water within 3 to 4 hours after DW was withheld overnight. The vaccine was administered to ducks 28 days old. Colony forming units (CFU) of CU vaccine consumed by each bird were calculated and ranged between 6.5 X 10^8 and 8.1 X 10^8 per bird. Secondary exposure via the same route was accomplished after 6 weeks by mixing the same inoculum in DW.

Wing web vaccination. Vaccination via WW puncture was performed by sticking a double needled pox applicator, dipped in the vaccine, through the ventral surface of the WW as close to the cord of the wing as possible. The 1000 dose vial of lyophilized vaccine was diluted in order to get approximately 4 X 10^8 organisms to adhere to the needle of the pox applicator. This procedure consisted of adding 3 ml sterile saline to the 1000 dose vial which resulted in a thick suspension[11]. The vaccinated ducks were observed daily for takes for the first 10 days after vaccination. Four weeks later revaccination was carried out using the same dose and route.

Serum antibody titer. A microtiter IHA test was used to measure the levels of serum antibodies[6]. The antibody titer was presented as log 2 of the end point dilution[1]. Blood was collected from ducks immediately before vaccination, 2, 4 weeks after primary vaccination,

then at 2,4,6,8 and 10 weeks after secondary vaccination. Serum was removed and stored at 20°C until assayed.

Polyacrylamide gel electrophoresis. Serum proteins were analyzed[9] and the percentages of serum protein fractions were estimated by densitometric scanning (Gelman DCD-16 Densitometer). The bands were identified as described elsewhere[12].

Criteria for evaluating protection. The effect of various routes of vaccination on the efficacy of the CU vaccine was evaluated using the following criteria (a) mortality, expressed as the % of ducks that survived. (b) Mean death time (MDT) of ducks that died after challenge. (c) Lesion score on post mortem examination of ducks succumbed to challenge infection. (d) Serum anti *P. multocida* antibody levels. (e) Total globulin (TG) levels and the albumin/globulin (A/G) ratio in sera of vaccinates.

Statistical analysis. Statistical analysis of the level of antibody and the survivability after challenge was performed using the analysis of variance[13].

RESULTS

Tables 1 and 2 show the immune response of white pekin ducks to vaccination by CU vaccine via D.W. The TG after an initial drop from the prevaccination level at the second week after primary vaccination showed a steady stepwise increase until it attained a peak level of 69.910 at 8 weeks after secondary exposure. The changes in TG levels at various intervals after vaccination were significant ($P < 0.001$, $P < 0.025$). *P. multocida* antibodies showed a similar pattern of significant ($P < 0.001$) increase starting from the fourth week after vaccination till reaching the highest GMT levels of 1372 at the eighth week after revaccination.

Bioassay revealed the efficacy of CU vaccine given via DW in significantly reducing mortalities among challenged ducks. 100 % protection was recorded at 6 and 8 weeks after revaccination.

As illustrated in Tables 3 and 4, the CU vaccine given two times by the WW method resulted in two peaks of TG and GMT of antibodies. The first peak at two weeks after primary vaccination revealed TG values of 70.6155 % and GMT of 680. The second peak was noted at 2 weeks after revaccination with TG levels of 71.917 % and GMT of 2744.

100% protection against challenge infection was observed at 2, 4 and 6 weeks after WW revaccination by the CU vaccine. Also it resulted in significant protection at various intervals against challenge infection that killed 100 % of control ducks of the same age.

DISCUSSION

Although performed on small groups in the experiments, the efficacy of CU vaccine, when administered to ducks by the DW or WW methods, appears to produce a good immune response and protective immunity against avian cholera. These experimental findings have been substantiated by serological responses, i.e., changes in serum protein fractions and anti *P. multocida* antibodies measured by the IHA test.

However, because the experimental populations were small a generalized conclusion cannot be made as to whether comparable results can be achieved under field conditions.

As far as could be determined, there are no references concerning the use of CU vaccine for vaccination of ducks against avian cholera, so it is difficult to make strict comparison of our results with those of others. However, in preliminary trials of vaccination against avian cholera in ducks with the attenuated strain 807 of *P. multocida*, there were immunological responders to such a vaccine[2]. Also White Pekin ducks developed immunity to avian cholera from an avirulent *P. multocida* vaccine given twice in the drinking water[3]; this vaccine gave considerably better protection against the disease than a killed vaccine. This vaccine was not the CU vaccine.

REFERENCES

1. Brough, M., Jr. A simple method for recording and analyzing serological data. Avian Dis. 22:362-365. 1978.
2. Cai, B.X., Z.J. Wu, L.Q., Wu, Y.M. Dou and S.C. Huang. Preliminary trials of vaccination against fowl cholera with attenuated strain 807 of Pasteurella multocida. Chinese Anim. Husb. and Vet. Med. 15:6-9. 1983.
3. Chang, C.F. Immunological response of ducks to an avirulent Pasteurella multocida vaccine in the drinking water. Taiwan J. of Vet. Med. and Anim. Husb. 43:33-39. 1984.
4. Daugherty, E. The efficacy of several immunizing agents for the control of fowl cholera in the White Pekin ducks. Cornell Vet. 43:421-427. 1953.
5. Derieux, W.T. Response of young chickens and turkeys to virulent and avirulent Pasteurella multocida administered by various routes. Avian Dis. 22:131-139. 1978.
6. Dua, S.K. and Maheswaran, S.K. Studies on Pasteurella multocida. VI. Nature of systemic immunity and analysis of correlation between levels of immunity induced by various fowl cholera vaccines and protection against challenge. Avian Dis. 22:748-764. 1978.

7. Gergis, S.M., I.M. Ghanem, S.M. Nanai and Nadia M. Ali. Evaluation of using autogenous bacterins in the same duck farms for consecutive years. Proceedings of the first Egyptian German Vet. Conference pp. 337-345. 1991.

8. Hilbert, K.F. and H. Tax. The value of chemically killed cultures for the control of cholera in ducks. Cornell Vet. 28:275-280. 1938.

9. Jovin, T.N., M.L.,Dante and A., Chramback. Some useful polyacrylamide gel electrophoresis systems. U.S. Gov. Res. and Dev. Reports, PB-196085 through PB-196092. 1971.

10. Lyton, H.W. Efficacy of broth-grown Pasteurella multocida bacterins in ducklings. Avian Dis. 28:1086-1095. 1984.

11. Schlink, G.T. and L.D. Olson. Vaccination of turkey breeder hens and toms for fowl cholera with CU strain. Avian Dis. 31:29-38. 1986.

12. Green, S.A., S.J., Jenkins and P.A., Clark. A comparison of chemical and electrophoretic methods of serum protein determinations in clinically normal domestic animals of various ages. Cornell Vet. 72:416-426. 1982.

13. Snedecor, G.W. and W., Cochran. Statistical Methods. 8th ed. Iowa State University Press, Ames, Iowa. 1967.

Table 1. Mean serum protein fractions, A/G ratio and average GMT of *P. multocida* antibodies at different intervals in sera of ducks vaccinated with CU vaccine administered by DW.

Weeks post DW vacc	Albumin[A]	Total globulins[B]	A/G[C] Ratio	Average[D] GMT
2	30.490	62.905	0.485	11
4	31.180	61.223	0.510	43
revacc.				
2	28.747	63.823	0.451	171
4	18.983	69.370	0.274	345
6	24.813	69.910	0.364	680
8	24.937	67.287	0.371	1372
10	26.697	66.937	0.399	894

[A,C]F test: (P<0.001) [B]F test: (P<0.025) [D]F test: (P<0.001)

Table 2. Results of challenge exposure of ducks vaccinated by CU vaccine administered by DW.

Weeks post DW vacc	Alive/dead[A] vacc.	% alive vacc.	MST[B]	Lesion[C] score
2	0/6	0	1.6	4.0
4	3/6	50	5.4	2.5
revacc.				
2	4/6	66.66	6.5	2.0
4	5/6	83.33	6.8	1.8
6	6/6	100	7.0	1.0
8	6/6	100	7.0	1.2
10	5/6	83.33	6.2	3.0

[A] No. alive 14 days post challenge / no. challenged.
[B] MST = mean survival time (days) for ducks that died.
[C] Scored on a 0 - 4 for all ducks on the group.
n.b. All control ducks (6/group) died after challenge.

Table 3. Mean serum protein fractions, A/G ratio and average GMT of *P. multocida* antibodies at different intervals in sera of ducks vaccinated with CU vaccine administered by WW.

Weeks post DW vacc	Albumin[A]	Total globulins[B]	A/G[C] Ratio	Average[D] GMT
2	30.490	62.905	0.485	680
4	31.180	70.615	0.366	345
revacc.				
2	28.747	65.397	0.440	2744
4	18.983	71.917	0.256	1280
6	24.813	67.347	0.364	755
8	24.937	66.600	0.405	680
10	26.697	63.770	0.451	394

[A]F test: (P<0.025) [B,C]F test: (P<0.05) [D]F test: (P<0.001)

Table 4. Results of challenge exposure of ducks vaccinated by CU vaccine administered by WW.

Weeks post WW vacc	Alive/dead[A] vacc.	% alive vacc.	MST[B]	Lesion[C] score
2	5/6	83.33	6.5	1.3
4	4/6	66.66	5.4	2.3
revacc.				
2	6/6	100	7.2	1.0
4	6/6	100	7.0	1.0
6	6/6	100	6.8	1.2
8	5/6	83.33	6.0	2.0
10	4/6	66.66	5.5	2.9

[A] No. alive 14 days post challenge / no. challenged.
[B] MST = mean survival time (days) for ducks that died.
[C] Scored on a 0 - 4 for all ducks on the group.
n.b. All control ducks (6/group) died after challenge.

THE ROLE OF DISSEMINATED INTRAVASCULAR COAGULOPATHY IN THE PATHOGENESIS OF FOWL CHOLERA IN TURKEYS

R. Friedlander, G. Johnson, L. Olsen, and M. Turrentine

School of Veterinary Medicine,
University of Missouri, Columbia, MO 65211

Fowl cholera is a highly infectious disease of poultry which manifests itself as either an acute septicemia or chronic debilitation. The etiological agent is *Pasteurella multocida* and its pathogenicity is due to an endotoxin. In mammals, endotoxins have been shown to activate the coagulative pathway by endothelial injury, activation of complement or the release of procoagulant substances from mononuclear cells[6]. Even though there has not been an abundance of recent studies on the avian hemostatic system, both the extrinsic and common pathways have been demonstrated to exist[1,3]. The common pathway is a prothrombinase complex encompassing Factors V and X, calcium and phospholipid[3].

A common component of disseminated intravascular coagulation (DIC) is a depletion of coagulation factors due to an increased rate of activation, inactivation or clearance which exceeds the biosynthesis of these factors. In humans, the Russell's viper venom time (RVVT) is a diagnositic test which is used to evaluate some clotting disorders. DIC has been associated with the pathogenesis of fowl cholera in chickens[5]. RVVT was modified to evaluate the role of DIC in turkeys exposed to *P. multocida*.

MATERIALS AND METHODS

Ten-week-old turkeys hens were intravenously challenged with virulent *P. multocida* (serotype 3). Blood samples were drawn from the brachial vein every 3 hours from both the control and experimental turkey hens and the plasma was harvested. Necropsy examination and histopathology were also performed on both groups of hens.

Russell's viper venom factor-X activating enzyme (RVV-X) was prepared by gel filtration chromatography[4]. Modified RVVT (mRVVT) measurements were performed on a semiautomatic clot timer. Citrated turkey plasma was mixed with rabbit brain cephalin and then incubated at 37°C for exactly 60 seconds. At this time, RVV-X and calcium chloride were added and the time interval to clotting was determined.

RESULTS

The experimental turkey hens developed a prolonged mRVVT within 3 hours following challenge (Figure 1). The mRVVT continued to elevate until death which occured 9 hours post-challenge. The results were statistically significant by 3 hours post-challenge and remained significant at subsequent time points. Histopathology indicated multifocal necrotizing hepatitis, renal and pulmonary congestion and lymphoid depletion in the spleen, bursa and thymus. Microthrombi were not found using phosphotungistic acid stain.

DISCUSSION

The prolonged mRVVT suggests that the pathogenesis of fowl cholera in turkeys is closely associated with DIC. The mRVVT was used to evaluate the hemostatic system of turkeys because the more common mammalian clotting time tests are ineffective for evaluating coagulation in avian plasma samples. The mRVVT results in this study indicates that the delay in coagulaopathy coincided with the clinical development of the disease. Even though the presence of microthrombi was expected in the examined tissues, the absence of microthrombi in mammals affected by DIC is not uncommon[2,6].

REFERENCES

1. Archer, R. Blood Coagulation. In Physiology and Biochemistry of Domestic Fowl. Volume II. Bell, J. and Freeman, B., editors. Academic Press, London. pp. 897-12. 1971.

2. Hinshaw, L. Overview of Endotoxic Shock. In Pathophysiology of Shock, Anoxia and Ischemia. Cowley,

A. and Trump, B., editors. Williams and Wilkins, Baltimore. pp. 219-38. 1982.

3. Johnson, G., Turrentine, M. and Swayne, D. Coagulation of Plasma from the Chicken: Phospholipids Influence on Clotting Rates Induced by Components of Russell's Viper Venon. Comp. Biochem. Physiol., 82B:647-53. 1985.

4. Kisiel, W. Molecular Properties of Factor V-Activating Enzyme from Russell's Viper Venom. J. Biol. Chem. 254:12230-34. 1979.

5. Park, N. Disseminated Intravascular Coagulation of Experimental Fowl Cholera in Chickens. Kor. J. Vet. Res. 22:211-18. 1982.

6. Ratnoff, O. Disseminated Intravascular Coagulation. In: Disorders of Hemostatsis. Ratnoff, O. and Forbes, C., editors. Gunne and Stratton, Inc. London. pp. 289-319. 1984.

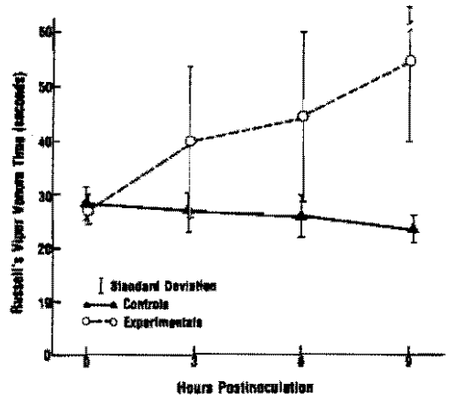

Figure 1. Average mRVVT in seconds of all experimental and control hens following intravenous injection of virulent *P. multocida*.

RESPIRATORY PATHOGENESIS OF FOWL CHOLERA: COMPARISON OF A VIRULENT STRAIN WITH VACCINE STRAINS

M. Matsumoto, M. Abrar, and J.G. Strain

College of Veterinary Medicine, Oregon State University, Corvallis, Oregon 97331-4802

Four strains of *Pasteurella multocida* were inoculated intratracheally in the order of 10^9 colony-forming-units (CFU) and their viable numbers in the respiratory tract and parenchymal organs were examined at 6 and 9 hours after inoculation. The four strains were; P-1059, a virulent strain; T-325, unencapsulated strain derived from P-1059; CU and M-9, vaccine strains. High numbers of organisms (10^5 to 10^9 CFU) were detected in the respiratory tract with all the strains. With M-9 strain, however, there was a significant decrease in the number of the organisms from 6 to 9 hours post inoculation (PI). All 4 strains invaded the blood stream by 6 hours PI. Strain P-1059 showed a highly significant increase in the numbers in the blood, liver, and spleen, while CU strain showed a significant increase only in the spleen. Strain M-9 decreased highly significantly in the blood from 6 to 9 hours PI. These results indicate; (1) all the 4 strains are highly invasive; (2) all strains but M-9 persist well in tissues; and (3) P-1059 multiplied rapidly in the organs, but CU showed limited multiplication.

FURTHER STUDIES ON THE IMMUNOGENICITY OF INFECTIOUS BURSAL DISEASE VIRUSES

Y.M. Saif, H.J. Tsai, and O. Tura

Food Animal Health Research Program,
Ohio Agricultural Research and Development Center,
The Ohio State University, Wooster, OH 44691

The antigenic diversity of infectious bursal disease viruses (IBDV) has been well documented. There are currently two recognized serotypes designated 1 and 2 and several subtypes of serotype 1 were detected. These antigenic differences were demonstrated by the use of the virus neutralization (VN) test.

The immunogenicity of IBDVs was also shown to vary significantly, depending on the strain of the virus. Other factors that influence the cross-protective characteristics of the virus are the dose of the virus used for vaccination and the dose of the virus used for challenge. In general, the cross-protective characteristics of the virus parallels the antigenic characteristics as indicated by the VN test. Briefly, there are 3 immunogenic types of the virus, two of which belong to serotype 1 "classic and variant viruses" and a serotype 2 group. Birds vaccinated or exposed to serotype 2 viruses are completely susceptible to serotype 1 viruses. Birds exposed to classic (isolated prior to 1984) serotype 1 viruses are protected from homologous viruses, and depending on the vaccine and challenge dose, could be protected from the variant (isolated since 1984) serotype 1 viruses. Birds exposed to variant viruses are fully protected from other variant viruses and from classic viruses. There are, yet, some quantitative difference in the degree of protection afforded by the classic viruses against variant virus challenge.

In recent studies, we investigated the effect of cell culture on the immunogenicity of two variant strains of IBDV. When the viruses were passaged (30 to 40 passages), they lost their pathogenicity but maintained their immunogenicity when used as inactivated vaccines. On the contrary, when these same viruses were used as live vaccines, they were not protective, leading to the speculation that the viruses might have lost their ability to replicate in the host although they maintained their full immunogenic potential.

It is of interest that viruses of serotype 1 "classic and variant" and serotype 2 cross-reacted similarly when examined by Western blotting and ELISA using polyclonal, monoclonal, and monospecific polyclonal antibodies to single viral proteins (VP2 and VP3). These results are helpful in understanding the previously obtained information on the cross-protective characteristics of the viruses. Obviously, there are common antigens that are detected by Western blot.

In other studies, we detected differences in molecular weights and in proportion of the proteins between the bursa-derived and tissue culture origin viruses. The bursa-derived viruses had a protein migration pattern similar to that described for tissue culture incomplete viruses. The biologic significance of these differences is currently not clear.

AN INFECTIOUS BURSAL DISEASE (IBD) VACCINE FOR DAY 18 IN OVO ADMINISTRATION IN CHICKENS

C.E. Whitfill[A], C.A. Ricks[A], D.G. Rowe[A], E.L. Owen[A],
E.E. Haddad[B], P.D. Andrews[B], J.K. Skeeles[B], and J.A. Thoma[C]

[A]EMBREX, Inc. P.O. Box 13989, Research Triangle Park,
North Carolina 27709,
[B]Department of Poultry and Animal Science,
[C]Department of Biochemistry and Chemistry,
University of Arkansas, Fayetteville, Arkansas 72701

INTRODUCTION

A new vaccine has been developed for IBD. The vaccine is composed of a mixture of an IBD vaccine and chicken serum (complex vaccine), and is administered *in ovo* at day 18 into the embryonated egg. The chicken serum contains a viral neutralizing factor (VNF). SPF birds were housed in isolation units for the study. Bursas were examined at 7, 9, 10, 11, 13, 15, 21, and 31 days of age and IBD antibody titers were evaluated at days 10, 21, and 31 of age. Birds were challenged with USDA:IBD at day 28 of age to test for immunogenicity.

RESULTS

Hy-Vac SPF embryonated eggs were injected *in ovo* at day 18. The complex vaccine is formulated to delay the emergence of the vaccine virus in the bird, and hence, bursal infection is delayed until approximately day 5-7 of age, at which time active immunity begins to develop.

All birds receiving the complex vaccine developed a protective IBD antibody titer, showed no body weight loss when compared to noninjected controls, and were protected against a USDA:IBD challenge given at day 28 of age. No mortality by day 10-31 of age was seen in any of the birds receiving the complex vaccine.

In contrast, chicks that received the IBD vaccine virus without chicken serum showed severe bursal atrophy by day 7 of age, indicating bursal infection began immediately after vaccination. These birds experienced body weight depression at days 10-31, as compared to the noninjected controls, had 23% mortality by day 10 of age, and did not develop IBD antibody titers by day 31 of age, due to early bursal infection and consequent immunosuppression.

CONCLUSION

In summary, the VNF:IBD complex vaccine can be safely administered *in ovo* at day 18 to Hy-Vac SPF embryonated eggs. The VNF in the vaccine delays virus infection of the bursa until approximately day 7 of age. At this time, active immunity against IBD begins to develop with increasing levels of IBD antibodies developing. No clinical manifestations resulted from the vaccination such as weight loss or mortality throughout growout.

PATHOLOGY AND CHARACTERIZATION OF IBD VIRUSES ISOLATED FROM WASHINGTON CHICKENS

Erina Alamsyah, A.S. Dhillon, and James Evermann

Washington State University-Puyallup,
Research and Extension Center, Department of Microbiology & Pathology,
Puyallup, Washington 98371

The pathology of 3 infectious bursal disease virus (IBDV) isolates of Washington poultry origin (WA-678, WA-770, and WA-994) and 7 other known IBDV strains (SAL, D-78, MO, OH, VAR-A, 2512, and IM) was studied in 3-week-old specific pathogen free chickens. The IM and 2512 strains resulted in illness and death in inoculated chickens. Macroscopic lesions were swollen gelatinous bursae with occasional hemorrhages in the plicae

followed by atrophy in the bursa of Fabricius. The WA-994 Washington isolate caused marked atrophy in the bursa of Fabricius. Moderate bursal pathology was elicited by the WA-678 Washington IBDV isolate. The WA-770 isolate resulted in a minimal atrophy. Clinical signs of morbidity or mortality were not present in groups inoculated with the 3 Washington isolates. Macroscopic lesions were limited to swollen gelatinous bursae with WA-994 at 4 days post inoculation necropsy. The IM and 2512 strains caused severe atrophy or bursa of Fabricius. Moderate atrophy was present with VAR-A and D-78 strains of IBDV. The SAL, MO, and OH strains of IBDV did not cause demonstrable atrophy of the bursa of Fabricius.

The serological relatedness of the 3 IBDV isolates was determined by virus neutralization test in chicken embryo fibroblast cells using the alpha method. The results of the cross-neutralization study showed that 3 Washington isolates were more closely related to serotype 1 than to serotype 2.

CHARACTERISTICS OF INFECTIOUS BURSAL DISEASE VIRUS-INDUCED IMMUNODEPRESSION IN CHICKENS

J.M. Sharma[A], Susan Belzer[A], and Jeff Rodenberg[B]

[A]Department of Veterinary PathoBiology,
College of Veterinary Medicine, University of Minnesota,
St. Paul, Minnesota 55108
[B]Solvay Animal Health, Inc., Mendota Hts., Minnesota 55120

Infectious bursal disease (IBD) is a common disease of chickens. The virus of IBD (IBDV) is widespread and tends to infect most commercial flocks of chickens early in life. Although the virus may cause mortality, the principal economic loss occurs because infected chickens become immunodepressed which leads to poor flock performance. IBDV-induced immunodepression leads to reduced ability of chickens to respond optimally to commonly used vaccines and enhanced susceptibility to opportunistic infections. Protecting commercial chickens against exposure to environmental IBDV continues to be a challenge because currently used vaccination strategies are not always effective.

Much effort has been expended to understand the characteristics of IBDV-induced immunodepression in chickens. Clinical and experimental data have shown that the virus infection results in reduced ability to produce antibodies to a number of infectious and non-infectious antigens. The virus has a predilection for bursa of Fabricius and causes marked atrophy of this organ. Acute bursal necrosis occurs within 24-48 hours of infection and abundant quantities of viral antigens can be readily detected in affected bursas. In a recent study, we used monoclonal antibodies to quantitate IgM-bearing cells in spleens of IBDV infected and virus free chickens. Our results revealed a marked reduction of IgM positive cells in virus-infected chickens. These data confirmed earlier indications that B lymphocytes bearing IgM receptors may be one of the main target cells for IBDV. Thus the humoral immunodeficiency following IBDV infection may be, in large part, due to the ability of the virus to infect and destroy antibody producing cells.

There is strong circumstantial evidence that IBDV may also cause depression of cellular immunity in chickens. The mechanism(s) of cellular immunodepression has been difficult to unravel. Previous studies have shown that the natural killer cell activity and certain macrophage functions remained intact in IBDV-infected chickens. Recently, we have attempted to examine the effect of the virus on T cells.

We noted that certain virulent strains of IBDV may cause extensive lesions in the thymus of infected chickens. Thymus is a primary lymphoid organ in chickens where T cell maturation takes place. The reason for the thymic lesion and its influence on T cell functions is not known. Immune fluorescent staining of frozen sections of thymic tissue from virus-infected chickens failed to detect evidence of virus antigen. Although we are currently examining the thymus by *in situ* hybridization for presence of viral replication, preliminary data with immune fluorescence indicate that the thymic lesions may not be caused by infection and lysis of T lymphocytes. We have also shown by cytometric analysis that IBDV did not alter the normal proportions of CT4 and CT8 subpopulations of T cells in spleen or circulations. Studies to understand how IBDV compromises the cell mediated immunity in chickens are continuing.

A COMMERCIALIZED DNA PROBE BASED DIAGNOSTIC TESTING SYSTEM FOR MYCOPLASMA GALLISEPTICUM IN CHICKENS AND TURKEYS

D. Ludtke, P. Tyrrell, A. Siefring, and I.D. Arnold

IDEXX Laboratories, Inc., One Idexx Drive,
Westbrook, Maine 04092

The global distribution and economic significance of Mycoplasma gallisepticum (MG) makes this organism one of the most important respiratory diseases in chickens and in turkeys[8]. In the United States there is a MG-control program within the National Poultry Improvement Plan which has been adopted by breeders to establish and maintain MG-clean chicken and turkey flocks[11].

A presumptive diagnosis of MG is made on the basis of a positive serologic test, together with clinical signs and historical information typical for this disease. A definitive diagnosis is made on the basis of culturing for isolation and identification of the organism.

Historically, researchers have documented the confusion surrounding the serological results for MG[1,10,11]. The rapid serum plate agglutination (SPA) test is designed to be a screening test. This test is intended to be very sensitive. The SPA is efficient at picking up positive birds, however, the specificity of this test is not as practical. False positive or non-specific reactions have been show to occur in birds vaccinated with inactivated, oil emulsion vaccines (*Staphylococcus aureus, Erysipelothrix,* and streptococcal bacterins) and clinical staphylococcal infections[10].

Hemagglutination inhibition (HI) test is used as a confirmatory test in flocks with reactors. The HI test is more specific than the SPA. However, it is less sensitive and develops 7-10 days later than the plate test. Additional problems encountered with the HI test, due mainly to antigenic variation, is a lack of uniformity among batch antigens and laboratory variation.

Commercially available ELISA test are more sensitive than the HI test and more specific than the SPA test, but are still prone to the non-specific reactor problems[1].

In the 1960's, molecular hybridization technology began its evolution[5]. The basis of molecular hybridization technology, the probe-target interaction, is not a new concept. For example, an antibody is a protein probe which interacts with a specific target antigen through a combination of hydrophobic, ionic and hydrogen bonding at certain sites. The nucleic acid probes interact with their complementary target sites, primarily through hydrogen bonding. The quantity of target sites ranges from tens to thousands, dependant upon the length of the hybrid. Researchers[2,3,4,6,7,9,12] have developed and used these molecular hybridization techniques to aid in the diagnosis of poultry diseases.

A specific and sensitive, non-radioactive DNA probe based method has been developed to detect MG in chicken and turkeys for commercial use, using the polymerase chain reaction (PCR). The test is designed to use a tracheal swab as the diagnostic sample. The MG DNA probes have a sensitivity to detect a minimum of 100 organism per tracheal swab sample. The MG DNA probes are specific to MG and do not detect DNA from *M. synoviae, M. gallinaceum, M. gallinarum, M. meleagridis, M. pullorum,* or *M. iowae.* The MG DNA probes also have the capability to discriminate the virulent strain of MG from the F strain of MG.

There are many benefits to using a DNA probe as a confirmatory test. The MG DNA probe does not have the problems that culture methods have such as overgrowth of *M. gallinarum* and *M. gallinaceum* or contamination with other organisms. Other problems experienced with culture such as no growth in subculture or a culture positive but immunofluorescent assay negative also do not occur with the MG DNA probe. The MG DNA probe test produces the results in a day and one half compared to the culture method which takes 5 days for immediate growth, or up to 28 days of holding the culture broth/or plate before proving negative. The disadvantages of the DNA probe test kit includes the low sample size, contamination of sample through careless handling, and the special equipment required to run the test kit (PCR machine, pipettors/tips, etc.).

In addition, the MG DNA probe test does not have the same problems as the serology tests, such as the time lag for seroconversion, the reagent problem, or infection without seroconversion. However, the disadvantage of the limited sample size and the cost when compared to the serological tests, makes the DNA probe test impractical as a screening test.

The speed and accuracy of a commercialized DNA based test for the definitive diagnosis of MG means significant economic benefits for the poultry industry.

REFERENCES

1. Avakian, A. P., S. H. Kleven, and J. R. Glisson. Evaluation of the specificity and sensitivity of two commercial enzyme-linked immunosorbent assay kits, the serum plate agglutination test, and the hemagglutination-inhibition test for antibodies formed in response to Mycoplasma gallisepticum. Avian Dis. 32:262-272. 1988.

2. Hasan, J. A. K., I. T. Knight, C. R. Tate, E. T. Mallinson, R. G. Miller, R.R. Colwell, and S. W. Joseph. Evaluation of Radiolabeled and Colorimetric DNA probes in Comparison with an Antigenic Screening Assay for the Detection of Salmonella from Poultry Farms. Avian Dis. 35:397-402. 1991.

3. Hyman, H. C., S. Levisohn, D. Yogev, and S. Razin. DNA probes for Mycoplasma gallisepticum and Mycoplasma synoviae: application in experimentally infected chickens. Vet. Microbio. 20:232-337. 1989.

4. Keam, L., J. J. York, M. Sheppard, and K. J. Fahey. Detection of Infectious Laryngotracheitis virus in chickens using a non-radioactive DNA probe. Avian Dis. 35:257-262. 1991.

5. Keller, G. H. and M. M. Manak. DNA Probes. Stockton Press, New York, New York. pp. 1-23. 1989.

6. Kleven, S. H., G. F. Browning, D. M. Bulach, E. Ghiolas, C. J. Morrow, and K. G. Whithear. Examination of Mycoplasma gallisepticum strains using restriction endonuclease DNA analysis and DNA-DNA hybridisation. Avian Path. 17:559-570. 1988.

7. 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.

8. 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.

9. Nascimento, E. R., R. Yamamoto, K. R. Herrick, and R. C. Tait. Polymerase chain reaction for detection of Mycoplasma gallisepticum. Avian Dis. 35:62-69. 1991.

10. Yoder, H. W., Jr. Nonspecific reactions to Mycoplasma serum plate antigens induced by inactivated poultry disease vaccines. Avian Dis. 33:60-68. 1989.

11. Yoder, H. W., Jr. Mycoplasma gallisepticum infection. 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. 198-212. 1991.

12. Yogev, D., S. Levisohn, and S. Razin. Genetic and antigenic relatedness between Mycoplasma gallisepticum and Mycoplasma synoviae. Vet. Microbio. 19:75-84. 1989.

HETEROGENEITY AMONG MYCOPLASMA GALLISEPTICUM STRAINS DETECTED BY DNA PROBE

M.Y. Johara, E.R. Nascimento, and R. Yamamoto

Department of Epidemiology & Preventive Medicine,
School of Veterinary Medicine, University of California,
Davis, California 95616

A common problem encountered in epidemiologic studies of *Mycoplasma gallisepticum* (MG) infections is the identification of the strains involved in different flocks. This study examined the applicability of two nucleic acid probes, namely an MG species-specific probe (pMG-1)[1] and an *Escherichia coli* ribosomal RNA (rRNA) probe[2] for differentiation of MG strains.

Twenty-two strains of MG were studied and they were grouped as (i) standard strains, (ii) F strains, (iii) variant strains, (iv) field F strains, and (v) field non-F strains. DNA from these strains were extracted by a miniprep procedure, digested with *Hin*dIII, and analyzed by restriction endonuclease analysis (REA) followed by Southern blot hybridization using the two ^{32}P-labeled nucleic acid probes.

Southern blot hybridization was able to detect strain variation that was not evident on REA. Hybridization patterns of the F strains were unique compared to all the other isolates. The strains tested could be classified into two broad clusters, namely F and non-F strains. Of the two probes tested by Southern hybridization, the patterns obtained with pMG-1 revealed differences within these two clusters that were not detected by the *E. coli* rRNA probe. Southern hybridization using pMG-1 seems promising as a molecular tool for epidemiologic studies of MG infections with regards to identification of strains in out-

break investigation, patterns of intra and inter flock transmission, and introduction of strains into new location.

REFERENCES

1. Khan, M.I.; B.C. Kirkpatrick, R. Yamamoto. *Mycoplasma gallisepticum* species and strain-specific recombinant DNA probes. Avian Pathol. 18:135-146. 1989.

2. Stull, T.L., J.J. LiPuma, and T.D. Edlind. A broad-spectrum probe for molecular epidemiology of bacteria: ribosomal RNA. J. Infect. Dis. 157:280-286. 1988.

THE 6/85 STRAIN—A NEW LIVE VACCINE FOR MYCOPLASMA GALLISEPTICUM

C. S. Roney, R. D. Evans, and P. K. Storm

Intervet Inc., 405 State Street,
Millsboro, Delaware 19966

INTRODUCTION

Mycoplasma gallisepticum (MG) is a disease of considerable historical significance in the poultry industry. MG causes respiratory disease in both young and mature chickens and turkeys and is referred to as chronic respiratory disease (CRD). In sexually mature birds, MG causes a drop in egg production that is costly to the egg producing industry.

Eradication of MG through test and slaughter is the preferred method of control. However, the emergence of multi-age complexes in the commercial layer industry makes depopulation and eradication impractical.

DISCUSSION

Vaccination for MG with inactivated cultures was reported as early as 1951[6] and further work was done using adjuvants and oil emulsions[4,5]. These inactivated bacterins are available today, however, they tend to be expensive and difficult to administer.

The F-strain, an attenuated live vaccine, was first used successfully in the late 1970's[1] and continues in use. The advantages of live vaccines are ease of administration and lower cost.

The 6/85 strain of MG is a patented, modified strain of a domestic field isolate (pat. no. 5,064,647). As a vaccine, the 6/85 strain has shown to protect chickens against clinical signs and egg drops from MG field challenge. It is apathogenic for chickens and turkeys and is genetically stable[2,3].

A disadvantage of the F-strain is that it is virtually fully pathogenic to turkeys. Table 1 shows essentially no airsac lesions in turkeys 10 and 28 days post-inoculation with the 6/85 strain. Both the F-strain and the R-strain produce considerable airsacculitis. Note that the 6/85 strain is not recoverable at 28 days, another safety benefit.

The ability of the 6/85 strain to protect against clinical signs from the R strain challenge is shown in Table 2. Compared to the F-strain, the 6/85 strain showed better protection at 4 weeks post-vaccination.

In a field situation, 100,000 pullets were vaccinated with the 6/85 strain by fine spray in a commercial pullet house. Eight weeks post-vaccination, 30 pullets were challenged with the virulent R-strain. Table 3 shows protection against airsac lesions in field vaccinated birds.

Since the 6/85 strain is a derivation of a virulent field isolate, genetic stability is essential. Table 4 shows average airsac lesion scores in chickens and turkeys inoculated with the R-strain, the 6/85 master seed and the 6/85 strain after 10 backpassages. There is no increase in virulence for the 6/85 strain after 10 backpassages[2]. Current work also shows no change in the organism electrophoretically after 10 backpassages[3].

Further studies show that the 6/85 strain is not readily transmitted either horizontally or vertically making it a safer product for use in heavily populated poultry areas. Vaccinated birds exhibit a modest antibody titer detectable on the ELISA test but are not positive on the serum plate agglutination test allowing differentiation between vaccinated birds and field challenge.

SUMMARY

The 6/85 strain of MG is a modified live MG vaccine that is safe for chickens and turkeys, provides protection against MG induced clinical signs and egg drop in

chickens and does not revert to virulence when backpassaged in chickens.

REFERENCES

1. Carpenter, T. E., K. F. Miller, R. F. Gentry, L. D. Schwartz and E. T. Mallison. Vaccination with F-strain Mycoplasma gallisepticum to Reduce Production losses in Layer Chickens. Avian Dis. 25:404-409. 1981.

2. Evans, R. D., and Y. S. Hafez. Evaluation of a Mycoplasma gallisepticum strain exhibiting reduced virulence for prevention and control of poultry mycoplasmosis. Accepted for publication. Avian Dis.

3. Evans, R. D., Hafez and C. S. Schreurs. Demonstration of the Genetic Stability of an MG strain following in vivo passage. Accepted for publication. Avian Dis.

4. Lin, M. Y., and S. H. Kleven. Evaluation of attenuated strain of Mycoplasma gallisepticum and vaccines in young chickens. Avian Dis. 28:88-99. 1984.

5. Panigvahy, B. L., L. C. Grumbles, and C. F. Hall. Immunogenic potency of oil-emulsified Mycoplasma gallisepticum bacterins. Avian Dis. 25:821-826. 1981.

6. Prier, J. E., and G. Dart. Immunological and serological studies on infectious sinusitis of turkeys. Cornell Vet. 41:38-46. 1951.

Table 1. Mean lesion scores and recovery rates of turkey poults 10 and 28 days post-challenge.

	10 Days Post-challenge		28 Days Post-challenge	
Challenge	**Airsaculitis***	**% MG Recovery**	**Airsaculitis**	**% MG Recovery**
R Strain	3.20	100	3.75	100
6/85 Strain	0.20	100	0.50	0
F Strain	2.60	100	2.60	100
Neg. control	0.40	0	0.20	0

*0 = bright clear airsacs; 1 = slight cloudiness; 2 = heavy clousiness; 3 = heavy cloudiness, or cheesy exudate, or thickening; 4 = any combination of (3)

Table 2. Protection v. R-strain challenge in chickens vaccinated with the F-strain and the 6/85 strain.

	4 Weeks Post-vaccination		12 Weeks Post-vaccination	
Treatment Group	**Mean Lesion Score**	**Protection***	**Lesion Score**	**Protection***
Neg. control	0.06	NA	0.40	Yes
F Strain	1.00	No	1.20	Yes
6/85 Strain	0.26	Yes	0.70	Yes
Pos. control	1.26	No	2.10	No

*Protection as compared to positive control (p = .05); challenge = R Strain 109/ml aerosol

Table 3. Mean lesion scores of chickens vaccinated with the 6/85 strain and challenged with the R-strain (M = 30).

Vaccination	Mean Lesion Score
6/85 strain	1.37*
un-vaccinated controls	2.37

*The difference is significant at p = .05.

Table 4. Mean lesion scores following infection with MG isolates.

	Turkey	Chicken
Neg. Control	0.0	0.0
R Strain	3.2	2.6
6/85 Strain	0.4	0.3
6/85 Strain P10	0.7	0.3

SAFETY AND EFFICACY OF THE TS-11 STRAIN OF MYCOPLASMA GALLISEPTICUM (MG) IN CHICKENS AND TURKEYS

Dennis E. Page and Nikki Kinney

Select Laboratories, Inc.
P.O. Box 2497, Gainesville, Georgia 30503

The safety of a live MG vaccine prepared with the ts-11 strain was examined in commercial broilers, turkeys, and leghorn chickens. In one study, poults and broiler chicks were inoculated directly in the thoracic air sac with 7.5 x 10^7 ccμ of ts-11. Hatchmate turkeys and broilers were inoculated in the same manner with 4.0 x 10^7 ccμ of a live F-strain vaccine.

Twenty-eight days later, all surviving birds were necropsied and scored for MG infection. The broilers receiving the ts-11 had 100% survive with no clinical signs of MG. The F-strain vaccinated broilers had 60% survive and 90% infection in the survivors.

The ts-11 vaccinated turkeys had 80% survive vaccination with 17% of the survivors infected. The F-strain vaccinated turkeys had 17% survive vaccination with 100% infection in the survivors.

Vaccination challenge studies demonstrated excellent challenge protection from R-strain challenge when compared to birds vaccinated with F-strain vaccine and challenge controls.

MYCOPLASMA GALLISEPTICUM F STRAIN ISOLATED FROM COMMERCIAL BREEDER TURKEYS

David H. Ley, Alan P. Avakian, and J. Edward Berkhoff

College of Veterinary Medicine, North Carolina State University,
4700 Hillsborough St., Raleigh, NC 27606

Mycoplasma gallisepticum (MG) is an important respiratory tract pathogen of commercial poultry and is regarded as an economically significant cause of disease confronting poultry production worldwide[4]. Strategies to reduce the adverse economic impact of MG disease in commercial poultry include: (1) surveillance, control and eradication programs as exemplified by the National Poultry Improvement Plan (NPIP); (2) use of antimicrobics; and 3) vaccination with modified live and/or killed products. F strain, a live naturally low virulent MG is widely used, particularly in areas of endemic infection, to reduce egg production declines in commercial layer chickens. However, F strain vaccinated flocks do not perform as well as MG clean flocks[1], and F strain is virulent for experimentally infected turkeys[2,3]. The possibility of F strain-caused outbreaks of MG in commercial turkeys has

existed since the vaccine became available. However, until recently this possibility was not easily investigated due to the difficulty of identifying MG isolates as F strain or wild type.

In February 1991, a flock of breeder turkeys experienced respiratory signs, sinusitis, airsacculitis, and increased mortality. MG was isolated in March, the North Carolina Department of Agriculture was notified, and appropriate control measures were initiated. Ultimately, however, this outbreak involved several breeder flocks of an integrated turkey production company before the last infected flock was identified in May 1991. During this time, MG was also isolated from a flock of commercial layer-type chickens in close proximity to the index turkey flock. SDS-PAGE (protein finger-printing) and restriction endonuclease analysis (DNA finger-printing) indicated that these isolates were identical with each other and examples of MG F strain. Additionally, MG isolates from two of the affected turkey breeder flocks were submitted to Dr. R. Yamamoto (Dept. of Epidemiology and Preventive Medicine, School of Veterinary Medicine, University of Calif., Davis, CA 95616). Both isolates were identified in Dr. Yamamoto's laboratory as MG F strain by use of an F strain-specific DNA probe and polymerase chain reaction. This case demonstrates: (1) the potential of MG F strain to cause severe disease in turkeys under field conditions, and (2) the application of new technologies for disease investigation and molecular epidemiology.

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. Lin, M. Y. and S. H. Kleven. Pathogenicity of two strains of Mycoplasma gallisepticum in turkeys. Avian Dis. 26:360-364. 1982.

3. Lin, M. Y. and S. H. Kleven. Evaluation of attenuated strains of Mycoplasma gallisepticum as vaccines in young chickens. Avian Dis. 28:88-99. 1984.

4. Yoder, H. W., Jr. Mycoplasma gallisepticum infection. 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. 198-212. 1991.

POSTERS

DRUG SENSITIVITY AND PLASMID PROFILES OF AVIAN ESCHERICHIA COLI IN MOROCCO

A. Amara, Z. Ziani, N. Assali, and K. Bouzoubaa

Institute of Agronomy and Veterinary Medicine,
Hassan II, Avian Diseases Department,
BP 6202 Rabat-Institute, Morocco

More than 250 cultures of *Escherichia coli* were collected. They were isolated from cases of avian colibacillosis in different areas of Morocco.

To determine the antibiotype profiles, drug sensitivity tests were conducted on these *E. coli* isolates using 16 antimicrobial drugs. The antimicrobial drugs used included all the families that are usually marketed to control avian colibacillosis in Morocco.

The results showed a high percentage of strains that were resistant to Erythromycin (94.19%), to Sulfonamides (79.84%), and to Oxytetracyclin (67.44%). Multiple resistance was also observed. Indeed, 45.61% of *E. coli* cultures in this study were resistant at least to 6 antibiotics. Five cultures were resistant to 13 antibiotics. Among 78 avian *E. coli* antibiotypes identified, 19 represented 60% of the isolates. Furthermore, 30% of the isolates included 5 antibiotypes. Thus, they can be used as epidemiological markers. More interestingly, about 20% of the *E. coli* cultures concerned with this study were resistant to quinolones (Nalidixic acid, Flumequine, Oxolinic acid, and Enrofloxacin).

For many strains of avian *E. coli*, we tried to extract and to purify the plasmids. To determine their plasmid profiles, agarose gel electrophoresis was performed.

The results showed, for the studied *E. coli* strains, at least one plasmid band was detected. Some of them contained more than one plasmid. Many samples had the same plasmid profile. Finally, a large plasmid was observed on most *E. coli* cultures under this study. Since most of the studied strains for plasmid profiling were resistant to Oxytetracyclin, Chloramphenicol, Erythromycin, Sulfonamides, and Trimethoprim-Sulfamethexazol, this large plasmid could be coding for resistance to these antimicrobial drugs.

SWOLLEN HEAD SYNDROME IN POULTRY FLOCKS IN BRAZIL

Clarice W. Arns[A] and H.M. Hafez[B]

[A]Inst. of Biology, Department of Microbiology and Immunology,
Unicamp, Campinas, Brazil
[B]State Veterinary Laboratory, 7000 Stuttgart 1, Germany

INTRODUCTION

A pneumovirus is known to be the primary causative agent of turkey rhinotracheitis (TRT) and appears to be associated with swollen head syndrome (SHS), egg production losses and respiratory diseases complex in chickens.

SHS has become a serious economic problem in many countries. The disease has been described and identified in

all countries where tests for it have been carried out[2,3,4,5,6,7,10,11,12,16,17,18].

The initial aetiological hypotheses were bacteria (*E. coli*), or coronavirus plus bacteria[10]. However, Picault et al.[13,14] did isolate a TRT-like virus from SHS diseased chickens in France. Since this virus was shown to be serologically related to TRT-virus, produced typical rhinotracheitis symptoms in SPF-turkey poults, and clinical coryza-like signs in SPF chickens and conventional guinea fowl after experimental infection, it was concluded that TRT virus is a common infectious agent affecting the upper respiratory tract of different poultry species.

Many broiler, broiler breeder and layer flocks reared in different areas in Brazil during 1991 showed clinical manifestation of SHS and serological evidence of infection. This paper presents a description of SHS disease symptoms and the serological results obtained in those flocks.

MATERIAL AND METHODS

Serological Survey. Serum samples collected from 10 broiler flocks were examined for the presence of antibodies to the TRT-virus using neutralization tests. Five flocks had SHS symptoms, 2 respiratory manifestations, and 3 were apparently healthy. Sera from 25 broiler breeder flocks were also collected. Fifteen flocks showed SHS clinical signs, 6 had respiratory symptoms, and 4 were apparently healthy. In adddition, 8 layer flocks were tested. Four flocks had SHS signs, 2 respiratory manifestations, and 2 were appparently healthy. All flocks were reared in the States of Saõ Paulo, Minas Gerais and Santa Catarina in Brazil. Serum samples were collected between May and November 1991.

Serum neutralization tests (SN). Tests were carried out in the CER-cell line[15] in microtitre plates using 100 $TCID_{50}$ of TRT virus strain STG 761/88[9]. Neutralisation titres were expressed as base 2 logarithmus of the reciprocal of the highest dilution of serum that prevented cytopathic effects. Sera with titre ≥ 3 $\log_2$ were considered positive.

TRT-ELISA. To confirm the results of the serum neutralization tests, serum samples from 3 broiler flocks were tested for the presence of antibodies to turkey rhinotracheitis virus using the ELISA method[8]. TRT-STG 761/88 virus isolate was used as an antigen.

CLINICAL OBSERVATIONS

Broilers. The disease was observed mostly in broilers between 4 and 6 weeks of age. The mortality rate was variable from negligible to 9%. The clinical signs identified were depression, decrease in food intake, nasal sneezing which progressed to conjunctivitis, followed by facial oedema starting around the eye, extending over the head, and descending to the submandibular tissue.

Post mortem examination found lesions generally located in the head region and revealed a caseous haemorrhagic exudate in the subcutis and mild rhinitis.

Bacteriological examination of internal organs revealed negative results. *Escherichia coli* could be isolated from the subcutaneous exudate over the head and from some tracheal swabs.

Broiler breeders. Symptoms similar to SHS signs were observed in breeder flocks between 32 and 62 weeks of age. The first signs were apathy, sneezing, conjunctivitis, and uni- or bi-lateral facial swelling which ascended over the head. These conditions were followed by cerebral disorientation, torticollis, and opisthotonus. Many of the affected birds, which showed nervous signs were unable to move, showed severe prostration, completely ceased food and water intake, and usually died as a result of starvation. The morbidity rate ranged between 8 and 10%, while the mortality was variable and relatively low (about 2-3%). The symptoms were mostly accompanied with a drop in egg production reaching 1-4% for 1 to 2 weeks.

Necropsy revealed localized lesions over the head as described in broilers. Animals with nervous manifestations and otitis of the middle ear with pus accumulation were constantly observed. Inflammation of the ovary, oviduct, and peritoneum was also detected.

Layers. Similar signs were observed in layer flocks. The morbidity was up to 8% and the mortality rate was variable from negligable to 2%. The signs were mostly accompanied with low egg production (2-4%) and in some cases poor shell quality was observed (2%).

RESULTS OF THE SEROLOGICAL SURVEY

Sera from 3 broiler flocks suffering from SHS tested positive for antibodies to TRT-virus. These results were confirmed in ELISA tests. No antibodies were found in sera collected from 7 flocks: 2 with SHS, 2 with respiratory signs, and 3 apparently healthy (Table 1).

Testing serum samples from breeder flocks revealed that antibodies to TRT-virus were present in all 15 flocks with SHS signs, 2 with respiratory manifestations and one with nervous symptoms. No antibodies were found in sera collected from 7 flocks: 4 without any disease history, and 3 with respiratory signs (Table 2).

Antibodies were detected in sera from 4 layer flocks with SHS signs (Table 3). However, examination of the sera collected from other layer flocks (with respiratory signs and apparently healthy flocks) revealed negative results.

DISCUSSION

The clinical signs observed and described in many broiler, broiler breeder and layer flocks accompanied with

the detection of antibodies to TRT virus confirm the presence of the pneumovirus infection in Brazil. The results are similar to those reported in the "Swollen head syndrome" in many countries worldwide.

The antibody to the TRT-virus was detected in serum samples collected from 3 broiler, 15 breeder and 4 layer flocks with SHS disease history. This indicates that infection with the virus is widespread in these areas in Brazil. These results are consistent with previous investigations which demonstrated that antibodies to TRT-virus were in the sera of broilers, broiler breeders, layers and conventional guinea fowl which were affected with SHS[1,8,13,14,17].

Further investigations on the distribution of infection in other areas in Brazil as well as trials on virus isolation are in progress.

REFERENCES

1. Cook, J.K.A., C.A. Dolby, D.J. Southee, and A.P.A. Mockett. Demonstration of antibodies to turkey rhinotracheitis virus in serum from commercially reared flocks of chickens. Avian path. 17:403-410. 1988.

2. Decanini, E.L., E.C. Miranda, and F.X. Le Gros. Swollen head syndrome in heavy breeders in Mexico. 40th West. Poult. Dis. Conf. Acapulco, Mexico. April 24-27, pp. 158-161. 1991.

3. Diaz de Espada, E. and M.E. Perona. Etiologia del Sindrome de cabeza hinchada. Revista da la seccion Espanola de La Asociacion. Mundial de Avicultura cientifica 40:36-42. 1984.

4. Drouin, P., J.Y. Toux, and J.P. Picault. L'Aviculteur 460:93-96. 1985. Cited after Picault et al (11).

5. El Houadfi, M., A. Hamam, J. Vanmarcke, and J.K.A. Cook. Swollen head syndrome in broiler chickens in Morocco. Proc. 40th West. Poult. Dis. Conf. Acapulco, Mexico. April 24-27, pp. 126- 127. 1991.

6. Goren, E. Een "nieuwe ziekte" bij de kip; diagnostische bevindingen. Tijdschr. Dier geneeskd. deel. 110:1076-1077. 1985.

7. Hafez, H.M. Swollen Head Syndrome: Potential risk for broiler and broiler breeders. XXVI-th Ann. Conv. Israel Branch WPSA, Zichron, Yaacov. pp. 39. 1988.

8. Hafez, H.M.,and U. Löhren. Swollen Head Syndrome: Clinical observations and serology in West Germany. Dtsch. tierärztl. Wschr. 97:322-324. 1990.

9. Hafez, H.M., and F. Weiland. Isolierung des Virus der Rhinotracheitis der Puten (TRT). Tierärztl. Umsch. 45:103-111. 1990.

10. Morley, A.J., and D.K. Thomson. Swollen head syndrome in broiler chickens. Avian. Dis. 28:238-243. 1984.

11. O 'Brien, J.D.P. Swollen head syndrome in broiler breeders. Vet.Rec. 117:619-620. 1985.

12. Perelman, B., M. Meroz, and Y.Samberg. Swollen head syndrome in broiler breeders in Israel. Vet. Rec. 123:444. 1988.

13. Picault, I.P., P. Giraud, P. Drouin, M. Guittet, G. Bennejean, L. Lamande, D. Toquin, and C. Gueguen. Isolation of a TRT-like virus from chicken with swollen-head syndrome. Vet. Rec. 121:135. 1987.

14. Picault, I.P., 1. Lamande, C. Gueguen, P. Giraud, P. Drouin, V. Kles, M. Guittet, and G. Bennejean. Etiology of swollen head syndrome. Joint Meeting of P.V.S.G. - E.E.C. - W.G. 1987. No. 7 - WPSA and WVPA Versailles, France, 12-13 Nov. 1987

15. Smith, A. L., G. H. Tignor, and K. Mifune. Isolation and assay of rabies serogroup viruses in CER cells. Intervirology 8:92- 99. 1977.

16. Wyeth, P.J. The rhinotracheitis, swollen head syndrome cause heavy loss. Poultry Digest. 49:16-18. 1990.

17. Wyeth, P.I., N.I., Chettle, R.E., Gough, and M.S. Collins. Antibodies to TRT in chickens with swollen head syndrome. Vet. Rec. 120:286-287. 1987.

18. Zellen, G.K. Case report: Swollen-head syndrome in broiler chickens. Proc. 37th West. Poult. Dis. Conf., Davis, California. pp. 139. 1988.

Table 1. Results of the neutralisation tests for turkey rhinotracheitis virus(STG 761/88 strain) in serum samples from broiler flocks.

State	No. of flocks	Clinical signs			No. of Sera	SN-results		No. of pos. flocks
		SHS	Resp.	No.		pos.	neg.	
SP	5	4*	1	0	114	82	32	3
MG	2	1*	1	0	26	0	26	0
SC	3	0	0	3	38	0	38	0
Total	10	5	2	3	178	82	96	3

SP = Saö Paulo
MG = Minas Gerias
SC = Santa Catarina
* = One flock showed negative serological results

Table 2. Results of the neutralisation tests for turkey rhinotracheitis virus(STG 761/88 strain) in serum samples from broiler breeder flocks.

State	No. of flocks	Clinical signs			No. of Sera	SN-results		No. of pos. flocks
		SHS	Resp.	No.		pos.	neg.	
SP	15	9	4*	2	289	134	155	11
MG	6	3	2**	1	108	35	73	4
SC	4	3	0	1	90	28	62	3
Total	25	15	6	4	487	197	290	18

SP = Saö Paulo
MG = Minas Gerias
SC = Santa Catarina
* = Two flocks showed positive serological results
** = One flock showed positive serological results and nervous manifestation

Table 3. Results of the neutralisation tests for turkey rhinotracheitis virus(STG 761/88 strain) in serum samples from layers flocks.

State	No. of flocks	Clinical signs			No. of Sera	SN-results		No. of pos. flocks
		SHS	Resp.	No.		pos.	neg.	
SP	6	3	1	2	48	14	34	3
MG	2	1	1	0	24	6	18	1
Total	8	4	2	2	72	20	52	4

SP = Saö Paulo
MG = Minas Gerias

AVIAN INTESTINAL SPIROCHETOSIS

I.G.M.I. Bosch

Poultry Health Institute Holland,
Box 43, 3940 AA Doorn, Holland

Intestinal spirochaetes were found in laying hens, in reproductive flocks and occasionally in broilers (Tompkins et al, 1986; Dwars et al., 1989). Clinical symptoms are retarded growth, loss in production, decreased yolk pigmentation and changes in consistency of both main intestinal and caecal droppings (Davelaar et al., 1986; Griffiths et al, 1987). In one study concerning a routine screening for spirochaetes in caecal feces from both laying and reproductive flocks, nearly all positive cases were related to intestinal disorders (Dwars et al., 1989).

The experimental infection with avian intestinal spirochaetes in broilers up to 3 weeks of age resulted in an increase of the amount of crude fat present in main intestinal feces, a decrease in the plasma caroten concentration, increased levels of alkalin phosphatase and growth retardation (Dwars et al., 1991). In practice, the most important economic losses may be expected in commercial laying and in reproductive flocks. Signs observed both in the field and under experimental conditions are; growth retardation, diarrhea, lowered egg production, and pale

egg yolk. The crude fat contents of main intestinal feces of chronically infected adult laying hens, kept on a commercial laying feed were also increased compared to control birds.

BACKYARD CHICKENS AS A RESERVOIR OF PULLORUM DISEASE AND FOWL TYPHOID IN MOROCCO

K. Bouzoubaa, J.G. Bell, and K. Lemainguer

Institute of Agronomy and Veterinary Medicine,
Hassan II, Avian Diseases Department,
BP 6202 Rabat-Institute, Morocco

An epidemiological study was carried out to determine the prevalence of pullorum disease and fowl typhoid in village chickens in Morocco. Seventeen villages were visited in each of the regions of Khemis Zmamra (region 1) and Tamelalt Sidi Rahal (region 2).

This study was based on serological analysis using two agglutination tests: seroagglutination (SA) and microagglutination (MA), and indirect-ELISA. Bacteriological analysis was carried out on cloacal swabs and samples from the litter.

The SA, MA, and indirect-ELISA revealed the percentage of seropositivity respectively of 7.6%, 29%, and 57% for region 1 and 4.4%, 18%, and 58% for region 2. Of these three tests, the indirect-ELISA was very sensitive. However, the possibility of cross reactions with the antigens of Gram-negative bacteria cannot be excluded.

Bacteriological analysis confirmed the existence of *Salmonella pullorum* and *Salmonella gallinarum* in the sample agent (10% positive in region 1 and 6% in region 2).

This study showed the existence of pullorum disease and fowl typhoid in village poultry farms in the two regions visited. The village chicken could play a role as a reservoir for *S. pullorum* and *S. gallinarum*. This constitutes a considerable threat for the poultry industry in Morocco.

NEWCASTLE DISEASE VIRUS AS A CAUSE OF ENCEPHALITIS AND INTRACYTOPLASMIC NEURONAL INCLUSION BODIES IN CHICKENS

A.J. Davis, S.J. Jenkins, and J.C. Rhyan

U.S. Department of Agriculture,
Animal and Plant Health Inspection Service, Veterinary Services,
National Veterinary Services Laboratories,
P.O. Box 844, Ames, Iowa 50010

During the course of an avian nephritis virus (ANV) pathogenicity study, four ANV-inoculated chickens housed together in the same isolator cage became sick. All of the other inoculated birds in different cages remained clinically normal. Two of the four sick birds were euthanized for pathological evaluation. Gross examination revealed bilateral conjunctival hemorrhages with bursal and thymic atrophy in one bird and a focal 2 mm pale area in the liver of the other bird. Light microscopy revealed lymphoid depletion in the bursa and thymus of both birds. Multifocally, within the kidneys of both birds were moderate interstitial infiltrates of plasma cells often associated with focal necrosis of tubular epithelial cells. The most striking changes, however, were present in the brain and were characterized by moderate, diffuse, lymphocytic meningoencephalitis with neuronal necrosis and mild multifocal spongiosis of the neuropil. Many neurons,

especially in the cerebrum, contained single and often multiple, eosinophilic, intracytoplasmic inclusion bodies.

Ultrastructural examination of brain tissue revealed numerous, granular and fibrillar, 18-20 nm diameter, intracytoplasmic, and less frequently intranuclear inclusions within neurons. These inclusions were morphologically consistent with a paramyxoviral etiology. Ultrastructural evidence of viral infection in the kidney was not seen.

Fresh tissues from these birds yielded Newcastle disease virus (NDV) on virus isolation. Avian nephritis virus was not isolated. Based upon these findings, it was concluded that these birds became ill due to accidental NDV infection. The source of infection was believed to be cross-contamination from nearby isolator cages that contained NDV-infected experimental chickens.

While encephalitis is not uncommon with NDV infection, intracytoplasmic inclusion bodies, especially in neurons, are infrequently reported. In this poster, we report morphologic details of such an unusual finding in the brain of two chickens.

STANDARDIZED BROILER FIELD NECROPSY REPORTING

Philip H. Davis[A] and Quinton J. Hanssens[B]

[A]Livestock Technical Service, SmithKline Beecham Animal Health Co.,
Rt. 5, Box 18, Florence, Alabama 35630
[B]Hoechst-Roussel Agri-Vet Co.,
811 Orleans Dr., Rogers, Arkansas 72756

The objective of the presentation is to suggest a standardized format for reporting the results of field necropsy sessions conducted by technical specialists for the broiler industry. While conducting necropsy sessions where large numbers of broilers are examined for coccidiosis, gross lesions of other conditions are observed. A standardized format for reporting these findings would be of benefit to the broiler production manager and other technical specialists who perform the same activities with that broiler company.

A survey was conducted and it was found that many professionals were using some type of form for collection of data from field necropsy sessions and that many of them were similar. Using these forms as an example, a composite was created that included the data on these forms. Field use of this form by the investigators to collect and report to production managers has proven of value. The form was placed on Symphony, Lotus 1-2-3 and Professional Write in order to facilitate the storage and retrieval of data and to graph the major groupings of gross lesions seen. Graphics programs were used to display these groupings.

SUMMARY

The use of this format can be personalized as required by the individual or reported without identifying the investigator or their employer.

A standardized reporting format will allow the broiler manager to review data from several sources and to benefit from information that might not otherwise be collected or reported.

The investigators presented this concept at the National Meeting on Health and Condemnations to a newly formed group, The Association of Avian Technical Service Veterinarians, and were encouraged to pursue efforts to develop a standardized reporting format. Comments on this type of activity may be made to either of the investigators and they will report these to this same group at its next meeting.

SEVERE MORTALITY IN BROILER CHICKENS ASSOCIATED WITH MYCOPLASMA SYNOVIAE AND PASTEURELLA GALLINARUM

R. Droual[A], H.L. Shivaprasad[A], C.U. Meteyer[A],
D.P. Shapiro[B], and R.L. Walker[C]

California Veterinary Diagnostic Laboratory System,
University of California, Davis
[A]Fresno Branch, 2789 S. Orange Ave., Fresno, California 93725
[B]Zacky Farms, 315 North H. Street, Fresno, California 93701
[C]Davis Central Laboratory, P.O. Box 1770, Davis, California 95617

Severe economic loss due to high mortality and condemnation rates occurred on two commercial broiler sites. Chickens from these sites had moderate to severe airsacculitis, pericarditis, perihepatitis, tracheitis and synovitis. *Pasteurella gallinarum* was most often isolated from these lesions. In addition, *Mycoplasma synoviae* was isolated from trachea and air sac. Although the lesions were suggestive of an *Escherichia coli* septicemia, *E. coli* was isolated only from trachea, one air sac with *P. gallinarum* and from one liver in pure growth. Chickens had been vaccinated against Newcastle disease and infectious bronchitis with live vaccine and a coronavirus was isolated from trachea and lung. Whether this coronavirus represented a vaccine or field strain of infectious bronchitis was not determined. These findings suggest that severe lesions were due to secondary infection by a strain of *P. gallinarum.*

EFFICACY OF HEMAGGLUTININ AND NEURAMINIDASE ANTIGENS PRESENTED IN ISCOMs AS VACCINE FOR INFLUENZA IN TURKEYS

Olufemi O. Fatunmbi[A] and John A. Newman[B]

[A]Department of Pathology, Michigan State University,
East Lansing, Michigan 48823
[B]Department of Veterinary Pathobiology, University of Minnesota,
St. Paul, Minnesota 55108

The protection induced in turkeys by an immunostimulating (ISCOM) subunit vaccine was investigated. Purified envelope glycoproteins—hemagglutinin (HA) and neuraminidase (NA) of the avian influenza virus strain A/Turkey/MN/1700/82—were absorbed on the glycoside Quil A to form an ISCOM subunit vaccine which was assayed in 3-week-old turkeys. Quil A (the adjuvant component) forms the matrix of the ISCOM. The HA and NA were incorporated into the matrix by hydrophobic interactions.

Groups of turkeys were administered with 1 or 2 vaccinations of ISCOM or oil-emulsion vaccine by the intranasal or subcutaneous route. Both types of vaccine induced seroconversion; the ISCOM vaccine consistently gave a superior serological response than the oil-emulsion vaccine. Serum antibody titers, as measured by the enzyme-linked immunosorbent assay, were greater following subcutaneous vaccination with either vaccine. However, the difference in serum antibody titers between the two routes of vaccination for the vaccine types did not correlate with the degree of protection. One or 2 intranasal administrations of the ISCOM vaccine gave the highest degree of protection as measured by virus recovery from the trachea and cloaca 96 hours post challenge. A similar result was obtained with 2 injections of ISCOM vaccine. The high level of protection induced against infection indicates the potential of HANA ISCOM as a subunit for turkeys.

EVALUATION OF SIMULTANEOUS VACCINATION AGAINST FOWL CHOLERA, NEWCASTLE AND FOWL POX DISEASES

S.M. Gergis, Ensaf, M. Khashaba, Fekria A. El-Bordeny
H.A. Hassan and A. Mechael

Veterinary Serum and Vaccine Research Institute, Abbassia,
Cairo, Egypt P.O. Box 131

INTRODUCTION

Fowl cholera (FC), Newcastle disease (ND) and fowl pox (FP) are the three important diseases affecting chickens and turkeys in the large Egyptian poultry breeding projects. They are also the biggest depressing factor on profit margin[1,2,6]. Vaccination programmes are always planned and carried out aiming to strengthen the host defense mechanisms so that they became able to withstand the constant attacks of the invading organisms of various kinds. Sueh programmes are now crowded by many viral and bacterial vaccines[3,4] which are either attenuated or inactivated. Thus, the birds must be handled frequently by labors and veterinarians, and this in and of itself may be one of the ways of introducing or transmitting diseases, beside being one of the important stress factors on vaccinated birds. The increasing number of vaccines makes it necessary to search for rational schemes for administration. The potential for simultaneous vaccination is now great because of the high quality of vaccines being produced. Efforts, funds and time could be saved by conducting vaccination campaign for more than one disease, provided that the protective response to each vaccine is not impaired[11].

The aim of this study is to evaluate the efficacy of synchronous vaccination of chickens with FC, ND and FP vaccines.

MATERIALS AND METHODS

In laboratory trials 450 white leghorn chickens were obtained from a laboratory hatchery as 1-day-old chickens. They were raised in an isolated facility till they reached 50 days of age then used experimently. Chickens were divided into 6 groups. The first 3 groups (50 each) received FC, ND and FP vaccines separately. The other 3 groups (100 each) received 2 vaccines simultaneously i.e (FC, ND), (FC, FP) and (ND, FP). Non immunized chickens were held as controls for each group and they were kept in separate places.

Vaccines used in this study were locally prepared by Veterinary Serum and Vaccine Research Institute, Cairo, Egypt. FC vaccine was a trivalent formalin-inactivated-oil-emulsion vaccine prepared from local isolates of *Pasteurella multocida*. ND vaccine was a lyophilized Komarov vaccine. The titer of this vaccine was 10^9 ELD_{50}/ml. The FP vaccine was lyophilized American Budette vaccine strain having a titer of 10^8 PFU/ml. Humoral immune response to ND was evaluated by measuring geometric mean titers (GMT) of HI antibodies[1]. For immunity to FC, GMT of anti *P. multocida* antibodies were measured by the IHA[11].

Bioassays were conducted by challenging vaccinated birds either with velogenic viscerotropic NDV (10^5 ELD_{50}) as previously recommended[1], FPV by stick wing method as described[6], or 10 LD_{50} of virulent *P. multocida* serotypes used in preparing the FC vaccine[2].

All experimental birds were observed for the development of either signs of the disease and or mortalities during the period of 14 days after challenge. In the field trials, simultaneous vaccination was conducted in a small native breeder (Balady) farm (5000 birds). The evaluation was carried out on random samples taken from this farm.

For statistical analysis the analysis of variance was conducted[12].

RESULTS

Non significant ($P>0.05$) differences, in GMT of NDV antibodies and in GMT of *P.multocida* antibodies, were noted between the groups of chickens vaccinated solely with ND or FC and the groups in which each of these 2 vaccines were inoculated simultaneously with another vaccine. Bioassay test results also revealed that the protection afforded by each of the vaccines used alone was not significantly ($P>0.05$) different from that afforded by simultaneous vaccination with another vaccine. Protection against FC reached 93% when FC vaccine was injected alone. While it was 91.5% and 93% respectively in the experimental and field trial groups vaccinated simultaneously. Differences in protection against challenge with virulent NDV were not significant ($P>0.05$), as protection was 90% in the groups vaccinated only with ND as well as the groups vaccinated simultaneously with the FC or FP vaccines. FP challenge results indicated no significant ($P>0.05$) differences in protection between the groups vaccinated only with FP (90.62%) and the groups vaccinated synchronously with ND (88.50%) or FC (90.50%).

DISCUSSION

Simultaneous vaccinations have been tried successfully in cattle[11] and in chickens[3,4,7,8,9] against some bacterial and viral diseases. Combined viral vaccines have also been tried in poultry[5,10,13]. Our experimental and field trial results indicated the safety and efficacy of synchronous vaccination of chickens against FC and ND or FP. Such a proposed scheme would be of value to overcome one of the major constraints in obtaining easy and adequate vaccination coverage. No competition or mutual enhancement was observed between the simultaneously inoculated vaccines in chickens. The results obtained in this study agreed with the previous findings observed by others[3,5,7,8,9].

REFERENCES

1. Allan, W.H., J.E. Lancaster and B. Toth. The production and use of Newcastle disease vaccines. Food and Agri. United Nations, Rome.P. 115.1973.

2. Bairy, M.H. Immune response to fowl cholera antigens. Am.J.Vet. Res. 36: 575-578.1975.

3. Balmasov, A.V., L.M. Erokhina, Y.S. Dukto, A.T Kuchner, V.V Chernyshev, V.L Burtsev and N.I. Arkhipov. Immunological response of chickens to vaccination against Newcastle disease, infections laryngeotacheitis and fowl pox. Veterinaryia, Moscow 4: 38-40.1978.

4. Cholakova, R. Combined poultry vaccine against infections bronchitis, Newcastle disease and infections bursal disease. Veterinaranomceditsninski Nouki. 22:32-38.1986.

5. Derieux, W.T and J.W. Dick. The response of broiler breeder chickens to parentral administration of avirulent Pasteurella multocida. Avian Dis. 24:743-750. 1980.

6. El-Sabbagh, A.H. Some studies on an Egyptian strain of fowl pox virus. Vet. Med. J. 8: 67-72. 1963.

7. Golubnichiic, V.P and R.D. Shusterman. Antigen interaction upon simultaneous immunization of fowls against Pasteurella multocida and Newcastle disease virus. Dostizheniya veterinavoi Naukii Peredovogoopyta zhivotnovodstvu 4: 42-44.1974.

8. Holubnychyi, V.P. Simultaneous immunization of poultry against Newcastle disease and fowl cholera. Veterinarya (Kiev) 39: 93-94. 1974.

9. Kodrnja, E.,B. Tunk/ and Z. Romic. Simultaneous immunization of poultry with Newcastle and fowl cholera vaccines. Vijesti serum 13: 275-278.

10. Nawathe, D.R and S.O. Ayoola. Experiments with a combined vaccine for poultry. Bulletim. Animal. Health and Production in Africa. 29: 62-72. 1981.

11. Osman, O.A, S. Abdel Rashid, S.M. Gergis, R.A. Dimitri, M.A. Mouaz and R.R. Youssef. The simultaneous inoculation of cattle with rinderpost and haemorrhagia septicaemia vaccines. Assiut Vet. Med. J.33: 45-57.1990.

12. Snedecor, G.W and W.G Cochran. Statistical Methods 8th ed. Iowa State University Press, Ames., Lowa, 1967.

13. Winterfield, R.W. Vaccination of chickens with Newcastle disease and infections bronchitis vaccines administered singly and in combination. Poultry Sci. 63:182-184. 1984.

14. Woolcock J.B. Resistance to microbial infection-vaccines in theory and practice. Austral. Vet. J. 49:307-315. 1973.

ASSESSMENT OF PROTECTIVE ANTIBODY LEVELS TO FOWL CHOLERA IN TURKEYS BY ELISA

C.J. Kelleher[A], M.B. Blankfard[A],
C.M. Lamichhane[A] and M.D. Ficken[B]

[A]Kirkegaard and Perry Laboratories,
2 Cessna Court, Gaithersburg MD 20879
[B]Department of Food Animal and Equine Medicine,
College of Veterinary Medicine, Raleigh, NC 27606

An in-direct enzyme-linked immunosorbent assay (ELISA) has been developed for specific detection of *P. multocida* antibodies in turkey serum samples. Briefly, the ELISA utilizes a highly purified *P. multocida* lipopolysaccharide antigen bound to plastic microtiter wells. The efficacy of the ELISA to detect anti-*P. multocida* antibodies and correlation with ELISA results and protection against challenge will be reviewed.

INFLUENCE OF ETHACAL® FEED COMPONENT (SYNTHETIC ZEOLITE A) ON MORTALITY OF BROILERS FED RESTRICTED OR AD LIBITUM DIETS AT HIGH ALTITUDES

S.M. Laurent[A], D.G. Barnes[A], and M. Berger[B]

[A]Ethyl Corp. P.O. Box 14799, Baton Rouge, Louisiana 70898
[B]Nutriologo, LaLuz 38, Cuernavaca, M.C.P. 62450, Mexico

A total of 2400 unsexed broilers were divided into two groups and fed for 56 days. One group was fed ad libitum, the other was given a restricted feeding program using controlled lighting. The groups were each further divided into two treatments, a control treatment and a 0.5% ETHACAL-fed treatment. There were 50 birds per pen replicated 12 times for each treatment group. The trial was conducted in Mexico at an altitude of 8200 feet (2500 meters) in a region where high ascites mortality is prevalent. Data were obtained weekly for mortality, body weight, feed consumption and feed conversion. Inclusion of ETHACAL in the diets of the ad libitum-fed birds resulted in a significant reduction ($p < 0.05$) in the cumulative mortality at 56 days. Significant reductions ($p < 0.05$) were also noted in the restricted-fed birds during periods 1-14 days and 36-56 days. Restricted feeding resulted in significant ($p < 0.01$) reduction in overall mortality when compared to ad libitum feeding.

IMMUNOGENICITY STUDIES OF A SALMONELLA ENTERITIDIS BACTERIN IN EGG TYPE CHICKENS

Joan D. Leonard and S.C. Schmittle

Biomune Co., Lenexa, Kansas 66215

Immunogenicity studies were initiated using a *Salmonella enteritidis* (SE) bacterin, with a tradename of LAYERMUNE SE™, in egg type chickens of varying ages. In all trials, 2 injections of the bacterin were given subcutaneously, 4 weeks apart. Challenge with virulent SE consisted of a dose far greater than would be encountered naturally. Nonvaccinated hens served as the controls and were challenged along with the vaccinates. These trials were designed to demonstrate the effectiveness of the bacterin to; (1)provide protection of the internal organs to prevent transmission of SE in the egg, (2)provide protection in the intestinal tract to prevent colonization and shed, and (3)provide protection across phage types.

Cultures of internal organs, including the reproductive tract and the intestine, were made to determine the incidence of SE colonization following challenge. Protection was determined by the failure to recover SE by standard culture techniques.

These studies demonstrated that this SE bacterin provided excellent protection which was statistically significant at $p < .05$ against infection of the internal organs including the reproductive tract. The protection of the internal organs, specifically the reproductive tract, reduced the risk of transmission of SE inside the egg.

The bacterin provided excellent protection of the intestine against colonization by SE. The ability of this bacterin to prevent infection and subsequent colonization of the intestine resulted in reduced SE fecal contamination on the egg shell. This bacterin was shown to significantly lower the incidence of SE fecal contamination on the exterior shell surfaces of eggs laid by hens challenged with an invasive SE. Subsequent to a high-dose challenge, this bacterin delayed the onset of SE colonization of the intestine and significantly reduced ($p < .05$) the incidence of SE contaminated eggs.

Studies demonstrated LAYERMUNE SE to be equally effective against challenge with phage types not contained in the bacterin (heterologous) when compared to homologous phage type challenge. *Studies* were conducted by the vaccination and subsequent challenge of highly susceptible young chickens. Phage types of SE not contained in the bacterin were used to challenge birds using the same challenge methodology as with homologous SE phage type challenge studies. In heterologous SE phage

type challenge studies, LAYERMUNE SE demonstrated equal or slightly better protection of the internal organs than the homologous SE phage type challenge. The results of these studies demonstrated that this bacterin was effective against both heterologous and homologous SE phage type challenge. While SE phage type is interesting from an epidemiological point of view, there appeared to be no influence of phage type on the ability of LAYERMUNE SE to impart immunity to the internal organs.

The use of LAYERMUNE SE provides the option of utilizing the chicken's immune system as a means to aid in the reduction of SE colonization in flocks. The immunogenicity studies demonstrated that LAYERMUNE SE significantly protected the internal organs and the intestinal tract against colonization by *Salmonella enteritidis*.

SALMONELLA ENTERITIDIS (SE) IN COMMERCIAL LAYER FARMS IN NEW YORK STATE; ENVIRONMENTAL SURVEY RESULTS AND SIGNIFICANCE OF AVAILABLE MONITORING TESTS

A. Mutalib, P. McDonough, S. Shin, V. Patten and D. Lein

Diagnostic Laboratory,
New York State College of Veterinary Medicine,
Cornell University, Ithaca, NY 14853

Seven hundred and fifty-one environmental samples were collected from 76 layer houses in a voluntary SE survey study carried out in New York State between January 15 and April 8, 1991. SE was recovered from 2 houses on the same farm. Sampling of manure pits and mice in hen houses was an effective method for SE screening. Phage types of SE from the environment, birds, and mice were identical. Rapid whole-blood test was unreliable and culture of cloacal swabs was inadequate for detection of SE carriers. Culture of organs from chickens did not correlate well with results of environmental samples.

THE INCIDENCE OF ENCYSTED STAGES OF PROTOZOAL PARASITES IN MUSCLE CELLS OF RAPTORS IN THE SOUTHEASTERN UNITED STATES

Paul C. Smith, David Lindsay, Byron Blagburn, and Fred Hoerr

Department of Pathobiology, College of Veterinary Medicine,
Auburn University, Alabama 36849

The College of Veterinary Medicine, Auburn University, maintains an approved raptor rehabilitation facility. Many of the birds presented during the fall-winter migration period have a history of severe trauma as a result of being hit by automobiles, gun shot wounds, or other accidents. During the fall of 1990 and the entire year of 1991, 50 raptors were submitted to the Alabama State Veterinary Diagnostic Laboratory for necropsy examination. Portions of pectoral and cardiac muscle were routinely processed for histopathologic evaluation and for a mouse inoculation bioassay for protozoal encystment. Muscle tissues were homogenized and subjected to acid pepsin digestion and inoculated subcutaneously into 6-8-week-old, female mice. Four to five weeks post inoculation serum was collected from each mouse and examined in an indirect fluorescent assay technique at 1:50 and 1:100 dilutions for antibodies to *Toxoplasma gondii*. Impression smears were made from the cerebrum cross-sections of the brain of each mouse and examined to *T. gondii* tissue cyst. Sub-inoculations were conducted to

confirm results. No isolates caused clinical disease in mice. Microscopic examination of hematoxylin—eosin stained, formalin fixed sections of pectoral and cardiac muscle were examined for protozoal encystment. Those reported here are morphologically indistinguishable from *Sarcocystis* or *Hemoproteus*. Findings are recorded in Table 1.

CONCLUSION

Toxoplasma gondii is a ubiquitous protozoal parasite that infects most species of mammals and birds but little is known about its relationship to clinical disease in wild, captive, or immunocompromised raptors. There is a single report of encephalitis in a Golden eagle due to sarcocystic species. Efforts to characterize these encysted protozoal parasites are continuing.

Table 1. Protozoal encystment in pectoral and cardiac muscle of raptors in the Southeastern United States.

Common Name	Total # Examined	# found positive for T. gondi	# found positive for Sarcocystic/ Haemoproteus
Red tailed hawk	14	4	6
Red shouldered hawk	5	5	3
Coopers hawk	2	0	1
Sharp shinned hawk	1	0	1
Kestrel (sparrow) hawk	2	1	0
Barred owl	6	2	2
Barn owl	4	0	0
Screech owl	4	0	2
Great horned owl	3	1	0
Vulture	5	0	2
Osprey	3	0	0
Golden eagle	1	0	0
Total	**50**	**13**	**17**

UPDATE ON USDA LICENSING REQUIREMENTS FOR MAREK'S DISEASE VACCINES

Ted Williams

Veterinary Biologics, BBEP, APHIS, USDA,
Rm 838 Federal Bldg., 6505 Belcrest Rd.,
Hyattsville, Maryland 30782

In the last 20 years, the United States Department of Agriculture (USDA) has licensed over 60 vaccines for the prevention of Marek's disease in chickens. These include monovalent, bivalent, and trivalent Marek's Disease Vaccines, as well as combination products containing both Marek's disease virus and either bursal disease virus or fowl pox virus. The purpose of this communication is to present the USDA's current licensing requirements for these products, with particular attention paid to those requirements for efficacy, field safety, and reversion-to-virulence. It should be emphasized that these requirements may change as new information concerning the disease is made available and new products for preventing the disease are developed.

Several licensing and postlicensing requirements for products for the prevention of Marek's disease are promulgated in the Standard Requirement for Marek's Disease Vaccine, located in section 113.330 of Title 9, Code of Federal Regulations (9 CFR 113.330). Master Seed Virus (X passage virus) purity and safety standards are provided, as are the purity, safety, and potency standards that a serial of vaccine must meet to be eligible for release. In addition, 9 CFR 113.330 stipulates that vaccine virus cannot be more than five passages from Master Seed Virus, although this restriction can be relaxed for a given Marek's disease virus strain if the USDA believes sufficient justification exists. It is important to note that the potency standard given applies specifically to those vaccines containing live turkey herpesvirus.

The indicated Standard Requirement, however, contains no description of the reversion-to-virulence, field safety, and efficacy issues that must be satisfactorily addressed by a license applicant before issuance of a license for a Marek's disease vaccine will be considered. The fact that standards for reversion-to-virulence and field safety are not specified is not unusual in that such standards have never been formally codified (included in a Standard Requirement) for any veterinary biological product type. Risk/benefit considerations as well as recognition of differences in strain characteristics and how prod-

ucts are recommended have led the USDA to determine that these standards should be developed on a product-by-product basis. This is not to say, however, that some standards for *evaluating* reversion-to-virulence and field safety for a given product type are not in place.

Reversion-to-virulence studies, otherwise known as backpassage studies, must be conducted prior to licensure for all vaccines containing live, attenuated microorganisms. Although in many instances herpesviruses used as Master Seed Viruses in the production of Marek's disease vaccines have not technically been considered "attenuated," almost invariably each has been scrutinized through some sort of backpassage study.

In an appropriate Marek's disease vaccine backpassage study, vaccine virus, at a titer similar to that expected for marketed product, is administered intraabdominally to 10 1-day-old genetically susceptible chickens. After 10 days, blood is drawn from each of the chickens and pooled. A sample is taken to confirm viremia and 0.25 ml of the remainder is intraabdominally administered to each of 10 additional 1-day-old chickens. This procedure is repeated for 10 serial backpassages. Each group of chickens is observed for 7 weeks postinoculation. At the end of the 7-week observation period all chickens are killed and examined for gross lesions of Marek's disease. Birds dying before the end of the 7-week period are also necropsied.

Field safety studies for licensure of a Marek's disease vaccine should include at least 35,000 birds in each of at least three States, preferably in three different geographic areas of the country. The vaccine used must be representative of product to be marketed postlicensure and must be administered in accordance with label directions. In most cases, vaccinated birds shall be observed through slaughter and slaughter condemnation rates shall be obtained and reported.

In contrast to the situation with reversion-to-virulence and field safety standards, efficacy standards for a number of types of live veterinary biological products *have* been codified, and it is the intent of the USDA to codify efficacy requirements for Marek's disease vaccines in the very near future. While some modifications may be made, it is likely that the efficacy-related material formally proposed for inclusion in 9 CFR 113.330 will be very similar to that presented in the following paragraphs.

An acceptable efficacy study shall include at least 30 maternal antibody negative chickens vaccinated by the prescribed route and at the minimum age recommended on the label, and at least 30 unvaccinated control chickens of the same source and hatch. Vaccine used in the study shall contain the proposed minimum protective dose of virus. In the case of a monovalent product containing the FC 126 strain of turkey herpesvirus, otherwise known as an HVT product, the vaccine shall contain 750 plaque forming units (pfu) of virus per dose.

Five days postvaccination, all chickens shall be challenged. For monovalent vaccines similar to those currently licensed, the challenge material may consist of a Marek's disease virus (MDV) of "regular" virulence, Georgia strain MDV, for example, or of a "very virulent" MDV such as the RB1B or MD-5 strains. A bivalent or trivalent vaccine, which is intended to protect chickens against very virulent MDV's, requires that a very virulent MDV be used in the efficacy study.

Seven weeks postchallenge, the birds shall be killed and examined for gross lesions of Marek's disease. Birds dying before 7 weeks shall also be necropsied. For a satisfactory demonstration of efficacy of an HVT product, at least 80 percent of the original number of vaccinates must survive the 7 week postchallenge period and carry no gross lesions of Marek's disease while such lesions must be found in at least 80 percent of the unvaccinated, challenged control birds.

In terms of efficacy, a manufacturer's licensed (or licensable) HVT product shall serve as the benchmark against which its other vaccines for the prevention of Marek's disease are compared. Licensure of a vaccine containing a different herpesvirus strain shall be contingent upon a demonstration that the new product is not significantly less efficacious than the manufacturer's HVT vaccine. The two products shall be tested "side-by-side" and the HVT vaccine shall contain 750 pfu per dose.

For licensure of a bivalent vaccine, a vaccine containing two herpesvirus strains, it must demonstrated that the bivalent product is significantly more efficacious than either of the monovalent products from which it was derived. The prototype bivalent product must have the same total titer as the monovalent products tested alongside it. For example, if the two monovalent products each contain 750 pfu per dose, a dose of bivalent vaccine should contain only 375 pfu of each virus strain. Trivalent vaccines are dealt with in a manner similar to that described for bivalent vaccines.