Proceedings & Technical Supplement of the Roche Turkey Coronavirus Workshop

co-editors
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May 4-6, 1998
Raleigh, North Carolina

Roche Animal Nutrition and Health

Dear Colleague,

We are pleased to offer this Proceedings from The Roche Turkey Coronavirus Workshop and 2nd Annual PEMS Workshop: Controlling the Disease. Roche sponsored this industry workshop in cooperation with the PEMS Task Force and North Carolina State University College of Veterinary Medicine. Roche has recognized the Turkey Industry's field frustrations and research endeavors with the coronaviral enteritis, and established this workshop to provide a scientific forum for professionals to exchange ideas on those relevant issues using a unique problem-solving format. Participants included industry and university veterinarians, nutritionists, and other technical individuals interested and involved with TCV.

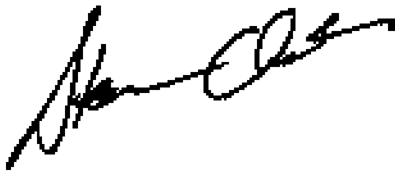
The primary goal of The Workshop was to provide a forum for professionals to discuss TCV. The format was selected to address the issue of TCV from an industry perspective and focus on current practical research, field experiences, diagnostics, and control programs.

A second purpose was to compile this detailed Proceedings, which will serve as an excellent reference to the Industry. This Proceedings book includes the case presentations and associated discussion, followed by discussions from the panel members. The last section is provided as a supplemental reference with abstracts from current TCV research and other technical information.

The PEMS Task Force and North Carolina State University College of Veterinary Medicine are acknowledged for their cooperation. We are especially thankful for the editorial assistance of Dr. Jean-Pierre Vaillancourt. In addition, we extend our appreciation all of the Turkey Industry participants.

I sincerely hope that you find this Proceedings useful. The Roche Turkey Coronavirus Workshop and 2nd Annual PEMS Workshop: Controlling the Disease and Proceedings is part of the Professional Services Series provided by Roche Animal Nutrition and Health as a service to their partners in the poultry industry.

Sincerely,



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Roche is committed to providing the Industry with quality products, sound scientific information and superior support services. The following are part of the Professional Services Series provided by Roche Vitamins Inc. as a service to their partners in the turkey industry.

- "Turkey Necropsy Procedures" video
- "Diagnosing Enteritis in Turkeys" video
- "Collecting Samples for Laboratory Analysis" video
- "Turkey Diseases, Volume 1" interactive CD-ROM
- Journal of Applied Poultry Research complimentary to veterinarians
- "The Turkey School" custom seminars
- The Turkey ORT Scientific Symposium and Proceedings
- The Turkey Coronavirus Workshop and Proceedings
- The Avian Pneumovirus Workshop and Proceedings

For more information regarding any of these items,
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AGENDA

TUESDAY, MAY 5, 1998

***REGISTRATION & REFRESHMENTS* (9:30a - 10:00a)**

Welcome Remarks & Introductions (10:00a - 10:15a)
Dr. Steven Clark, Roche Vitamins Inc.

Case reports, presented by Industry professionals, focusing on field issues relating to the control of TCV. Panel participants focused on their research and areas of expertise to supplement each case report. Audience participation will be encouraged. Pertinent highlights relating to TCV control will be summarized.

Panel participants include Dr. Deen (NCSU, expertise: swine), Dr. Saif (OSU, expertise: infectious diseases), Dr. Wu (Purdue University, expertise: molecular virology/TCV diagnostics), Dr. Gelb (University of Delaware, expertise: broiler coronavirus). Moderators will be Dr. Clark (Roche Vitamins) and Dr. Vaillancourt (NCSU).

Case Reports (10:15a - 11:30a)

LUNCH (11:30a - 12:30p)

Case Reports (12:30p - 2:30p)

BREAK (2:30p - 3:00p)

Case Reports (3:00p - 5:00p)

DINNER specially catered by the NCSU Poultry Science Club

WEDNESDAY, MAY 6, 1998

***WRAP-UP BREAKFAST* (8:00a - 10:30a)**

- Closing remarks by Panel participants
- Research remarks by other investigators
- Brainstorming break groups: identifying what we learned during The Workshop. We focused on what we knew about TCV, what we thought we knew about TCV and what we needed (i.e., research goals, future plans, etc.).

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WORKSHOP OUTLINE

The Turkey Coronavirus Workshop and 2nd Annual PEMS Workshop: Controlling the Disease

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The following summary was compiled by the participants on the last day of The Workshop following a brainstorming session, highlighting what we discussed over the two days.

The purpose of The Workshop was to provide a unique problem-solving format for professionals to exchange ideas:

- Industry perspective
- Practical solutions

I. What we know

A. Biosecurity procedures
  1. Dead bird disposal & management
  2. Litter movement & management (composting, etc)
  3. Traffic control
  4. Importance of the human element is the key to success: requires strict enforcement and commitment

B. Maturity factors of the gut are important for virus infection and disease

C. Risk factors

1. Distance to litter, farms, ponds, etc.
2. NCSU and Industry have details
3. Increased geographic density

D. Litter movement

1. High risk
2. Proper procedures

E. Vaccine is being tried and experimented

F. Ventilation procedures: dry litter

G. Serology: IFA test works

H. TCV is a significant economic problem

1. Hens are affected more than toms (because they are sold younger)
2. Poor performance

I. Young affected worse than older

J. TCV can be cleaned up

K. Regionally reducing turkey density helps control TCV

II. What we think

A. Infection may not be seasonal but

1. IFA+ results are seasonal
2. Clinical signs (disease) are seasonal

B. Litter treatment (low moisture, composting, time) should help

C. TCV causes disease

1. but in lab settings, TCV alone does not cause mortality

D. Transmission

1. Via flies and other vectors, and aerosol
2. Major transmission is fecal-oral

E. Different strains exist

F. Stress causes increase in breaks

G. Bird individual variability affects disease outcome

III. What we need

A. Communication

1. Industry-Academia
2. Notification system

3. Newsletter
4. Proceedings

B. Where does the virus go in the bird (beside enterocyte)?

C. Relationship of PEMS and TCV?

D. Is there a different pathotype of TCV?

E. What is (are) the reservoirs(s)?

F. Transmission (other than fecal-oral)?

G. Non-turkey vectors?

H. Role of feed in controlling/preventing (modifying) disease?

I. Better diagnostic tests
   1. Serology tests' sensitivity & specificity

J. Relationship among TCV, TGE, BCV, IBV?

K. Gut immunity for TCV?

L. Litter conditions and survival of agent

M. Bovine origin issue

N. Prophylaxis/treatments

WORKSHOP HIGHLIGHTS

The Turkey Coronavirus Workshop and 2nd Annual PEMS Workshop: Controlling the Disease

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"The Turkey Coronavirus Workshop and 2nd Annual PEMS Workshop: Controlling the Disease", sponsored by Roche Vitamins Inc. in cooperation with the PEMS Task Force and North Carolina State University College of Veterinary Medicine was held May 5-6, 1998, in Raleigh, North Carolina. Coronavirus is a known cause of enteric problems in turkeys and has been found to be associated with PEMS (Poult Enteritis and Mortality Syndrome). The primary goal of The Workshop was to provide a scientific forum for professionals to exchange ideas on the relevant issues related to controlling Turkey Coronavirus (TCV).

The agenda utilized a unique problem-solving format: Industry participants who are directly and actively involved with coronavirus infection presented case reports related to the control of TCV. A panel of 4 University participants used their knowledge, experience and research to supplement the case reports and to advance the development of the best approach to solve coronavirus—related problems. The purpose of this format was to address the issue of TCV from an Industry perspective and focus on current practical research, field experience, diagnostics (including serology and virology), and control programs (including biosecurity and management).

The meeting concluded with a brainstorming session to identify what was learned and what further work is needed. A total of 47 people participated. The audience consisted primarily of veterinarians and researchers. Industry representatives from North Carolina, South Carolina, Virginia, Arkansas and Texas, as well as academic participants from North Carolina, Virginia, Iowa, Ohio, Indiana and Delaware, attended.

Nine case reports were presented by Industry technical representatives on biosecurity programs, economics of TCV, epidemiology of field breaks and evaluation of diagnostic tests. During The Workshop, the information and issues related to the control of coronavirus were divided into three categories:

Knowledge

The first category was "what we know" about TCV. Biosecurity was identified as a priority for controlling TCV. Biosecurity procedures include management of dead bird disposal, litter management and movement of used litter. Proper biosecurity procedures need to focus on controlling traffic patterns, especially of people and vehicles connected to dead bird disposal and litter management. The key to a successful biosecurity program is total commitment from everyone within a company and geographic region, and strict enforcement of procedures.

Maturity factors of the intestine are important for virus infection and disease; as the bird gets older and the intestine matures, the bird is less susceptible to severe clinical disease. Previous epidemiology has identified potential risk factors for contracting TCV, including close proximity to other farms, ponds, contaminated litter and cattle. High geographic density was identified as a key risk factor. Movement of contaminated litter is also considered a high risk, and proper handling procedures were identified.

It is also known that TCV "vaccines" are being evaluated in the field. The indirect fluorescent antibody test (IFAT) works well in the field for identifying TCV—infected flocks, and it is an important tool in controlling the spread of disease. TCV is a significant economic problem, mainly due to poor flock performance, causing financial losses for both growers and processors. Hens economically are more affected than toms. The age at time of infection is an important factor; the younger the flock at time of infection, the more severe the clinical expression. We also know that TCV can be eradicated from a farm with proper biosecurity programs, identifying TCV—positive flocks and reducing the density of turkeys grown in a region.

Assumptions

The second category focused on "what we think" we know about coronavirus, that is, assumptions we may use in the field or questions we should consider. Though field observations suggest that seropositivity of iFAT results is seasonal, and that clinical signs also appear seasonally, the virus infection per se may not be seasonal. The actual time of year varies in different geographical regions of the United States; that is, in the southeast the season is summer through fall, while in the west TCV was diagnosed in the winter. Litter management, such as maintaining dry litter, composting used litter and long downtime, may help control TCV. Unlike most laboratory research that does not reproduce clinical enteric disease from TCV infection alone, it is thought that in the field, TCV does cause disease. Though there is not much scientific evidence, it is thought that coronavirus is transmitted by flies and other insect vectors and via aerosol transmission. Major transmission is fecal-oral, such as, contaminated litter and equipment. Stress appears to "trigger" outbreaks of clinical disease. The variability in clinical presentations

suggests that individual bird variability affects the outcome of the disease, and different strains of TCV may exist. Other factors, such as management and weather, could also affect the severity of the clinical outcome.

Needs

The third category represents "what we need" to control coronavirus. Communication was identified as a critical need. Improved communication between Industry and academia, continuing the PEMS Newsletter, a Proceedings of The Workshop, and a coordinated notification system identifying TCV—positive farms and contaminated litter are needed. We need to better understand the movement and locations of the virus inside the bird besides the enterocyte. Also, there is a need to better understand intestinal immunity to TCV. This would improve diagnostics and help improve our understanding of the disease.

Also, there still needs to be a better understanding of the relationship between PEMS and TCV, and the relationship of TCV, TGE (transmissible gastroenteritis of swine), BCV (bovine coronavirus) and IBV (infectious bronchitis virus in chickens). Understanding the mode of transmission, the possible role of reservoirs and nonturkey vectors needs to be further researched. It is presumed that there may be different pathotypes of TCV, complicating diagnosis and varying clinical disease.

In addition, our knowledge of the role of feed in controlling, preventing or modifying the disease needs to be increased. Improved quick diagnostic tests are needed. We should increase our understanding of litter conditions and the survival of the agent in litter. Though most believe its priority is low, there is the issue of whether TCV is of bovine origin. Moreover, there is a need for efficacious prophylaxis.

Summary

Dr. Charles Corsiglia, staff veterinarian for Plantation Foods, presented a case of TCV recently identified in Texas. Dr. David Rives' (staff veterinarian for Prestage Farms) presentation demonstrated the epidemiology of a TCV case associated with contaminated litter. Mr. Parker described situations where farms turned TCV-negative after successive TCV-positive flocks, without depopulation. Dr. Shannon Jennings, staff veterinarian for Carroll's Foods, reviewed data showing the seasonal effects of TCV outbreaks and possible associations with rainfall, temperature and fly populations. A review of biosecurity procedures was given by Dr. Hugo Medina, veterinarian with Wampler Foods. Dr. Eric Gonder, veterinarian with Goldsboro Milling Company, reviewed depopulation and biosecurity procedures to eliminate TCV in a geographical area. Dr. Dan Karunakaran, staff veterinarian for Shady Brook Farms, reviewed economics of TCV outbreaks. Several other veterinarians and poultry scientists from academia, government and industry also contributed to each presentation with timely questions and/or comments.

Panel participants included Dr. John Deen from North Carolina State University College of Veterinary Medicine (NCSU-CVM). Dr. Deen's expertise is in swine management and economics. Dr. Y.M. Saif is from The Ohio State University, specializing in infectious diseases. Dr. Ching-Ching Wu is from Purdue University.

Her qualifications are in molecular virology and TCV diagnostics. Another panel participant was Dr. Jack Gelb from the University of Delaware, who has conducted research in broiler coronavirus (infectious bronchitis). Chairpersons and organizers for The Workshop were Dr. Steven Clark of Roche Vitamins Inc. and Dr. Jean-Pierre Vaillancourt of NCSU-CVM.

In addition to sponsoring The Workshop, Dr. Steven Clark and Mr. Ed Seed, director of Roche Animal Health North America, on behalf of Roche Vitamins Inc., presented a donation for $5,000 to support the PEMS Task Force. Dr. Oscar Fletcher, Dean of North Carolina State University College of Veterinary Medicine, accepted this donation on behalf of the Task Force. The mission of the PEMS Task Force is to obtain and coordinate research funding and activities to control PEMS. The Task Force is to gather information concerning excessive early mortality in turkey poults and to formulate a plan to diagnose, treat, control, and ultimately prevent future episodes from occurring. A second concern addressed by the Task Force is the cause, control, and prevention of poult enteritis complex.

"The Turkey Coronavirus Workshop and 2nd Annual PEMS Workshop: Controlling the Disease", is part of the Professional Services Series provided by Roche Vitamins Inc. as a service to their partners in the poultry industry. Roche graciously acknowledges the cooperation of the PEMS Task Force and North Carolina State University College of Veterinary Medicine.

CASE REPORT

Coronavirus in South Texas Turkey Flocks

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ABSTRACT

A limited number of turkey farms in Texas, confined to the southern growing region, have been identified with TCV. The "can't happen here" attitude is gone. TCV positive growout farms are associated with two specific brood farms, implicated as the source of this outbreak. The current surveillance program and all positive diagnoses are based on the IFA test. Confirmed positive farms go through an extensive cleanout and disinfection program to eliminate the disease. Education of company and contract personnel on biosecurity procedures has been a priority.

BACKGROUND

Plantation Foods produces approximately 8.5 million birds per year. The production system is set up as "brood and move". There are three growing areas and one breeder area. The southern growing area is densely populated with poultry. The northern area and central growing areas are well isolated. The breeders are located in the north.

INTRODUCTION

The first confirmed case(s) of coronavirus—positive flocks occurred in February of 1998. Evidence suggests that it may have gone undetected for 3-6 months previous to that.

The first confirmed flock was 10 days old when the clinical signs and mortality suggested a viral enteritis. The second confirmed flock was 21 days old when the clinical signs and mortality suggested a viral enteritis. Both flocks were 100% BUTA and consisted of a 50:50 mix of hens and toms.

To date only two brooder farms have been classified as positive. However, since we brood and move all our farms, we have identified 8 growout farms as positive (based solely on IFA blood work). Positive growout farms where either: (a) the recipient of the birds from

the previous brood flock from the above mentioned brooder farms or (b) are in a group of farms that are owned by one individual who received birds from one of the confirmed positive brood farms and subsequently spread it to their other growout farms.

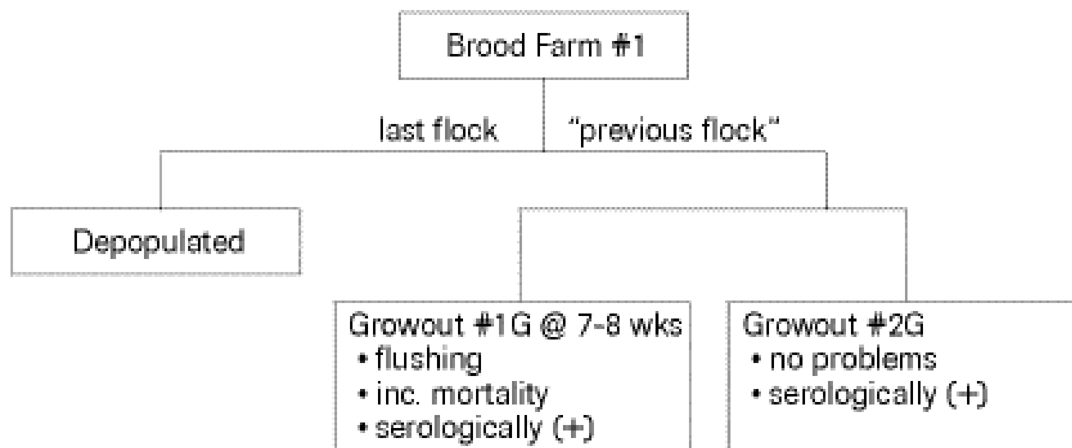
There are currently 18 growout houses (on 4 farms) with positive corona IFA serology. Of the two brood farms initially infected one is 5 weeks into the next cycle without signs of reinfection and one is 4 weeks into the next cycle with signs suggesting reinfection.

HISTORICAL INFORMATION

Both farms are located in a densely populated poultry—growing region of South Texas, Gonzales County. Other poultry production in the area includes Tyson (approx. 1.2 mil./wk), Cal-Maine (approx. 2.5 mil.), Lester Bros. (approx. 500,000/wk), Smith (approx. 1.5 mil.), Maxum (approx. 1.5 mil.), independent layers (approx. 200,000), Buddy's Natural Chicken (approx. 100,000/wk), Plantation (4 million) in south Texas. The oldest growing area is about 30 years old. One of the implicated brood farms is within a mile of a growout farm. The other brood farm is within a mile of two growout farms and 150,000 layers. Both farms use built up litter (rice hulls) and usually clean out only one time per year.

Farm #1 was depopulated at 3 weeks of age due to a diagnosis of TCV. The previous flock (i.e., "2 flocks ago"), once moved, was serologically positive for TCV at the growout. Part of the flock was moved to Growout #1G and experienced flushing and mortality. The other part of the flock from Brooder Farm #1 was moved to Growout #2G and experienced no clinical signs. (see Diagram 1).

Diagram 1. Epidemiology for Farm #1



Brood Farm #2 flock was diagnosed with TCV. The previous flock (i.e., "2 flocks ago"), was also serologically positive in the Growout Farm (see Diagram 2). Note that Growout #9G was confirmed TCV—positive, but did not receive poults from Brood Farm #2 or #1. Growout #9G is located across the road from Brood Farm #2 and it is suspected that coronavirus was somehow tracked to this Growout. Also Growouts #7G and #8G were confirmed serologically positive for TCV, with no clinical signs, but neither received

poults from Brood Farm #2 or #1. Growouts #7G and #8G do have the same farm manager as #6G, and #6G was TCV—positive, receiving poults from #2. It is suspected that the farm manager tracked coronavirus from #6G to the other Growout barns.

Diagram 2. Epidemiology for Farm #2

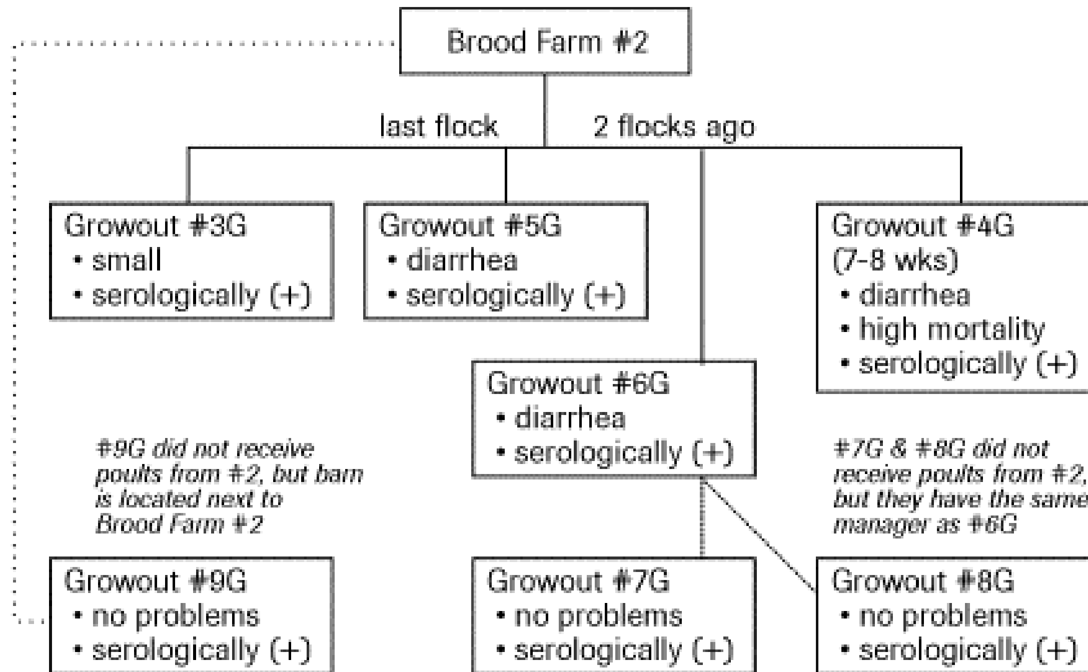


Table 1. Farm performance for the TCV—positive Brood Farms

	TCV+ Brood Farm #1	TCV+ Brood Farm #2
Livability	• Historically: 96.6% moved (#1 of 36 in ranking) • 1997: 97.1% moved (#1 of 30 in ranking)	• Historically: 92.0% moved (#24 of 36 in ranking) • 1997: 91.5% moved (#24 of 30 in ranking)
Weight (lbs)	• Historically: 3.30 @ 5 weeks (18 of 36 in ranking) • 1997: 3.41 @ 5 weeks (#5 of 30 in ranking)	• Historically: 3.08 @ 5 weeks (31 of 36 in ranking) • 1997: 2.77 @ 5 weeks (#30 of 30 in ranking)

CLINICAL SIGNS

Both farms experienced similar clinical signs, though at different ages.

Table 2. Clinical signs for the TCV—positive Brood Farms

Farm #1 began at 10 days	Farm #2 began at 3 weeks
• hyperactivity and vocalization • "raking" feed out of feed pans onto the floor • eating litter • huddling, seeking heat, piling under brooder stoves • loose droppings • mortality	• hyperactivity and vocalization • off-feed, eating litter • huddling, seeking heat, acting cold • loose dropping, diarrhea (pronounced) • mortality

PRODUCTION RESULTS

Table 3. Production results for the TCV—positive Brood Farms

Farm #1	Farm #2
Depopulated (destroyed) at 3 weeks of age	Moved to growout farm at 6 weeks of age
% Mortality at time of depopulation, by house (# dead birds/# birds placed) • #1 (4,564/17,645) = 25.8% • #2 (4,934/18,453) = 26.7% • #3 (9,426/19,096) = 49.3%)	% Mortality at time of move, by house (# dead birds/#birds placed) • #1 (8,922/28,990) = 30.7% • #2 (7,757/26,563) = 29.2%
No other parameters available	• Weight at 6 weeks: 3.52 lbs (avg) • Feed conversion: 2.23 • Total livability: 70.27%

DIAGNOSTICS

Table 4. Diagnostic results for the TCV positive Brood Farms.

	Farm #1	Farm #2
Serology for MG, MM, MS	Negative	Negative
Serology: IFA (Dr. T. Bryan/ T. Hooper - SIPAC-ADDL)	• 17-days-old serum 5 pooled samples: 1 (positive), 4 (suspect) • 27-day-old serum from birds in isolation at Texas A&M: 6/6 (positive) • 35-day-old serum from birds in isolation at Texas A&M: 6/6 (positive)	• 21- day-old serum: 1 (positive) & 1 (suspect) • 35-day-old serum: 6/6 (positive)
Bacteriology	• Salmonella • E. coli • Klebsiella • Proteus	• Salmonella • E. coli • Klebsiella • Proteus
Intestinal scrapings	• trichomonas-like organisms • cochlosoma	• trichomonas-like organisms • cochlosoma
Histopathology	• intestine: lymphoplasmacytic infiltrate of lamina propria, necrotic enterocytes, villi thickened and blunted, villous atrophy • thymus: cortical thinning • bursa: lymphoid necrosis	• intestine: lymphoplasmacytic infiltrate of lamina propria, necrotic enterocytes, villi thickened and blunted, villous atrophy • thymus: cortical thinning • bursa: lymphoid necrosis
Virus isolation from intestinal material		Isolated TCV (at TVMDL, Gonzales; confirmed SIPAC-ADDL)

MANAGEMENT

Sanitation

Both confirmed positive farms have gone through an extensive cleanout program with down time as close to 4 weeks as possible. Houses were pretreated for flies, beetles and rodents. They were then washed with a hot water/high pressure spray system. Litter was completely removed from the farm and trucked off site. Medication tanks, water lines, and drinkers were washed with a strong base (sodium hydroxide) followed by a strong acid (sulfamic acid) solution. The houses, feed tanks, feed lines were

washed again with hot water, high pressure, and detergent. Houses were allowed to dry and then the curtains and floors were sprayed with a compound containing proprionic acid, phosphoric acid and polyoxyethylene polyoxypropylene block polymer-Iodine complex. After a second dry—down period, the floors were treated with cresylic acid. When the cresylic acid—treated floors were completely dry, new litter was brought into the houses and the rings built, after which the houses were fumigated with formaldehyde and allowed to stay empty. The outside of the houses are mowed and the area a few feet from the sidewalls are treated with weed killer and cresylic acid. Medication sheds, work rooms, and similar buildings are cleaned, washed, and formaldehyde fumigated. All vehicles used in the cleanout procedure were washed and disinfected prior to the next step in the process. Pest control programs for flies, beetles, and rodents were evaluated and updated if needed (in relation to active ingredient, proper placement, rotation, and efficacy). Boots and coveralls used in the cleanup process were cleaned or discarded.

Biosecurity

Growers have been encouraged to put gates on all farms and a wash station for all vehicles. Service personnel have been told to visit farms only when asked or if necessary. Boots and coveralls are being provided to each farm to attempt to eliminate the chance of farm–to–farm contamination by service personnel. All feed trucks that deliver to the southern growout area were washed and disinfected after feed deliveries. The poult moving equipment is completely washed and disinfected after moving birds off farms, as well as the crew truck and personnel. Live haul trucks and cages that pick up birds in the southern area were double washed after unloading, evaluated for cleanliness, and rewashed if necessary. The discovery of corona in the southern growing area has heightened both Plantation Foods personnel and contract growers' awareness for isolation, traffic control, and sanitation on the farms.

Litter Management & Disposal

All farms diagnosed as positive for coronavirus exposure were required to be cleaned out completely. Litter is moved or spread as far away as possible from turkey houses, some driven as far away as San Antonio (90 miles). Most of the growers are using a contract cleanout service that is required to tarp and clean all trucks that are used in the cleanout process. We attempt to route the litter–hauling trucks away from turkey farms as much as possible. All equipment used in the disposal of litter is cleaned and disinfected prior to using it again.

Dead bird disposal

All birds on the farm that was depopulated were buried and the area around the pit was treated and burned. Growout farms that were serologically positive either composted the birds or used a rendering service.

Treatment

Treatment in both cases has been palliative. Prior to depopulation of the first flock, the birds were given high levels of vitamin E in the water, a commercial vitamin B complex pack, roxarsone, powdered milk, sulfadimethoxine, and electrolyte packs. Various methods to entice the birds to eat were attempted: feed was placed in egg flats and distributed throughout the house, corn chops were used to top dress the feed (limited success), green food coloring was used to attract the birds to the feed (limited success), paper was rolled down the center of the house and feed placed on top (limited success), a smaller crumbled feed was used, store–bought feed in bags was tried (various brands, no differences observed). The laundry list of attempts mentioned above was helpful for some of the birds, but the majority of the flock was irreparably damaged and therefore destroyed.

The second flock that broke at three weeks (though at the same basic time as flock #1) was treated similarly. There was more roxarsone used as an attempt to knock down the high levels of protozoal organisms seen on intestinal scrapings. The flock was treated with oxytetracycline for 3-4, days which seemed to exacerbate the problem. Most of the treatment consisted of supportive care including high levels of vitamins (E, C, B-complex), electrolytes, and milk. The floor conditions became unmanageable, so bagged shavings were brought in and spread over the old litter a half house at a time over a few days. In the areas of the house with new litter, the mortality seemed to drop as compared to the areas that had not been top dressed. Pelleted feed was dumped and replaced with crumbled feed and corn chops in an attempt to stimulate eating (relatively good success). Particular attention was paid to ventilation and air quality. The farm was given an unlimited gas supply in an attempt to keep the air quality acceptable and dry out the litter.

CASE REPORT

Biosecurity Measures at Wampler Foods' North Carolina Complex

HUGO MEDINA

Wampler Foods, Inc.
Harrisonburg, Virginia, USA

SUMMARY

A strict biosecurity program was initiated to control Turkey Coronavirus (TCV), Poult Enteritis and Mortality Syndrome (PEMS), and various other diseases. The biosecurity program included the following:

- Conversion of multiage facilities to single-age production
- Complete depopulation of all diseased farms
- Use of detailed cleaning and disinfection procedures
- Use of insecticides
- Implementation of a rodent control program
- Use of waterline sanitation procedures
- Initiation of biosecurity meetings with growers
- Improving barn ventilation
- Complete evaluation of management practices
- Controlling flies and wild birds
- Modifying dead bird disposal practices
- Controlling other animals (cattle, dogs, cats, etc.) near barns

The subsequent suspension of several growers in the immediate area helped to reduce the disease load. Another major factor in controlling TCV was the availability of an indirect fluorescent antibody (IFA) diagnostic serology test for TCV. The results were used as tools to schedule placements and/or initiate depopulation. The biosecurity program was deemed a major factor in the ultimate elimination of TCV from the western North Carolina operation.

DISCUSSION

Tom Knapp, former Turkey Live Production Manager, Wampler Foods Inc. North Carolina Division, provided the following information on implemented biosecurity measures and comments on their results.

The following are the major factors that contributed to the reduction, control, prevention and elimination of TCV, PEMS and other turkey diseases at Wampler Foods Inc. complex located in western North Carolina.

The PEMS complex was first described in North Carolina in the summer of 1991.

Due to major disease problems, a strategic decision was made to transform facilities to a single-age production. North Carolina Division growing housing was 80% two-age style of farm, 10% were three–age and the remaining were single-age, all-in—all-out facilities. Within 8 months, 72% of the multiple-age farms were converted to single-age facilities. A single-age facility encompasses either brood-and-move flock schedules or all-in—all-out production. This was a critical component to breaking the disease cycle at Wampler Foods Inc.

In addition, with that transition, it was mandatory that any farm that had PEMS, TCV and/or Fowl Cholera be completely depopulated, cleaned out and sanitized. Clear, uniform standards of inspection were used on each facility before the authorization for placing poults was given.

Every farm was cleaned, sanitized and inspected. Wampler Foods Inc. North Carolina Division was the first to reintroduce the use of formaldehyde by spray application in the East Coast. Formaldehyde 37% concentration was applied at the maximum legal concentration for poultry house application: 20 oz per 1,000 cubic feet. The insecticide cyflutain (Tempo® or Commodore®) was also applied for the control of flies and darkling beetles. The use of these insecticides in conjunction with the disinfectant influenced the reduction of the common insects in the poultry houses, even though the role of these insects as vectors for PEMS and/or TCV had not been determined.

Steps were taken to improve rodent control using rodent baiters (bait stations) donated to our producers. Many baiters were located in and around the houses. Also, the type of baits (rodenticides) were rotated quarterly. This practice was initiated in 1996; the impact of this practice can be measured by the reduction of not only PEMS and TCV, but also Fowl Cholera outbreaks:

Year	***No. of cases of Fowl Cholera***
1995	29
1996	26
1997	5
1998 (Jan.-April)	0

The waterline sanitation program required the presence of free chlorine at 3 to 5 ppm between and during the presence of flocks. This contributed in reducing bacterial loads in the birds' drinking water.

Wampler initiated tracking and monitoring incoming traffic to each farm with mailboxes and sign-in sheets. Service vehicles were routinely inspected to make sure that they were clean, both inside and outside, and in the bed of the truck. Mobile (portable) disinfectant units were installed in the service vehicles and feed trucks to spray a quaternary ammonium disinfectant on wheels. The driver, prior to entering and leaving any live production facility, activated these units. The disinfectant solution was approved by the N.C. State pesticide division and complied with all the state laws for application.

We initiated biosecurity meetings with producers in small groups to inform them of what we were attempting to do. We also met with equipment, shavings, propane and electric service suppliers. Everyone was requested to signin on cards prior to entering any live production facility; to wear plastic boots at the time anyone steps out of the vehicle; and to never enter the facility without a specific purpose and authorization from the grower and company personnel.

There were up to 8 different shavings suppliers. They were screened for quality and quantity and reduced to two. These suppliers were trusted for their biosecurity practices. This had a dramatic impact on litter quality.

Fan standards were increased for all producers requiring the installation of fans in the brooder houses and making the houses "tight" to improve ventilation. Improvements in ventilation, such as better air exchange, dry litter and keeping flies outside of the houses, are believed to have a positive effect on controlling TCV.

No new vaccination programs were implemented. On the contrary, vaccinations were reduced. Less than six farms were vaccinated for Turkey Coryza (Bordetella avium) and Newcastle Disease Virus. Medication costs of 0.8 - 1.0 cent per pound were reduced to less than 0.25 cent per pound. It was observed that, in the presence of PEMS or TCV, medication did not reduce the problem, but actually exacerbated it.

A major breakthrough in February 1997 was the availability of an IFA diagnostic test in North Carolina for TCV. We initiated the serological monitoring of all flocks at 6 and 11 weeks and market. The results of this screening indicated that 70 to 100% of the flocks were serologically positive at processing time. The results were used as tools to schedule placements and/or initiate depopulation, cleaning and disinfection. It took about a year from the start of the monitoring to have zero serologically positive flocks. By March of 1998 the N.C. Turkey Division was serologically free of TCV.

Grower education was important. They were all convinced of the need to initiate strict biosecurity procedures. Growers were provided with information on performance results from both healthy and sick flocks. After depopulation and conversion to single-age farms, flocks began to show some improvement in productivity, and healthy birds could be grown.

Without specifically knowing the source or mode of transmission of PEMS and TCV, probably the most influential factor for TCV control was for growers to manage their facilities again with personal ownership. The service managers, feed truck drivers, live-haul crew employees, supplier personnel and anyone else connected with live production were involved. One of the concerns was the presence of absentee owners who had other business activities besides producing turkeys.

Company personnel encouraged growers to have Best Management Practices for the bird health and husbandry practices.

Flies were a major problem; this was so bad that even during the winter months, flies were in the brooder houses. Fly manure covered electrical cable and/or water system tubing present in the turkey house. Flytraps outside the buildings along the eves (with fly bait) were used. Flytraps were kept clean and fresh for effectiveness.

Any producer that owned cattle, sheep or goats were asked to keep them at least 100 feet away from the facilities. This guideline was to prevent these animals from defecating next to the houses or to prevent feces from splashing inside the turkey houses. Growers were asked to control grass and weeds by spraying herbicide along the building's drip line and by mowing the rest. Cats and dogs were not allowed in the buildings at all. These animals were suspected vectors or carriers of TCV.

Too many of the facilities had wild birds inside the houses. Growers were asked to repair and maintain end doors, wire mesh and screens. Use of a shotgun to eliminate wildbirds inside the houses was allowed. The use of mice glue-boards was also effective in the elimination of wild birds due to the location of these traps.

The suspension of 44 growers was due to their poor performance ranking. Thirty-two out of the 44 growers (73%) were located in Union County, which is a densely poultry–populated area. This had a positive impact on disease control, with the average distance from the feed mill being 12 miles. The suspension of these growers helped reduce the disease load within the area.

The disease problem was so great and growers were in such an economic disadvantage that they were open to any changes. A complete evaluation of management practices was performed. Company personnel questioned how we were exposing the birds to disease and how susceptible they were due to poor biosecurity practices.

The presence of two dead bird disposal facilities (rendering plants) was a concern. To make sure that all producers followed biosecurity practices, the company hired a person to monitor the rendering facilities and to ensure that the growers disinfect their vehicles as they left the premises. Growers that practiced daily dead bird delivery to the rendering plants were encouraged to get chest freezers and deliver frozen birds only when their freezers were full; this minimized the trips and exposure to these facilities.

All TCV–positive farms were depopulated, cleaned and disinfected, followed by 3 weeks downtime. It was observed that if birds were placed on litter from a previous TCV positive flock with less than 10 days downtime, the flock had a greater chance of breaking with TCV again. It was not known if the presence of the problem was due to a short down time

or its reintroduction in the facility. Some flocks broke with TCV after depopulation. Most TCV outbreaks occurred in flocks about 4 weeks of age or older. Flocks did not seroconvert until 6 weeks of age.

With the implementation of the above measures, the severity and number of problems were reduced. PEMS by definition was not present on any of our facilities in 1997. There were TCV outbreaks, but PEMS outbreaks were reduced by ventilation, water quality sanitation, litter and temperature management, and TLC (Tender Loving Care) for the birds had a major impact on the reduction and elimination of these diseases.

CASE REPORT

The Goldsboro Milling Company Turkey Coronaviral Enteritis Outbreak at Albertson, North Carolina: Epidemiology and Biosecurity

ERIC GONDER

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SUMMARY

A turkey coronavirus (TCV) outbreak occurred between February 1997 and October 1997, in the Albertson area, located in eastern North Carolina. The 17 farms involved in the outbreak were single-age finishers, which made the logistics of controlling the disease easier. Biosecurity practices included depopulation and cleaning of positive farms, controlling all traffic in the area by routing, driver education and vehicle inspection, and disinfection. All flocks in this geographic area were serologically tested every 2 weeks using the indirect fluorescent antibody test (IFAT). Intestinal samples were submitted for direct fluorescent antibody testing (DFAT), histopathology and viral isolation whenever possible. Details of the outbreak, biosecurity procedures and comments on diagnostic tests will be reviewed.

THE OUTBREAK

The outbreak occurred between February 1997 (first case) and October 1997 (last quarantine lifted). The TCV outbreak eventually involved 17 farms out of the 41 on the map area, and one off the map, but related to the area. All farms involved were single-age finishers, with two exceptions, neither of which were epidemiologically significant. This was a tremendous help in controlling the disease.

We were notified by another company that TCV was in the area, and had been testing all flocks south of NC Highway 55 for a couple months. We had detected and depopulated two farms south of Goldsboro successfully during this period, and found retrospectively that Farm #15 was TCV–positive retrospectively (after the birds were slaughtered), in December 1997. The farm was cleaned, disinfected and restocked following normal procedures and the subsequent flocks were normal.

Companies in the immediate area instituted a cooperative "Eastern North Carolina Turkey Biosecurity Program" (see Addendum 1). All vendors and growers attended TCV educational meetings and were asked to follow strict biosecurity guidelines. We attempted to control all traffic by routing, driver education, vehicle inspection and disinfection (see Addendum 2). Compliance was a problem in several cases. Routinely, memos were sent to all of the involved farms and all levels of company management to report the status of the outbreak, quarantines and specific biosecurity procedures relating to traffic control, movement of litter and cleaning.

Farm 23+24 showed clinical signs, some seroconversion to TCV, and TCV was isolated late in the flock's life. By the time we found it, eggs for the next brood had been set (the farm was on a 15-week cycle), and the choice was to try a rapid clean-out under supervision with formaldehyde fumigation, or kill 75,000 hen poults. I chose to place back and lost. Clean-out was satisfactory in all respects; we found later there was significant traffic between #23+24, and #22.

Due to the high density of farms in the area, we monitored all flocks serologically every 2 weeks, whether clinically suspicious or not. Samples were submitted for direct fluorescent antibody testing (DFAT), histopathology and viral isolation whenever suspicious clinical signs were seen.

Whenever possible, we attempted to repeat positive serology on several occasions and at different laboratories to reassure both ourselves and our growers of their disease status; this testing is summarized in the table. The indirect fluorescent antibody test (IFAT) was invaluable in determining which birds with diarrhea had TCV and which didn't. The DFAT was occasionally useful, especially in confirmation, but we had problems with sample quality during shipping, interference from conditions damaging intestinal epithelium, and possible lack of sensitivity in older birds. Virus isolation took longer, but was more sensitive, but subject to cross-contamination during sample collection.

Our effort to control TCV in this dense area was eventually successful, but took longer than everyone would have liked, primarily due to scheduling difficulties, failure to follow recommended procedures and fatigue. One key element may have been that we were eventually successful in depopulating Farms #23+24, #22, #25, #20, #27, #28, #26, #11 and #35 throughout the entire month of July. We had some cases appear in this area later, sometimes with unclear clinical signs in older birds, but could detect these with IFAT surveillance and respond accordingly.

Figure 1. Map of the Albertson area, located in eastern North Carolina. Goldsboro Milling Company turkey farms are indicated.

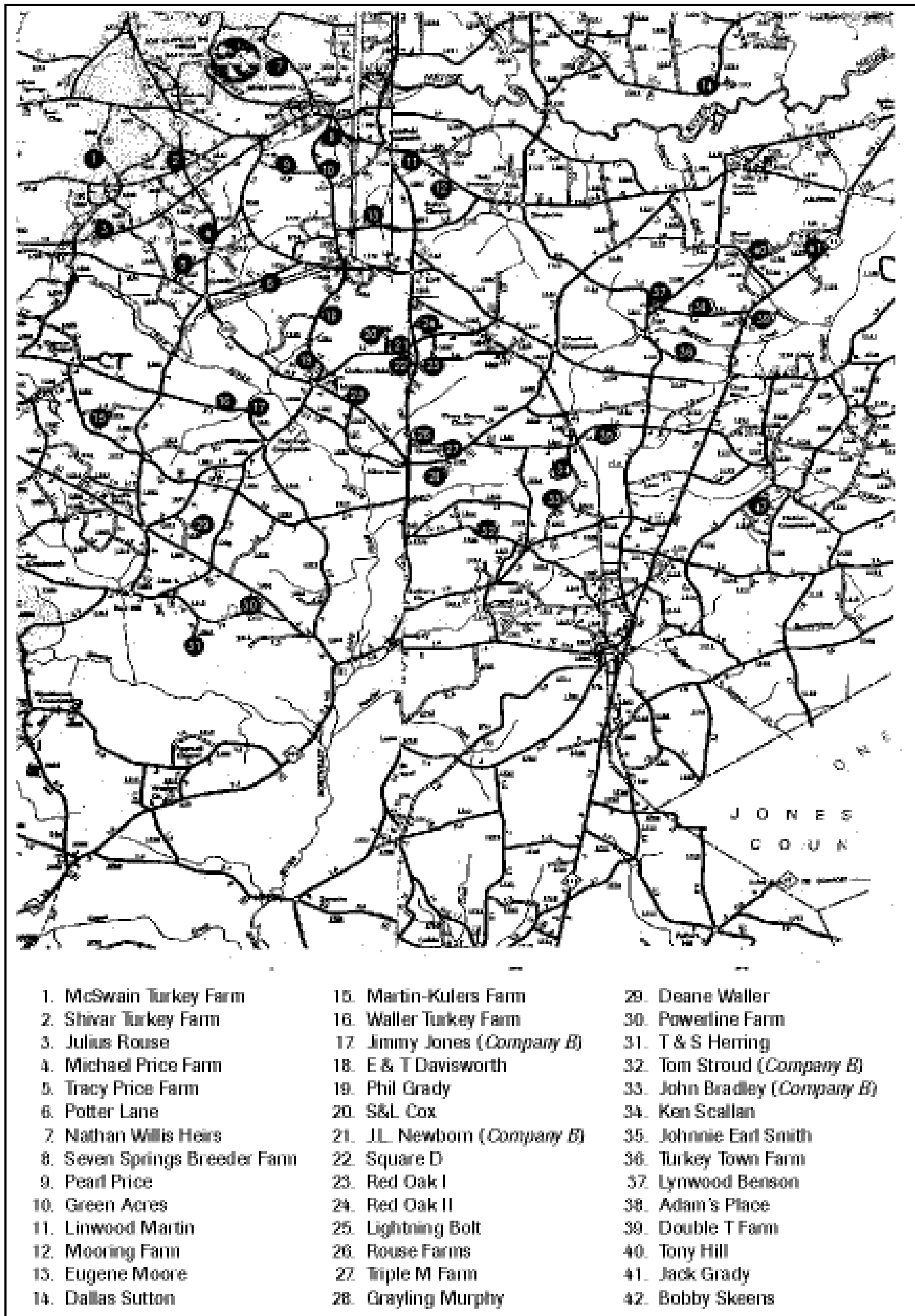


Table 1. Flocks involved in the "Albertson" TCV enteritis outbreak in 1997 at Goldsboro Milling Company

	Farm/ Flock	Date Taken	Age (weeks)	Date IFAT	Date DFAT	Date ISOL	Date QUAR	Date Sold	Quar Off	Repop Date	Histopath/Notes
1	23+24/1	2/13	11	2/18		2/24	2/21	2/25	3/17	3/20	No lesions. Sold. Cleaned. Rebroke (on next flock after cleanout).
2				1/6": 4 of 6S		POS					
3	23+24/2	4/1	2	4/9	4/9	4/14					2nd pass isolation.
4				6/6-	ENT	SUSP: sample thawed					Positive. Possible sample switch with another flock. 4/18 Dx: bursal atrophy (on histology).
5		4/3	2		4/9	4/14					
6					ENT	ENT: sample thawed					
7		4/9	3		4/15	4/28					
8					ENT	ENT					
9		4/21	5	4/23			4/23	6/21	7/14	8/7	
10				HSE1: 5 of 5-							
11				HSE2: 5 of /6+							
12				HSE4: 4 of 6S							
13				HSE5: 2 of 6S							
14		5/7	7	5/14							Houses #2, 4, 5 Positive @ CVM and ADDL. Negative at UGA.
15				ADDL: POS CVM: POS							
16		6/18	12	6/26	7/1	7/11					Dx: Trich/Cocci (on histology).
17				HSE1: 6 of 6+	NEG	NEG					Cattle negative: both control feces and farm cattle sera are weakly positive.
18				HSE4-6/6+							

	Farm/ Flock	Date Taken	Age (weeks)	Date IFAT	Date DFAT	Date ISOL	Date QUAR	Date Sold	Quar Off	Repop Date	Histopath/Notes
19	22/1	2/26	13	2/28	2/28	3/10	2/28	3/28	5/7	5/8	Traffic between 23/24. Next flock broke (after complete cleaning).
20				5 of 6+	NEG	POS					
21		3/21	17	3/2							
22				6 of 6+							
23	22/2	5/11	8	5/8							
24	22/2	5/11	8	5/8							
25				NEG							
26	6/11	11	6/19				6/19	7/11	8/18	9/3	Flock moved in 5/8 at 6 wks of age (after complete cleaning).
27			6 of 6+								
28		6/23	13	6/26							
29				ADDL: 4 of 4+ CVM: 3 of 4+							
30	25/1	3/4	14	3/7	3/6	3/17	3/6	4/1	5/7	5/8	Dx: Viral enteritis. Across the woods from #22. Next flock broke (after cleanout).
31				2 of 2+	POS	POS					
32		4/21	19	4/2							
33				6 of 6+							
34	25/2	6/11	11	6/19			6/19	7/11	8/18	9/3	Flock moved in 5/8 at 6 weeks of age.
35				6 of 6+							
36	MIL023	3/4	10	3/6	NEG thereafter						
37				1 of 6S							
38	20/1	3/7	10	3/19	3/17	3/24	3/17	4/3	5/14	6/10	Dx: Viral enteritis. Rebroke on next flock (after cleanout).

	Farm/ Flock	Date Taken	Age (weeks)	Date IFAT	Date DFAT	Date ISOL	Date QUAR	Date Sold	Quar Off	Repop Date	Histopath/Notes
39				3 of 6+, 6 of 6S	POS	POS					
40		3/13	11	3/19	3/19						
41				6 of 6+	SUSP						
42		4/9	14	4/9							Different house
43				2 of 6+, 3 of 6S							
44	20/2	6/10		6 of 6-	NEG	NEG: sample thawed					Ds: Hexamita (on histology)
45		6/24	8	7/7			7/7	7/27	8/18	8/20	See JJO036: from same brooder
46				HSE2: 6 of 6+							
47				HSE3: 4 of 6+, 2 of 6S							
48				HSE4: 6 of 6+							
49		7/8	10	7/15	7/17	7/29					
50				HSE3: 2 of 6S	SUSP	ISOL					
51	19/1	3/17	8	3/19	3/19		3/19	4/27	6/12	6/15	Chronic viral enteritis
52				6 of 6+	POS						
53	27/1	3/17	8	3/19	3/19		3/19	6/11	9/16	9/26	Mild viral enteritis
54				6 of 6+	POS						
55		4/7	11	4/17							
56				4 of 5+							
57	28/1	3/17	7	3/19	3/19		3/19	6/24	9/16	9/30	No diagnosis
58				4 of 4+	SUSP						

	Farm/ Flock	Date Taken	Age (weeks)	Date IFAT	Date DFAT	Date ISOL	Date QUAR	Date Sold	Quar Off	Repop Date	Histopath/Notes
59	036D1	4/4	4			4/14					Suspect switch w/ROF02. These are breeder hen replacements
60						POS					
61		4/14	5	4/15	4/15	4/28					
62				6 of 6-	NEG	NEG					
63		6/9	13	6/16							
64				6 of 6-							
65	16/1	4/9	12	4/17			4/18	4/29	6/12	6/17	Suspect live haul traffic. Farm near road to processing plant
66				5 of 6+							
67	26/1	4/12	8	4/19							
68				6 of 6-							
69		4/21	9	4/23			4/23	6/19	7/22	7/25	Dx: Cochlosoma (by histology)
70				HSE1: 6 of 6+							Dx: Worms
71				HSE4: 6 of 6+							
72		6/15	16	6/16							
73				HSE1: 3 of 6+ 2 of 6S							
74				HSE4: 2 of 6+, 2 of 6S							
75	11/1	4/29	11	5/5	5/5	5/9	5/7	6/18	7/14	7/24	Dx: Bacterial enteritis. Litter from 22/1 was spread nearby
76				3 of 5+, 1 of 5S	NEG	NEG					

	Farm/ Flock	Date Taken	Age (weeks)	Date IFAT	Date DFAT	Date ISOL	Date QUAR	Date Sold	Quar Off	Repop Date	Histopath/Notes
77		5/7	12	5/14							
78				CVM: 6 of 6+, ADDL: 6 of 6+							
79				6/1							
80				NEG: UGA							
81		5/22	14	5/29							
82				HSE1: 6 of 6+							
83				HSE2: 6 of 6+							
84				HSE3: 6of 6+							
85	34/1	4/16	14	4/22							
86				5 of 5-							
87		5/6	16	5/14			5/14	5/23	6/12	6/18	TCV suspect. Suspect traffic from dead haul. Next flock broke (after cleanout)
88				ADDL: 6 of 6+, CVM: 6 of 6+							
89				6/1							
90				UGA: NEG							
91	34/2	7/7	9	7/24							
92				1 of 6S							
93		7/10	10	7/21			7/22	9/5	10/14	10/18	Suspect traffic dead haul
94				2 of 6+							
95		7/15	10	7/24							
96				6 of 6+							
97		8/27	15	8/29							
98				6 of 6+							
99	35/1	4/28	11	5/2							

	Farm/ Flock	Date Taken	Age (weeks)	Date IFAT	Date DFAT	Date ISOL	Date QUAR	Date Sold	Quar Off	Repop Date	Histopath/Notes
100				6 of 6-							
101		5/8	13	5/14			5/14	6/3	7/14	7/30	Chronic viral enteritis. Suspect traffic from dead haul. Next flock broke (after cleanout)
102				ADDL: 6 of 6+, CVM: 6 of 6+							
103				6/1							
104				UGA: NEG							
105	35/2	8/27	9	8/29	8/31	9/11					Suspect traffic from dead haul. (The cleaning was worst of any we repopulated)
106				6 of 6-	NEG	NEG					
107		8/29	9	8/29			9/2	9/29	10/30	11/24	
108				1 of 6+, 3 of 6S							
109		9/3	10	9/9							
110				6 of 6+							
111	12/1	6/11	7	6/16	6/16						
112				6 of 6-	NEG: sample thawed						
113		6/26	10		?	7/11	7/14	8/12	9/16	9/26	Dx: Necrotic enteritis. Litter from 22/1 was spread nearby
114						POS					
115		7/9	11		7/17	7/29					Chronic viral enteritis
115					SUSP	POS					
117		7/10	11	7/14							
118				3 of 6+, 2 of 6S							
119				2 of 6S							

	Farm/ Flock	Date Taken	Age (weeks)	Date IFAT	Date DFAT	Date ISOL	Date QUAR	Date Sold	Quar Off	Repop Date	Histopath/Notes
120		7/8	19	7/14							
121				6 of 6-							
122	JJO036	6/26	9	7/14							Mates moved to SLC003. Moved from same brooder
123				3 of 6S, 3 of 6-							
124		7/10	10	7/14			7/15	8/21	9/16	9/30	
125				4 of 6+, 1 of 6S							
126		7/22	12	7/25							
127				5 of 5+							
128	13/1	7/14	13	7/17			7/18	8/7	9/16	9/17	
129				6 of 6+							
130		7/22	14	7/25							
131				6 of 6+							
132	10/1	6/10	7	6/16	6/16	6/16					
133				6 of 6-	NEG	NEG: sample thawed					
134		7/15	13	7/21			7/22	8/5	9/16	10/1	Sample switched with LBE053? No signs. Quarantined on suspicion. Retested. Released.
135				6 of 6-							
136		8/4	15	date?							
137				ADDL: 2 of 4+, 2 of 4S; CVM: 6 of 6+							
138		8/11	16	date?							No explanation
139				ADDL: 6 of 6+ CVM: 6 of 6+							

	Farm/ Flock	Date Taken	Age (weeks)	Date IFAT	Date DFAT	Date ISOL	Date QUAR	Date Sold	Quar Off	Repop Date	Histopath/Notes
140	37/1	7/15	17	7/21			7/28		8/11		Samples switched with GAF083? No signs. Quarantined as a precaution. Retested. Released.
141				2 of 4+, 2 of 4S							
142		7/29	18	date?							
143				All HSES: NEG							
144		8/4	19	8/7							
145				ADDL: NEG; CVM: NEG							
146	18/1	7/24	8	7/21							
147				6 of 6-							
148		7/29	9	7/31							
149				2 of 6S, 4 of 6-							
150		8/5	11	8/8	8/14	8/22	8/11	9/25	10/27	11/4	
151				ADDL: 5 of 5+ CVM: 5 of 5+	Neg: sample thawed	POS					
152	048D1	12/23/97	23	1/7/98							No clinical signs. Breeder hen replacements
153				CVM: POS							
154		1/8/98	26	1/13/98							
155				CVM: POS; ADDL: 2 of 6S							
156		12/29/97	24	1/13/98							Killed vaccine related: non-specific reaction
157				ADDL: 1 of 12S							
158				11 of 12-							
159	650G	12/17	97	24	1/7/98						Clinical signs; looseness
160				CVM: POS							
161		1/8/98	27	1/15/98							
162				ADDL: 6 of 6-; CVM: NEG							Killed vaccine related?; non-specific reaction?

Footnotes for Table:

- The farms are identified by number in the left column of the table, and are identified by the same number on the map. The number following the slash in the first column refers to the sequential flock on the farm (i.e., 23+24/1 refers to the first flock on farms 23 and 24 involved in the outbreak, 23+24/2 refers to the second flock involved on these same farms, etc.).
- All units are finishers except #10 (it is a two-age farm), and #23 (single-age farm) and breeder replacement farms.
- All results are from the Animal Disease Diagnostic Lab (ADDL-SIPAC), Purdue University unless otherwise indicated. CVM: College Of Veterinary Medicine, North Carolina State University. UGA: University Of Georgia.
- Farm/Flock: Farm is the number on the map; Flock is sequential flock affected.
- Date taken: is the date sampled.
- Date IFAT: is the date results of indirect fluorescent antibody test were reported for sera. S: suspect. Pos: positive result. Neg: negative result.
- Date DFAT: is the date results of direct fluorescent antibody test were reported for intestinal samples. S: suspect. Pos: positive result. Neg: negative result.
- Date Isol: is the date results of virus isolation were reported for intestinal samples.
- Ent: is enterovirus.
- Date Quar/Quar Off: is the dates quarantine was imposed/released.
- Date (mm/dd): is reported as month/day
- Repop. date: is the date the farm was repopulated. (Finishers are repopulated with 5 to 6 week old birds).

Addendum 1. Eastern North Carolina Turkey Biosecurity Program

The steps below must be followed for every farm visit. Remember, steps 3-8 apply to negative as well as positive farms.

1. Determine the disease status of the farm prior to your visit by contacting the grower or the company representative listed below. Do not go to a positive farm unless absolutely necessary.
2. Schedule positive farms last each day. Do not go from a positive farm to a negative farm for any reason without thoroughly cleaning vehicles and equipment. Personnel should shower and change clothes.
3. Put on plastic boots as you exit your vehicle. The boots should not touch the floor of your vehicle and your shoes should never touch the ground.
4. Do not go into a house unless absolutely necessary.
5. If you must enter a house, put on disposable coveralls, a hair net, and an additional pair of plastic boots before doing so.
6. Use foot dip pans, wash stations, and/or sprayers where available.
7. When you are ready to leave, remove outer boots, coveralls and hair net and dispose of on farm. Clean up your equipment. Wash hands and arms. Remove inner boots as you re-enter your vehicle and deposit them in container provided or drop on ground. Again, the boots should not touch the floor of your vehicle and your shoes should not touch the ground.
8. Vehicles should be cleaned inside and out at the end of the day.

Thank you for you cooperation in this program. Working together, we can go a long way toward preventing the spread of turkey coronavirus and other potentially devastating diseases. If you have questions about the program, contact______.

Addendum 2. Memo: Traffic Control and Cleaning for Feed Truck Drivers

Delivering feed right is important. Part of that delivery that gets more and more important as we add more flocks is getting from one place to another without carrying any disease along. Take time to read this sheet and follow these procedures. We will answer any questions you have about them and will watch to see how they work.

1. Before leaving the mill with feed, check with the dispatcher to see if a certain route must be followed during your delivery. Generally: breeder feed is delivered before feed for meat birds; feed for birds in houses is delivered before feed for range birds; feed for younger flocks is delivered before that for older flocks.
2. Check your truck for plastic boots, bug spray, and Alcare hand cleaner. You need one pair of boots each time you get out of the truck.
3. Don't splash mud on your truck; drive easy through puddles.
4. IF THE FARM HAS A WASH STATION (USUALLY BREEDER FARMS) USE IT! USE THE HOSE TO CLEAN THE FLOORBOARDS AND STEPS FOR THE CAB. If the station doesn't work, use the hose in the pumphouse to clean all mud from the undercarriage and wheels and make your delivery. If neither works, come back without delivering. Tell the dispatcher either way.
5. At the farm, put on plastic boots before you get out of the track.
6. Shut the cab door and keep the windows closed so bugs don't get in.
7. Unload the feed. Bag any spills. Stay away from the house, dead birds, mud, or manure. Stand upwind of dust. Keep as clean as possible. Don't wander around; we have routes around the farms and it'll mess us up if you wander off the route we have for you, which is farm gate, feed tank, farm gate, with you moving ONLY from your truck to the feed tank and back.
8. NEVER GO INTO A HOUSE UNLESS SOMEONE IS GETTING HURT OR THERE'S A FIRE. If you want a tour of a turkey house, we'll arrange one.
9. Stand at the driver's door, throw your boots away right there, and get in. Don't wear the plastic boots into the truck. Use the Alcare to clean your hands, and the bug spray to kill any flies that get in.
10. If there's a wash station on the farm, repeat #4 on the way out.
11. Return to the mill, and drive SLOWLY through the wash station, including the second (disinfectant) cycle. Wash the floorboards and steps with the hose.
12. Keep the windows and doors closed when at the mill.
13. DON'T STOP ON ROUTE. DON'T STOP ANYWHERE PEOPLE MEET AND THEN GO TO A FARM WITHOUT WASHING AND DISINFECTING THE TRUCK AND FLOORBOARDS.
14. Questions/comments to the dispatcher, serviceman or _______________.

Addendum 3. Letter to vendors

Dear Vendor:

A new disease is moving into our area; we intend to keep it from spreading further. We have imposed company quarantines on [X] farms to date. It will be difficult for us to notify you of each specific farm, since the situation will change rapidly. Our growers, servicemen and veterinarians will always know which farms are quarantined.

Effective immediately:

1. When called to a farm, always ask if it is quarantined. If a farm is quarantined and you are also going to other farms, make sure that you go to the quarantined farm last.
2. When you go on any of our farms, we expect you to be in clean plastic or clean disinfected boots when you step out of your vehicle.
3. If you go into the houses, we expect you to be in boots and clean coveralls, with clean equipment, especially anything that contacts litter or manure. We expect you to clean that equipment when you leave.
4. If you can conduct your business by telephone, do so.

We appreciate your cooperation. A copy of the information packet that we sent to our growers is attached for your review.

Sincerely,

Staff veterinarian

CASE REPORT

Seasonal Effects of Turkey Coronavirus Outbreaks

SHANNON JENNINGS

Carroll's Foods, Inc.
Warsaw, North Carolina, USA

INTRODUCTION

Personal observations show a definite seasonal trend of new TCV outbreaks based on serology. Conversations with some other local company veterinarians also suggest a similar trend. No definite explanations are available, but there are some interesting possible associations with local rainfall, temperature and fly population.

DISCUSSION

Diagram 1 shows the number of new breaks of turkey coronavirus as diagnosed by indirect fluorescent antibody test (IFA) over the past 3 years, 1996 through 1998 to date. (The graph represents when IFA test results were received; one should assume on average a 4–week turnaround time from time of submission to time of receiving results. Carroll's Foods has about 200 farms.) We first started testing for turkey coronavirus (TCV) in February of 1996, and I think it had been going on in those particular farms for several months prior to that. In September 1996 Hurricane Fran hit North Carolina, and it seemed after that we had clinical signs at different places. That is when we started testing. Instead of testing just in a particular geographic area, we started testing across the board. So our true baseline of what was actually TCV positive in the field probably doesn't come until September or October of 1996. Then we started working with the three other turkey–producing companies in this area, and they did pretty much the same thing, testing all flocks to see where TCV was. After that we worked on trying to clean it up. 1997 was probably a better year to observe the true trends. TCV positive trends are notable, with a low occurrence rate in the winter months and a high occurrence in late summer and fall. In 1997 we continued depopulating farms, cleaning up, until we got down to only 2 positive flocks in the field in June, and then the number of positive cases started going up. Other companies started seeing breaks. You can see that trend as it peaks in the October and November months. This year (1998) we have had

only one case in January and we don't have any current TCV–positive flocks for our company in the field right now.

Diagram 1. Occurrence of Turkey Coronavirus positive flocks (via IFA), for Carroll's Foods, by month for 1996-1998

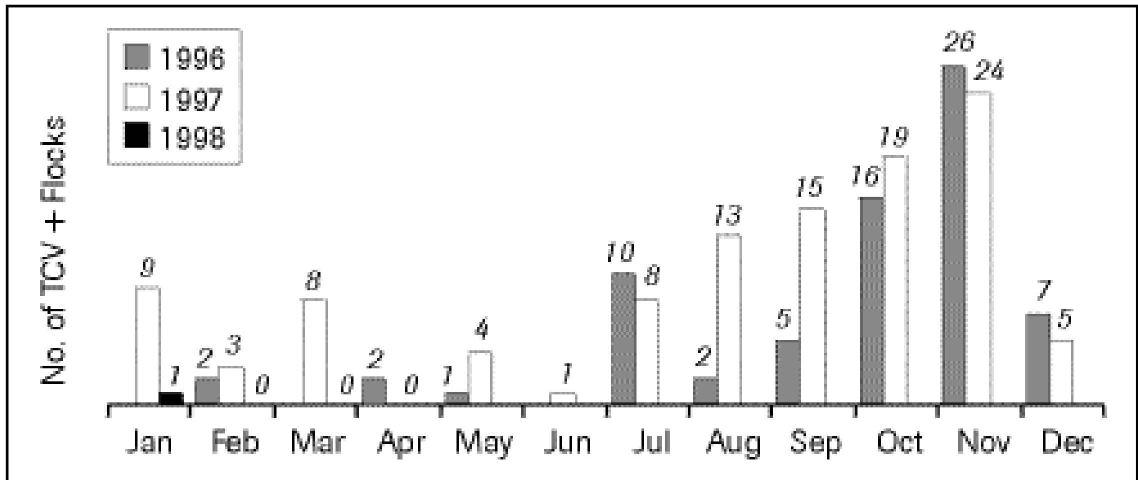
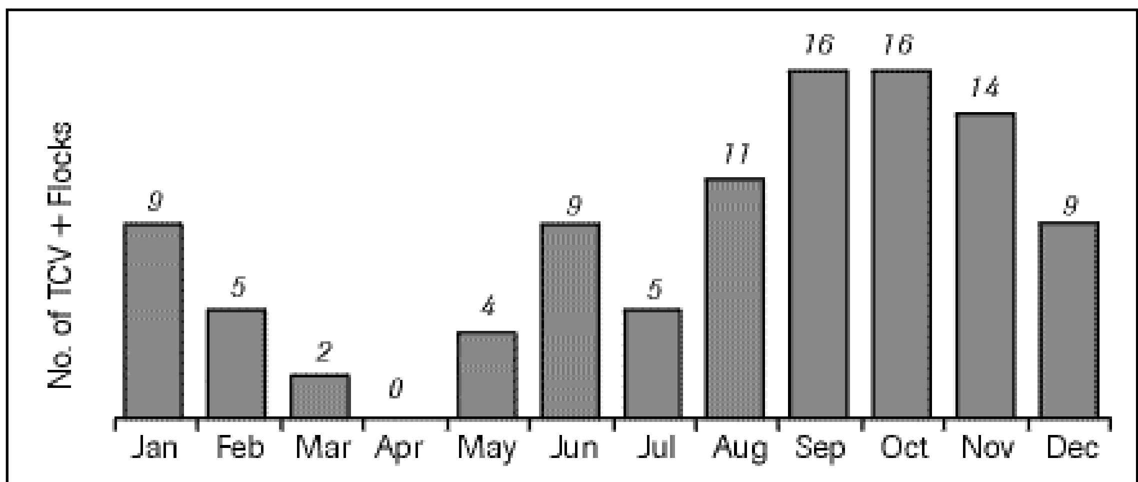


Diagram 2 represents another local company's TCV–positive flocks over the past year (1997). The same trend occurred. From the end of the summer through the fall of the year, we get these explosive outbreaks. Then in the winter months the occurrence of positive flocks seemed to calm down. In the early spring of the year, things are calm.

Diagram 2. Occurrence of Turkey Coronavirus positive flocks (via IFA), for Company #2, by month for 1997



Just a field observation regarding infectivity, it appears that in the spring you can have cases where you have positive farms that don't "blow up" and cause a lot of associated outbreaks nearby. But in the late summer and early fall, if you have a TCV–positive farm in the field and there are other turkey farms within 1 mile of it, they are going to turn positive. There's nothing you can do about it.

I attempted to see if there was an association between rainfall (Diagram 3) or temperature (Diagram 4) and TCV–positive cases. It appears that through the fall of the year we are actually on a declining temperature trend, as TCV cases are trending upward. Temperatures peak in July. In July 1997 we had a tremendous amount of rain in Sampson and Duplin counties. Rainfall totals for the past year are more variable. I do not see any association between rainfall, temperature and TCV cases, other than that the TCV breaks do follow the months of high temperatures and high humidity (i.e., rainfall).

Diagram 3. Total rainfall by month, in inches, for 1997 through March 1998

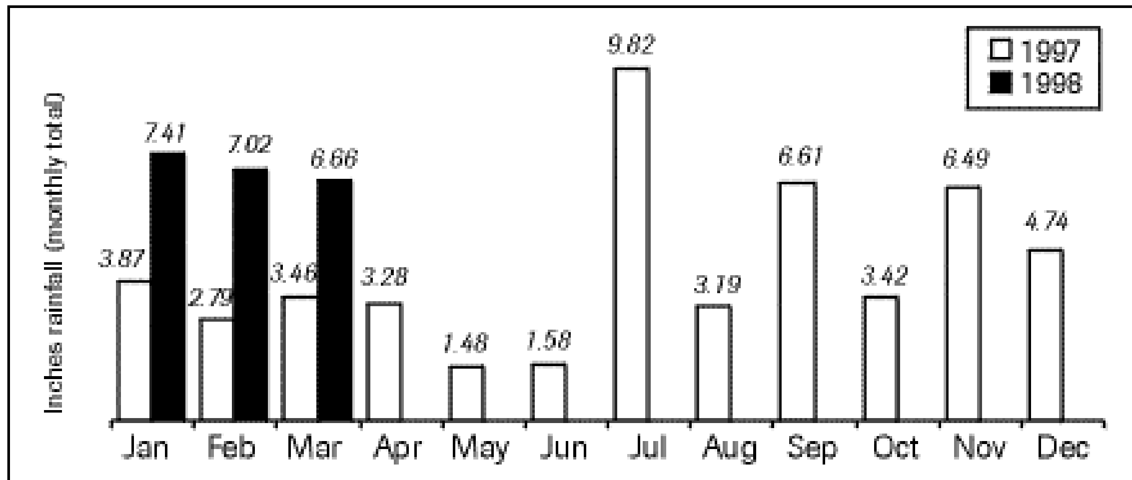
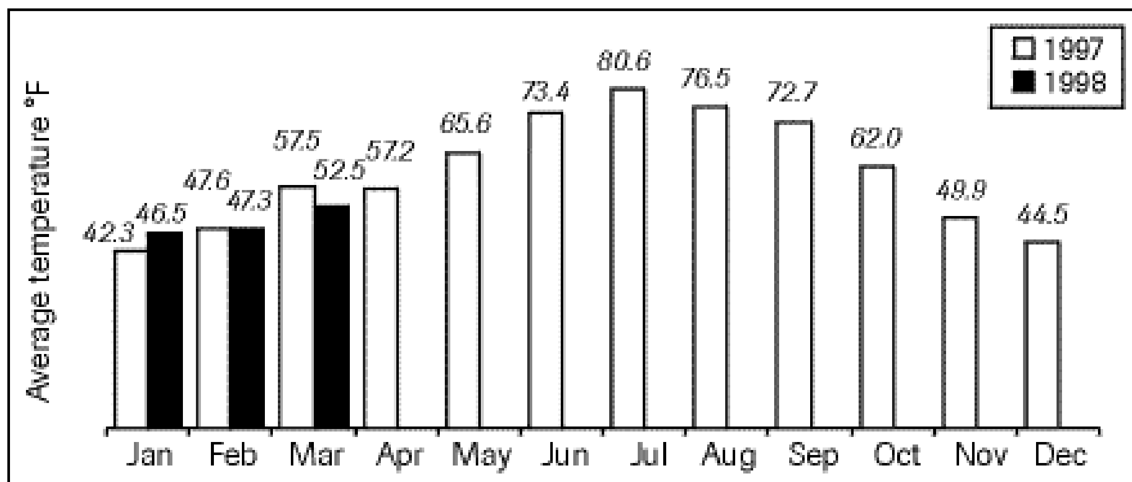


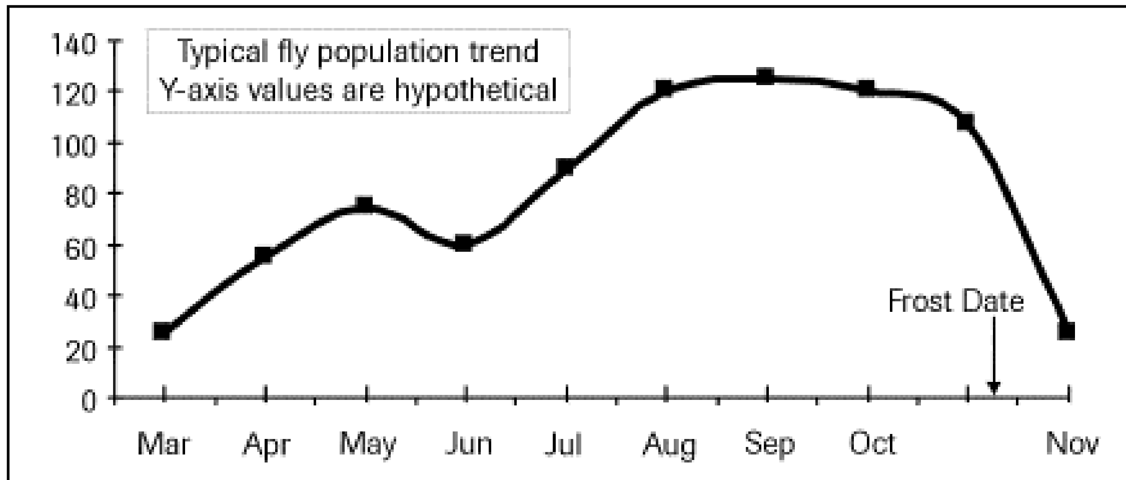
Diagram 4. Average temperature by month, for 1997 through March 1998



This brings up other questions: Are there carrier states or latent infections? Could there be carrier states or latent infections where a stress right in the heat of the summer triggers disease? Then you get these explosive breaks and with every break you get many more millions of virus that can affect other farms. Just as an example, with pseudorabies in hogs, during late stages of the infection, they are not shedding virus, but given a stress (or high doses of dexamethasone) they'll start shedding again.

There also is the question of possible insect vectors. Diagram 5 is a typical fly population trend from Dr. Mike Stringham (NCSU, Department of Entomology). It should be noted that this graph is hypothetical; it does not represent fly sampling in turkeys. It only illustrates relative house fly densities through a typical summer. These values can be much more or less pronounced from month to month. Comparing this to Diagram 1 and Diagram 2, we observe that the largest number of explosive outbreaks occur in October and November, immediately following the peak in the fly season. So there could be an association between TCV and flies.

Diagram 5. Typical fly population trend. (Y-axis values are hypothetical)



SUMMARY

Personal observations show a definite seasonal trend of new TCV outbreaks based on serology, with a higher occurance of TCV in the late summer to early fall months. A similar trend was observed with another company in eastern North Carolina. There is no direct association among rainfall, temperature and TCV cases, other than the TCV breaks do follow the months of high temperatures and high humidity (i.e., rainfall). A graph of typical fly populations does correlate with TCV breaks, suggesting that flies may be associated with TCV.

It is also observed that an active TCV case in the field, in the spring, will remain contained and will not spread. But an active field case during July, August, or September (in eastern North Carolina) is highly transmissible. That is to say, "It will cross company lines".

CASE REPORT

The Economic Impact of Turkey Coronavirus

DAN KARUNAKARAN

Shady Brook Farms
Harrisonburg, Virginia, USA

INTRODUCTION

Our company produces about 17 million turkeys in Virginia, with about 240 growers. Of our production, 70% is hens.

All of the flocks slaughtered in our processing plant are tested for turkey coronavirus (TCV). We do a composite sample, taking 20 sera samples and then we pool them into 1 composite sample. So far it has been working well for the purposes of identifying TCV–positive flocks. Purdue-SIPAC Lab (Tom Bryan) and Virginia Tech (Bill Pierson) have basically done all of our laboratory diagnostics, using indirect Fluorescent Antibody (IFA) and virus isolation. We started out performing some direct FA but we converted everything to IFAs and virus isolation. From June 1996 through November 1997, during that period of 17 months, we tested about 5000 sera using IFA and performed 239 virus isolations. During 1997 we had 49 TCV–positive farms, of which 25 were consumer hen farms (representing 76 flocks) and 24 were heavy tom farms (representing 46 flocks).

I want to show you how the whole corona picture looks in our organization. First will be a review of the TCV–positive hens, then the heavy toms, followed by summary comments on coronavirus. I will also give some observations on enterovirus.

DISCUSSION

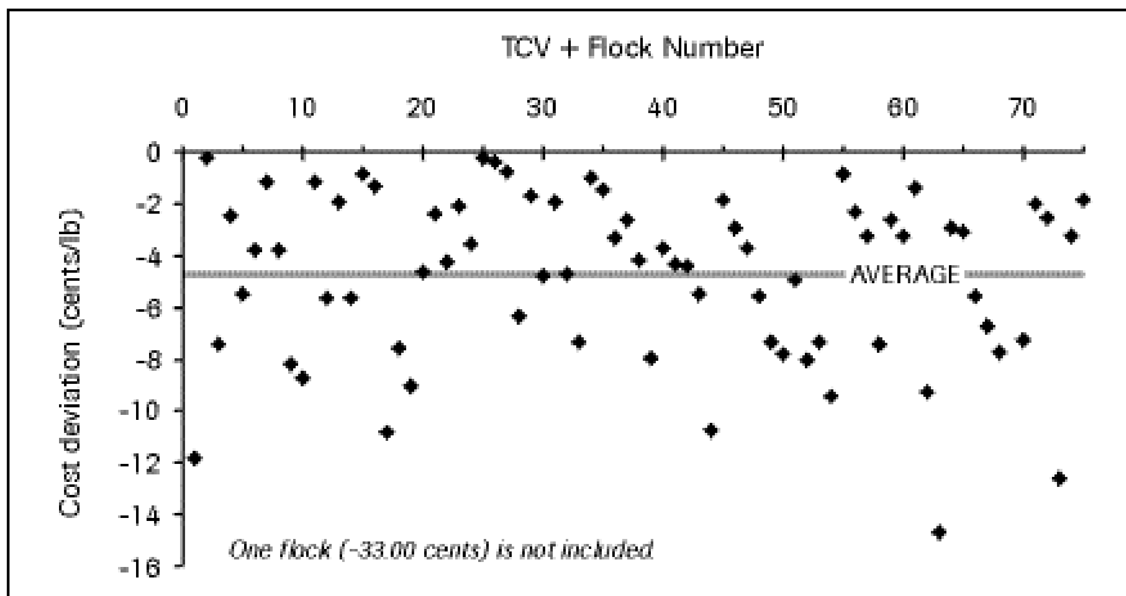
Our company raises four categories of birds: consumer size hens (15.5 pounds), heavy hens (21.0 pounds), light toms (25.5 pounds) and heavy toms (31.0 pounds). This discussion will focus on the effects of TCV on consumer hens and heavy toms.

CONSUMER HENS

In summary, there were 75 hen flocks positive for TCV in 1997, representing 8.8 million pounds of turkey meat. Every pound produced from TCV–positive hen flocks, in our company, costs us 5 cents more than average. Part of that is medication cost, which averaged 1.3 cents. TCV results in poor flock performance, as noted by the poor average daily gains (0.1338 pounds per day), body weights (13.7 pounds at 103 days), and below standard feed conversion (2.49) and livability (86.6%). For a normal TCV–negative hen flock, the body weight would average 16 pounds in 100 days, with a 2.2 feed conversion and 93% livability.

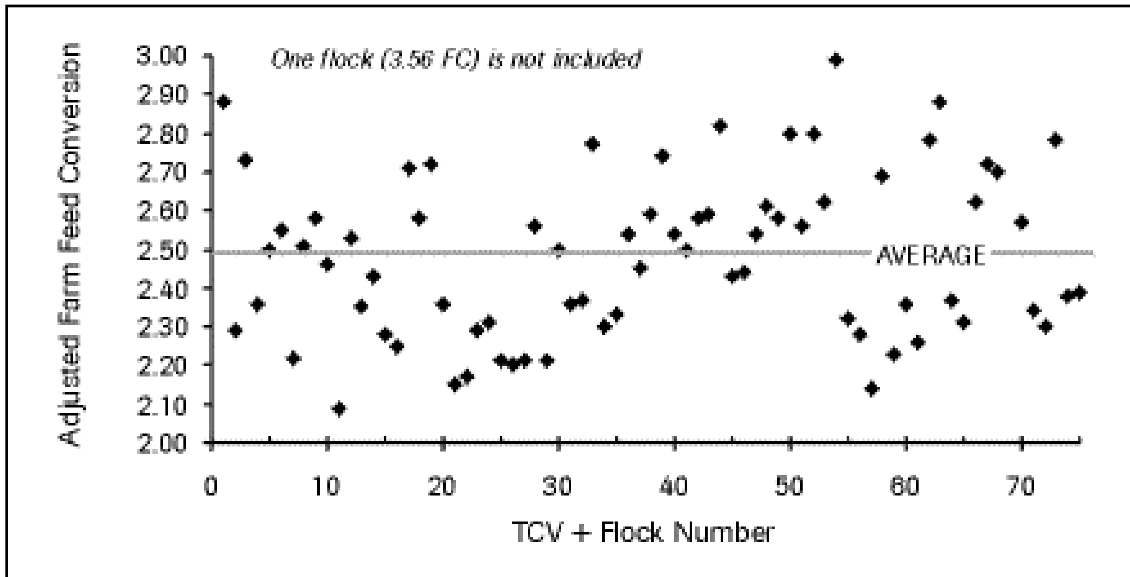
Diagram 1 is the distribution of cost deviation ("Ingradient Cost Factor") for 75 hen flocks that were TCV positive in 1997. The average company cost is shown as "0"; these TCV hen farms averaged 4.74 cents higher cost than average. All of these flocks are higher cost flocks than our normal flocks, ranging up to 14.5 cents higher cost compared to our normal flocks. So, in our situation, all of these flocks cost the company a lot of money.

Diagram 1. Cost variance for 75 commercial hen flocks, TCV positive, in 1997



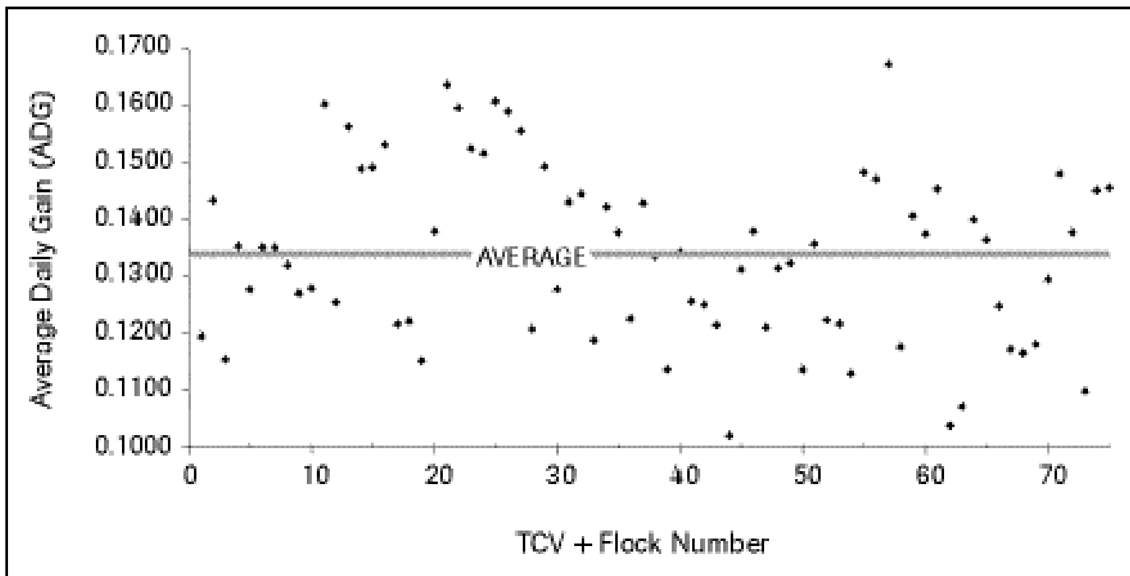
The adjusted feed conversion (Diagram 2) is adjusted to a 14.5–pound bird. For the 75 farms, the average is 2.49. This graph demonstrates the severity and variability TCV can have on flocks. For normal flocks the average feed conversion is budgeted for 2.22 and 2.24 after adjustment.

Diagram 2. Adjusted farm feed conversion for 75 commercial hen flocks, TCV positive, in 1997



Average daily gains (ADG) are very poor, averaging 0.1338 pounds per day. Some of the better flocks, a noncorona flock, may have ADG of 0.1600 to 0.1700. There is a lot of variability in the distribution of ADG (Diagram 3).

Diagram 3. Farm average daily gain (ADG) distribution for 75 commercial hen flocks, TCV positive, in 1997



The average livability (Diagram 4) for these 75 flocks was 86.6%. Average body weight (Diagram 5) was 13.7 pounds. TCV has variable effects on livability and body weight.

Diagram 4. Livability distribution for 75 commercial hen flocks, TCV positive, in 1997

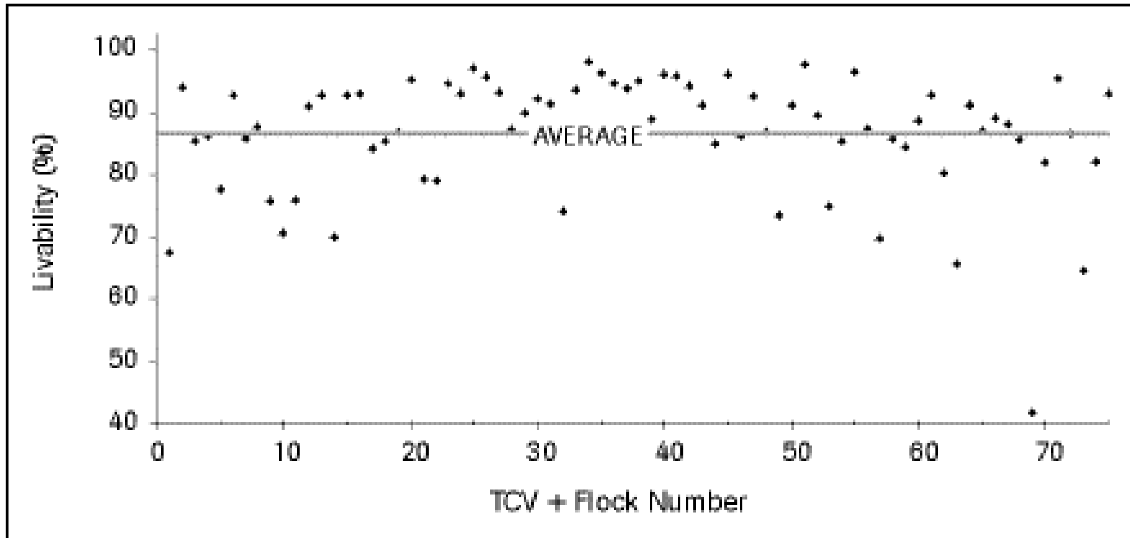
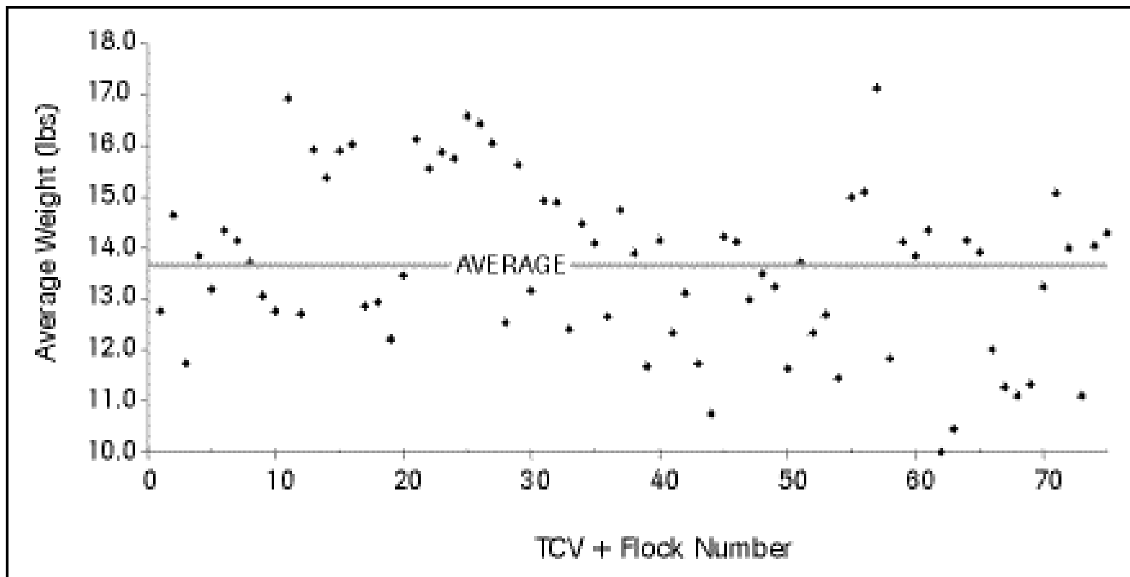
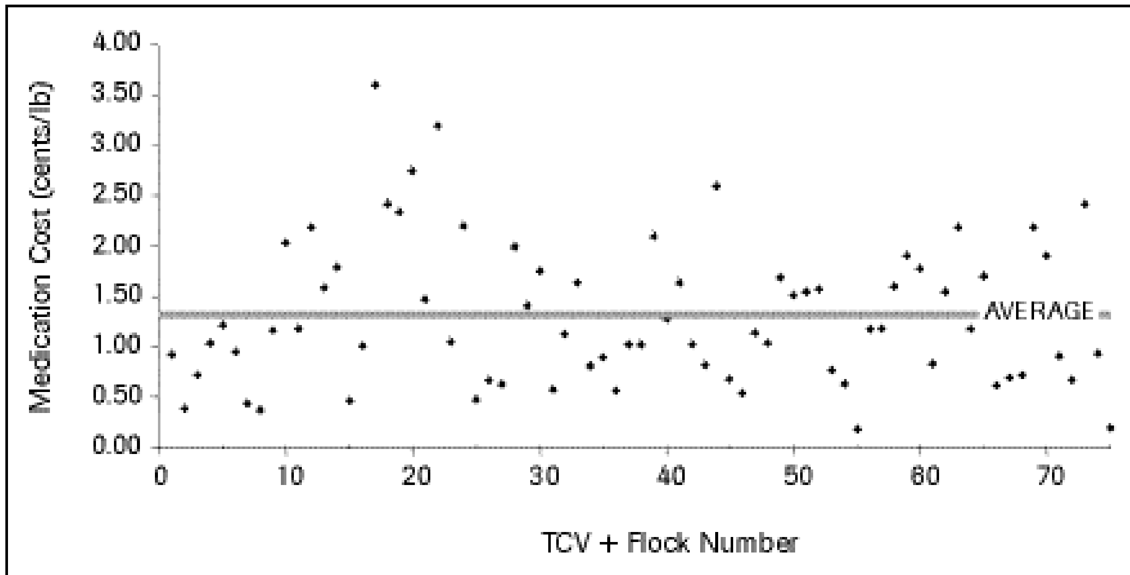


Diagram 5. Average weight distribution for 75 commercial hen flocks, TCV positive, in 1997



The medication cost (Diagram 6) for these TCV hen flocks is high. It is difficult to access the benefits associated with some medications on these infected flocks. Medication cost averaged 1.3 cents per pound.

Diagram 6. Medication cost (in cents per pound of meat) distribution for 75 commercial hen flocks, TCV positive, in 1997

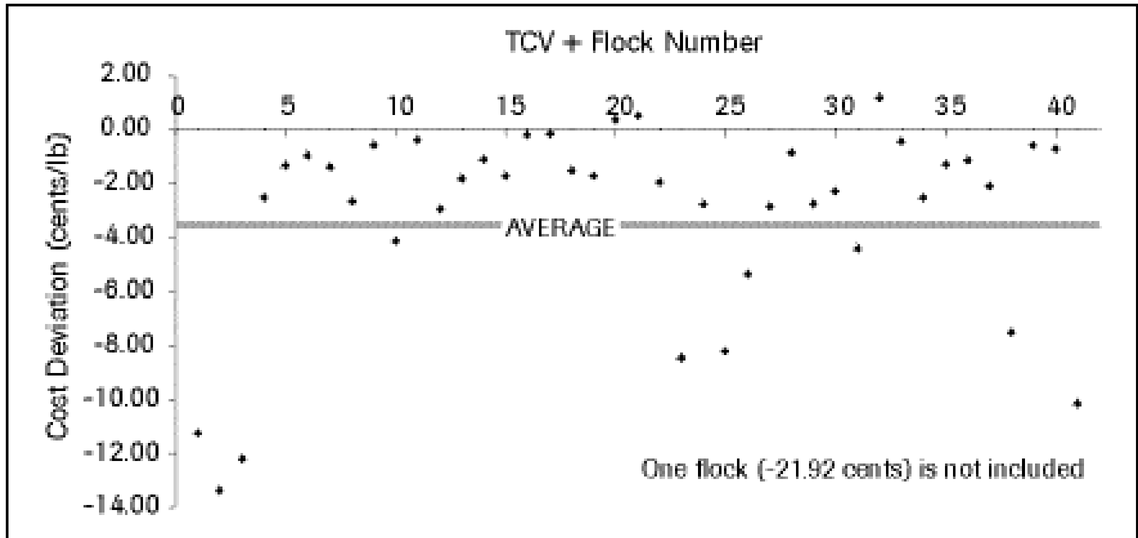


HEAVY TOMS

The heavy–tom category are for 31–pound toms at market. In summary, during 1997 we had 24 heavy tom farms (representing 46 flocks) that were positive for TCV. These 46 flocks accounted for 5.8 million pounds of production. The average cost of production was 3.51 cents per pound higher than the company goal. Medication in excess of expected costs accounted for 1.16 cents. TCV results in poor flock performance, as noted by the poor average daily gains (0.2051), body weights (25.3 pounds at 127 days), and below standard feed conversions (2.53) and livability (83.35%). For an average TCV–negative heavy tom flock (approximately 130 days of age) ADG would be 0.2300 to 0.2400, livability would be 88%, with a feed conversion of 2.40 for a 30–pound tom.

As with the hens, the cost deviation (Diagram 7) for these 46 TCV–positive heavy tom flocks averaged 3.5 cents higher than the company goal. Some flocks were up to 20 cents.

Diagram 7. Cost deviation for 46 heavy tom flocks, TCV positive, in 1997



The adjusted feed conversion (Diagram 8) and livability (Diagram 10) were both poor, averaging 2.53 and 83.3% respectively. Some flocks experienced up to 50% mortality. The ADG was 0.2051 pounds per day (Diagram 9), ranging from 0.1700 to 0.2400. We really did not have a single corona flock that had acceptable performance.

Diagram 8. Adjusted farm feed conversion for 46 heavy tom flocks, TCV positive, in 1997

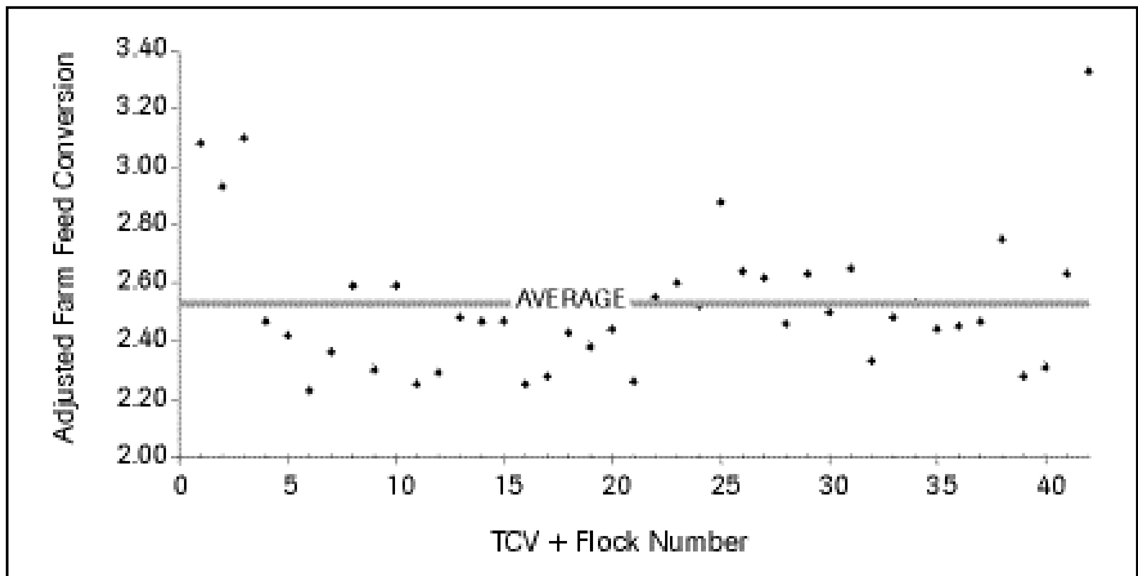


Diagram 9. Farm average daily gain (ADG) distribution for 46 heavy tom flocks, TCV positive, in 1997

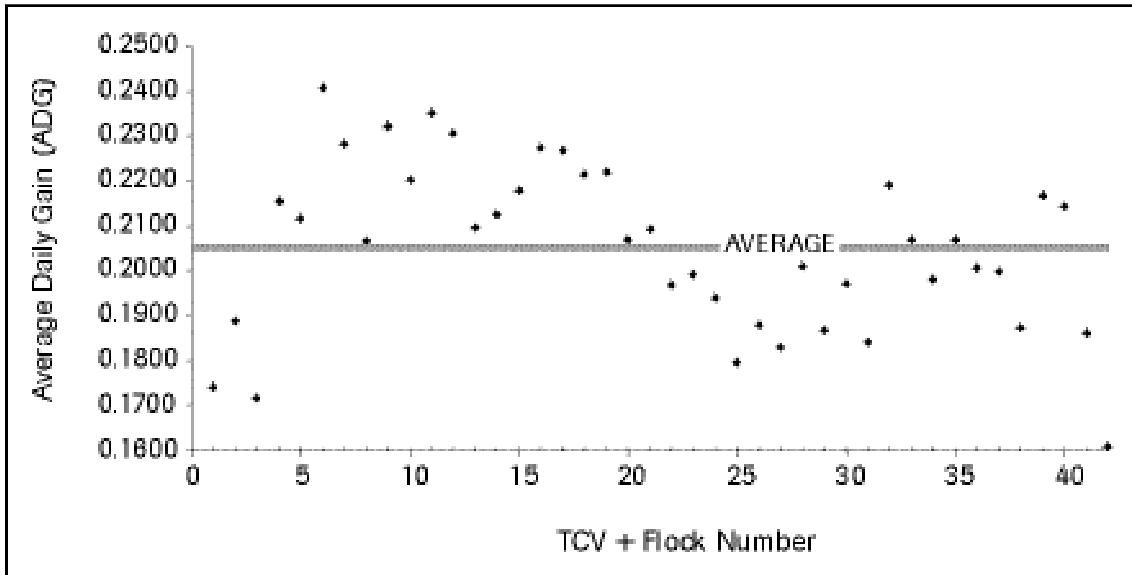
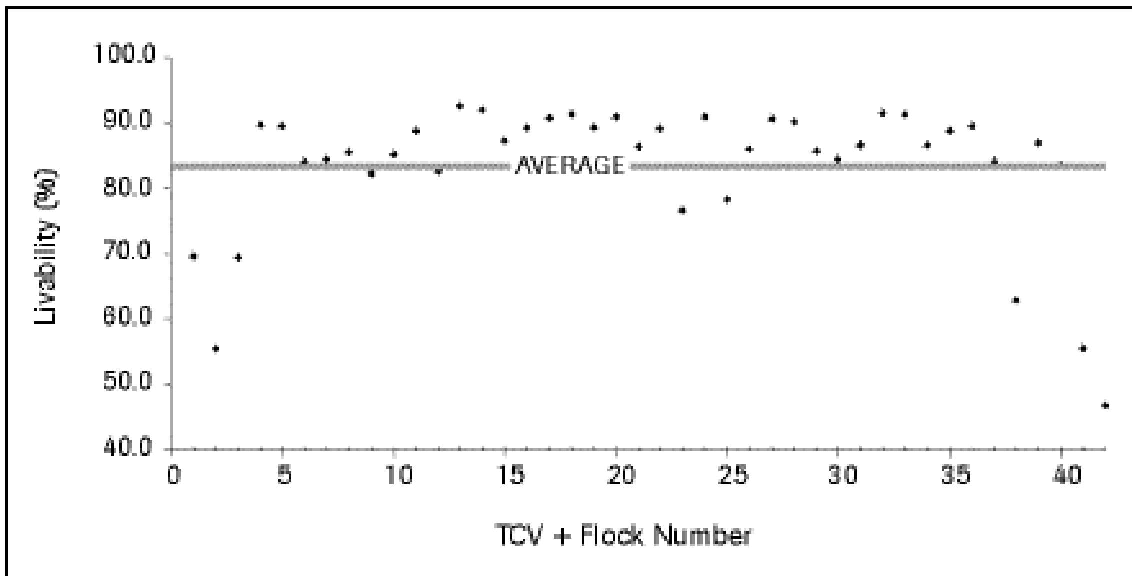
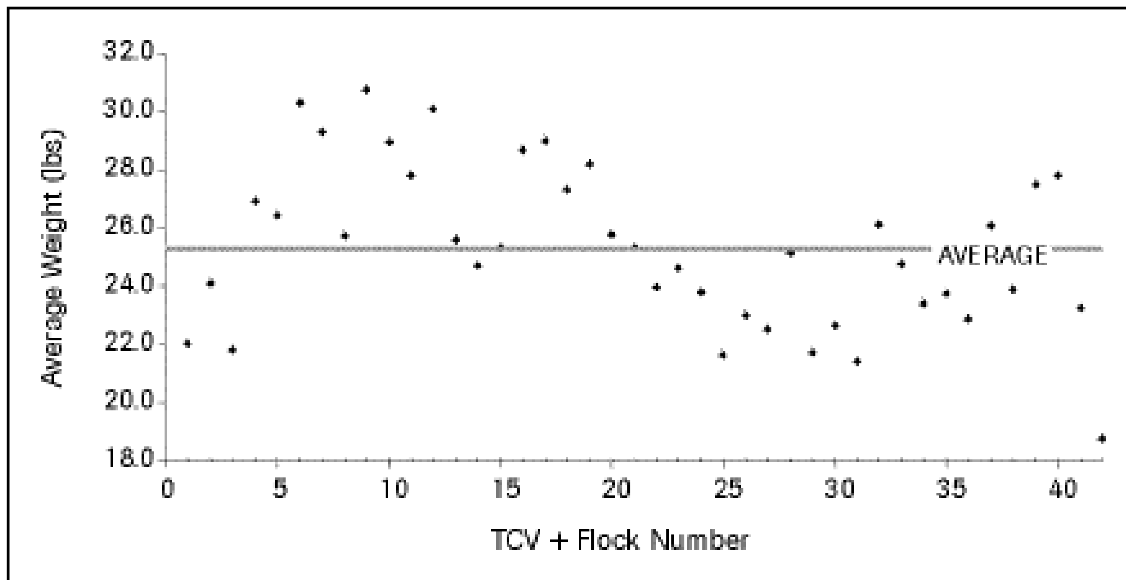


Diagram 10. Livability distribution for 46 heavy tom flocks, TCV positive, in 1997



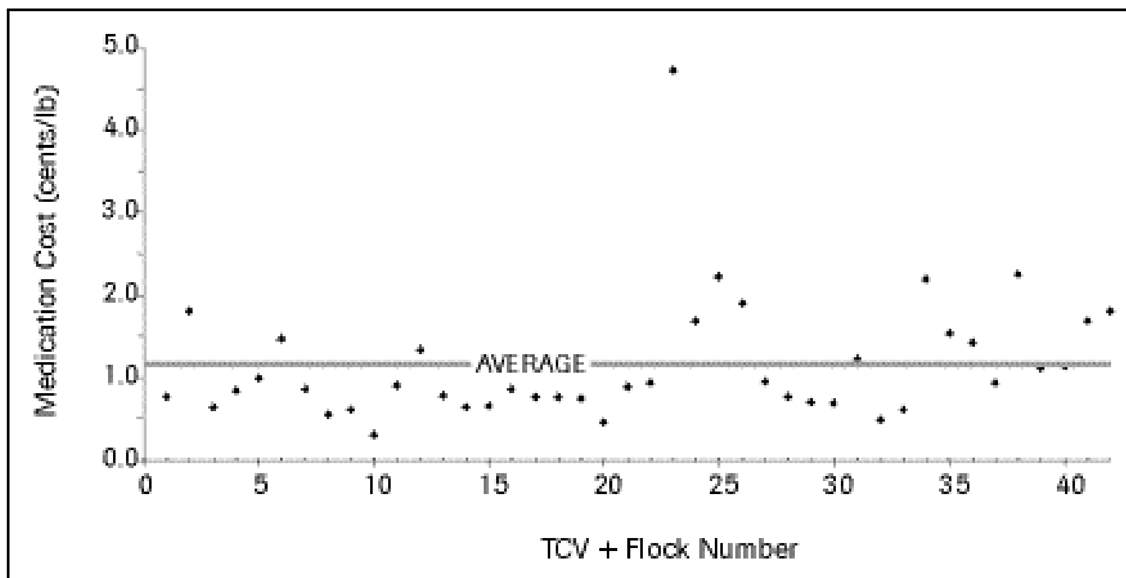
Average body weights (Diagram 11) for these 46 flocks were severely affected, averaging only 25.3 pounds at 18 weeks of age. The expected weight for the same age bird in a normal flock is 30 to 32 pounds. None of the flocks were able to reach an average of 30 pounds.

Diagram 11. Average weight distribution for 46 heavy tom flocks, TCV positive, in 1997



Medication cost (Diagram 12) averaged 1.16 cents per pound, ranging from 0.31 to 4.73 cents.

Diagram 12. Medication cost (in cents per pound of meat) distribution for 46 heavy tom flocks, TCV positive, in 1997



SUMMARY

TCV did cost the entire company quite a bit of money. The actual losses for 1997 to the contract growers were $243,000. TCV cost the company's growout division, with increased feed cost and performance losses, approximately $729,000. The processing plant had increased cost of $641,000 associated with processing small birds, uneven

birds, increased time. Total losses to the company attributed to TCV in turkeys amounted to $1.6 million for 1997 alone. This did not include 5 or 6 flocks that had to be destroyed. This did not include several TCV–positive flocks identified in late 1997 and scheduled to be slaughtered in early 1998.

In summary, in 1997 there were 75 consumer hen flocks, 46 tom flocks and 9 heavy hen flocks (data not shown) for a total of 130 flocks positive for TCV. The average loss for the grower was $1,900 per flock. The growout division lost an average of $5,600 per flock, and the processing plant lost on average $4,900 per TCV positive flock. The total loss was over $12,000 per flock.

CASE REPORT

Comments on In-House Studies on Turkey Coronavirus

DAN KARUNAKARAN

Shady Brook Farms
Harrisonburg, Virginia, USA

INTRODUCTION

The following is a review of several studies we have conducted in an attempt to better understand turkey coronavirus (TCV). Studies involved:

- Cattle and sheep manure
- Beetles
- Meckel's Diverticulum
- Poultry meal samples and litter samples
- Flock evaluation over time

DISCUSSION

We took 10 different farms where the cattle were in close proximity to corona–positive flocks and took 5 cattle manure samples and made composite samples. These samples were put into embryonated eggs to recover corona virus. Two different laboratories failed to isolate TCV. We also similarly evaluated sheep manure, but never isolated TCV.

Beetles from positive farms were evaluated for the presence of TCV. Beetles were collected from a farm that was confirmed corona positive. Beetles were separated from the litter. Twenty-five beetles were emulsified and this homogenate was put into embryonated eggs. TCV was never isolated.

Meckel's Diverticulum has been implicated as a site for coronavirus. Mid-gut samples, including the Meckel's Diverticulum, from 30 to 50 birds were collected at the processing plant. One flock was turkeys that followed a positive flock without depopulation, but were serologically negative and clinically negative. Body weights were good. This was a flock that was in the brooder house while the older growout flock was positive. These

brooder–house birds were TCV negative and remained negative through market. The homogenate was put into embryonated eggs and was passed twice. We could never isolate TCV.

We also evaluated two farms that broke with TCV after depopulation. After depopulation, we had 2 successive TCV–negative flocks followed by a TCV–positive flock. Samples of Meckel's Diverticulum were submitted for virus isolation. The homogenate was put into embryonated eggs and was passed twice. We could not isolate TCV.

Growers ask if poultry meal could be a source of TCV contamination. Samples were collected and tested in embryonated eggs. TCV was not isolated.

Litter samples from positive flocks were tested for "infectivity". One-day–old poults were gavaged with litter samples. We could not reproduce weight suppression. Nor could we isolate TCV out of the litter from positive flocks. Our company also has a broiler operation. In an attempt to see if broilers could be carriers for TCV, 6 broiler breeder flocks were tested. Sera were tested by IFA and they were negative.

Depopulated farms usually became positive after 2 successive negative flocks. Depopulation was found to be 60% effective. Indeed, only 60% of those farms depopulated in 1996 remained negative in 1997.

CONCLUSION

The biggest questions are where does TCV come from and what is the source of infection? TCV in the field can be highly infectious. Vectors and transmission are big issues.

Have we ever seen any TCV–positive flock turn TCV–negative? Yes; it was a breeder flock that went positive at 18 weeks of age. While routinely testing for TCV, we found all our breeders positive at one time, but this particular flock broke at 18 weeks with clinical signs, and then we force–molted these hens. During the force–molt period, they turned negative, and they stayed negative until they went to market. I do not know exactly how to explain this situation. Does the virus just come and never really reside in the turkeys? We placed sentinel birds in the breeder flock at force–molt time, but never isolated the virus. So does that mean the virus is not there? Based on that test, it appears to be the case.

We have initiated "biosecurity audits" (Table 1). Biosecurity is a "misused and abused" word. We have selected 18 different aspects of biosecurity that we feel should be a priority. To ensure compliance, we then audit farms for biosecurity.

Table 1. Farm Biosecurity Audit Form

Farm Name: ____________________

Date: ____________________

	Items	Yes	No	Corrective Action	Notes
1	Flock management to specific individuals: no visitors.				
2	Do you have proper clothing and footwear for all visitors?				
3	Clean boots worn in poultry houses?				
4	Disinfect station available at every entrance?				
5	Are the shared or rented farm equipment clean and disinfected?				
6	Do you visit other poultry farms?				
7	Do you have family members in poultry-related jobs?				
8	Are the dead birds disposed of properly? Are they covered transported?				
9	Do dogs & cats have access to the v icinity of turkey houses?				
10	Are the beetles & flies under control?				
11	Do you use rodent baits regularly?				
12	Any "backyard poultry" on the farm?				
13	Cleanout equipment disinfected?				
14	Litter leaving the farm: Is it always converted?				
15	Where does the litter go?				
16	Are the doors kept locked?				
17	Equipment used in cattle must be disinfected.				
18	Is the grass and overgrowth trimmed properly?				

CASE REPORT

Comments on Enterovirus in Turkeys

DAN KARUNAKARAN

Shady Brook Farms
Harrisonburg, Virginia, USA

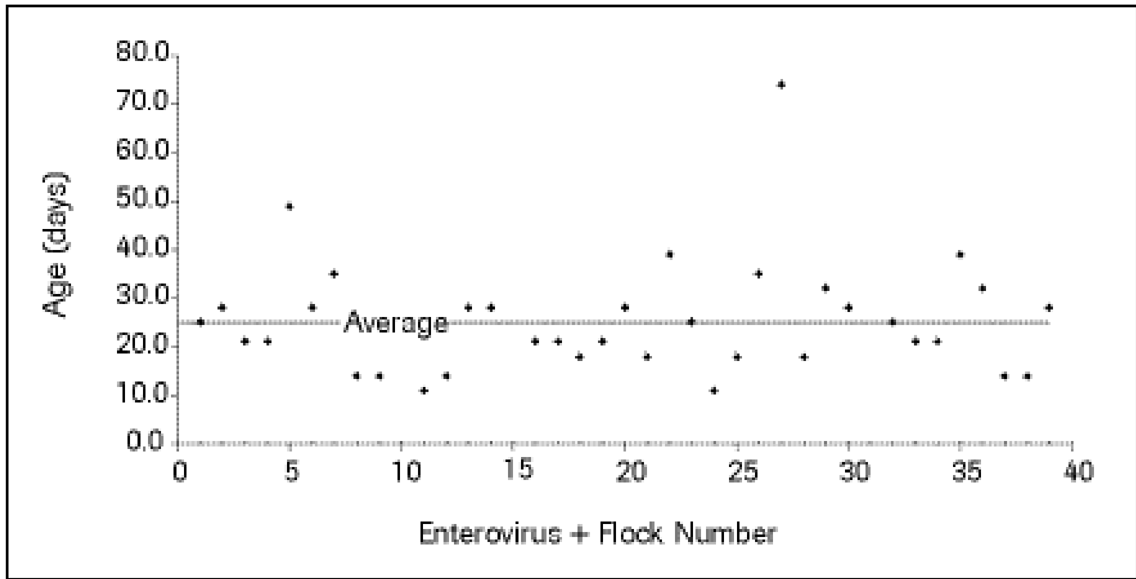
INTRODUCTION

Flocks with flushing are often suspected of being affected with coronavirus. For any flock that is flushing we routinely collect 3 to 4 mid-gut samples, freeze them, and ship them on dry ice. Intestinal samples are sent to Indiana (SIPAC Laboratory) for virus isolation. The lab can put these samples into embryos and give you 3 possible results: (1) no virus isolation, (2) coronavirus isolation or (3) or enterovirus isolation. We have confirmed several enterovirus isolations. I am unaware of a serologic test available for detecting enteroviruses.

DISCUSSION

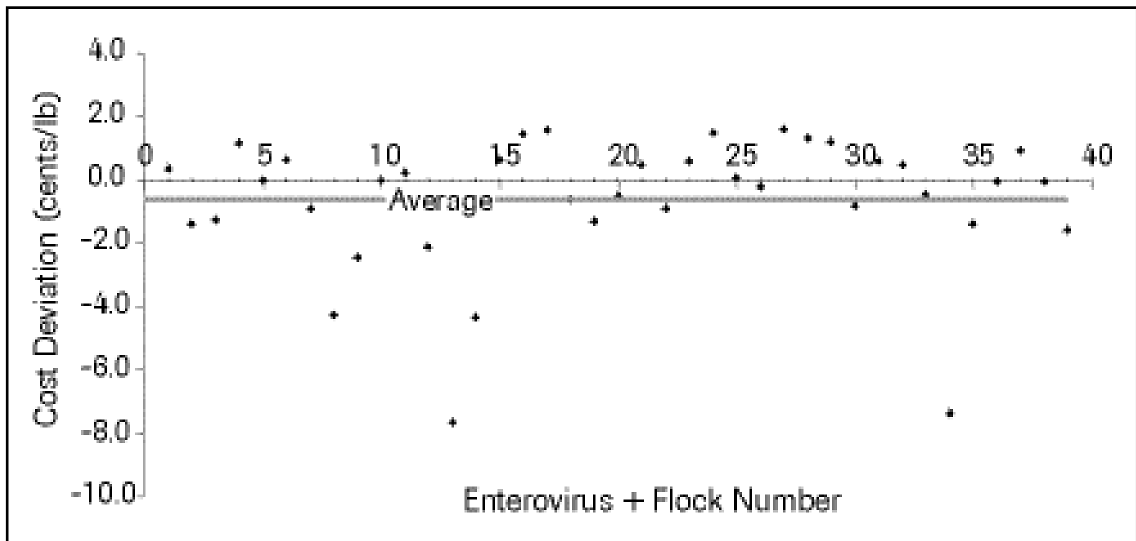
Age distribution (Diagram 1) of confirmed enterovirus–positive flocks is quite spread out. This represents 39 flocks. The average age was 25 days, with the majority of flocks between 14 to 42 days of age. It is basically a brooder house problem. These flocks were confirmed enterovirus positive, coronavirus negative and all flocks presented clinically with flushing.

Diagram 1. Age distribution (in days) for 39 turkey flocks confirmed enterovirus positive (+) and coronavirus negative



The average cost deviation for these 39 enterovirus positive flocks was -0.63 cents, ranging from +1.61 cents to -7.65 cents. Some flocks were devastated. Flocks presented with flushing and off-feed and had a wide variation in body weight. The only difference is that these enterovirus flocks appeared to recover somewhat better than TCV flocks.

Diagram 2. Cost deviation (in cents per pound of meat) for 39 turkey flocks confirmed enterovirus positive (+) and coronavirus negative



CASE REPORT

Turkey Coronavirus in a "Remote Brood" Heavy Tom Facility

DAVID RIVES

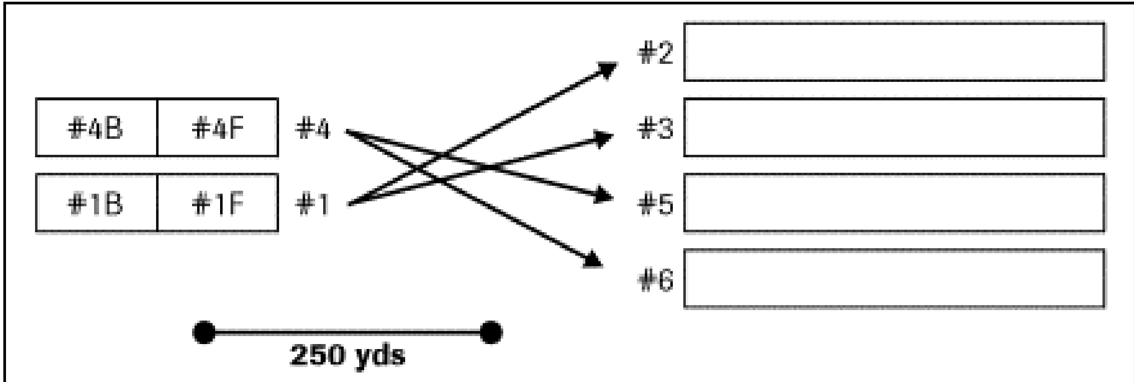
Prestage Farms
Clinton, North Carolina, USA

INTRODUCTION

This case of turkey coronavirus occurred between November 1997 and February 1998, in 6–week–old male turkeys. The affected farm consisted of two Brooder Houses and four Growout Houses. House #1, a Brooder barn, placed 11,283 Nicholas turkeys. The other Brooder barn, House #4, placed 4,100 Nicholas and 7,300 Hybrid turkeys. This case involved two farms, one farm that was affected (22,683 birds) and another farm that was most likely the source of the infection.

The affected farm is a double tom unit with two Brooder Houses and four Growout Houses (see Diagram 1). The Brooder Houses are located approximately 200 yards to the east of the Growout barns. Flocks are placed every 14 to 15 weeks. Birds are moved on a trailer at 6 weeks of age to the Growout barns. Brooder House #1 has nipple drinkers and supplies birds to Growout Houses #2 and #3. Brooder House #4 has bell drinkers and supplies birds to Growout Houses #5 and #6. Each Brooder House has a separate caretaker.

Diagram 1. Farm layout



not drawn to scale

CLINICAL SIGNS

Approximately two weeks prior to the onset of clinical signs, the caretakers were involved in removing litter from Brooder Houses on another farm that was later determined to be coronavirus positive. The caretaker for House #4 drove the spreader truck.

House #4 flushed severely at 6 weeks of age. Birds were extremely depressed. Flushing began in the rear of the House (#4B) and moved into the front (#4F) over the course of a week. Birds from House #1 did not show any clinical signs until 13 weeks of age (6fi weeks after transfer to Growout Houses #2 and #3).

PERFORMANCE

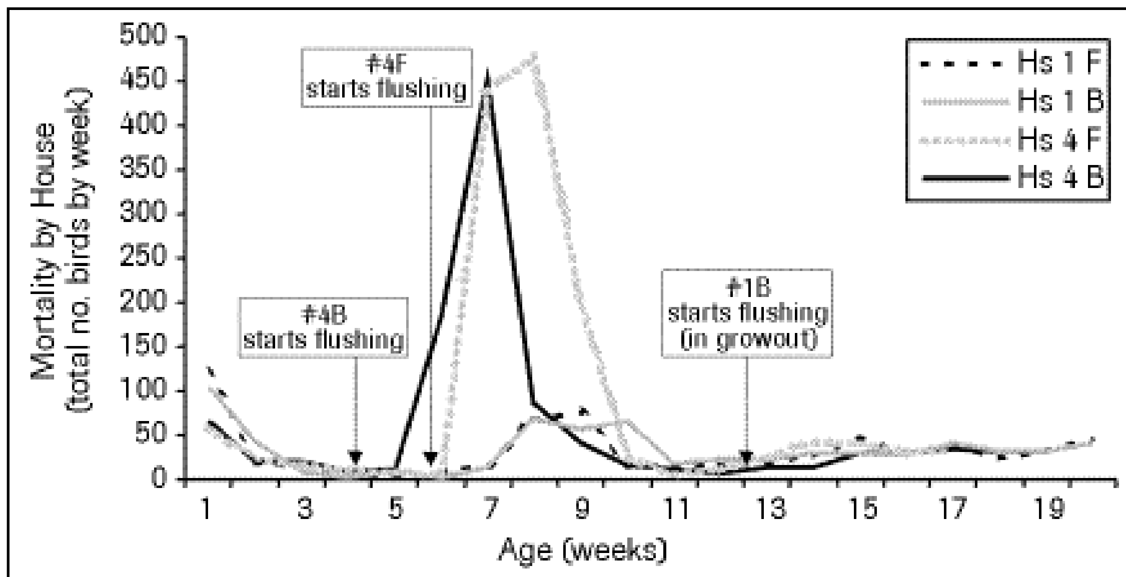
Feed conversion: 0.12 higher than the company 2—week average (0.42 higher than the farm's previous 3 flocks).

Mortality: 6% higher than the company 2—week average (7% higher than the farm's previous 3 flocks).

Avg. daily gain: 0.02 pounds less than the company 2—week average.

Total mortality at 20 weeks of age for the respective Brooder Houses: House #1F was 11.3%, #1B was 12.4%, #4F was 25.8%, #4B was 18.9%.

Diagram 2. Mortality by week (House 1 placed 11,283 birds. House 2 placed 11,400 birds)



DIAGNOSTICS

The previous flock on this farm tested TCV negative at 19 weeks by IFA at the time the present flock was 5 weeks old. It (Brooder House #4) was later confirmed TCV positive (see Table 1 and 2).

Table 1. Summary of TCV serology

Date	No. of birds (age)	House	Test (Lab)	Result
November 11, 1997	6 birds (6 wks)	#4B	IFA (Purdue)	Negative
December 4, 1997	6 birds (7 wks)	#4B	IFA (NCSU)	Positive
	6 birds (7 wks)	#4F	IFA (NCSU)	Negative
	6 birds (7 wks)	#1F	IFA (NCSU)	Negative
February 8, 1998	6 birds (16 wks)	#3	IFA (Purdue)	Positive
	6 birds (16 wks)	#5	IFA (Purdue)	Positive

Results of samples collected when the flock was 7 weeks of age (by Dr. Dennis Wages, NCSU-CVM) indicated that House #4 was TCV positive.

Table 2. Summary of diagnostic tests performed at 7 weeks of age

	House #1F	House #4F	House #4B
Serology: TCV IFA	Negative	Negative	Positive
Feces: TCV EM	Negative	Positive	Negative
Feces: Salmonella	Negative	Negative	Negative
Feces: Cryptosporidia	Negative	Negative	Negative
Feces: Coccidia	1+	2+	2+
Histology	No significant lesions in most tissues. 1 bursal core. Intestine was WNL (within normal limits).	Villus atrophy and fusion. Bursal lymphoid depletion and necrosis. Thymic atrophy.	Villus fusion and blunting. Severe lymphoid atrophy and necrosis of bursa. Thymic atrophy.

MANAGEMENT

Litter from Brooder House #1 was used to top-dress Growout Houses #5 and #6. Litter remained in House #4 for 3 weeks before being spread. Litter in the Growout Houses remained for 3 weeks after the birds were sold before removal. Dead birds were picked up for rendering on a coronavirus–positive route dedicated only to TCV–positive farms. Poults were placed back in the Brooder Houses prior to the sale of the coronavirus–positive flock.

SUMMARY

This case involved a 2-age farm facility that was diagnosed positive for TCV in the brooder house. The affected farm consisted of two Brooder Houses and four Growout Houses. Clinical signs correlated with diagnostic tests. IFA was used to confirm the

diagnosis of TCV. Clinical signs progressed as the birds got older and correlated with the infected brooder house. One brooder house was suspected as the source of the initial infection. It is suspected that the farm was contaminated with TCV by caretakers who cleaned out a distant TCV–infected farm.

Following an extensive cleanout and disinfection program to eliminate the disease, the subsequent flock has remained TCV negative. The subsequent flock was placed on a normal rotation schedule and was not delayed due to depopulation.

CASE REPORT

Epidemiology of an Interesting Outbreak of Turkey Coronavirus in Western North Carolina

RONNIE PARKER

Circle S Ranch
Monroe, North Carolina, USA

SUMMARY

Eight farms in a 3-mile radius were positive for turkey coronavirus (TCV). All 8 were repopulated while TCV positive birds were still on the farm (due to logistics, a complete depopulation was not possible). Seven of the eight farms have remained TCV negative as of May 1998. Rendering trucks were implicated in the initial spread.

DISCUSSION

As of May 1, 1997, we had no serologically positive cases of TCV. By June 1, 1997, we had 11 confirmed positive farms by blood test. Basically this TCV outbreak went through the summer with only 5 or 6 more positive farms and by the fall of the year we were hit with about 19 more, giving us 36 positive farms for the year, which is about 36% of Circle S farms.

We depopulated every farm but 7 that were all in one area. We wanted to do an area depopulation, but we had already placed birds on 2 farms in that area, so we elected to place all farms and depopulate the entire area of positive farms on the next placements. On the other 29 farms we did an extensive clean-up and had at least 4 weeks down time after manure was removed from farm. Where we had several farms in an area, we waited and placed all back together requiring some growers to be empty 6 weeks longer than they would have been. On 85% of our TCV–positive farms we converted to single-age operations by either all-in/all-outs or brood-and-move production. We created an extensive biosecurity program with employees and growers, including: installing disinfectant units on service trucks and feed trucks, writing biosecurity programs for each department, writing step-by-step depopulation procedures for growers, distributing letters and having meetings with all vendors that may visit farms, conducting round-table discussions with department heads. Service people and growers made sure everyone was involved in the biosecurity plan.

On the 7 farms that were TCV positive (with turkeys placed back on without depopulating or removing manure), only 1 was serologically positive for the subsequent flock. Although most of the flock feed conversions were higher than average, almost all flocks still gained very well. A few flocks performed better than average. Three of the flocks were uneven in size and had clinical signs of TCV. All were tested 3 times (at 8, 12 and 18 weeks of age) but still only 1 showed serologically positive. We had several flocks that tested TCV positive at one lab and negative at another lab. Further testing at both labs continued to report negative results. The birds never had any TCV clinical signs.

Average livability on TCV–positive flocks was 8% worse than on TCV–negative flocks, although some TCV–positive tom flocks went to market with 90% livability. Average cost difference between positive and negative flocks was 4 to 5 cents per pound.

It has been observed over the past few years that generally the clinical signs associated with coronavirus are more severe May through October. The first flock that is positive on a farm is usually not affected except for feed conversion; the second presents with a combination of increased mortality, continued medication treatments and poor performance. The third flock is a disaster. We have had enteritis problems on numerous flocks that respond to treatment and go on to perform well. We also have severe enteritis problems that cause the birds to grow unevenly and perform at a substandard level but do not test positive for TCV, even though they have signs suggestive of TCV enteritis. Is this another type of virus or a type of TCV that can not be serologically identified (at this time)? We feel that most of our PEMS cases are TCV–related and, for some unknown reason, from November through April, birds are not affected as severely as for the remainder of the year.

In our operation we feel that single-age production with a total downtime is the only way to eliminate coronavirus. We are working toward our goal of all single-age farms. We will depopulate any TCV–positive farm we may have and do our best to single–age that farm. In 1998 we will off-site brood (brood-and-move) for 27% of our farms and 5% will be all-in/all-out. We have already seen substantial improvements on farms where single age has been implemented. We also feel that incineration for dead bird disposal has been a major advantage for reducing all health problems. Continued rodent control is a must to ensure a corona-free farm. The "explosion" of TCV in the fall of the year could be attributed to the increased movement of rodents due to cooler weather. We are not putting much faith in a cure for TCV other than by total eradication, but we would love to see a tool that would help us cope with TCV on a farm until we could depopulate it.

CASE REPORT

Epidemiology: Two Interesting Cases of Turkey Coronavirus

Anonymous Cases Presented by
JEAN-PIERRE VAILLANCOURT

North Carolina State University College of Veterinary Medicine
Raleigh, North Carolina, USA

SUMMARY

Clinical and production information are presented for two farms. Farm A had 4 flocks and Farm B had 3 flocks. On farm A, the negative impact of coronavirus on productivity is obvious. However, on the second farm (Farm B), the TCV–positive birds were heavier at processing time. Interestingly, half of the last flock on Farm B was TCV positive and half was TCV negative. Both groups were raised in the same brooder house and at the same time, separated only by a wire fence. If coronavirus is so contagious, why did half of one barn stay corona–negative?

DISCUSSION

Farm A (Diagram 1)

Flock #1 was tested for coronavirus in February 1997 via IFA and 6/6 samples were positive. Flocks #2 and #3 also tested positive. Flock #4 tested negative for TCV. The negative impact of coronavirus on productivity is obvious (Table 1).

Table 1. Production data for Farm A

Production parameter	*Flocks #1, #2, #3 (average)*	*Flock #4*
Livability	92.4%	86.8%
Mean weight at slaughter	26.52 lbs	37.67 lbs
Gain	2210	2672
Feed conversion	2.60	2.38
Feed cost/lb[a]	2.2¢/lb higher than Flock #4	–
Medication cost/lb	0.35¢/lb higher than Flock #4	–
Grower pay/head	$1.14	$2.18

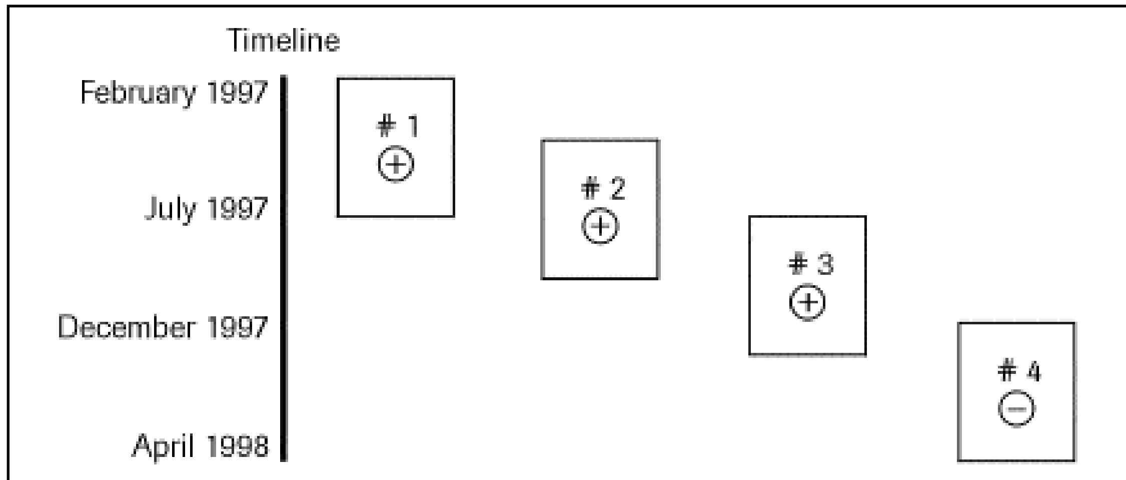
[a]Adjusted to same ingredient cost

This is a two-age farm with the brooder house separated by about 100 feet from the growout houses.

Flocks #1, #2 and #3 flushed in the brooder house and somewhat in the growout houses. Livability was normal; feed conversion, average daily gain and medication cost were very similar for these three TCV–positive flocks.

The farm was not depopulated. Flock #4 was two weeks old when Flock #3 was sold. The growout houses were cleaned and new shavings spread prior to moving Flock #4.

Diagram 1. Schematic of timeline and TCV diagnosis for Farm A, by flock.



Farm B (Diagram 2)

Flock #1 was TCV negative at 16 weeks and was sold at 20 weeks.

Flock #2 was placed when Flock #1 was 14 weeks of age. Flock #2 flushed at 4 to 5 weeks of age and tested positive to coronavirus at 7 weeks.

Flock #3 was placed the same day from various breeder flocks. Interestingly, half of Flock #3 was TCV positive and half was TCV negative. The back section (#3B) flushed at 5 weeks of age and tested positive at 8 weeks. (Routinely, flushing flocks are tested 2 - 3 weeks following a flushing episode, in order to allow the TCV–suspect flock to seroconvert). The front section's (#3F) floor stayed dry and the birds did not flush. At 6.5 weeks of age, the birds were moved to two growout houses 80 feet away from the brooder house.

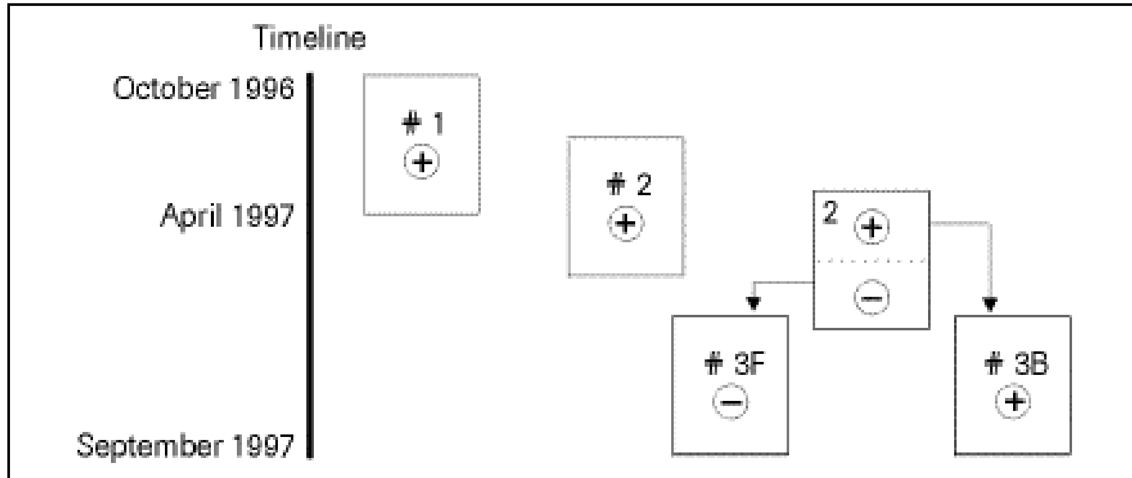
The birds from the #3F remained clinically normal and the growout house stayed dry throughout to market. These birds tested negative to coronavirus when bled two days prior to market. The mean weight of these birds was 29.24 lbs. Of 7,700 birds placed, 876 died (88.62% livability).

The birds from the back section of the brooder house (#3B) flushed for a short period of time after moving to the growout house. They tested positive to coronavirus when tested 2 days prior to market. These birds weighed on average about one pound more

than the birds from the (TCV negative) front section (30.27lbs). Of 7,800 birds placed, 934 died (88.03% livability).

Note that the same person took care of both sections during brooding and during growout. During the brooding period, this person always serviced the TCV–positive birds first and the negative ones second. No "between section" biosecurity was implemented.

Diagram 2. Schematic of timeline and TCV diagnosis for Farm B, by flock



CASE REPORT

Epidemiology of Turkey Coronavirus in a Commercial Turkey Operation

Anonymous Cases Presented by
STEVEN CLARK

Roche Vitamins Inc.
Parsippany, New Jersey, USA

SUMMARY

Birds placed in known corona–positive premises have remained corona–negative for 2 consecutive flocks up to the present time (13 weeks). The growout premises have not been cleaned or disinfected. If coronavirus is so contagious, why has this premise stayed corona–negative?

DISCUSSION

Whereas the farm is approximately 4 years old, since testing for coronavirus began in early 1997, it had never been positive. The nearest poultry neighbor is approximately 3 miles to the east. Other than that, a major concentration of turkey farms lies approximately 5 miles north on a straight line. There is no known traffic between these turkey farms and the study farm.

A flock of 14,000 toms was placed in mid-June 1997 ("June Flock"). At 23 days, birds started becoming restless. Twenty–four hours later, birds were apparently backing off feed. No excessive morality was seen at this point in time, but the servicemen medicated with penicillin. During transfer, the servicemen noted a lot of unevenness and ruffled feathers. Two weeks after transfer to the growout buildings, the birds became apparently loose, causing the floor to be "slick". At this point, despite medications, the mortality rose dramatically (Diagrams 1 and 2).

Birds were tested at 13 weeks and found positive for coronavirus, based on an indirect fluorescent antibody (IFA) test. By the time the results were returned to the company, preparations had been made to place birds in the brooder house. The operations manager decided to go through with the placement since the hatchery had already been informed of the order. The June Flock was harvested on the first of November

(20 weeks and 3 days) with an average body weight of 30.15 lbs, an FCR of 2.99 and a livability of 77.95%. Condemnations at the plant were 3.85%.

The brooder barn was thoroughly cleaned and disinfected with formaldehyde. Birds were placed in the brooder barn on the first of October ("October Flock"). The brooding was uneventful, with a few mortalities (2.1%, cumulative 7-day mortality) early in the brooding stage.

The growout house was not thoroughly cleaned, because of the short downtime between flocks. An entire tractor trailer load of new shavings was delivered due to the poor condition of the litter, but this was enough to merely top–dress two entire range houses. The water line was sanitized using household bleach, and the feed lines were "blown" empty.

When the October Flock was transferred to the range shed, the birds weighed 3.5 lbs at 35 days. This is slightly below the breed standard for that age. No major disease problems were noticed. At around 10 weeks of age, a blip in mortality was observed due mainly to "heart attacks"/ruptured aortas (Diagrams 1 and 2).

Because of circumstances, coronavirus testing was ordered at 6 weeks and then, most recently, at 12 weeks. Both results for the October Flock were negative.

The October Flock was harvested in mid-February 1998. Birds were 19.5 weeks with an average weight of 37.8 lbs, a feed conversion of 2.63, a livability of 88.86% and a plant condemnation of 2.1%.

When the October Flock was 13 weeks old, another scheduled flock was placed. This flock was also corona–negative up to the present time (13 weeks, May 1998). The growout premises have not been thoroughly cleaned or disinfected. If coronavirus is so contagious, why has this flock stayed corona–negative?

Diagram 1. Weekly Mortality

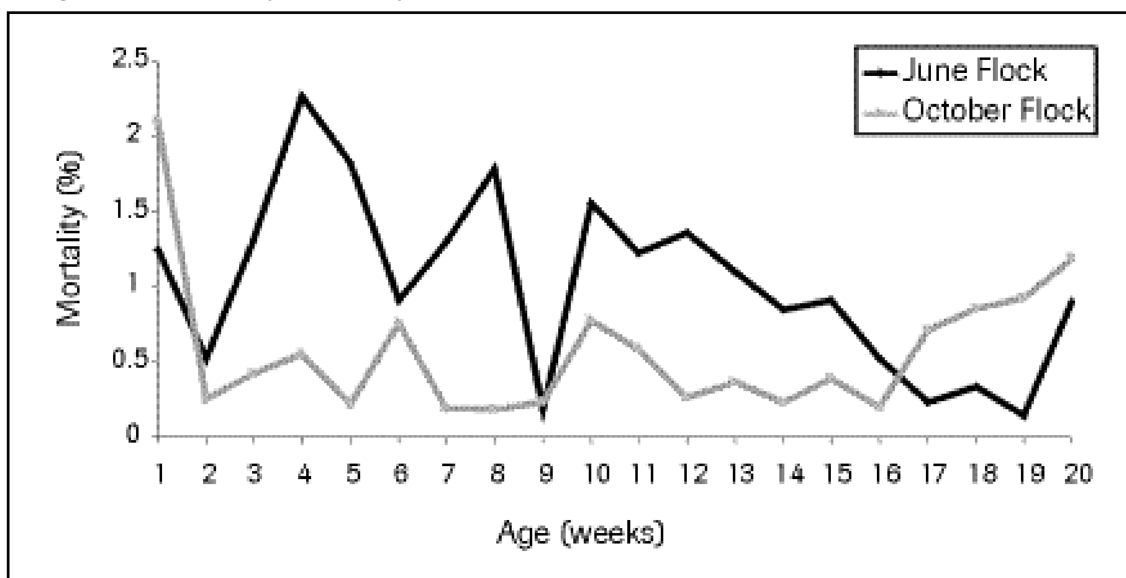
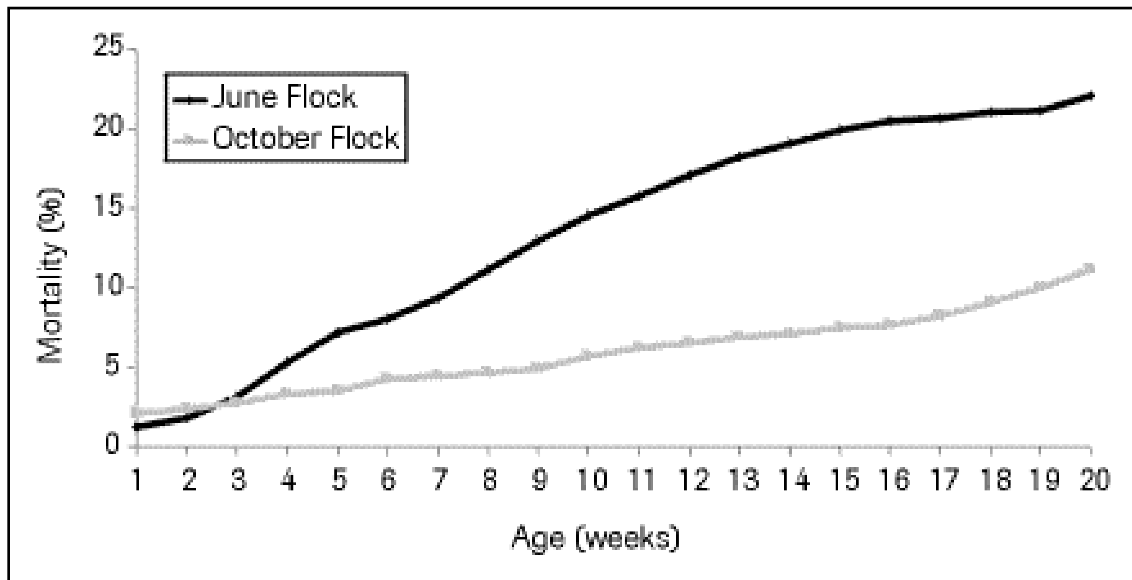


Diagram 2. Cumulative Mortality



PANEL COMMENTS

A Commentary on Turkey Coronavirus

JOHN DEEN

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This article was edited from comments and discussions during The Workshop. Dr. Deen's area of expertise is swine health and management.

COMMENTARY

In swine we [the swine industry] also have a few coronavirus agents, and the most important one is called transmissible gastroenteritis virus (TGEV). It is relatively uncommon, though we are seeing outbreaks of it in the last few years in North Carolina. It has a high pathogenicity in younger animals, especially in naive herds, and it can result in up to 100% mortality rates in 0– to 21–day animals. TGEV is less pathogenic in older animals. In some cases we are seeing predisposition to other agents, including some causing diarrhea, but especially those causing respiratory diseases. The other major pathogen is called porcine respiratory coronavirus (PRCV), which is relatively new. Probably in the last 10 years, it has spread relatively quickly through the populations of swine and, interestingly enough, you can not differentiate these two on serology, even though this one is almost nonpathogenic. If PRCV is pathogenic, it is working through the respiratory system, not as an enteric agent. We have success now differentiating these two on a differential ELISA, but it is only good at the herd level, not at the individual level. So we are struggling with these two and now there is discussion about different strains of TGE occurring as well.

In summary, swine have two different coronaviral infections. TGEV is relatively uncommon, yet is highly pathogenic in young pigs in naive herds and is often undiagnosed in older animals. PRCV is very common, yet has low (or unmeasured) pathogenicity. So, in some ways these are the same, and in some ways these are different.

I think the swine industry needs to improve dealing with coronaviral infections in these areas:

- Biosecurity
- Epidemic control
- Vaccination
- Serological differences
- Mixed infections “drive” the epidemiology of these infections.
- Herd size

BIOSECURITY

As discussed with turkeys, biosecurity has been a real problem. I commend the turkey industry for going back to the basic fundamentals of biosecurity and disease control. It is not as easy to do (in the swine industry) when the prices are high and we are “stuffing every part of the system with pigs”. Maybe the current low market price will help us get back to some of the basics.

VACCINATION

Vaccination has been tried a lot, probably more so in the swine industry than in any other industry. The immediate call with a new disease is a new vaccine and most of these vaccines are not very efficacious. These were probably counterproductive in controlling a disease outbreak, and actually lengthened them. It is really frowned upon now to use either modified live vaccines or otherwise.

SEROLOGY

We found it important to differentiate the types—either serologically or otherwise, whether or not we had mixed infections, especially the PRCV and TGE combination; this was totally different than one agent at a time. I would add as well that we have been fighting a disease called porcine respiratory and reproductive syndrome (PRRS). We have come to conclude that really we are talking about a number of agents interacting in that herd, and it really changes what we see going on within that herd. I would suggest that turkeys may have different coronaviral agents. The clinical expression may be different than what you expect when combinations of viruses occur.

HERD SIZE

The other problem for us has been the size of swine herds. Over the years, we have increased our herd size very quickly, and eradication and control has been much more difficult, and also biosecurity, especially with aerosol spray, has become much more complicated. I think we are paying for some of that.

EPIDEMIC CONTROL

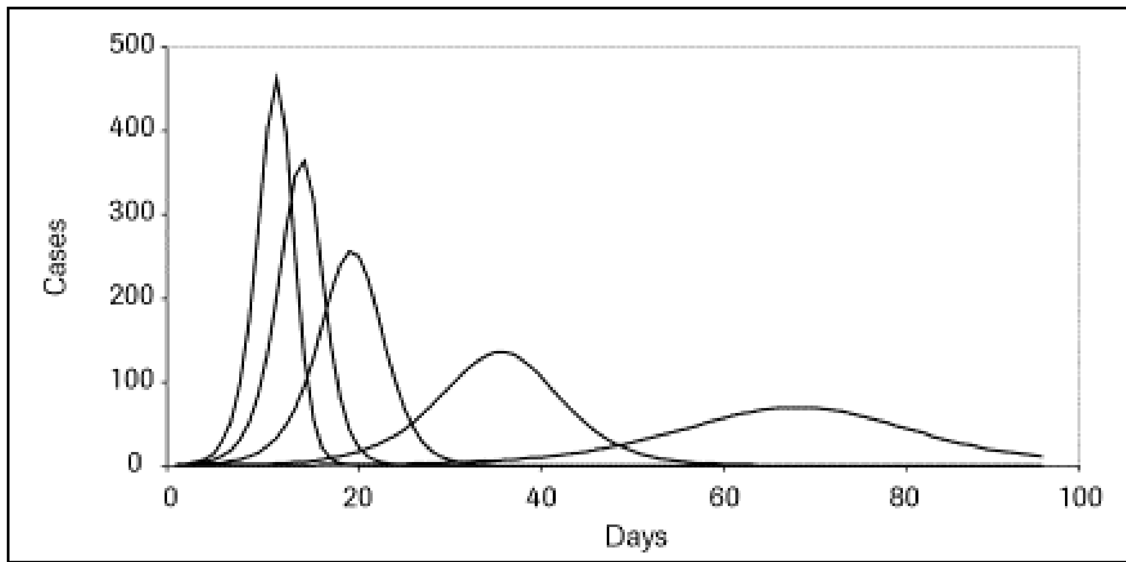
We learned how to control our epidemics. Epidemic control is performed through:

- The ability of the disease to pass from one animal to the other.
- The time it takes for an animal, once infected, to pass the disease on to the next animal.
- The introduction of noninfected animals to infected animals before the outbreak is over.

If we are going to control the epidemic, we have to control the ability of the disease to pass from one animal to the next. I think what I am hearing in your discussions is some difference in the ability of the agent to pass bird to bird. For instance, consider the moisture level in the litter. Is there the propensity for the agent to survive longer and, therefore, spread through the flock more quickly? Also, there is a difference between serotypes, and also between diseases. Once an animal is infected, how long does it take it to secrete the virus and pass it on to the next animal? It is longer than we think sometimes when we see an outbreak. Very few of these agents have the ability to infect and start secreting within 12 or 24 hours, which is needed to get some of the outbreaks going.

The last point is, if we have this disease going on and we want to stop it from spreading, the worst thing we can do is introduce more animals that are negative into a positive herd. Here is what we call the Reed Frost immunologic model of disease outbreaks (Diagram 1). Swine coronavirus, TGE, fits into this model quite well. We can either have a very acute outbreak that spikes really quickly, or if we slow down the spread of disease from animal to animal, we get a slower outbreak with less animals involved and which can spread over a longer time, to the point where the disease spreads so slowly that it does not infect all the animals in the herd. That sounds attractive in some ways, but we have learned over time that the best thing we can do is spread it as quickly as possible. What we do in swine, especially in reproductive herds, is create our own crass vaccination, which is taking the intestine of affected piglets, putting it through a sink grinder, and feeding it back to the animals. So we get all the animals positive, and surprisingly what happens is the immunity is good. It appears to be lifelong, although the serology is not. They do not seem to get reinfected, and if we keep new animals from entering the herd over about a 60-day period, we can prevent—especially in smaller herds—the disease from reinfecting any animals, and the herd actually becomes negative.

Diagram 1. The Reed Frost model of disease outbreaks



In other words, by "negative", we mean there may be serologically positive animals but we see no signs of virus transmission, and after 60 days the newly introduced animals no longer become infected. This does not always work, especially in larger herds, but our practice is driving towards that. In some turkey flocks, do you get such a slow outbreak that it is of very low prevalence, hard to pick up, but it is sitting at a low level within some of these flocks due to good management, other factors, or maybe the strain of the virus? I am not sure, but regarding swine, these are the types of herds that we have difficulty picking up and actually controlling. In certain seasons, especially in winter, these outbreaks occur suddenly, where we think we have had good biosecurity. But in retrospect, we had a very low prevalence of coronavirus.

DISCUSSION

Question: Any further comments on disease outbreaks?

Answer: The most common time to have an outbreak is in January/February, and it seems to be much more transmissible during that period. Or if the spread is slow in winter it can be a herd that is partially immune. For instance, if we have PRCV–positive swine and we get TGE into it, we end up with a slow spread. Conversely, we have had an outbreak previous and a proportion of the herd may still be immune. We call these chronic herds or chronic TGE. Once you're in this situation, it is really hard to get all the animals immune simultaneously. This sort of situation continues.

Question: Any comments on maternal immunity?

Answer: Maternal immunity is not as strong as we assume it is sometimes. In some management circumstances, it is strong. However, we have problems with a lot of movement of piglets now and we are seeing some slip through the management procedures.

Question: How does this management strategy of early segregated weaning, contribute to the TGE problem?

Answer: Not really anything significant. The only thing it does is allow us to remove part of the population of animals off to another site, and essentially have a smaller population on the same site. Also, it allows us to more easily stabilize that population. Using stability is the other thing we are talking about. Often in swine, we are looking for immune populations that are not shedding virus, rather than always naive populations as being the endpoint.

Question: Are you trying to get them immune before you leave the premises?

Answer: Yes. In some cases, even in a nursery, we will infect all the pigs currently on that nursery site, and not introduce more animals, for 60 days on the grow-finish site, then allow the group to stabilize.

Question: Would not feeding this to the sow keep the sow immune until she's not spreading it to the pigs?

Answer: Yes. That is not the situation in poultry because turkey coronavirus (TCV) is not vertically transmitted so we are coming out of the hatchery with naive, but uninfected birds.

Question: Whenever these sporadic outbreaks of TGE occurred on premises where it has not occurred previously or has not occurred for a substantial period of time, what is believed to be the source of the introduction of the agent to those particular units? Is it about to be these chronically infected animals that cannot be detected, or what is thought to be the source for the initial outbreak?

Answer: Two major sources: the first is infected breeding stock from breeding stock suppliers. Again, we have difficulty with PRCV–positive stock and the ability to detect TGE–positive animals within that stock. The second one is just poor biosecurity; this is not so much assuming aerosol transfer, but simply the transfer of trucks and other equipment. Mostly it is the movement of relatively fresh manure from one site to the next.

Question: I can see that if there is an outbreak going on, one farm gets it first, then the disease spreads, but what is the primary introduction?

Answer: There is a possibility of aerosolized transfer or other methods (starlings or aerial transfer). All of those are possible. Initial outbreaks are the spot outbreaks that can occur, but once these get started in an area, you can explain the secondary spread.

Question: The swine industry does not clean the live–haul trailers at the plant. Is that done somewhere else? I think that has real potential for a number of things.

Answer: I think the swine industry is rather fatalistic about its growout facilities, compared to the turkey industry. We have had circulation of TGE virus in grow/finish facilities that we have not detected at all, and in some cases we have seen cull trucks picking up pigs

from grow/finish facilities and from our multiplying herds simultaneously, so it is rather crass.

Question: How much of these problems in baby pigs are reduced by the feeding of creep feeds?

Answer: There has been some observations that the faster you get them off the sows, the better. So if you have a high quality creep feed or actually alternate milk/feed, they do much better off the sow than on the sow. These are piglets which have been taken away from the sow at 5 or 6 days. This creep feed is specialized feed. It is mostly milk powder, spray dried blood plasma and similar things. In a few cases, we have done some work on hyperimmunizing cattle and collecting colostrum from cattle; that has worked quite well. The big discovery in swine production, regarding enteric disease, was spray dried blood plasma. It has a low antigenicity, but on top of that it appears to limit feed intake, so the piglets do not engorge. They do much better on this than on any other product we have seen so far. It is highly digestible.

Question: If you have TGE in a nursery, are there piglets that do not seem to be bothered by it?

Answer: Oh, yes. There are very few diseases that affect all the animals in a population. My major research area is studying why disease differentiates in a population; and therefore, affects average daily gain in a concentrated portion of the animals. TGE is a bit more prevalent than most, but still, it follows the "80/20 Rule": 80% of the problems are in 20% of the animals. Swine coronavirus has a short-term effect upon growth rate. They are not "chronic"; they do not cause a chronic low feed intake in the pig. In other words, if they get over it without any secondary disease, they are not going to become "tail enders". Other diseases appear to step in and produce whatever syndrome it is to depress their appetite.

Question: If I have a sow that farrows and I prevent her pigs from suckling the colostrum, how does that compare to pigs that suckle for 4–5 days, then are put on creep feed? Does that first exposure to colostrum have a lasting effect?

Answer: Yes. The colostral immunity is quite an important part of the defense of the pig. They are pretty naive. Colostral immunity is permanent, even if they are exposed later in life.

Question: When we first started running into these turkey enteric problems, the question was asked, "are we transferring this from dam to offspring via the egg?". The one thing we did was to collect cull eggs from PEMS–positive/TCV–positive and PEMS–positive/ TCV–negative breeder flocks. We collected the yolk material and freeze dried it. Then we fed it back to the day-old poults to see if we could produce this "colostrum effect". Whether it was positive egg yolk material or negative egg yolk material, it had a definite impact on protecting those animals from exposure to turkey coronavirus (TCV). The logic I guess is that you're bathing those enterocytes with IgG or preventing a portal of entry for this virus. It also appears that if this is the case, your concept of the "nasty vaccine"

could possibly be developed for something like this. Is it not beneficial to have an agent that could protect the enterocyte site, which is the primary site, and still allow the primary agent (viral or other) to produce this serological or immunological effect, without causing the disease?

Answer: I think that this is fairly applicable, but my training is in epidemiology and my first response is, let's get rid of the bug instead.

A Commentary on Turkey Coronavirus

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This article was edited from comments and discussions during The Workshop. Dr. Gelb's area of expertise is Infectious Bronchitis (a broiler coronavirus).

COMMENTARY

The case reports yesterday were very well presented. I commend the industry people that are attending this meeting for their work in pulling this material together.

CARRIER STATE

Carrier state was discussed yesterday in addition to turkey coronavirus (TCV) persistence and infectious bronchitis virus (IBV). A carrier state or persistent infection is very common in birds that are infected with field strains of IBV and also vaccine strains of IBV. These also become persistent in the cecal tonsillar tissue. This is important for diagnostic reasons, and for potential transmission to other flocks that may be susceptible. For IBV alone, this is important for long-term, low–level shedding from the alimentary tract in clinically normal birds, which are birds that are not sneezing or coughing. Whether this occurs in TCV–infected birds is unknown, but it certainly would be something that should be investigated. This can occur for many weeks, or even months in the case of IBV, and we have seen it. This has also been documented by other researchers studying IBV, and these coronaviruses apparently may have this type of unique behavior in the bird.

Viral shedding, in my opinion, should be considered to be the most important means of disease transmission between farms because viruses are continually introduced into the environment via the feces. Not all birds are infected, but it is a percentage, which may be 5, 8, 10, 15%. However, these birds are continually pumping out (fresh) virus at a low rate into the environment, and they are clinically normal. So we may

track this virus around on our shoes or clothing, and by moving contaminated equipment or cleaning out a poultry house. We should consider that the litter and this equipment is contaminated.

VECTORS

We have discussed potential insect vectors, arthropod vectors, rodents, pets and other creatures. My general comment: I certainly believe that these vectors may help spread the disease, but I guess that this is mechanical transmission rather than biological, because there probably is no additional amplification or growth of the virus in these vectors. However I consider the continual excretion of the virus (fresh virus being introduced into the environment) to be a far greater potential for transmission than insect and rodent vectors, particularly between different farms and different premises.

Coronaviruses are generally thought to be highly host-specific. This may not be the case with TCV and the apparent (published) relationship to bovine coronavirus (BCV). The jury apparently is still out on the relationship of TCV to BCV: how similar it is, if it is basically the same virus or not, etc. If I were thinking about vectors and organisms other than those mentioned, I would obviously be looking at cattle as potential transmitters; many of you already are thinking along those lines, which is good. So I would try to minimize contact with cattle and I think you are already doing this.

SPREAD OF THIS DISEASE

Spread of the disease is related to the carrier state. Spread of the disease by movement of used litter from TCV–positive farms is noted. In the case of IBV, we have very strong evidence that certain variant serotypes of IBV are transmitted by moving contaminated manure. In 1992, we first saw the variant serotype 072 in the Delmarva region, and it decimated our broiler flocks there. It was clearly pathogenic, causing respiratory symptoms, predisposing birds to *E. coli* (colibacillosis), high airsac condemnations, etc. When we isolated many strains of this virus, they were all of the same serotype. We looked retrospectively and found that in 1990, 2 years earlier, it was basically the same 072 virus that we isolated in commercial layers.

This layer facility was multi-age, it was never cleaned out and depopulated, which is typical of the commercial layer industry. The manure from this commercial layer flock was routinely spread in the Delmarva region to the south. These layers were located more to the north of the Delmarva peninsula; the broiler–growing regions were more to the south, and this is where the manure was spread on croplands as fertilizer. We believe that this is a primary way that this may have spread. It may not have occurred exactly in 1990 when we first isolated the virus. The virus was probably in the layer complex long before that because they had ongoing egg production problems. However, statistically, things will catch up with you. If it does not catch up immediately, the more you spread manure, the greater the risks.

CLEANOUT AND DISINFECTION PROCEDURES

There is one large integrated broiler company in the US that is going back to basics, like the turkey industry. I commend you for doing the good cleanout, sanitation and disinfection methodology that we all probably learned in school. The broiler company did this because they just could not be profitable with infectious bronchitis virus (IBV). They were dealing with a variant serotype of their own from which there was no vaccine. So they did not have much choice other than to go back to basics and clean out this infection. Basically, they do the same things that you are doing: removal of the old litter. They clean out about every third flock, and they have this system working quite well to keep the level of challenge manageable. They remove the litter and birds' nests and all the remaining feed in the feed lines and feed pans. They remove all the litter from the farm on covered trucks. They have created a company to compost the litter. They resell it to gardeners, and it is profitable for them, just in that respect.

The broiler company washes down initially with soap, 1 gallon per 1,000 gallons of water. They disinfect with a high–pressure sprayer using a combination of disinfectant and pesticide. (This may or may not work or may not be the best way to go. I understand that some research shows decreased effectiveness of compounds if pesticides and disinfectants are mixed, but nonetheless they do this and it apparently does work for them.) They make four passes in the house, two on each side, and they also emphasize the importance of cleaning out storage buildings and related buildings where personnel work, and where there may be bathrooms, etc., and where records are kept. Those must be cleaned as well.

When they visit farms, they do the same things that you are doing, using coveralls, hairnets, plastic boots, etc., maintaining signs outside indicating the importance of animal health. They also try to deal with dead bird disposal by composting those birds on the farm site in a location that is removed from the immediate vicinity of the poultry house. However, I do think that dead bird pickup is a significant health risk and should be considered at the same level as litter removal.

FINAL COMMENTS

I commend the turkey industry for attempting to control TCV by eradication. I definitely think that this is the correct direction. Thinking about developing a vaccine or using a controlled exposure approach, I think is a major step backwards for the turkey industry. You have been sophisticated enough to realize that this is the way to profit, and should continue thinking this way. Coronaviruses are not easy to eradicate, and if you are successful doing it, again, I think this is the best way to go. To live with them in the future is just going to present additional problems.

DISCUSSION

Question: Is infectious bronchitis transmitted by aerosol or by people?

Answer: Certainly aerosol transmission occurs through coughing and sneezing. However, direct contact and transmission by humans and equipment are also very significant ways that the virus can be spread.

Comment (Dr. Gelb): Looking at infectious bronchitis virus (IBV), another important avian coronavirus, we see it is very well recognized that the virus replicates persistently in the cecal tonsil for many weeks after clinical disease signs have resolved. So a flock can go through a break, sneeze, cough, etc., and then recover. You can demonstrate a rise in serum antibody production, but the birds will shed virus in the feces for a prolonged period of time. Also it is not all birds, but a significant fraction, which may be 10, 15, 20% of the birds, so you can transmit the disease very effectively in that fashion. Whether or not it is a true carrier state requires discussion—some people define carrier state differently. I use persistence, which is an academic term, but we know that IBV behaves in a similar fashion, which is important to recognize. We see this very commonly in commercial multi-age egg laying operations, where birds over a period of weeks and months in those facilities apparently are reinfecting or re-exposing themselves over that period of time. This is reflected in antibody production, where antibody titers rise and fall. You can monitor this very easily, in spite of no vaccinations being given. So transmission certainly is via field exposure, which most likely results from the virus that's being released via the feces and then inhaled.

Question: Do I understand that IBV virus also appears in the kidneys quite a bit, and this could be a source of virus shedding, rather than the gut?

Answer: Yes, that has been more difficult to pin down, i.e., the role of the kidney is unknown. The kidney as a source of virus is possible. A number of investigators have shown that the cecal tonsil is clearly a source of virus, but the kidneys may also be involved.

Comment (Dr. Gelb): I offer these comments on some IBV research that we have been involved in relative to persistent infections. This work attempts to demonstrate the infectivity of previously infected flocks in the field using sentinels, and also attempts to demonstrate infectivity on experimentally infected birds maintained in a laboratory setting. We have yet to find any evidence of persistent infections that are transmissible to other birds. That does not mean that there may not be antigen-producing cells or something that can be demonstrated by immuno-histochemistry, but the longest period that we have been able to demonstrate infectivity in previously exposed birds has been 12 weeks. So the virus does hang around for a long period of time, but it does not seem to persist forever, at least as far as its ability to transmit. We have not found that litter is a source of the organism when taken from previously infected flocks.

Comment (Dr. Gelb): Chickens are most definitely susceptible to infection with turkey coronavirus (TCV); however, they show absolutely no clinical signs. Dr. Guy's (from

North Carolina State University) attempts to demonstrate antibodies in the chickens were confused by the cross reaction of the serological test(s) with IBV. So until we have a test that can discriminate between IBV and TCV in infected chickens, there's no way to tell which one we may be dealing with. I think chickens need to be scrutinized very closely, as they can be silent carriers. They can also transmit the disease very readily to sentinels that are placed in contact with experimental birds. We have put turkey sentinels into 6 broiler flocks in the TCV/PEMS endemic area in western North Carolina, and all of these failed to yield any mortality-producing agents or coronavirus. So we do not have any evidence that natural transmission from chickens to turkeys can occur. That is another part of the puzzle that still needs to be solved.

PANEL COMMENTS

A Commentary on Turkey Coronavirus

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This article was edited from comments and discussions during The Workshop.
Dr. Saif's area of expertise is infectious diseases of poultry.

COMMENTARY

It is obvious that progress has been made in the control of turkey coronavirus (TCV) infection. We have heard about some operations that had gotten rid of the infection. Various measures that were taken might be drastic, but these measures were some of the basic things that we have previously learned about biosecurity and what to do about eliminating and preventing a disease. So, for the few that were able to get rid of the infection, congratulations for a great job.

EPIDEMIOLOGY

Listening to some of the case presentations, evidently there are considerable gaps in our knowledge about a variety of aspects of TCV infection. There is still a great deal to be learned about the epidemiology, specifically about persistence, viral shedding, possible reservoirs, origin and vectors. Once it is in the area, it is relatively easy to determine how the virus is spread, but how the index case gets exposed is unclear. Determining whether cattle play a role requires more research. We need to compare some of the bovine isolates with some of the turkey isolates to determine the differences and similarities. Coronaviruses are thought to be species specific; they just do not move between species very easily, but there are always exceptions in a biologic system. We need more data about that particular aspect to find out whether there really is transmission from cattle to turkeys. More research is needed comparing isolates from different species, including chickens, turkeys, cattle and swine. There is a need to experimentally infect turkeys with some of the bovine and swine isolates to determine whether they will cause a disease or an immune response. I think this information would be quite valuable.

Dr. Jim Guy at North Carolina State University has shown that there is a definite antigenic relationship between TCV and infectious bronchitis virus (IBV). This work is being corroborated in a variety of other laboratories, with similar conclusions. The current classification of IBV is in serogroup 3 coronavirus and TCV is serogroup 4. These might be combined into one group.

DIAGNOSTICS

One of the problems we have working with TCV is that we have not been able to efficiently grow these viruses in tissue culture systems; therefore, we do not have large pools of virus for use in serologic testing. All the testing that we have discussed uses the indirect fluorescent antibody (IFA) test. The IFA has served the turkey industry very well so far. It has been very useful for those who have been trying to find out what is going on and to eliminate the infection. However, I think we need something besides the IFA test with equal or better sensitivity and specificity. Certainly I would not want to use the IFA only to compare strains.

Only Dr. Tom Brown at the University of Georgia has some viruses that grow in tissue culture. Most researchers that work with these viruses can get the virus going in some tissue culture systems, but after a few passages, the virus disappears. Consequently, we do not have large pools of the virus. An alternative is to grow the virus in eggs, harvesting these eggs and using this to develop an ELISA (enzyme-linked immunosorbent assay) test. But this procedure is cumbersome. There is quite a bit of room for improvement, but once we get an ELISA, I think we can then have an antigen capture test and also an antibody test. I believe this would be quite useful.

IMMUNITY

The immune response currently needs more investigation. One question: do flocks go from positive to negative status serologicall?.

With regards to cattle, most cows in the Midwest have antibodies against coronaviruses. There is a background antibody titer in most cattle. Most likely, every broiler and layer in the U.S. also has antibodies, even if they were not vaccinated. This is something to keep in mind when we discuss the possible relationships between cattle, chicken and turkey strains.

OTHER INFECTIOUS AGENTS

There are other viruses and infectious agents that affect the gut of the growing turkey. The commercial turkey is plagued with a variety of enteric infections. In some of the samples that our laboratory receives from the field, we can find evidence of 4 or 5 virus infections. This is common, not the exception, and is virtually the rule. Dr. Schultz-Cherry, Dr. Reynolds, Dr. Guy and our laboratory have detected some of these viruses. We still do not know what some of them are, but we do know that these agents infect turkeys and some cause stunting.

If you have problems and do not have any evidence for coronavirus, then you should start looking at what else is there. There are a variety of small viruses, such as astroviruses and enteroviruses, and there are at least 3 serogroups of rotaviruses and reoviruses that infect turkeys. There are other agents besides viruses that can cause enteric disease in commercial turkeys. The concurrent presence of these infections with or without TCV infection could result in a multitude of clinical signs and lesions that will vary depending on the nature of these agents.

DISCUSSION

Question: Any comments on the small virus that you've been working with?

Answer: We know it is not an enterovirus or an astrovirus. Possibly, it might be a calicivirus. There are other caliciviruses that infect other species. The one that we know very well is the Norwalk agent that affects humans; it causes severe enteric disease. Some of the other caliciviruses act in the gut, but some act systemically and produce a variety of disease signs. We do not know whether this turkey isolate is a calicivirus or not yet. Some of the electron micrographs look like it might be. We are working with some folks at CDC (Center for Disease Control) trying to get a better definition of what it is. We put it twice into turkey poults and our SPF birds, and they get stunted. So, we are pursuing this and trying to figure out what is going on.

Question: If we want to profile these flocks that fit into this TCV/PEMS (Poult Enteritis and Mortality Syndrome) category, what are the appropriate samples to verify the presence of these other viruses?

Answer: Frozen intestinal samples. Since we like to do immune electron microscopy, we request acute serum at the time of the outbreak, and then blood collected about 3 weeks later. We use this convalescent serum for the immune electron microscopy.

A Commentary on Turkey Coronavirus

CHING-CHING WU

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This article was edited from comments and discussions during The Workshop.
Dr. Wu's area of expertise is molecular virology and turkey coronavirus diagnostics.

COMMENTARY

I concur with all the points that Dr. Gelb and Dr. Saif just mentioned concerning turkey coronavirus (TCV). In addition, I am very pleased to see the joint efforts of academia and industry in dealing with TCV enteritis.

My comments will focus on what our laboratory can do in diagnosing and controlling the disease. We all know that Purdue University's Southern Indiana Agriculture Center (SIPAC) in Dubois initiated the TCV indirect fluorescent antibody (IFA) test for the diagnosis of Poult Enteritis and Mortality Syndrome (PEMS) several years ago when most scientists were debating its etiology. A $14,000 fluorescent microscope was purchased to provide the industry with accurate test results. Dr. Tom Bryan and Mr. Tom Hooper, both at SIPAC, conduct the service on an ongoing basis. The West Lafayette Laboratory supports SIPAC with electronmicroscopy (EM), virus isolation, PCR (polymerase chain reaction) test and production of polyclonal antisera.

IFA AND ELISA

The IFA is widely used and appreciated. It provides good correlation with the clinical picture of TCV. However, its sensitivity is only around 60%. Our research group, consisting of pathologists, virologists, avian specialists and full–time graduate students, realized the need for a more sensitive and economical test with a higher throughput. In order to provide the industry with a sound evaluation of the flock status, a sample size which can give a small confidence interval is required. ELISA (enzyme-linked immunosorbent assay) seems to meet all criteria listed above. We proposed to develop an antigen-capture and an

antibody-detection ELISA. The antibody-detection ELISA should be an improvement over the current IFA test.

Presently the industry typically submits 6 samples per flock for IFA testing, which, in our evaluation, does not provide sound prediction. Since the practice of pooling sera further decreases the sensitivity of IFA, it should be discouraged. Actually this may explain some of the "mystery cases" that we have discussed. The following two examples are offered to illustrate these points.

Example #1: IFA titers

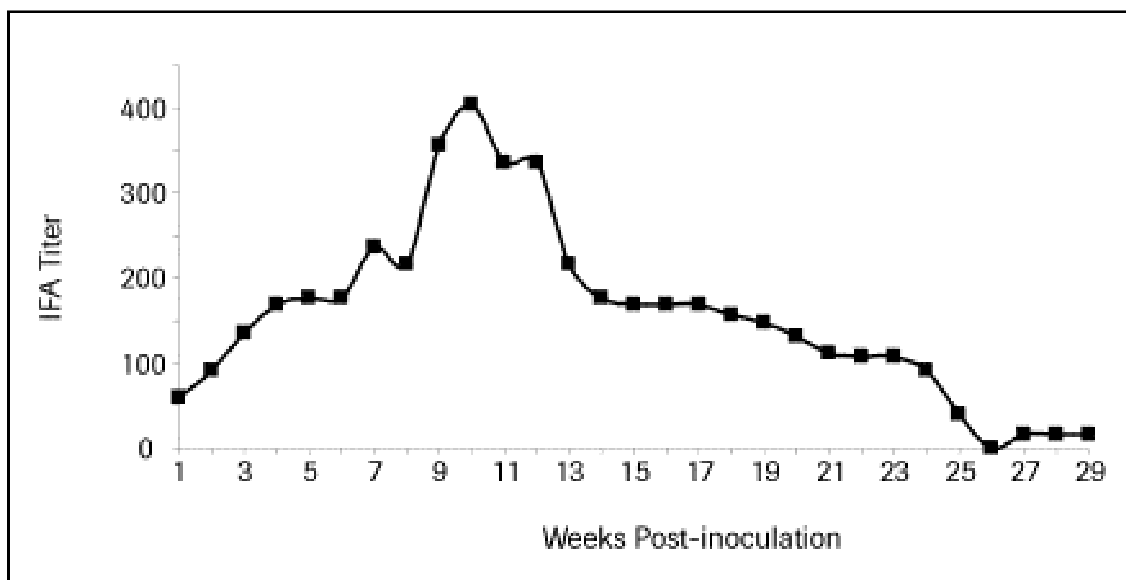
In order to raise antisera to TCV, we infected 10 turkey poults at three weeks of age. We collected sera before inoculation and weekly post-inoculation (PI) up to 30 weeks of age, and monitored with the IFA test (Figure 1). We observed the following:

There was a peak titer period (10 - 14 weeks PI) and the rest of the time the titers were low (< 150).

Two out of ten poults never seroconverted even though all ten birds were inoculated with virus. This shows individual bird variation. Antibody could be present for a long period of time but may not be detectable at all times.

Based on the data, we can easily see that 6 sera samples from a house of turkeys would not predict the serology of the entire population in this house. In addition, the timing for sampling and testing is also debatable. It is not impossible to see that a chronic infection with very low antibody titers could be beyond the detection range of IFA. Or the titer could be at the borderline of the detection limit; thus, pooling the samples could dilute the titer to an undetectable level.

Figure 1. Time course of TCV IFA test antibody titers



Example #2: PCR

We have developed a TCV PCR test, and have applied it to field samples. Table 1 shows PCR results from samples provided by Dr. John Barnes, North Carolina State University. This proves the poor sensitivity of IFA and the associated "mystery cases".

Please keep in mind that these are intestinal homogenate samples collected at the end of Dr. Barnes' study at approximately 14 days post-exposure to material prepared from flocks with unknown enteritis. Since the PCR is used to detect viral RNA, it is an antigen detection test. PCR provides better sensitivity if it is used during the early viral shedding period at 2 - 3 days postexposure. However, Dr. Barnes requested that I test these samples anyway, since some of the them were positive to the TCV IFA test but some were not.

Table 1. PCR results of intestinal homogenate samples from North Carolina

Group #	*Identified as*	*PCR + results*
1	"Control"	0 of 3
2	"TCV [IFA] -"	0 of 5
3	"moderate stunting"	0 of 5
4	"high mortality, PEMS +/TCV [IFA] -, entero +"	2 of 5
5	"TCV [IFA] +"	0 of 5
6	"TCV [IFA] -, rota +"	1 of 5
7	"TCV [IFA] +, entero +, rota +"	0 of 5

TCV [IFA]—/+ = the indirect fluorescent antibody test result for Turkey Coronavirus was negative/positive
PEMS = Poult Enteritis and Mortality Syndrome
Rota = rotavirus
Entero = enterovirus
—/+ = negative/positive diagnosis

The first group is the "control" group, so we did not detect anything on PCR. The second group, based on Dr. Barnes' record, was "TCV [IFA] negative", and we did not detect anything either. Group 3 was identified as "moderate stunting" and PCR negative. The 4th group was identified as "high mortality, PEMS positive/TCV [IFA] negative", had 2 PCR positives out of the 5 samples, was negative to coronavirus, and enterovirus was isolated. Group 5 was "TCV [IFA] positive", so the PCR will not detect TCV. This is not surprising because it is the late stage of infection called "TCV positive", which means TCV serology [IFA] positive. At the very end-stage, the virus is periodically (intermittently) shed; therefore, the PCR is not extremely effective. Group 6 is identified as "TCV [IFA] negative", virus isolation (VI) negative, and rotavirus positive; PCR detected only 1 positive. Group 7 is identified as "TCV [IFA] positive", enterovirus positive, and rotavirus positive; this was PCR negative on gut samples.

We did not detect any viral RNA from the control group. However, we did identify several samples that were IFA negative, PCR positive (Groups 4 and 6). It was interesting that "not

all of the samples from the same group provided the same results". This indicated significant individual differences and further stressed the importance of sampling size. In some cases, we noted that IFA was more sensitive than the PCR (Groups 5 and 7). This was due to the stage of the infection. At the end stage of most infectious diseases, the more favorable diagnostic tests are serological rather than antigen detection; the number of the etiological agent has already peaked and fallen, but the antibody titers are just optimal. Nonetheless, PCR was able to pick up some samples with negative TCV IFA.

PCR

Finally, I would like to refer to the data presented at the 1997 American Association of Avian Pathogists meeting [T.L. Lin, S. Tsai, C.C. Wu and T. Bryan, Sequence analysis of spike protein S1 subunit of turkey coronavirus isolates] to assure the inclusiveness, or the "detection power", of the primers used for the PCR. The primers (small pieces of DNA that are complementary to TCV nucleic sequences) used were able to amplify sequences of Indiana, eastern North Carolina, western North Carolina, Minnesota and Virginia isolates. The sequences were compared to each other for similarities and differences. They were also compared to the published sequence of bovine coronavirus (BCV). Several differences were detected between TCV and BCV. However, the major significant difference was a mutation of one nucleic acid which formed a stop codon. This change translates into the production of a smaller piece of spike protein with a potentially different structure. This may explain why BCV can replicate in HRT cells and TCV cannot.

SUMMARY

In summary, we understand very little about TCV. The highest florescent antibody (FA) titer was observed at 10 weeks postinoculation. A more sensitive and reliable test is needed for accurate diagnosis of TCV. At the present time, FA, PCR and embryo propagation are useful to confirm TCV infection. There are variations detected between TCV and BCV with possible structural variations of the spike protein. Our laboratory efforts will focus on ELISA development, strain characterization and detection of variations. Our endeavors in understanding the disease process will include studying the host—TCV interactions.

DISCUSSION

Comment (Dr. Wu): At this time, PCR is offered as a diagnostic test, though we ask the submitter to call ahead of time to inquire about PCR. We only use PCR to verify other tests. It is more important to run the test on samples collected during early stages of the infection rather than at the end.

Question: Dr. Barnes, is Group 7 our hottest group?

Answer (Dr. Barnes): Yes. This type of PEMS was established from sentinels placed into an affected flock in South Carolina in 1997. Mortality often reaches 100% in weekly passages.

Question: Dr. Barnes, what were your experiences with Group 4?

Answer (Dr. Barnes): Group 4 is the group that is most interesting to us. It is PEMS positive/TCV negative. It was established from sentinels placed into 4 flocks in eastern North Carolina in 1996. The flocks in the field remained TCV negative (IFA test), and this group has been negative by conventional coronavirus testing for about a year and a half.

Typically the mortality pattern for Group 4 was slightly different than the TCV-positive groups. Mortality tends to occur a few days later. Also, the bursal lesions seen in TCV-positive birds are not seen in these birds. We call the group "coronavirus negative", but that really needs to be qualified as negative based on current techniques (FA).

Group 3 also is interesting because it was established by sentinels placed in a flock from which you previously received some PCR positives. This group has also remained negative for TCV using current tests. Clinical signs in this group are intermediate between PEMS and poult enteritis.

Question: Dr. Barnes, what do you think of the Group 4 with the positive PCR?

Answer (Dr. Barnes): I would like to propose that there is a separate coronavirus, which is not being detected by our standard IFA tests. It is difficult to believe that there is another enteric coronavirus in turkeys because the IFA test tends to be very broad and detect most viruses. Material from Group 4 was also sent to Dr. Saif. He observed coronavirus using immune electron microscopy from that group as well. We just had all our groups retested about 4 weeks ago, and their TCV status has not changed. Group 4 has been negative on every test that we have had conducted on it over about a year and a half. Consequently, I suspect that there very well may be a separate coronavirus. Another possibility is that it could be the coronavirus-like agent that Dr. Reynolds has identified. By the way, that is the same group from which Dr. Saif observed the small virus that he is working on. In the system that we use, which magnifies the problem, we typically have 70–100% mortality in each passage with that particular material, so it is highly lethal.

Question: Is it possible that these birds had TCV to start with, but then PEMS agent took over? If the TCV and PEMS agents are together, which one takes over? It appears to happen in the field.

Answer (Dr. Gonder): It depends on the test used for the initial diagnosis. Apparently, coronavirus [TCV] actually took over in a couple of our cases. Several of our field cases were negative or suspicious on direct FA, and subsequently positive by IFA test or virus isolation. In these cases, it appeared that there was something else going on causing considerable gut epithelial damage, such as necrotic enteritis or enterovirus, which may have destroyed the epithelial cell integrity necessary to interpret the direct FA test.

Clinical Studies on Poult Enteritis Mortality Syndrome

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This article was condensed from a progress report presented to the PEMS Task Force on June 12, 1998.

OBJECTIVES

1. Continue sentinel program to determine:
   a) status of flocks on specific control programs
   b) status of those with acute enteric disease during finishing
   c) possibility of vertical transmission of PEMS agent(s)
   d) potential reservoir(s)
2. Determine accuracy and usefulness of new diagnostic tests using sentinel-derived PEMS.
3. Correlate presence or absence of putative agents in naturally infected flocks or sentinel-derived birds by experimental reproduction of the disease using standardized methods and/or serology.
4. Identify nutritional, management, and poult factors that affect the disease and determine their usefulness experimentally.
5. Determine if controlled exposure to turkey coronavirus (TCV) and/or other infectious agents could be a useful interim procedure for reducing mortality and, if so, to use additional approaches to minimize stunting.

PROGRESS YEAR-TO-DATE

1. Maintenance of PEMS.
   Purpose - Maintain PEMS by weekly passage.
   A. Four types of PEMS recovered from sentinels placed into affected flocks have been maintained by weekly passage in poults. Types and passages are:
      - R9A (established 1996) - Eastern NC, TCV-, 41 passages in current series, high virulence

- R27 (established 1994) - Eastern NC, TCV+, 134 passages in current series, high virulence
- R 3-5 (established 1997) - South Carolina, TCV+, very high virulence, 35 passages
- R3-6 (established 1997) - Western NC, TCV+, 31 passages, high virulence

B. Infective material from these groups has been supplied to other investigators.

C. Confirmed occurrence of PEMS without detectable TCV.

D. Source material for identification of new agents associated with enteric disease in turkeys.

2. Effect of freezing on PEMS infectivity.

Purpose - Determine a method to store PEMS infective material without loss of mortality agents.

A. Maintaining PEMS by weekly passage requires considerable resources preventing other work from being done.

B. In several previous studies, no consistent method of storing samples for extended periods has been determined; however, a current study is looking promising (see below).

3. Infectivity of litter from TCV seropositive flocks.

Purpose - Determine if exposure to litter from TCV seropositive flocks immediately after they are removed for processing results in TCV infection.

A. Two groups of sentinels were placed into houses immediately after 4 TCV seropositive flocks had been removed for processing. One group was left overnight; the other remained in the environment for 3-5 days. Two back-passages were done to magnify any agents.

B. Results - no TCV infection occurred in any of the groups. Stunting occurred in 3 groups. Mortality in one group was associated with coccidiosis involving ileum and ceca.

C. Findings indicate TCV does not persist in the environment of recovered flocks when infection occurs early in finishing during the winter. It is concluded that litter is an unlikely source of TCV, but it still needs to be handled appropriately because other enteric agents are present.

4. Relationship between stunting and PEMS.

Purpose - Determine if stunting may be a mild form of PEMS.

A. A stunting syndrome was established in sentinels placed in contact with affected poults from two flocks. Mortality did not occur after several back-passages.

B. It is concluded that PEMS is a distinct disease and that there are a number of poorly defined milder enteric diseases that affect young poults.

5. Effect of single and combined infections with TCV and a reovirus isolated from poults with PEMS.

Purpose - Determine if TCV and reovirus isolated from PEMS–affected poults interact to cause the disease.

A. A sequential clinical, pathologic, and immunohistochemical study was done. TCV caused stunting but no mortality. No interaction was found with reovirus and PEMS did not occur in dually infected poults.
B. Evaluation of histologic changes are in progress. Identification of lesions will aid in diagnosis of infections with these viruses.

6. In vivo lymphoblastogenesis test.

Purpose - Develop an easy, rapid test for determining cell mediated immunity in turkeys.

A. Normal poults were injected intravenously with various mitogens in different concentrations. Spleens were removed and weighed between 24 and 96 hrs postinoculation.
B. No consistent differences in spleen size were found among the various groups.
C. It is concluded that approach did not work.

7. Efficacy of controlled exposure to TCV NC95.

Purpose - Determine if exposure of day-old poults to different amounts of TCV NC95 might minimize the direct impact of the virus on the poults and confer resistance against subsequent PEMS challenge.

A. The apparent positive benefits found in previous studies using this approach were not repeated. Controlled exposure did not provide protection against challenge. Although the normal seeder (low) challenge was done, mortality was still high, making the possibility of overchallenge likely. It appears that immunization may be difficult to achieve if it is even possible at all.

CURRENT STUDIES & INTERIM FINDINGS

1. Investigation of PEMS outbreaks.

Purpose - Determine etiologic agents involved in PEMS outbreaks during brooding using currently available techniques. Study epidemiology of disease transmission.

A. Outbreak in Sampson Co 6/9/98 in 2.5 wk hens. TCV positive. Epidemiologic investigation being initiated.
B. Samples from a possible outbreak in Colorado received 6/10/98 are being processed.

2. Effect of Freezing on PEMS infectivity.

Purpose - Develop a method of maintaining types of PEMS other than weekly passage in poults.

A. Different method of handling samples and exposing birds used. Two of 4 PEMS types readily reproduced; others uncertain at this time. TCV+ groups more easily reproduced compared to TCV- group. Stunting in all groups. Mortality generally higher in birds given fresh inoculum compared to those given frozen inoculum.

3. Backpassage of TCV NC95.

Purpose - Determine if sequential passage and/or increased exposure affects TCV infection.

A. Stunting but little mortality has occurred through 3 full and 2 partial backpassages. 10 backpassages will be done.

PLANS FOR 1998/99

1. Continue to maintain types of PEMS and provide infective material to investigators.
2. Intensively evaluate different types of PEMS, especially TCV- PEMS, for novel infectious agents.
3. Develop techniques to isolate and identify Campylobacter and determine their role in PEMS.
4. Continue to try to freeze infective material without loss of agents that cause mortality.
5. Conduct sequential studies to determine the pathogenesis of PEMS following infection with specific putative causative agents.
6. Place sentinels on litter of additional TCV–seropositive flocks, especially flocks that become infected during brooding.
7. Study naturally occurring outbreaks of PEMS during brooding, especially how flocks may have become exposed.
8. Develop a reproducible low–challenge procedure that can be used to evaluate resistance.
9. Determine if in ovo inoculation with TCV NC95 will adversely affect the poult and provide any resistance against subsequent challenge.
10. Study cell dynamics in the bursa of Fabricius of TCV–infected and PEMS–infected poults.
11. Experimentally infect birds to assist in epidemiologic studies and/or studies on reservoir - vector hypothesis.
12. Review previous studies and seek future funding to continue the work.

Develop FA Test for GA (TCV-GA) and NC (TCV-NC) Isolates of SMT-Associated Turkey Coronavirus (TCV)

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This article was condensed from a progress report presented to the PEMS Task Force on June 12, 1998.

JUSTIFICATION

Intestinal coronaviral infections in turkeys cause diarrhea, decreased gain, and are statistically associated with Spiking Mortality, the most severe form of PEMS. We have isolated in turkey embryos and cell culture systems a turkey enteric coronavirus from 3–week–old turkeys affected with SMT/PEMS in the Monroe area of North Carolina. Purified cultures of this isolate, when back–passed in turkeys, induce 30 to 60% mortality, severe diarrhea, bursal cores, bursal atrophy, and thymic atrophy identical to that seen in naturally occurring SMT/PEMS. From feces of clinically normal adult cattle resident on SMT/PEMS–affected turkey farms, we have also isolated two enteric coronaviruses in turkey embryos and cell culture systems. These bovine-origin turkey enteric coronaviruses induce 50 to 70% mortality in exposed turkey poults. A fourth field isolate has been cultivated in the same systems from mildly diarrhetic 2–week–old turkeys in Georgia. This isolate is less virulent than those listed above, induces no mortality, and produces slight decreases in gain and conversion. For laboratory comparisons, enteric coronaviruses of other species also have been propagated.

Nucleic acid coding regions show differences between our turkey coronaviral isolates. These differences lead to surface antigenic differences between isolates, and the failure of infection with the less virulent isolates to protect against the more virulent isolates.

Polyclonal antibody developed against virulent TCV cross reacts with non-pathogenic enteric coronaviruses. Thus, present polyclonal antibody based assays cannot differentiate turkey infection with the highly pathogenic TCVs from infections with less pathogenic TCVs, or nonpathogenic coronaviruses of other species. These cross reactivities may be

part of the cause for the statistical correlation of coronavirus presence with SMT/PEMS as shown by Goodwin, *et al.*, but less than a 1:1 as shown by Barnes, *et al*.

PROGRESS TO DATE (START DATE 5-1-96)

1. TCVs were grown in pure culture, sucrose gradient purified, and repropagated in cell culture.
2. Viability of repropagated virus was determined by cytopathic effect in cell culture.
3. Coronaviral identity of repropagated isolates was confirmed by RFLP-PCR assays.
4. Titer of these purified isolates in cell culture was determined and optimized.
5. Animals were hyperimmunized with cell culture origin TCV.
6. Resultant polyclonal antibody was titered.
7. IgG fraction was precipitated and concentrated.
8. Polyclonal antibodies were used in indirect and direct FA tests to detect TCV infections.
9. Cross reactivity with BCV, some IBV isolates, and less pathogenic TCVs was detected.
10. Indirect Fluorescent Antibody Tests for anti-TCV antibody have been developed. These have been modified for use in 96–well plates.
    a. Virus is grown in cell culture in flat–bottomed 96–well plates.
    b. TCV positive and negative cell cultures are included on each plate.
    c. Each plate allows testing of 24 samples with each sample run in duplicate against 2 positive and 2 negative wells. This number includes room for 4 positive and negative serum controls per plate.
    d. Plates, reagents list, positive and negative control sera, and protocol are available on request.

Develop PCR Test for SMT-Associated Turkey Coronaviruses

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This article was condensed from a progress report presented to the PEMS Task Force on June 12, 1998

JUSTIFICATION

Intestinal coronaviral infections in turkeys cause diarrhea, decreased gain, and are statistically associated with Spiking Mortality, the most severe form of PEMS. We have isolated, sucrose gradient purified, and repropagated in cell cultures a highly pathogenic turkey enteric coronavirus from 3–week–old turkeys affected with SMT/PEMS in Monroe, North Carolina. This isolate induces 30 to 60% mortality, severe diarrhea, bursal cores, bursal atrophy, and thymic atrophy. We have also isolated, purified, and recultured turkey enteric coronaviruses from normal adult cattle resident on SMT/PEMS affected turkey farms, and another less pathogenic turkey enteric coronavirus. For laboratory comparisons other avian and mammalian coronaviruses have also been grown.

Using nucleic acid sequences coding for viral proteins we currently can detect enteric coronaviruses in cell culture systems and will shortly develop assays for detection in feces.

PROGRESS TO DATE (START DATE 5-1-96)

1. A RFLP-PCR assay was developed that detects turkey intestinal coronaviruses.
    a. To develop this assay we sequenced genes coding for the S-1 surface glycoprotein of 5 isolates of TCV.
    b. Nucleic acid sequences conserved between these 5 isolates were identified, and 2 primer sets designed to anneal to these sites were constructed. These

primers were designed to produce a PCR product of either 1070 base pairs or 710 base pairs.

2. Using either set of PCR primers, a PCR product was produced that would detect and confirm the identity of TCV in cell cultures at a concentration down to 10 4.5 TCID/mL.
3. No cross reactivity with any Infectious Bronchitis Virus tested was found.
4. Detection limits in feces with added cell culture TCV and incubated for 1 hour, the detection limit rose to 10 5.0 TCID/mL.
5. Detection limits in mucosal scrapings of turkey intestines allowed detection of 10 5.5 TCID/mL (scrapings from turkeys experimentally infected 6 days earlier with TCV).
6. Detection limits in feces allowed detection of 10 6.0 TCID/mL (feces collected from pens containing poults experimentally infected 6 days earlier with TCV).
7. Restriction mapping of the PCR products produced by these 2 primer sets will be presented at AAAP this summer with an eye toward identification of specific restriction patterns for each isolate. This will allow identification of a specific strains of TCV for epidemiological tracking and correlation of specific strains with traits of high or low pathogenicity. Additional funds are requested for completion of this restriction mapping of isolates.

Develop Enteropathogen Detection Profile

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This article was condensed from a progress report presented to the PEMS Task Force on June 12, 1998.

JUSTIFICATION

SMT/PEMS has resisted determined attempts to correlate occurrence with a single etiologic cause. Specific *E. coli* isolates, cryptosporidium, hexamita, alphavirus, and strains of intestinal coronavirus have been proposed as putative causes. Most turkey flocks meeting the original clinical definition of SMT/PEMS have intestinal coronavirus either by presently employed IFA assays (Barnes, *et al.*) or by electron microscopic assays of droppings (Goodwin, *et al.*). However, some flocks that have mortality patterns meeting the clinical definition of SMT/PEMS are negative by these existing assays for intestinal coronavirus. Conversely, some flocks that do not meet the clinical definition are positive for intestinal coronavirus by these assays. This may be due to cross reactivity of presently used IFA assays with nonpathogenic coronaviruses in turkeys, slow seroconversion after exposure, antigenic diversity of intestinal coronaviruses, and transient shedding of intestinal coronavirus in numbers detectable using direct or immunoelectron microscopy.

An alternate possibility is that flocks meeting the clinical definition of SMT/PEMS have a multifactorial disease due to simultaneous or subsequent infection with combinations of multiple enteropathogens. If this is true, no single etiologic agent would ever show 1:1 correlation with SMT/PEMS occurrence, but statistical examination of test results for these individual agents may show combinations that represent the greatest risk for turkey producers. Control methods may then be targeted at these high–risk combinations.

This project was designed to test application of existing procedures, and to develop new procedures, for pathogens suggested to be involved in SMT/PEMS. These assays will be applied to samples from naturally occurring and experimentally reproduced SMT/PEMS

to determine the involvement, or lack thereof, in this disease. New procedures will be added as they are developed or new pathogens are suggested to be involved. As pathogens are found not to be involved in SMT/PEMS, agents will be dropped from the protocol.

PROGRESS TO DATE (START DATE 5-1-96)

1. Acquired equipment for PCR and FA assays.
2. Employed and trained personnel to perform services.
3. Tests developed for use in enteropathogen profile:

***Viral identification*-**

1. Direct and immunoelectron microscopy.
   - Samples: Frozen/chilled feces, cell culture fluids, amnioallantoic fluid from eggs, or tissue homogenates.
   - Cost: $40.00/sample
   - Turnaround time: within 5 working days.
2. Primary isolation.
   - Samples: Frozen/chilled feces, cell culture fluids, amnioallantoic fluid from eggs, or tissue homogenates.
   - Cost: $50.00/sample
   - Turnaround time: 1 week per passage, for 5 passages.
3. Coronavirus IFA identification.
   - Samples: Frozen/chilled isolates in cell culture.
   - Cost: $40.00/sample
   - Turnaround time: within 5 working days.
4. Coronavirus PCR identification.
   - Samples: Frozen/chilled isolates in cell culture.
   - Cost: $50.00/sample
   - Turnaround time: within 10 working days.

Serologic assays-

1. Turkey coronavirus antibody by AGP qualitative assay.
   - Samples: Chilled serum.
   - Cost: $0.25/sample
   - Turnaround time: within 5 working days.
2. Turkey coronavirus antibody by VN quantitative assay.
   - Samples: Chilled serum.
   - Cost: $10.00/sample
   - Turnaround time: within 10 working days.
3. REO virus antibody by ELISA quantitative assay.
   - Samples: Chilled serum.
   - Cost: $1.00/sample
   - Turnaround time: within 5 working days.

4. SMT-associated REO virus antibody by VN quantitative assay.
   - Samples: Chilled serum.
   - Cost: $10.00/sample
   - Turnaround time: within 10 working days.
5. Alphavirus antibody by VN quantitative assay.
   - Samples: Chilled serum.
   - Cost: $10.00/sample
   - Turnaround time: within 10 working days.
6. IBD antibody by ELISA quantitative assay.
   - Samples: Chilled serum.
   - Cost: $1.00/sample
   - Turnaround time: within 5 working days.
7. IBD type specific antibody by VN quantitative assay.
   - Samples: Chilled serum.
   - Cost: $10.00/sample
   - Turnaround time: within 10 working days.

Bacteriologic assays-

1. Primary isolation and identification.
   - Samples: Chilled swabs or intestines/tissues.
   - Cost: $10.00
   - Turnaround time: within 5-10 working days.
2. *E. coli* typing and characterization by RAPD analysis.
   - Samples: Chilled swabs or tissues, or isolates.
   - Cost: $50.00/sample
   - Turnaround time: within 10 working days.
3. *E. coli* pathogenicity assay by embryo lethality assay.
   - Samples: Chilled swabs or tissues, or isolates.
   - Cost: $50.00/sample
   - Turnaround time: within 30 working days.
4. Salmonella culture and typing by agglutination.
   - Samples: Chilled swabs or tissues.
   - Cost: $10.00/sample
   - Turnaround time: within 10 working days.

Histopathologic assays-

1. Routine lesion description, interpretation, and protozoa identification.
   - Samples: Formalin–fixed tissues/intestines.
   - Cost: $10.00/sample
   - Turnaround time: within 5 working days.

2. Findings based on use of existing profiling assays listed:
    1. Alphavirus infection was determined to cause intestinal disease in 0- to 14–day–old turkeys, but there was age resistance to enteric disease after this age. No increased incidence of alphavirus antibody was present in turkey flocks with experimental or naturally occurring SMT/PEMS. No increased incidence of alphavirus was detected in turkeys with SMT/PEMS. We recommend this pathogen be dropped from further study.
    2. *E. coli* Types 1 and 2 were examined using culture methods, congo red binding, RAPD nucleic acid assays, embryo lethality assays, and animal inoculation studies. They were found to be indistinguishable from each other, and from *E. coli* isolated from routine healthy turkey flocks in Georgia and North Carolina. No clinical signs were detected after inoculation of these agents into turkeys, and they were avirulent in the embryo lethality assay. We recommend these agents be dropped from further study.
    3. IBD type 2 was isolated from turkeys with SMT/PEMS, but was also isolated from most normal healthy turkeys over 4 weeks of age. All turkeys over 4-6 weeks of age have seroconversion to IBD by ELISA and this is most often, but not always, type 2. Exposure to IBD type 2 alone produced mild decreases in weight gain. Simultaneous exposure to IBD type 2 and turkey intestinal coronavirus produced a synergistic effect that was more than additive.

       We recommend IBD type 2 be dropped from further current study at this time, but this decision may have to be revised in the future due to the synergistic effect with coronavirus.
    4. Turkey intestinal coronaviruses (TCVs) are present in the majority of cases of naturally occurring and experimental SMT/PEMS. TCVs isolated from 2- to 3–week–old turkeys with clinical SMT/PEMS are highly pathogenic. TCVs isolated from flocks without SMT/PEMS are less virulent. These isolates have different nucleic acid sequences and surface antigens from each other and from previous isolates of TCV.

       However, some cases and models fitting the clinical definition of SMT/PEMS have been detected that are negative for TCV using existing detection methods. We recommend that work on differentiation of highly pathogenic and less pathogenic/nonpathogenic intestinal coronaviruses remain part of future profiling efforts, and that more sensitive diagnostic assays be pursued for these pathogens as part of the profiling effort.
    5. Cryptosporidium and hexamita were found to be frequently present in some but not all cases and models of SMT/PEMS. This is likely due to the cell–mediated immunosuppression that is present in this disease. No evidence for a primary nature for these pathogens has been detected. We recommend that the pathogens be considered secondary invaders in immunosuppressed birds and dropped from present profiling.

6. Salmonella frequency and serotypes detected in SMT/PEMS flocks show no difference from those present in non-SMT/PEMS flocks and we recommend these pathogens be dropped from future profile efforts.
7. Reovirus isolates from SMT/PEMS flocks are not antigenically different from those present in non-SMT/PEMS flocks so we recommend examination for these isolates be dropped from future profiling efforts.

Production of Type- and Strain-Specific Monoclonal Antibodies to Turkey Intestinal Coronaviruses

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This article was condensed from a progress report presented to the PEMS Task Force on June 12, 1998.

JUSTIFICATION

Turkey intestinal coronaviruses (TCVs) produce enteric disease and are associated with naturally occurring SMT/PEMS. These pathogens differ from each other genetically, antigenically, and in the severity of the intestinal disease they produce. Presently available polyclonal antibodies cross react between these TCVs and do not allow differentiation of highly pathogenic TCVs (TCV-NC, TCV-BO#1, TCV-BO#2) from less virulent TCVs infecting turkeys (TCV-GA, BCV). They also cross react with other unrelated coronaviruses such as IBV-Mass and IBV-072. Since chickens and cattle serve as subclinical reservoirs for TCVs, differentiation of TCV from these cross-reacting coronaviruses in their natural hosts is critical to eventual control and elimination of pathogenic TCV from areas of turkey production.

We have cultured, sucrose gradient purified, and repropagated in cell culture systems isolates of 6 TCVs (TCV-NC, TCV-BO#1, TCV-BO#2, TCV-GA, TCV-MN, and TCV-QU), BCV, and multiple strains of IBV. We have sequenced the viral nucleic acids coding for the S-1 viral surface protein of all these isolates, and found there is significant variation between the TCVs associated with the current outbreak and the historical TCV isolates from Minnesota and Quebec, Bovine coronaviruses, and Infectious Bronchitis viruses of chickens. These nucleic acid differences translate into antigenic differences in the resultant S-1 viral surface protein. These antigenic differences will allow design of monoclonal antibodies that differentiate turkey isolates from contaminating mammalian and chicken isolates. Furthermore, strain-specific monoclonal antibodies will allow differentiation of pathotypes within the TCV group and allow epidemiological, cross-protection, and vaccination studies to be conducted and correctly evaluated. These

monoclonal antibodies will allow increased sensitivity and specificity in the IFA, VN, and AGP tests currently in use and will allow development of more sensitive ELISA screening assays and immunohistochemical techniques for diagnosis and determination of pathogenesis.

We propose to extract the S-1 viral surface protein from each of the TCV isolates presently in cell culture and use this as an immunogen to produce type– and strain-specific monoclonal antibodies in mice. Clones resulting from these fusions will be screened colorimetrically using 96-well plates coated with strains of whole virus of each of the TCV isolates, as well as BCV and IBV strains to assure specificity. We have experience in these techniques and have used the same procedures to develop monoclonal antibodies to IBV strains, Pasteurella isolates, and other avian pathogens. The Monoclonal Antibody Production Facility at University of Georgia does these fusions and clone productions routinely.

OBJECTIVES FOR FY 1997-98

Develop type- and strain-specific monoclonal antibodies against the SMT-associated intestinal coronaviruses.

SPECIFIC AIMS FOR FY 97-98

1. Extract the S-1 viral surface protein of each of the 4 TCVs that have distinct nucleic acid patterns coding for S-1 (TCV-NC, TCV-BO#1, TCV-BO#2, TCV-GA). Forward these proteins to the UGA Monoclonal Antibody Production Facility (UGA-MAPF).
2. Identify 1 resultant clone that reacts only with all 6 turkey intestinal coronaviruses (4 above plus TCV-MN and TCV-QU) and no mammalian or chicken coronaviruses.
3. Use UGA-MAPF to produce strain-specific monoclonal antibodies that react with each of the 6 turkey intestinal coronaviruses.
4. Produce sufficient volume to supply these to other laboratories servicing the corporate sponsors.

PROGRESS FOR FY 97-98

1. Viruses have been cultivated in sufficient volume and the first 2 forwarded to the UGA Monoclonal Antibody Production Laboratory.
2. Production of large volumes of monoclonal antibodies for the first 2 viruses (TCV-NC and TCV-GA) are complete.
3. We have in storage 2-3 liters of 7 different monoclonal antibodies as well (as the clones of cells to produce more) against TCV-NC and TCV-GA. These monoclonals can be shipped to any other laboratory upon request.
4. We are in the process of characterizing these monoclones. They react in IFA to cell culture virus, and all have neutralizing activity against TCV. Detection of subgroup (IgG vs. M, etc.), and examination of any hemagglutination-inhibiting activity is in progress.
5. Separation of viral proteins by Western blotting and identification of the specific protein reacting with each monoclone is planned.

Descriptive Epidemiology of Turkey Coronavirus (TCV) and Poult Enteritis Mortality Syndrome (PEMS) in Turkey Flocks Monitored in 1997

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This article was condensed from a progress report presented to the PEMS Task Force on June 12, 1998 and from information presented at the American Association of Avian Pathologists meeting July 27 - 29, 1998

ABSTRACT

Poult Enteritis Mortality Syndrome (PEMS) is an economically devastating disease. To date, many questions remain unanswered about the syndrome, including its cause, transmission of causative agent(s) and control methods. PEMS has been linked to Turkey Coronavirus (TCV), with areas having a higher prevalence of TCV having an increased incidence of PEMS. An epidemiologic investigation was conducted to delineate the role of TCV in PEMS and to identify risk factors associated with PEMS.

In summary:

- Mortality was increased in both TCV(+) and PEMS(+) flocks relative to healthy flocks.
- Mortality patterns differ between TCV(+) flocks and PEMS (+) flocks.
- PEMS was not caused by TCV in turkey flocks monitored.
- 43% of PEMS(+) flocks were also TCV(+).
- 41% of PEMS(-) flocks were TCV(+).

OBJECTIVES

- Delineate the role of TCV in turkey flocks experiencing excess mortality.
- Identify risk factors associated with turkey flocks experiencing excess mortality (in progress, data not available).

The TCV Status was determined as follows: six serum samples were obtained from each flock at 7 weeks (brooder house exposure) and again at 11-14 weeks (growout house exposure) and tested using an indirect fluorescent antibody test (IFAT). The

PEMS Status was determined by a flock experiencing mortality, of an unknown cause, greater than 2% during any 3–week period during brooding.

DISCUSSION

Figure 1 illustrates the different mortality patterns prior to 14 weeks of age associated with PEMS-only positive and TCV-only positive flocks. Flocks with PEMS experienced increased mortality during the brooding phase (0 - 5 weeks of age) of production. Flocks infected with TCV experienced increased mortality during the growout phase (after 5 weeks of age) of production. Flocks with TCV and PEMS (mixed infection) experienced increased mortality levels throughout the life of the flock (Figure 2). Combining the mortality curves of flocks with only PEMS and flocks with only TCV results in a mortality pattern (Figure 1) that resembles that of flocks with mixed infections (Figure 2).

Figure 1. Weekly percent mortality for flocks with TCV or PEMS versus healthy flocks

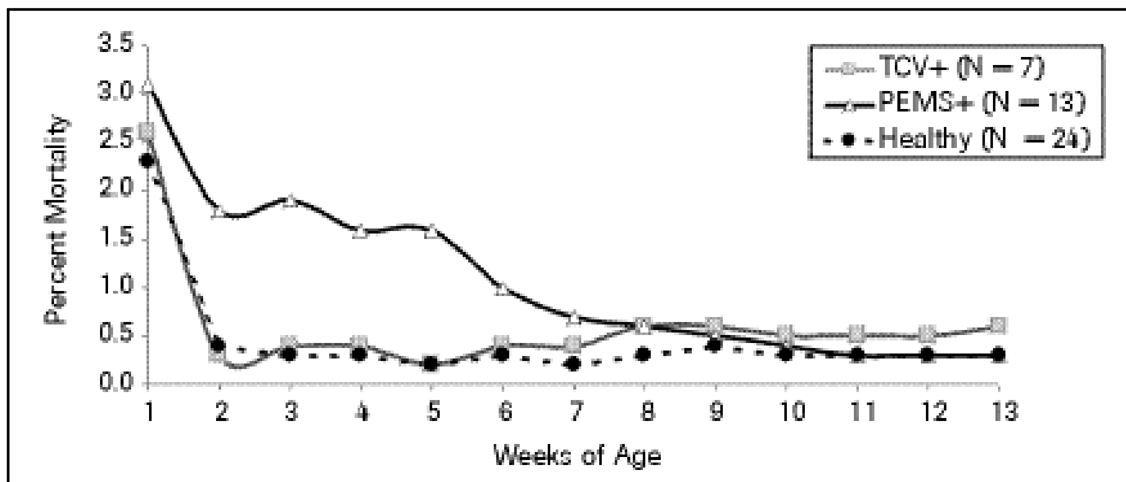
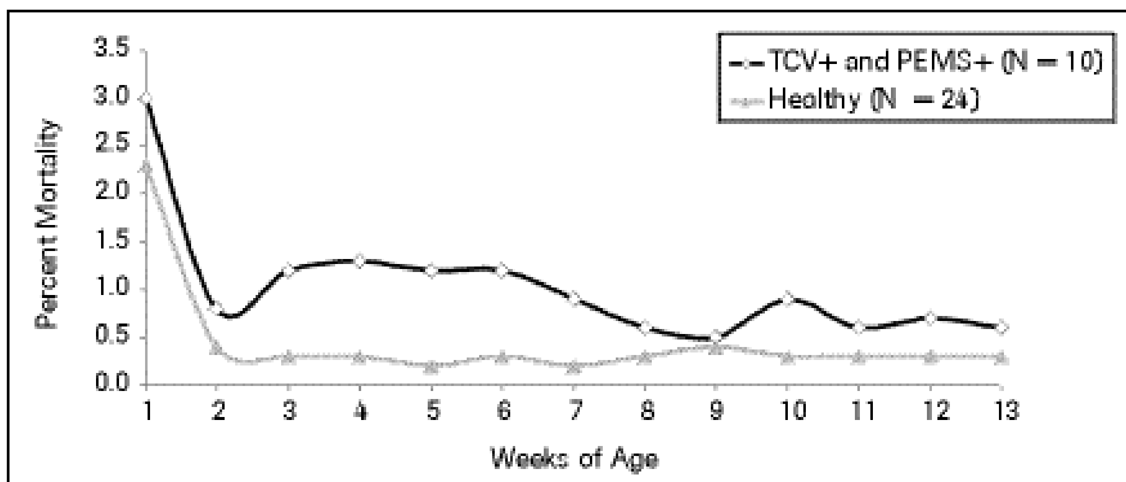


Figure 2. Weekly percent mortality for flocks with TCV and PEMS (mixed infection) versus healthy flocks



Induction of Poult Enteritis and Mortality Syndrome Due to the Interaction Among Bacterial and Small Virus Isolates from PEMS Infected Poults

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This article was condensed from a research proposal presented to the PEMS Task Force on June 12, 1998.

DISCUSSION

Over the past three years, scientists in the Poultry Science Department have concentrated on the possibility that PEMS (Poult Enteritis and Mortality Syndrome) can be induced by bacteria, specifically, atypical *Escherichia coli,* which have been isolated in virtually every PEMS infected poult that has been sampled by us. Several *E. coli* isolates have been characterized extensively and have been shown to be infective under conditions of normal brooding temperatures on very high moisture litter. These *E. coli* can cause clinical signs very similar to PEMS under these conditions, causing high rates of mortality as well as all the other signs of PEMS. These isolates also can cause diarrhea when given to poults in high temperature brooding environments with very dry litter, but mortality is minimal under these conditions. Furthermore, we have evidence that suggests that a plasmid in these atypical *E. coli* isolates may be the cause of this increased virulence. We also have evidence to show that these atypical *E. coli* isolates have the ability to develop resistance to the fluoroquinalone antibiotics after only a single exposure, and that development of resistance is also associated with a change in serotype of the bacterial isolates. Additionally, it has been shown that the common bacterial isolates associated with PEMS, which include *E. coli*, *Salmonella, Klebsiella, Staphylococcus* and *Streptococcus*, are all largely antibiotic–resistant.

Recently, Dr. Y. M. Saif, Dr. K. A. Schat, and Dr. Stacy Schultz-Cherry have all isolated small–diameter viruses from PEMS–infected poults that are roughly 25 to 65 nm in diameter. Whereas it is not yet known if these viruses are the same or different, it is important that these three laboratories have independently isolated small round non-enveloped icosahedral capsid viruses from PEMS–infected poults. Dr. Schat believes

that his virus is a DNA virus, but Dr. Saif believes that his virus is a RNA virus and has tentatively identified it as a very rare Calicivirus that has never been reported in turkeys previously. The small virus that Dr. Schultz-Cherry has isolated from the bursa of Fabricius and the thymus has not yet been classified as a DNA or a RNA virus. However, based upon its very small diameter (20 to 25 nm) it could be an Enterovirus, but additional work must be conducted before definitive identification can be made.

The Calicivirus is characterized as a diarrheal virus, and in humans, it is usually contracted through exposure to contaminated fish that have been in waters contaminated by sewage. This virus can cause mini-epidemics in families, hospitals, on board ships, etc., and is potentially very dangerous to people who may be immunocompromised. The Calicivirus contributes to the massive mortality caused by infantile diarrhea in developing countries. This virus is poorly understood because of the difficulty in propagating it in tissue culture. The Calicivirus is commonly identified via immunoelectronmicroscopy of feces. Currently, there is no animal model for the study of these viruses. The Calicivirus requires roughly two to four days for its incubation before frank signs of diarrhea are seen. The only supportive therapy for afflicted humans is rehydration. Fowl Calicivirus has been rarely observed in chickens, but it has never been reported previously in turkeys. At this point in time, Dr. Saif feels strongly that a Calicivirus has been isolated from the feces of PEMS–nfected turkey poults.

The PEMS-associated virology conducted by Drs. Saif and Schat was sponsored by a grant from the PEMS Task Force to the Poultry Science Department and subcontracted to the individual investigators. Contained in that funded project was a description of the required interaction studies that would need to be conducted if a novel small virus(es) was found. That work described the testing of the virus(es) for infectivity and virulence. It called for the determination of interactions between the virus and several *E. coli* isolates and other predominant PEMS-associated species of bacteria that were being characterized at that time. This work was necessary in order for us to determine if a virus was causing immune dysfunction, thereby leading to increased susceptibility and increased mortality that may be attributed to bacterial infection.

In the summer of 1997, Schat made a breakthrough by identifying in vitro the cell-culturable small virus from a PEMS–positive, coronavirus–negative PEMS infection. Saif has identified his Calicivirus from both coronavirus–positive and coronavirus–negative PEMS–infected poults. Schultz-Cherry has identified her small virus from a coronavirus–positive PEMS infection. When there were active investigations of the bacteriology associated with PEMS, scientists within the Poultry Science Department emphasized that here were characteristic bacterial isolates that were nearly always isolated from PEMS–infected poults regardless of the Coronavirus status. These observations have significant supporting documentation. Therefore, it is vital to the understanding of the etiology and pathogenicity of the putative etiological bacterial and viral agents that their interaction be included to aide the determination of their combined potential to induce PEMS mortality.

In November of 1997, the North Carolina Agricultural Foundation provided a one-time grant to establish a small isolation facility at the Dearstyne Poultry Research Center for the express purpose of studying the interaction between bacterial and small virus

isolates associated with PEMS. That facility is nearing completion after some small modifications to the isolators are made by Standard Safety in Chicago, IL. We will have the capacity to run eight different isolators at one time. The isolators will have 12 to 15 air exchanges per hour and will receive from a central fan coil-thermostatically controlled heater unit all prewarmed air that will be passed through HEPA filters before entering the individual isolators. The exhaust air will also pass through HEPA filters before being exhausted into a collecting duct that will be terminated with another HEPA filter before being exhausted through the flu–associated with the gas-fired boiler system. The isolators will remain sealed with collection of wastes in four–inch–deep pans under each cage unit in the isolators. Movement of dead birds or replacement of feed or water can be made through an air lock on each isolator, thereby preventing any breaks in isolator security. Each time the air lock is used, it will be subjected to chemical disinfection and sterilization procedures. As a safety feature, we are also adding an automatic generator to ensure against power failures that may suddenly terminate these crucial experiments. These isolator units have been fashioned, with few modifications, after those used by Dr. Saif at the Ohio Agricultural Research and Development Center in Wooster, Ohio.

Etiology and Pathogenesis of Poult Enteritis and Mortality Syndrome

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This article was condensed from a research proposal presented to the PEMS Task Force on June 12, 1998.

ABSTRACT

Poult Enteritis and Mortality Syndrome (PEMS) is a transmissible, infectious disease of young turkeys of unknown etiology. The disease occurs in flocks of young turkeys, 1-4 weeks of age; affected flocks exhibit depression, diarrhea and excessive mortality. Total mortality attributable to PEMS generally is greater than 10%, and may be as high as 60%. Severe economic losses occur as a result of mortality, depressed growth, poor feed utilization, increased time to market, and increased susceptibility to infectious diseases.

The cause of PEMS has not been determined. Several different infectious agents have been associated as causes of the disease, including *Campylobacter* spp., *E. coli, Cryptosporidium* spp., turkey coronavirus, reoviruses, rotaviruses, and enteroviruses. The etiology of this disease needs to be determined. The failure to identify the cause of the disease is the principal impediment to development of appropriate prevention and control procedures.

Studies in our laboratory have focused on viruses identified in PEMS-affected turkeys, particularly turkey coronavirus. Experimental studies have been conducted using several different viruses, including turkey coronavirus, reoviruses, adenoviruses, and enteroviruses. However, experimental infection of young turkeys with these viruses did not reproduce the disease, either alone, or in combination with other pathogens (*E. coli*, *Campylobacter* spp., *Cryptosporidium* spp.). Interestingly, the disease could be reproduced by using bacteria-free filtrates in combination with sterilized *Cryptosporidium* oocysts or in combination with *E. coli* and *Campylobacter* spp. These studies strongly suggest that (1) PEMS is caused by the interaction of two or more infectious agents and (2) one of the agents likely is a virus.

The proposed studies will continue our search for the putative PEMS virus using immunohistochemical and electron microscopic studies. If this line of investigation is successful, studies will be done to propagate the identified virus, characterize it, develop appropriate diagnostic tests and provide epidemiologic and experimental evidence for its involvement in PEMS. A second line of investigation will continue our studies on turkey coronavirus. These studies will aim to (1) improve laboratory diagnostic procedures for this virus, (2) determine the role of the virus in PEMS, (3) evaluate the interaction of turkey coronavirus and other viruses, bacteria and protozoa in the pathogenesis of PEMS, () identify other reservoirs and vectors of the virus. It is anticipated that these studies will lead to a better understanding of the etiology and pathogenesis of PEMS that will ultimately lead to development of effective control procedures.

OBJECTIVES

Our studies strongly indicate that poult enteritis and mortality syndrome (PEMS) is caused by the interaction between a virus and one or more other infectious agents (most likely bacteria or protozoa). The overall objectives of the proposed research will be to identify the virus and its copathogen.

JUSTIFICATION

Poult enteritis and mortality syndrome (PEMS) is a transmissible, infectious disease of young turkeys of unknown etiology. During the past several years, turkey producers in North Carolina, Indiana, Georgia, South Carolina, Virginia and Texas have experienced severe financial losses due to this disease. The disease tends to occur suddenly in flocks of young turkeys, 1-4 weeks of age; affected flocks exhibit depression, diarrhea and excessive mortality. A sharp rise in mortality typically occurs in affected flocks and generally exceeds 1% for a period of 3-5 days. This characteristic mortality pattern is responsible for the name initially given to the disease: "spiking mortality" of turkeys. In most affected flocks, total mortality attributable to PEMS exceeds 10%; however, some flocks have experienced mortality as high as 60%. Whereas mortality is the most prominent clinical effect and the principal cause of economic loss, other less prominent effects in recovered flocks include depressed growth, poor feed utilization, increased time to market and increased susceptibility to infectious diseases.

The cause of PEMS has not been determined; however, studies have conclusively determined that infectious agents are responsible for causing this disease. A wide variety of infectious agents has been identified in turkeys experiencing PEMS, and associated as causes of the disease. The infectious agents most commonly identified in PEMS-affected turkeys in North Carolina include *Campylobacter* spp., *E. coli* strains, *Cryptosporidium* spp., reoviruses, turkey coronavirus, rotaviruses, enteroviruses, and adenoviruses. The etiology of PEMS needs to be determined. The failure to identify the cause of the disease is the principal impediment to development of appropriate prevention and control procedures.

Previous studies in our laboratory have been focused on the viruses associated as causes of PEMS. Experimental studies have been conducted with several different viruses that we have been able to prepare as pure inocula: turkey coronavirus, reoviruses, adenoviruses, and turkey enteroviruses. Experimental attempts to reproduce PEMS have been unsuccessful using these viruses. PEMS was not reproduced when young turkeys were inoculated with these viruses, either alone, or in combination with other pathogens (*E. coli*, *Campylobacter* spp., *Cryptosporidium* spp.). However, the disease could be reproduced using bacteria-free filtrates in combination with Cryptosporidium oocysts or in combination with *E. coli* and *Campylobacter* spp. These studies strongly suggest: (1) the interaction of two or more infectious agents in the pathogenesis of PEMS, and (2) the likely involvement of a virus.

The proposed studies will utilize fluorescent antibody procedures to identify virus(es) in PEMS-affected turkeys that previously have eluded detection. Studies subsequently will be done to propagate identified viruses, characterize them, and provide epidemiologic and experimental evidence for their involvement in PEMS. In addition, experimental studies will examine the interaction of turkey coronavirus with bacterial and protozoan agents identified in PEMS-affected turkeys.

LITERATURE REVIEW

Poult enteritis and mortality syndrome (PEMS) is a recently described disease of 1- to 4–week–old turkeys (1). The disease is characterized by sudden, sharp increases in mortality (greater than 1% daily mortality) lasting for 3 to 5 days (2). Affected turkeys exhibit depression, diarrhea, and tend to die quickly after development of clinical signs. Flocks that have recovered from the disease exhibit growth depression, increased time to market, decreased feed efficiency and increased susceptibility to infectious agents. The cause of the disease has not been determined; however, mycotoxins, nutritional deficiency, and a variety of infectious agents have been suggested as causes (1,2). *Cryptosporidium* spp., *E. coli*, *Campylobacter* spp., turkey enterovirus, rotavirus, reovirus, infectious bursal disease virus and turkey coronavirus have been identified in PEMS flocks. The most commonly identified agents in episodes of PEMS in North Carolina are *Cryptosporidium*, turkey coronavirus, enterovirus, reovirus and rotavirus, and it is not unusual to find all of these agents together in droppings collected from PEMS-affected turkeys (J. Guy, J. Barnes, unpublished observations).

Experimental studies using young turkeys have been conducted with several different viruses that we have been able to prepare as pure inocula: reoviruses, adenoviruses, turkey enteroviruses and a turkey coronavirus. However, experimental infection of young turkeys with these viruses did not reproduce the disease (3), either alone, or in combination with other secondary pathogens (*E. coli*, *Campylobacter* spp., *Cryptosporidium* spp.). The disease could be reproduced by using bacteria-free filtrates in combination with sterilized Cryptosporidium oocysts or in combination with *E. coli* and *Campylobacter* spp. (3).

Turkey coronavirus (TCV) was associated as the cause of PEMS based on immuno-fluorescent studies (4). Turkey coronavirus was identified in several sites of tissue

damage observed in PEMS-affected turkeys: epithelium of duodenum, jejunum, ileum, and bursa of Fabricius. However, turkey coronavirus could not be detected in other lesional sites associated with the disease (lymphoid areas of the bursa of Fabricius, spleen, or thymus).

Epidemiological studies were undertaken to determine the involvement of turkey coronavirus in PEMS outbreaks using immunofluorescent detection procedures (T. Weaver, J. Barnes, J. Guy, unpublished). Eighty-seven flocks were examined; these were classified as normal (23), and PEMS-affected (64). Representative birds from these flocks were examined for presence of TCV. These studies indicated that TCV is widespread in North Carolina; however, the studies suggested that TCV was not the cause of PEMS. No association was found between the presence of the virus and the presence of the disease. This was further supported by the finding of PEMS flocks that were TCV-negative, and normal flocks that were TCV-positive.

Pathogenicity studies using TCV also suggest that this virus is not the cause of PEMS. Turkey–embryo-propagated TCV (NC95 strain) failed to produce mortality in orally inoculated turkeys, but did produce moderate growth depression. Turkey–embryo-propagated TCV (Minnesota/bluecomb strain) obtained from the American Type Culture Collection did not produce mortality and did not produce growth depression.

The TCV identified in PEMS-affected turkeys in North Carolina (TCV [NC95]) has been determined to be a strain of turkey enteric "bluecomb" coronavirus based on antigenic analyses (5). This virus is identical to the virus identified in Indiana turkey flocks. In addition, these studies demonstrated an antigenic relationship between TCV and infectious bronchitis virus, and suggested the possibility that chickens are susceptible to TCV infection and are potential reservoirs of infection (5).

PROGRESS YEAR-TO-DATE

1. Specific immunohistochemical procedures have been developed for detection of turkey coronavirus (TCV) using a monoclonal antibody-based immunoperoxidase (IP) procedure. A monoclonal antibody (MAb) specific for TCV has been developed. Specificity of the MAb was verified by positive reaction with five epidemiologically distinct TCV strains (recent isolates from North Carolina, Indiana, Arkansas and Virginia, and the original Minnesota "bluecomb" virus), but not with other avian and mammalian viruses including infectious bronchitis virus, bovine coronavirus, avian adenoviruses, reovirus, and Newcastle disease virus. Immunoblotting indicated that the MAb specifically recognized a 28 kD TCV protein, presumed to be the viral matrix protein.

   The MAb-based IP procedure was compared with direct immunofluorescence (FITC-conjugated polyclonal antibodies specific for TCV) for detection of TCV in infected turkeys. The IP procedure was slightly more sensitive for detecting TCV-infected birds; the IP procedure detected infection on days 1-14 postexposure and direct immunofluorescence detected infection on days 2-14 postexposure. Bursa

of Fabricius and ileum were the best sites (highest number of positives) for detecting TCV antigens in tissues of infected birds.

2. Serological procedures for TCV. An indirect immunofluorescent antibody (IFA) procedure was developed for serological detection of TCV-infected flocks. Antigen for this test consists of exfoliated epithelial cells collected from bursae of Fabricius of infected turkeys. The test was validated by examination of sensitivity and specificity. The test was determined to be specific for TCV based on positive staining using TCV-specific antisera and the absence of positive staining using a variety of antisera specific for avian viruses (Newcastle disease virus, reovirus, rotavirus, adenovirus, enterovirus). Sensitivity was deemed to be acceptable based on detection of antibodies in 100% of experimentally infected birds, beginning at 14 days postexposure and continuing through 42 days postexposure (end of trial). Seropositive birds on day postexposure: 0/20 positive on day 0 PE; 1/20 positive on day 7 PE; 20/20 positive on days 14, 21, 28, 35 and 42 PE.
3. A reverse transcriptase, polymerase chain reaction (RT-PCR) procedure has been developed for detection of TCV in infected turkeys. The RT-PCR procedure amplifies an 1100 base-pair DNA segment corresponding to the TCV matrix/nucleocapsid gene region. Diagnostic use of this RT-PCR procedure is currently under investigation. The RT-PCR successfully detected TCV in pooled dropping samples collected from experimentally infected turkeys on days 2-8 PE. Specificity of the procedure was verified by the absence of PCR products using pooled dropping samples collected from six different groups of uninfected control birds.
4. Molecular characterization of turkey coronavirus. The RT-PCR procedure has been used to amplify the 1100 base-pair gene region of three TCV strains (NC95, Indiana and Minnesota "bluecomb"). The RT-PCR products have been cloned into pUC19 plasmids, and sequenced. The sequence of these viruses were compared with published nucleotide sequences of infectious bronchitis virus (IBV) and bovine coronavirus. Based on these comparisons, TCV is closely related to IBV having a base homology of approximately 85% and distantly related to BCV having a base homology of approximately 45%. Phylogenetic analyses were done based on TCV nucleotide sequences and published sequences of other coronaviruses. These analyses divided the avian and mammalian coronaviruses into three distinct genotypes, with the avian coronaviruses, IBV and TCV, comprising one distinct genotype.
5. Development of a TCV–specific enzyme-linked immunosorbent assay for detection of TCV-specific antibodies. Using the nucleotide sequence information derived from TCV strains and the deduced amino acid composition of the matrix protein, we are attempting to produce an ELISA procedure using TCV-specific peptides. These studies are in progress.
6. Susceptibility of SPF chickens to TCV infection. Three-day-old SPF chickens were inoculated by combined oral/intratracheal routes and examined for clinical signs, weight gain and presence of TCV in various tissues. TCV infection resulted in a clinically inapparent infection (normal weight gain, no mortality). TCV was isolated from pooled droppings on days 1, 3 and 6 postexposure (PE) and the virus was detected by monoclonal–based immunoperoxidase staining in apical villous epitheli-

um of the duodenum, jejunum and ileum on days 2-8 PE. These findings indicate that chickens may be inapparent hosts and potential sources of TCV for turkeys.

7. Characterization of small round virus identified in PEMS-affected turkeys. A "small round" virus, approximately 24 nm in diameter and lacking apparent surface structure, has been identified in PEMS–affected turkeys. The virus has been tentatively identified as an enterovirus–like virus (ELV) based on size and morphology. It is distinct from an earlier described turkey enterovirus (TEV) (Guy and Barnes, 1991) in that it is not recognized by TEV-specific antisera and it replicates in embryonated chicken eggs inoculated by either the yolk or CAM route. Specific immunohistochemical procedures were developed for detection of this ELV; these procedures indicated that this virus was not likely involved in PEMS. The virus could be detected in only 1/6 PEMS(+) groups maintained by H.J. Barnes. Further characterization of the virus is in progress.

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Physiological and Immunological Manifestations in the Diagnosis of Poult Enteritis and Mortality Syndrome: 4 Studies

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This article was condensed from a progress report presented to the PEMS Task Force on June 12, 1998.

STUDY 1. PEMS 100 NM FILTRATE TRIAL

INTRODUCTION

Previous trials indicated that PEMS symptoms could be reproduced in turkey poults by oral inoculation with 100 nm filtrate of fecal material from PEMS poults. In January 1998, another trial was conducted to confirm these results. Five treatment groups were included in this trial: PBS control (poults inoculated with PBS only), 100 nm Control (poults inoculated with 100 nm filtrate of fecal material collected from uninfected control poults), Crude Control (poults inoculated with crude fecal material from uninfected control poults), 100 nm PEMS (poults inoculated with 100 nm filtrate of fecal material collected from PEMS poults), and Crude PEMS (poults inoculated with crude fecal material from PEMS poults). Each treatment group consisted of 3 replicate pens of 10 poults/pen. Poults were orally gavaged at 7 days of age with the appropriate inocula. The results of the trial are as follows:

TOTAL MORTALITY (%)

PBS Control	10.0
100 nm Control	3.3
Crude Control	6.7
100 nm PEMS	3.3
Crude PEMS	13.3

BODY WEIGHT (WEEKLY)

By 21 days postinoculation (DPI), the average body weight of the 100 (m PEMS group was significantly lower ($P<0.05$) than the average body weights of the PBS Control, 100 (m Control, and the Crude Control groups. The average body weight of the Crude PEMS group at 21 DPI was significantly lower ($P<0.05$) than all of the other treatment groups.

LYMPHOID ORGAN WEIGHT (21 DPI)

No significant difference in spleen weight between the groups was measured at 21 DPI. However, the average bursa weight of the 100 (m PEMS group was significantly lower ($P<0.05$) than that of the 100 (m Control and Crude Control groups. The average bursa weight of the Crude PEMS group was significantly lower ($P<0.05$) than in all of the other groups. In addition, the average thymus weight of the 100 (m PEMS group was significantly lower ($P<0.05$) than in all 3 control groups and the average thymus weight of the Crude PEMS group was significantly lower ($P<0.05$) than in all other groups.

CONCLUSIONS

PEMS mortality observed in a previous trial with a 100 (m filtrate of PEMS fecal material unfortunately was not reproduced in this trial. However, body weight was significantly lower in the 100 (m PEMS group as compared with the control groups. In addition, significant decreases in bursa and thymus weights were observed in the 100 (m PEMS group as compared with the controls. From these experiments, it seems evident that an agent associated with PEMS is present in a 100 (m filtrate of PEMS fecal material.

STUDY 2. NATURAL KILLER CELL EXPERIMENTS

Two experiments have been performed using natural killer (NK) cells obtained from the spleens of Control and PEMS poults (from 2 separate hatches) in 51 chromium release assays. In the first experiment, NK activity was reduced in the spleens of PEMS poults as compared with uninfected controls at 24 h post–contact-exposure to PEMS. By 48 h post–contact-exposure, NK activity was slightly higher in PEMS poults as compared with uninfected controls. However, in the second experiment, NK activity was higher in PEMS poults as compared with controls at 24 h post–contact-exposure and, by 48 h post–contact-exposure, NK activity in the PEMS group was equal to that of the control group. Further investigation concerning the effects of PEMS on NK cell activity is planned.

STUDY 3. LYMPHOCYTE SUBPOPULATIONS IN PEMS

Two experiments were conducted at 14 DPI employing blood and splenic lymphocytes from control, coronavirus-negative PEMS poults and coronavirus-positive PEMS poults. In both experiments, the CD4+:CD8+ lymphocyte ratio in the blood of coronavirus-negative PEMS poults was lower than both uninfected and coronavirus-positive PEMS poults. This result was significant when the coronavirus-negative PEMS group was

compared to the control alone. In addition, in two separate experiments at 14 DPI, the CD4+:CD8+ ratio in the spleen of coronavirus-positive PEMS poults was higher compared with both the uninfected and the coronavirus-negative PEMS poults. These findings clearly suggest that PEMS exposure induces alterations in the incidence of lymphocyte subpopulations which in turn may divert the immune responses towards either inflammatory or humoral pathway.

STUDY 4. CYTOKINE ALTERATIONS

Further studies examining cytokine (and other macrophage products) alterations associated with PEMS are currently being planned, but experiments to date have demonstrated: increased interleukin-1 (IL-1) and interleukin-6 (IL-6), increased total transforming growth factor-b (TGF-b) and TGF-b1, decreased TGF-b2, decreased tumor necrosis factor-a (TNF-a), and increased nitrite.

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The Interaction of "Novel Small Viral Agents" with the Immune System of Young Turkey Poults

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This article was condensed from a research proposal presented to the PEMS Task Force on June 12, 1998.

SUMMARY

In efforts to identify the etiological agent(s) of Poult Enteritis and Mortality Syndrome (PEMS), three different laboratories have isolated viral agents from gut contents (Ohio and Cornell) and bursa (Southwest Poultry Research) of PEMS poults. These agents are being propagated. Preliminary indications are that these agents are very small (23 to 50 (m range), and, when inoculated in germ-free poults, induce certain signs similar to the ones exhibited by PEMS poults. All prior experimental studies on immune function in PEMS poults have been conducted by using contact or oral/fecal exposure to undefined inocula. With the availability of these novel viral agents, there is an immediate need to test whether these viral agents can induce immune alterations in poults.

A Strategy for the Prevention and Control of Stunting Syndrome

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SUMMARY

For the past several years, researchers at Iowa State University have been investigating an enteric disease condition that occurs in young turkeys and is referred to as stunting syndrome (SS). A major objective of this research has been directed towards isolating and identifying the etiologic agent of stunting syndrome (SSA). Recently, we have been successful in isolating the causative agent. We have also learned that there is age susceptibility associated with SS. That is, when day-old birds are exposed to the SSA, severe SS results. However, if poults are not exposed to the SSA until 7 days of age, the effects are much less severe. Very mild (if any) SS is produced if birds are exposed beyond 2 weeks of age. Therefore, we postulate that if we can prevent the SSA from infecting birds within the first 1 to 2 weeks following hatch, then we can have a major impact on decreasing SS. Recently, we conducted a preliminary trial (Table 1) in which we injected superconcentrated antibody preparations against SSA into day-old poults prior to placing them into stunting syndrome contaminated isolators. The results of this preliminary trial indicated that poults were partially protected from the effects of the SSA. We hypothesize that administering SSA–specific antibody to poults, either by subcutaneous injection or in the feed, or both, can protect them from stunting syndrome.

DISCUSSION

We have recently developed a neutralization assay for the SSA and found that antisera directed against the SSA will neutralize the virus. Additionally, we have learned from our experiences with SS that there is an age susceptibility to SS. That is, when day-old birds

are exposed to the SSA, severe SS results. However, if poults are not exposed to the SSA until 7 days of age, the effects are much less severe. Very mild (if any) SS is produced if birds are exposed beyond 2 weeks of age. Therefore, we hypothesize that if we can prevent the SSA from infecting birds within the first 1 to 2 weeks following hatch, then we can have a major impact on decreasing SS. We propose to employ two strategies to test this hypothesis. The first strategy will involve concentrating serum antibodies from turkeys which have been hyperimmunized against the SSA. The concentrated antibodies will be injected into poults prior to exposure to the SSA to evaluate the effect of passive immunity. If this strategy is successful, then it may be feasible to either inject poults at the hatchery with antibody preparations or vaccinate breeder hens to convey high levels of maternal antibody naturally to poults. Preliminary studies to test this strategy (Table 1) have been very encouraging. If the injection of sera antibody proves successful, we will extend this strategy by determining if antibody derived from the egg yolk of chickens vaccinated with SSA can be utilized as a source of antibody. The rationale for using chicken egg yolk antibody is that it would be easier and much more feasible to produce large quantities of antibodies for poult injection by this method. An additional strategy is to provide the antibody in the feed as a dietary supplement. Our approach will be to hyperimmunize laying hens (chickens) with the SSA and use egg yolk with high levels of specific anti-SSA antibodies as a feed ingredient in the starter diet of turkey poults. The rationale is to provide specific SSA antibody within the lumen of the bird's intestinal tract (via the feed) so that it may bind with SSA and neutralize the virus thereby alleviating infection. This passive immunization strategy has been successfully used for combating other enteric virus diseases of neonates such as rotavirus infections.[12,13]

LITERATURE REVIEW

A disease (or diseases) of undetermined etiology that occurs in young poultry has been commonly referred to as "viral enteritis," but has a variety of names depending upon the avian species involved. The disease in turkeys has been frequently termed "turkey viral enteritis", "poult enteritis", "malabsorption syndrome" and "maldigestion syndrome."[6,7,8] Additional terms, such as "stunting syndrome", "poult enteritis complex" and "spiking mortality,"[8] have been used to describe similar enteric disease conditions of unknown etiology. Typically, birds are noticeably affected within the first two weeks of life and usually display clinical signs of diarrhea, nervousness, growth retardation (stunting), litter eating and excessive drinking.[6,7,8] Experimental findings in turkeys have documented that poor feed utilization, depressed weight gains, decreased feed consumption, maldigestion (due to decreased disaccharidase activity) and malabsorption are manifestations of stunting/malabsorption syndrome[7,8]. A sequela to the initial onset of clinical signs has been reported to be poor feather development involving the primary wing feathers, resulting in the "helicopter chick/poult".[9] An additional sequela is the development of a rachitic condition,[10] which has been reported to occur more frequently in affected poults than chicks.

Malabsorption syndrome of poults has been reported to induce an early osteoporotic lesion associated with hypocalcemia, which progresses into a rachitic lesion associated

with vitamin D depletion and hypophosphatemia.10 Birds which recover from poult malabsorption have an increased incidence of angular limb deformities.[11] Post-mortem findings of affected turkeys typically include distention of the gastrointestinal tract with gas and fluid, loss of tone of the intestinal tract and dilated ceca with frothy contents.[7,8] Increased mortality has been observed in turkeys (especially in spiking mortality cases). In those cases in which mortality has been low, morbidity, resulting in decreased growth (stunting) and flock unevenness, is of greatest concern. Generally, there is little compensatory growth in stunted birds following the course of the disease; therefore, birds remain stunted throughout the growout period to market age. For the past several years, researchers at Iowa State University have been investigating an enteric disease condition that occurs in young turkeys and is referred to as stunting syndrome.[2,3] A major objective of this research has been directed towards isolating and identifying the etiologic agent of stunting syndrome (SSA). Recently, we have been successful in isolating the causative agent.[4] Initial electron microscopic observations of the SSA indicated that it varied in size and shape. It also appeared to have a membrane. In appearance it was similar to a coronavirus. In fact, our initial impression was that we had isolated a coronavirus. Many of these particles have distinct peplomeres protruding from their surface similar to coronaviruses, while other particles have a curved (kidney bean) morphology and resembled torovirus particles. Hemagglutination experiments revealed that the SSA only hemagglutinates rat erythrocytes at cold and room temperatures (4° and 25° C). This is a distinguishing characteristic of SSA and coronaviruses. Coronaviruses (the bluecomb agent) are known to hemagglutinate both rabbit and guinea pig erythrocytes. The ability of the SSA to hemagglutinate rat erythrocytes at 4° and 25° C is more consistent with the hemagglutination profile of toroviruses.[5]

INVESTIGATIONS

We have been successful in propagating the SSA in 24-day-old embryonating turkey eggs. By this technique, eggs are inoculated with the SSA by the amnionic route. The inocula (SSA) requires activation with trypsin prior to embryo inoculation. Typical lesions of fluid-filled, ballooned intestines are induced in the embryos by 4 days postinoculation. This technique has allowed a method of propagating the SSA and obtaining large quantities of purified virus. Additionally, we have used this in vitro method of propagation for serum neutralization studies in which homologous SSA antisera and antisera produced against other agents have been assessed.

We have determined that the SSA is serologically distinct from the turkey enteric coronavirus (bluecomb agent) and the other agents (antisera) that were evaluated. These included bovine coronavirus, TGE of swine, avian bronchitis virus, bovine breda virus 1 and 2, avian influenza virus and Newcastle disease virus (NDV). We consider the SSA as a newly identified viral agent that causes stunting syndrome of turkeys. We are currently involved in studies to further characterize and properly identify (classify) the SSA.

A preliminary study was performed in which blood sera was collected from turkeys hyperimmunized with SSA and NDV. The antibodies were concentrated by ammonium

sulfate precipitation. The antibody preparations were injected subcutaneously into poults prior to placement into either an isolator previously contaminated with SS or a negative control isolator. Two duplicate trials were conducted on different dates. Nearly identical results were obtained in each trial. The results of the first trial are displayed in the Table 1.

Table 1. Results of first trial

Group	*Isolator*[1]	*Treatment*[2]	*N*	*Avg. Body Wt*[3]
1	Neg	TPB	14	183[A]
2	Neg	NDV	16	184[A]
3	Neg	SS	14	191[A]
4	SS	TPB	15	137[C]
5	SS	NDV	16	128[C]
6	SS	SS	16	155[B]

[1] Isolator to which poults were placed. Neg. = Negative control. SS = Stunting Syndrome contaminated.
[2] TPB = Tryptose Phosphate Broth. NDV = Newcastle disease virus concentrated antiserum.
SS = Stunting Syndrome Agent concentrated antiserum.
[3] Average body weight at 12 days postexposure (placement in isolators).
[A,B,C] Values with different superscripts indicate statistical difference ($p < 0.01$).

The results of these preliminary trials suggest that the SSA–specific antibody preparations, injected into day-old poults prior to placing them into contaminated stunting syndrome isolators, had a positive effect in lessening the severity of stunting syndrome. For several years members of this research team have expended much time and effort on numerous methods, strategies, practices, etc., to treat, prevent and/or in some way have a positive impact on stunting syndrome. The results of these preliminary studies represent the first time we have definitely been successful. We are very encouraged and enthused by these results.

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Studies on Poult Enteritis and Mortality Syndrome at OSU

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OBJECTIVE 1.
IDENTIFICATION AND CHARACTERIZATION OF INFECTIOUS AGENTS ASSOCIATED WITH THE DISEASE

Natural infections. We continued to examine field samples "gut contents and bursas" from affected flocks using immune electron microscopy (IEM). Similar to our early findings, we detected several serogroups of rotavirus, reovirus, coronavirus and a small round virus.

OBJECTIVE 2.
REPRODUCING THE DISEASE IN SPF AND CONVENTIONAL POULTS

In earlier studies we have shown that inoculation of SPF poults with the PEMS inocula, which contained a variety of agents, resulted in severe clinical signs and high mortality.

During the latest period, we initiated two experiments using the small round virus (the only agent of unknown identity) to study the pathogenicity of the virus in 7– and 32–day–old SPF poults. In each trial, there were 3 experimental groups, a noninoculated control, an inoculated group, and a contact group placed with the inoculated group. The birds were euthanized at predetermined intervals and the experiments lasted 3 weeks.

All the inoculated and contact poults were depressed, had enteritis and their body weights were significantly below the noninoculated controls, but no mortality occurred. The virus was detected in gut contents of inoculated and contact group birds. The results clearly indicated the potential pathogenicity of the small virus.

OBJECTIVE 3.
DEVELOPING DIAGNOSTIC REAGENTS AND TESTS

- We were able to propagate the small virus in SPF turkey embryos and passage it successfully in these embryos. High titers of virus were obtained, which will facilitate the production of reagents for different tests.
- We are also able to propagate coronaviruses in SPF turkey embryos.
- The small virus size was estimated to be 35-40 nm.
- Currently we are proceeding with a variety of tests (buoyant density, protein profile, nucleic acid typing, etc.) to identify the small virus.

Characterization of Agents Associated with Poult Enteritis Mortality Syndrome

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ABSTRACT

Outbreaks of poult enteritis mortality syndrome (PEMS) continue to cause financial losses to the turkey industry. The etiologic agent of PEMS remains an enigma. However, many investigators propose that turkey coronavirus (TCV) is the causative agent and is most likely involved in outbreaks. However, outbreaks without TCV have been identified and the disease has been experimentally reproduced without TCV. This suggests that TCV is not the causative agent. The uncertainty may be in part due to the diagnostic tests. Currently, TCV is diagnosed by identification of the virus using immunofluorescence, ELISA or viral isolation. These tests are cumbersome and rely on the antibody detecting the circulating TCV strains. The ability of TCV to evolve through genetic recombination may make detection of emerging strains difficult with current reagents. This would have devastating effects on commercial turkey producers. Our laboratory's aim is to determine the role of TCV in PEMS and develop new diagnostic tools to examine TCV in commercial flocks.

Currently, circulating strains of TCV (from PEMS-free and PEMS-affected areas) are being characterized by nucleotide sequence analysis. The Minnesota strain (bluecomb) of TCV is also being sequenced as a reference isolate. Peptide antibodies to conserved and divergent regions of the virus will be produced based on amino acid sequences and tested for diagnostic use. The data generated will be used to develop a TCV database that will allow one to predict emerging strains and examine the relationship of circulating strains to previous outbreaks. We can also compare TCV to mammalian strains of coronavirus, including bovine strains. The peptide antibodies will be crucial to determine if emergent strains of TCV are present in PEMS-free flocks. These data will further our knowledge of the role of coronavirus in PEMS.

Poults infected with PEMS, independent of the presence of coronavirus, exhibit lifelong immunosuppression. Studies are underway to identify the immunosuppressive

virus(es). To isolate potential agents, filtered tissue homogenates from PEMS and control birds were added to cultured cell lines and examined for cellular differentiation or cytopathic effect (CPE). Cell supernatants and monolayers were then analyzed by electron microscopy (EM). EM analysis indicated the presence of a "small round" virus in a mammalian epithelial cell line. Studies are underway to characterize the virus and determine its role in PEMS pathogenesis.

Finally, we are taking two novel, molecular approaches to identify viral agents involved in PEMS: phage display and subtractive hybridization. Both techniques were successful in identifying the previously "unculturable" agents hepatitis C and herpesvirus-7 (the causative agent of Kaposi's sarcoma). The basis of phage display is that foreign DNA is inserted into a virus that infects bacteria (bacteriophage). Bacteria are then infected with the recombinant phage and numerous proteins are expressed on the phage surface. The phage can then be screened with the antibody of interest (biopanning) and positive viruses can be isolated and identified. If DNA from PEMS-infected tissue is inserted into the bacteriophage, unique proteins are made that can be identified with convalescent sera from PEMS-infected or control birds. Any unique proteins identified can than be used to develop serological assays to detect circulating antibodies in commercial turkey flocks. Presently, libraries from the bursa, thymus, and intestine/feces from PEMS-infected birds are being screened with PEMS-specific antibodies and analyzed by nucleotide sequence analysis.

Subtractive hybridization is a powerful technique that allows one to compare two populations of mRNA and obtain clones that are expressed in one population but not in the other. This novel technique was used to successfully identify a new human herpesvirus in AIDS-associated Kaposi's sarcoma. Briefly, mRNA from control and infected tissues are converted to complementary DNA (cDNA) using an oligo d(T) primer and reverse transcriptase. The cDNA will be enzymatically digested with Rsa I to generate large fragments of DNA containing blunt ends. The "tester" cDNA (infected tissue cDNA) is then hybridized to the "driver" cDNA (control tissue cDNA), and the hybrid sequences are removed. Consequently, the remaining unhybridized cDNAs represent genes that are expressed in the "tester" or infected tissues, but are absent in the "driver" or control tissue. To determine the efficiency of the subtraction, RNA from infected and control tissues will be run on formaldehyde gels and transferred to nylon membranes, and then probed with the subtracted cDNAs. If the technique was successful, the subtracted cDNA should primarily recognize RNA from infected tissues. The selected "tester" cDNAs will then be amplified by polymerase chain reaction (PCR), cloned into the T/A blunt cloning vector, and amplified in bacteria. These unique sequences will then be subjected to nucleotide sequence analysis.

Through a multifaceted approach, the viral agents involved in PEMS will be identified. Future studies will focus on the role of TCV and other viruses on PEMS pathogenesis and development of therapeutic interventions.

RESEARCHER ABSTRACTS

Mechanical Transmission of Turkey Coronavirus (TCV) in Young Turkeys by Adult Darkling Beetles, *Alphitobius diaperinus*

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This article was condensed from a research proposal presented to the PEMS Task Force on June 12, 1998.

SUMMARY

The turkey industry in North Carolina has been confronted with Poult Enteritis Mortality Syndrome (PEMS), an enteric disease of turkeys of an unknown etiology. PEMS emerged in young turkey flocks in North Carolina in 1991. This disease is characterized by an abrupt, sudden mortality and a marked stunting in birds that survive the disease. Surviving birds have impaired immunity and are susceptible to other infectious diseases. PEMS has since spread throughout North Carolina and into neighboring states, with North Carolina losses of $133 million since it first appeared. PEMS research has focused on the cause of disease and identifying methods of prevention, control and treatment. Studies have demonstrated the infectious nature of the disease and discovered that turkey coronavirus (TCV) is often, but not always, associated with the disease. Traditional efforts to control the disease have been successful in part. Insects may be involved in the epizootiology of this disease. This ongoing project evaluates the role of the darkling beetle, *Alphitobius diaperinus*, in the transmission of turkey coronavirus (TCV).

OBJECTIVE

Examine the potential of adult beetles to transmit TCV virus to healthy turkeys under laboratory conditions.

Early experiments were conducted under controlled environments using commercial turkeys and laboratory–reared adult darkling beetles. One-day-old turkeys were raised in heated brooders until 7 days of age. Turkeys were then weighed and divided into 8 groups of 15 birds each.

Two strains of turkey coronavirus were selected for study. Virus isolate (NC95) is a highly infectious strain with low mortality. NC95 was collected from a commercial turkey brooder house and embryo propagated. Guts of NC95–infected embryos were fed to turkeys to establish a source of infected fecal material. The second turkey coronavirus strain was isolated from a PEMS outbreak (PEMS+). It is a highly virulent, high–mortality strain of virus that has been maintained in live birds by repeated backpassages.

Adult darkling beetles were divided into 5 groups of 200 each. The beetles were deprived of food and water for 3 days. Beetles were fed virus-infected feces mixed with chicken feed. Two groups of beetles were given fresh NC95–infected feces. Two other groups of beetles were fed PEMS+infected feces.

The negative control beetles were given chicken feed and water. One group of beetles from the NC95 and the PEMS+ beetle groups were surface sterilized by immersion in a 50% sodium hypochlorite and Tween 80 solution for 10 minutes, then rinsed once in 70% ethanol, and 3 times in sterile distilled water. Whole beetle homogenates were suspended in 20 ml DMEM tissue culture medium. Clarified homogenates were held on ice. Following centrifugation, 1 ml of the suspended virus supernatant was administered to the turkeys by gavage.

Turkey treatments were as follows:

A. Sham-inoculated-negative control with DMEM (1 ml/bird)
B. Inoculated with unsterilized beetle homogenate from TCV–negative beetles
C. Inoculated with unsterilized beetle homogenate from TCV–positive beetles
D. Inoculated with surface sterilized beetle homogenate from TCV–positive beetles
E. Inoculated with unsterilized beetle homogenate from PEMS+ beetles
F. Inoculated with surface sterilized beetle homogenate from PEMS+ beetles
G. Inoculated with laboratory strain of NC95–infected feces
H. Inoculated with PEMS+ infected feces

Three days postinoculation, 5 birds were randomly selected from each treatment group. These birds were necropsied and bursa tissues collected for fluorescent antibody tests for the presence of TCV. Growth depression was measured by weighing the birds on days 7, 14 and 21. Birds were bled for TCV serology tests on day 21 and euthanized.

RESULTS

Birds in the negative control groups and birds fed uninfected beetles were TCV negative in both FA and serology tests (Table 1). One bird was FA positive in the PEMS+, unsterilized beetle group, while 2 and 3 birds were FA positive in the NC95 and PEMS+ positive control groups, respectively. Day 21 serology test results were TCV positive for the one bird in the NC95 group fed unsterilized beetles and 6 birds for the PEMS+ fed unsterilized beetles. Both NC95 and PEMS+ control groups were positive for TCV serologically. Bird weight was lower in the PEMS+ fed unsterilized

beetles, and both NC95 and PEMS+ control groups. Mortality occurred in the PEMS+ unsterilized beetle group and the PEMS+ control group. Adult darkling beetles may act as mechanical vectors of TCV and the agent(s) responsible for PEMS in turkeys.

Table 1. Results of fluorescent antibody and serology tests of turkey poults fed TCV–infected adult darkling beetles

Treat-ment	*Inoculum*	*Mortality*	*Avg. Daily Wt. Gain (grams)*	*FAT Serology (Day 3)**	*FAT Serology (Day 21)**
A	DMEM	0	39.8	0/5	0/6
B	Neg. Beetles: control	0	40.7	0/5	0/6
C	TCV Beetles: Un-sterilized	0	37.1	0/5	1/6
D	TCV Beetles: Surface sterilized	0	37.1	0/5	0/6
E	TCV Beetles: Un-sterilized	3	16.4	1/5	6/6
F	PEMS+TCV: Surface sterilized	0	41.2	0/5	0/4
G	TCV Feces	0	23.0	2/5	6/6
H	PEMS+TCV Feces	2	17.7	3/5	6/6

* Number of positive birds/total number of birds examined

Development of Enzyme-Linked Immunosorbent Assays and PCR for Diagnosis of Turkey Poult Enteritis

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PROGRESS YEAR-TO-DATE

This project was proposed to obtain turkey coronavirus (TCV) antigen and antibody to TCV that can be used to coat ELISA plates. Research progress is summarized below.

1. For propagation of TCV, 18–, 20– or 22–day–old turkey embryos were inoculated with TCV- containing intestinal homogenates via the amniotic route. Inoculated turkey embryos were incubated for 3, 5, or 7 days. Inoculation of 18– or 20-day-old turkey embryos and incubation of inoculated turkey embryos for a longer period of time (5 or 7 days) did not improve the propagation efficiency of TCV. Propagation of TCV in turkey embryos via the allantoic sac was attempted, but not successful. TCV was successfully propagated in 22-day-old embryonated turkey eggs by inoculation of TCV-containing intestinal homogenate via the amnionic route.
2. For concentration of TCV, supernatants obtained from centrifugation of infected turkey embryo intestinal homogenates at 3,000 x g for 10 min were further ultracentrifuged and three different approaches (30% w/v sucrose solution, 60% w/v sucrose solution, or 50% saturated ammonium sulfate) were tried and compared. It appeared that concentration of TCV from the supernatants could be better achieved by ultracentrifugation of the supernatants at 100,000 x g through a cushion of 60% sucrose solution at 4° C for 1 hr.
3. For purification of TCV, concentrated materials from ultacentrifugation through a 60% sucrose cushion were subjected to further purification process by sucrose gradient, tartrate gradient, or size-exclusion column chromatography. The results indicated that purification of TCV from concentrated materials was better achieved by a continuous 40 to 60% sucrose gradient ultracentrifugation or Sephacryl S-1,000 size-exclusion column chromatography.

4. TCV isolates from other investigators, bovine coronavirus (BCV), infections bronchitis virus (IBV), pololyclonal antibody to TCV, monoclonal antibody to BCV or IBV are being obtained for evaluation of antigenic relationship among different TCV isolates and different coronaviruses. The purpose is to find suitable antigen and antibody for the development of ELISA for TCV.
5. A nested PCR procedure was developed to detect TCV in the turkey intestines. Some FA-negative intestine samples were positive for TCV by PCR amplification. This indicated that a more sensitive and reliable test is needed for accurate diagnosis of turkey coronaviral infection. At the present time, FA, PCR, and embryo propagation are all needed to confirm turkey coronaviral infections.
6. Ten 2-week-old turkey poults were orally inoculated with filtered intestinal homogenate prepared from infected turkey embryos. Turkeys were bled weekly postinoculation (PI) for 29 weeks. The highest FA titer was observed at 10 weeks PI. The presence of FA titer lasted until 25 weeks PI.
7. Currently, isolates of TCV from different geographical locations were obtained and S1 gene of spike protein was sequenced. Data were compared to published BCV sequences. Preliminary analysis indicated a significant amino acid sequence change in S1 between TCV and BCV. Minor sequence changes were also detected among TCV isolates. More data are being analyzed. The significance of these changes will be evaluated.

1997 AAAP Scientific Abstracts Relating to Turkey Coronavirus

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STUDIES ON CORONAVIRUSES AND POULT ENTERITIS

Donald L. Reynolds, Joan Oesper, and Sevine Akine

A comparative pathogenesis study was performed to determine the pathogenic effect of various coronavirus isolates in turkeys. The bluecomb isolate (from ATCC), a coronavirus from a recent field outbreak and bovine coronavirus were evaluated. Each virus was propagated in embryonated eggs and purified prior to inoculating poults. One-day–old commercial poults were inoculated orally and observed for 2 weeks. Viruses were passaged 5 times in these poults. Clinical signs, lesions and body weights were assessed. Sera samples were collected pre- and postexposure. Clinical signs, lesions and weight gain were not different between infected and noninfected control poults.

SEQUENCE ANALYSIS AND COMPARISON OF THE S1 REGION OF TURKEY ENTERIC CORONAVIRUSES WITH AVIAN MAMMALIAN CORONAVIRUSES

Holly S. Sellers, Tom Brown, Mark Jackwood, Laura Kelley and AnaPatricia Garcia

Reverse transcriptase-polymerase chain reaction (RT-TCR) was used to amplify the S1 region of turkey enteric coronaviruses from several naturally occurring cases of spiking mortality of turkeys (SMT) and a bovine-origin coronavirus. Amplified products will be sequenced, and phylogenetic analyses of the nucleotide and deduced amino acid sequences will be performed. Sequences will be compared with previously published sequence data from selected avian and mammalian coronaviruses.

PROPAGATION, TITRATION, AND VIRUS NEUTRALIZATION OF TURKEY ENTERIC CORONAVIRUSES IN HRT-18 CELLS

Tom P. Brown, Laura A. Kelley, Holly S. Sellers, and AnaPatricia Garcia

Turkey enteric coronaviruses (TCV) from naturally occurring cases of spiking mortality of turkeys (SMT) and a Minnesota reference strain were propagated in HRT-18 cells. Turkey enteric coronavirus isolates were titrated by serial dilution of virus in HRT-18 cells to endpoints, as visualized by cytopathic effect. A virus neutralization test using these propagation techniques in 96 well plates was developed using TCV antigen and sera taken from infected turkeys, vaccinated turkeys and suspected carriers of TCV.

IN VITRO ACTIVITY OF SERUM, YOLK AND CHEMOTHERAPEUTICS AGAINST SPIKING MORTALITY ASSOCIATED TURKEY ENTERIC CORONAVIRUSES

Tom P. Brown, Laura Kelley, Holly Sellers, and AnaPatricia Garcia

Multiple turkey enteric coronaviruses have been isolated from naturally occurring cases of spiking mortality of turkey/poult enteritis mortality syndrome (SMT/PEMS). These pathogens have been propagated, titrated and optimized in HRT-18 cell cultures. Acute and convalescent sera and yolk from naturally occurring and experimental cases of SMT/PEMS were examined for individual activity by use of virus neutralization assays in this cell culture system. Putative chemotherapeutic agents were also examined for anticoronaviral activity in vitro.

TURKEY FLOCKS WITH HIGH SPIKING MORTALITY THAT ARE NEGATIVE FOR TURKEY CORONAVIRUS

H. John Barnes, James S. Guy, J. Todd Weaver, and Shannon R. Jennings

Although turkey coronavirus (TCV) commonly has been identified in flocks with spiking mortality of turkeys (SMT) – an acute, infectious, transmissible disease characterized by high mortality, marked stunting and immunologic dysfunction — its role in the disease remains uncertain. Spiking mortality in turkeys was recovered from 4 flocks by sentinels, and the disease has been passed weekly by contact. Extensive immunohistochemical and serologic testing has failed to demonstrate TCV, even though stunting and effects on lymphoid organs have been similar between TCV-negative and TCV-positive SM1 groups. Mortality in the TCV-negative SMT groups has been somewhat lower, less consistent, and later than in the TCV-positive SMT groups. However, combining the 4 groups together has resulted in highly virulent SMT. A variety of potential enteropathogens has been identified, but none has been determined to be the cause of the disease. An attempt to reproduce the disease using filtered feces was unsuccessful. These results suggest that 2 distinct diseases with similar clinical appearances exist or the TCV is a secondary agent that is not essential for SMT to occur. Until these possibilities are resolved, it is recommended that the TCV status of SMT outbreaks be determined and the disease be referred to as TCV-positive and TCV-negative SMT.

SEQUENCE ANALYSIS OF SPIKE PROTEIN S1 SUBUNIT OF TURKEY CORONAVIRUS ISOLATES

T.L. Lin, S.C. Tsai, C.C. Wu, and T.A. Bryan

Turkey coronavirus isolates from Indiana, North Carolina and Minnesota were subjected to RNA extraction, followed by reverse transcription. Nucleotides encoding the S1b gene fragment of spike protein were amplified by polymerase chain reaction and then were sequenced. A high degree of nucleotide sequence homology without any deletions or insertions was seen in the isolates studied. One or 2 nucleotide changes were

noted in the polymorphic region. Results indicated that S1b gene fragments of turkey coronavirus isolates were highly conserved.

CLINICAL AND HISTOPATHOLOGICAL FINDINGS IN TURKEY POULTS INOCULATED WITH BOVINE ENTERIC CORONAVIRUSES

AnaPatricia Garcia, Tom Brown, Pedro Villegas, and Laura Kelley

One-day-old turkey poults were inoculated orally with cell-adapted bovine-origin intestinal coronaviruses and backpassaged 10 times. For each passage, effects on clinical signs, body weight, feed conversion and pathologic changes were determined. Three days after inoculation, birds were lethargic and many had moderate to severe diarrhea, but none had significant gross lesions. Histopathologic examination of thymus, bursa of Fabricius, and small and large intestine revealed moderate to severe bursa atrophy and villus atrophy of the ileum. Marked differences were found in body weight and feed conversion between control and inoculated birds.

CELLULAR AND BIOCHEMICAL LESIONS ASSOCIATED WITH POULT ENTERITIS AND MORTALITY SYNDROME

Frank W. Edens and Rachel E. Doerfler

Malabsorption and malnutrition are major causes of death associated with poult enteritis and mortality syndrome (PEMS). This is not alleviated with water supplements of sucrose (0.5 to 5.0%) or potassium phosphate (0.5%). Decreased serum inorganic phosphate and glucose, increased activity of hepatic glucose-6-phosphate and decreased hepatic glycogen in PEMS-infected poults indicated decreased nutrient absorption and use for energy metabolism and growth. Loss of intestinal epithelial cell integrity observed as multinucleated, giant, foamy cells with violated tight junctions, degenerating microvilli, subcellular membrane disintegration, and mitochondrial edema were associated with hyperdense eosinophilic mucosal inflammation in PEMS-infected poults.

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EFFECT OF TURKEY CORONAVIRUS INFECTION ON COMMERCIAL TURKEY FLOCK PERFORMANCE

D.V. Rives and D.B. Crumpler

Turkey flocks produced by a southeastern US integrator are being monitored by indirect fluorescent antibody test for exposure to turkey coronavirus. Performance parameters such as daily gain, feed conversion, body weight and livability are being compared for corona–positive and –negative flocks. Concurrent exposure to B avium and Newcastle disease virus and their effects on performance are also being monitored. Results from 1997 indicate a 3.86% advantage in livability and a 0.21 feed conversion advantage for corona–negative consumer hens. Corona negative heavy toms showed a 3.35–lb weight advantage. Corona-positive hens and toms cost $0.02 more per lb. to produce.

EXAMINING THE CAUSE(S) OF POULTRY ENTERITIS MORTALITY SYNDROME

Stacey Schultz-Cherry, H. John Barnes, Matthew Koci and Bruce Seal

Poultry enteritis mortality syndrome (PEMS) is an infectious, transmissible disease that affects young turnkeys. Poult enteritis mortality syndrome is characterized by high mortality, diarrhea, severe growth depression and lifelong immune suppression. Currently the causative agent of PEMS is unknown. Our goal is to identify the PEMS agent. Our preliminary results suggest that PEMS-infected birds undergo lymphocyte suppression prior to clinical evidence of disease. Additionally, an unidentified small round virus isolated from the bursa of infected birds by replication in cell culture also induces lymphocyte suppression in poults. Studies are underway to identify the immunosuppressive agent using novel molecular, biochemical and immunologic techniques.

VIRUSES ASSOCIATED WITH POULT ENTERITIS IN CALIFORNIA FLOCKS

Peter R. Woolcock and H.I. Shivaprasad

Poult enteritis is one of the most common diseases seen in turkey flocks aged between 1 and 7 weeks. Clinical signs include loose droppings and increased mortality.

Grossly, the small intestines have pale serosa, segmental dilation, and watery contents. The incidence of cases diagnosed as poult enteritis since 1992, and the viruses associated with them, will be examined. Viruses that have been detected include rotaviruses, small round viruses ranging in size from 18 to 30 nm in diameter, reoviruses, adenovirus and birnavirus.

DETECTION OF NATURALLY OCCURRING TURKEY INTESTINAL CORONAVIRUS (TCV) IN TURKEY FECES USING RT-PCR

Laura A. Kelley, Tom P. Brown, Doris H. D'Souza, AnaPatricia Garcia, and Saad Gharaibeh

Samples of feces were obtained from turkeys with experimentally produced turkey intestinal coronavirus (TCV) infection (positive control), naturally occurring spiking mortality of turkeys/poult enteritis mortality syndrome, or no TCV infection (negative control). Samples were diluted with PBS, passed through a 0.2 micron filter, and the RNA extracted. These nucleic acid extracts were analyzed using reverse transcriptase-polymerase chain reaction primers designed to detect a gene region coding for S1 surface glycoprotein of TCV.

ANALYSIS OF RT-PCR PRODUCTS OBTAINED USING S1 PRIMERS FOR THE DETECTION AND COMPARISON OF TURKEY INTESTINAL CORONAVIRUS (TCV) ISOLATES

Doris H. D'Souza, Tom P. Brown, Laura A. Kelley, Saad Gharaibeh, and AnaPatricia Garcia

Turkey intestinal coronavirus isolates were propagated in HRT-18 cell cultures, their RNA extracted, and the region coding for turkey intestinal coronavirus (TCV) S1 surface glycoprotein was sequenced. Primers specific for the S1 sequences conserved among coronaviral isolates were constructed, and these yielded reverse transcriptase polymerase chain reaction (RT–PCR) products that were separated by electrophoresis in 1% agarose gels. These products were examined by sequencing and restriction mapping to determine similarities or differences between the turkey intestinal coronavirus isolates and bovine enteric coronavirus isolates. Sequencing of the RT–PCR products was carried out in both directions with the S1 primers that were used to obtain the RT–PCR products.

IMMUNOHISTOCHEMICAL AND POLYMERASE CHAIN REACTION (PCR) PROCEDURES FOR DIAGNOSIS OF TURKEY CORONAVIRUS INFECTIONS

J. Guy, L. Smith, H.J. Barnes, and J. Breslin

A monoclonal antibody (4.24) specific for turkey coronavirus (TCV) was produced for immunohistochemical detection of TCV in infected turkeys. Specificity of 4.24 was verified by indirect immunofluorescence; 4.24 reacted strongly against 5 epidemiologically distinct TCV strains, but not against other viruses. A Mab-based immunoperoxidase procedure detected TCV antigens in experimentally infected turkeys on days 1 to 13 after inoculation; no staining was observed in sham–inoculated turkeys. A polymerase chain reaction (PCR) procedure was developed for detection of TCV in dropping samples. The

procedure utilized primers developed for PCR amplification of infectious bronchitis virus RNA. Sensitivity and specificity of the PCR procedure is being assessed.

DETECTION OF INTESTINAL CORONAVIRUS RNA IN TURKEYS USING DNA IN SITU HYBRIDIZATION

AnaPatricia Garcia, Tom P. Brown, Doris H. D'Souza, Laura A. Kelley, and Saad Gharaibeh

Intestines, thymuses, and bursae from turkeys experimentally infected with a turkey coronavirus isolated from cattle were processed for in situ hybridization, using a digoxigenin–labeled DNA probe complementary to a conserved region of group II-coronavirus RNA. Coronavirus nucleic acid was detected in formalin–fixed tissues. Results were compared with histopathologic findings, and virus presence was confirmed using electron microscopy. This technique is useful for detection of turkey intestinal coronavirus infection when fresh tissues are not available.

PRODUCTION OF MOUSE MONOCLONAL ANTIBODIES REACTIVE FOR TURKEY INTESTINAL CORONAVIRUS (TCV) ISOLATES AND THEIR USE IN TCV DETECTION

Tom P. Brown, Doris H. D'Souza, Laura A. Kelley, Saad Gharaibeh, and AnaPatricia Garcia

Turkey intestinal coronavirus (TCV) isolates were propagated in HRT-18 cell cultures, purified by sucrose gradient centrifugation, recultured and concentrated by centrifugation. Whole virus was used to immunize mice, fusions performed and resulting clones were screened for anti-TCV reactivity, using an indirect fluorescent antibody test in 96–well plates. Reactive clones were expanded and used to detect TCV in cell culture and histologic sections. Clones have been stored and are available for use in TCV detection.

IMMUNOHISTOCHEMICAL LOCALIZATION OF TURKEY INTESTINAL CORONAVIRUS (TCV) IN HISTOLOGIC SECTIONS FROM EXPERIMENTALLY INFECTED TURKEY POULTS

Saad Gharaibeh, Tom P. Brown, Laura A. Kelley, Doris H. D'Souza, and AnaPatricia Garcia

Four-day–old turkey poults were orally administered either cell culture origin turkey intestinal coronavirus (TCV) UGA-APN, intestinal homogenate containing TCV from naturally infected poults, or no inoculum. Poults were killed on days 4, 5, and 6 after inoculation, and tissue samples were obtained and fixed in 10% neutral-buffered formalin for 6 hours. Turkey intestinal coronavirus infected HRT-19 cells and noninfected HRT-18 cells were formalin fixed, pelleted and served as positive and negative controls, respectively. All samples were embedded in paraffin, sectioned and stained for TCV antigens, using peroxidase-antiperoxidase techniques. A CSA system (DAK) was used to increase antigen detection sensitivity.

AN ASSESSMENT RELATING PEST DENSITIES WITH PEMS AND TURKEY CORONAVIRUS OUTBREAKS IN NORTH CAROLINA TURKEY FLOCKS

S. Michael Stringham, Donna K. Carver, Karen Burns, Miles Hall, Jean-Pierre Vaillancourt, H. John Barnes, and Carri Williams

Fly (*Musca domestica*), lesser mealworm (*Alphitobius diaperinus*), and rodent populations were monitored in turkey breeder houses during the summer of 1997. Turkey breeder houses on farms located in the major production regions of North Carolina were randomly selected for the study. Fly and lesser mealworm populations were monitored weekly. Rodent populations were measured once at the beginning of the study and again once turkeys were moved from breeder houses. Pest density was compared with the disease status of each farm to determine relationships between the severity of pest infection and the occurrence of poult enteritis mortality syndrome and turkey coronavirus. [No significant association was found between fly or lesser mealworm densities and poult enteritis and mortality syndrome (PEMS) or turkey coronavirus. Data was not sufficient to assess associations between rodent densities and disease status.]

DESCRIPTIVE EPIDEMIOLOGY OF CORONAVIRUS IN COMMERCIAL TURKEYS IN NORTH CAROLINA

Donna K. Carver, Jean-Pierre Vaillancourt, and S. Michael Stringham

A prospective epidemiology study was conducted to study field cases of turkey coronavirus (TCV) in North Carolina. Fifty-two turkey farms were monitored and the serologic status for TCV was assessed at 6, 13, and 20 weeks of age. Data are to be analyzed using univariate methods, and the descriptive epidemiologic traits of TCV will be discussed including mortality patterns, flock performance and management schemes.

THE USE OF CONTROLLED EXPOSURE OF TURKEY CORONAVIRUS TO CONTROL MORTALITY ASSOCIATED WITH PEMS

Dennis P. Wages, James S. Guy and H. John Barnes

Poult enteritis mortality syndrome (PEMS) is characterized by high mortality, immunosuppression, enteritis, and moderate to severe stunting. A variety of viruses and secondary bacterial and protozoal agents have been incriminated in contributing to mortality. Turkey coronavirus can be a significant component in the severity of PEMS. An embryo–propagated turkey coronavirus was used in a controlled exposure experiment to reduce mortality in a coronavirus–positive model of PEMS. Turkey poults were inoculated orally at 1 day of age with 0.1ml of an embryo–propagated turkey coronavirus. Mortality was reduced in the coronavirus-controlled exposed group compared with unvaccinated controls from 52.7 to 22.2%. Another study using embryo–propagated turkey coronavirus given orally at 1 day of age also reduced mortality compared with unvaccinated controls, from 58 to 11%. Even though reduction in mortality resulted from a controlled exposure to turkey coronavirus

at 1 day of age, the mortality may still be unacceptable from a commercial industry standpoint. It does, however, demonstrate that early exposure to turkey coronavirus does induce some degree of resistance to coronavirus–positive PEMS infection.

METHODS FOR PREVENTION AND CONTROL OF TURKEY STUNTING SYNDROME

Don Reynolds, Sevine Akine, Ali Akbar, and Joan Oesper

Stunting syndrome is an enteric disease of young turkey poults that results in impaired feed efficiency, decreased weight gains and stunted growth. The agent that causes this disease has been termed the stunting syndrome agent (SSA). To determine if passive immunity would be of benefit in preventing stunting syndrome, antibodies were purified and concentrated from adult turkeys that had been hyperimmunized with SSA. Poults were injected with either anti-SSA antibody preparations or sterile phosphate buffered saline solution and then exposed to SSA. Birds receiving SSA–antibody preparations had higher weight gains than did those birds that received no SSA antibodies.

BIOSECURITY IN PEMS–AFFECTED REGIONS: MEASURES TAKEN AND COMPLIANCE

Jean-Pierre Vaillancourt, Donna K. Carver, S. Michael Stringham, Carri Williams, Karen Burns, and Miles Hall

A prospective study was conducted to monitor biosecurity measures put in place by turkey companies in North Carolina. A registration station was set up on 15 farms to collect information about visitors. Validation was performed by collecting used plastic boots left on the farm. A hidden camera was also used to monitor 4 farms for five 24–hour periods. Finally, a survey was conducted on 52 farms. Results indicated that basic biosecurity procedures (e.g., putting on coveralls, using hand sanitizer or gloves, washing cars/trucks, etc.) are still not implemented on many farms. Validation data also show a lack of compliance by visitors, including company employees.

SUSCEPTIBILITY OF CHICKENS TO POULT ENTERITIS AND MORTALITY SYNDROME

H. John Barnes, James S. Guy, J. Todd Weaver, and Jean-Pierre Vaillancourt

Clinical disease did not develop when chickens were orally inoculated with infective droppings or commingled with infected turkeys; however, antibodies to turkey coronavirus (TCV) were demonstrated. Turkeys contracted poult enteritis mortality syndrome (PEMS) when they were commingled with clinically normal chickens from the third successive blind passage following contact with infected poults. Exposed chickens developed antibodies to TCV and antigen was demonstrated in bursal epithelium. Antibodies to TCV in chickens could not be demonstrated because of cross-reaction with infectious bronchitis virus. Disease or evidence of TCV infection did not develop in sentinel and backpassaged turkeys following placement into 6 broiler

flocks in a PEMS endemic area. These findings indicate that chickens are susceptible to experimental infection with TCV and organisms that cause PEMS, but they remain asymptomatic, and PEMS is not a threat to commercial broiler flocks. Failure to demonstrate natural infection of broilers suggests they are unlikely to be a reservoir of the disease.

Flock Characteristics of Southern Indiana Turkey Coronaviral Enteritis Cases Diagnosed by Immunofluorescence

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First presented by Dr. Bryan at The 47th North Central Avian Disease Conference, in Columbus, Ohio, September 29 - October 1, 1996.

INTRODUCTION

Turkey coronaviral enteritis (TCE) was a devastating burden to the southern Indiana turkey industry during 1994. It is suspected to have been a major cause of summer mortality and poor wintertime feed conversion in previous years, based upon electron microscopy (EM).

Immunofluorescent diagnostic tests for TCV were begun in 1994 using methodology based upon early trials at University of Minnesota (Patel *et al.*, 1975, 1976, 1977) and turkey coronavirus antisera obtained from Dr. Saif.

In 1994, Porter et al. reported on a feeding trial comparing weights of inoculated vs. control poults as well as crumble vs. mash fed poults. Inocula consisted of filtered cecal content known to contain coronavirus. Weights at 21-days were markedly reduced after inoculation at 7 days of age. In 1995, a second trial was reported with similar results. In 1996, Lin et al. reported purifying the coronavirus from a diagnostic southern Indiana case, measuring its performance effect in SPF turkeys, and noting similarities of Minnesota and southern Indiana coronaviruses. Goodwin et al. reported association of production losses and presence of coronavirus using TEM.

ANALYSIS

Our objective is to share observations concerning flock performance of 1994 turkey flocks that were TCE–positive as determined by immunofluorescence. Distribution of

the TCE–positive flocks is listed in Table 1 along with a list of those flocks where revenue met or exceeded expenses.

Table 1. Immunofluorescent TCE positive flocks by month

		Total no. of flocks	*Economics were reported, no. of flocks*	*Revenue ≥ expense, no. of flocks (%)*
1994	Jan	0	0	NA
	Feb	0	0	NA
	Mar	1	1	1 (100%)
	Apr	0	0	NA
	May	1	1	0 (0%)
	Jun	10	9	3 (33%)
	Jul	18	8	1 (13%)
	Aug	29	14	6 (43%)
	Sep	19	11	4 (36%)
	Oct	15	2	1 (50%)
	Nov	4	0	NA
	Dec	7	2	2 (100%)
1995	Jan	0	0	NA
	Feb	1	0	NA

Of all turkey flocks diagnosed with TCE by immunofluorescence (105), partial records were available on 48, as listed in Table 2, as of September 1996.

Table 2. Summary characteristics, 48 of TCE–affected flocks

	*Male (31)**	*Female (17)*
Age range (TCE +)	6 to 126 days	9 to 101 days
Total mortality, range	7.88 to 66.77% (31)	3.63 to 40.10% (16) (1 flock @ 101 days)
Average mortality	27.18%	13.83%
Fed crumble/pellet	14.50% (17)	11.49% (12)
Fed mash/mash	35.54% (14)	38.97% (6)
Market age, range	118 to 143 days (20)	90 to 148 days (11)
Market weight, deviation from std	-7.43 to +0.14 lbs (20)	-4.61 to +1.12 lbs (11)
Feed conversion, deviation from std, by diet type		
Fed crumble/pellet	-0.104 (15)	-0.118 (12)
Fed mash/mash	-0.446 (14)	-0.258 (5)
Condemnation		
Range	1.24 to 4.70% (30)	0.47 to 2.04% (15)
Average	2.49%	1.13%
Revenue ≥ expense	11 (7 fed crumble)	7 (4 fed crumble)

* Parenthesis encloses the number of flocks per category.

The last question to be examined concerns the flocks where revenue met or exceeded expenses. The data are placed in Table 3.

Table 3. Summary of characteristics of "break even" flocks

	*Male (11)**	*Female (7)*
Age range (TCE +)	6 to 126 days	9 to 101 days
Total mortality, range	7.88 to 39.32%	3.63 to 29.4%
Average mortality	18.00%	14.57%
Market age, range	118 to 143 days (20)	95 to 148 days (11)
Feed conversion, deviation from std, by diet type		
Fed crumble/pellet	+0.025 (7)	+0.088 (4)
Fed mash/mash	-0.245 (4)	-0.147 (3)
Feed conversion , deviation from std		
Range	-0.41 to +0.14	-0.26 to +0.26
Average	-0.09	-0.02
Condemnation		
Range	1.24 to 4.60%	0.56 to 1.31%
Average	3.10%	1.17%

* Parenthesis encloses the number of flocks per category.

SUMMARY

TCE–positive flocks had increased mortality, decreased weight gain and poor feed conversion. Poor financial return of TCE–positive flocks indicates that TCE is a financially significant disease.

The data suggest that in future cases, use of a crumble/pellet diet will improve livability of infected birds. The case for medication was purposefully avoided, as it often becomes bogged down in discussion of secondary or concomitant infection causes. Nor were we able to make an 'airtight' case for continued TCE monitoring, regardless of mortality. It would be hoped that future tests would be simple and inexpensive enough to encourage routine monitoring.

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Identification of Turkey Coronavirus in Commercial Turkey Flocks

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Presented at the 47th Western Poultry Disease Conference
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Turkey coronavirus (TCV) was identified in field samples collected from turkeys in six states in the midwestern and southeastern United States, including Arkansas, Indiana, Minnesota, North Carolina, South Carolina and Virginia. Diagnostic procedures that were used to identify TCV infection included fluorescent antibody (FA) techniques to detect viral antigen or serum antibodies, virus isolation and electron microscopy.

FA staining of frozen tissue sections detected TCV antigens in epithelial cells of the intestinal mucosa and bursa of Fabricius of infected turkeys. FA staining employed FITC-conjugated polyclonal antibodies in a direct FA procedure, or unconjugated polyclonal or monoclonal antibodies in an indirect FA procedure. Viral antigens were detectable in experimentally infected turkeys for up to 14 days postexposure (PE).

TCV-specific antibodies were detected in convalescent serum using indirect FA procedures. Antigen–laden slides were prepared by two different procedures. Antigen slides were prepared using frozen sections of turkey embryo intestines collected 24-48 hours after embryo inoculation with embryo-adapted strains of TCV. Alternatively, antigen slides were prepared using exfoliated epithelial cells collected from bursae of Fabricius of 4-week-old turkeys, 4 days after TCV inoculation. Bursae were harvested from infected turkeys and rinsed in cell culture media. Bursae were incubated at 4°C for 24 hours with gentle stirring to exfoliate epithelial cells; cells then were spotted onto glass slides. TCV-specific antibodies could be detected in turkeys within 10-14 days PE, and turkeys infected early in the brooder house remained serologically positive throughout the growout period.

TCV was isolated from intestines of infected turkeys by inoculation of 22– to 24–day–old embryonated turkey eggs by the amniotic route. Embryo intestines were harvested 3 days PE and FA identified the virus in frozen sections. Electron microscopy was utilized in selected cases to identify coronavirus particles in intestinal contents and droppings; however, the sensitivity and specificity of this procedure compared with other diagnostic procedures was considered to be poor.

Serology was considered to be the fastest, least expensive and most sensitive of the diagnostic procedures employed to detect TCV infection.

Turkey Coronavirus (TCV): Cleanup and Prevention of TCV Enteritis

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This article was presented at the 21st Annual North Carolina Turkey Industry Days Conference, October 22 - 23, 1998. Raleigh, NC.

Prevention and eventual eradication are possible. It has been done in Minnesota and other Midwest areas, including Indiana. Regardless of the money, effort and time it takes, it is the best and most economical approach to the problem, both for individual farms and the turkey industry. Four weak links in the chain of this disease help in prevention and eradication.

1. Turkeys appear to be the only ready host and are possibly the only host for the disease.
2. The virus is present only in the intestine and feces.
3. It is not egg-transmitted.
4. It does not survive well outside the host.

Prevention means stopping turkey coronavirus (TCV) from being ingested by susceptible turkeys. Any movement of fecal material from infected flocks and farms must be controlled (biosecurity).

The most important control is when infected flocks are moved to market. Prior to moving, the coronaviral status of the flock should be determined. If the flock is infected, virus is shed for the life of the birds, and especially with the stress of feed withdrawal, loading, hauling, etc. Fluorescent antibody testing or sentinel turkeys can be used to test flocks of unknown status. Because antibodies do not develop for approximately 14 days after infection, prior activity on a farm that is found to have a serologically positive flock needs to be examined. Tremendous amounts of virus may have been shed and may have been transmitted to other flocks and farms during that time. As soon as it is determined that a flock is infected, it should be placed under quarantine, and minimum contact with those birds and their feces should be maintained. Essential contact should be controlled, using a separate caretaker if possible. At the very least, a

separate pair of boots and coveralls should stay in the infected house and be worn while working with that flock. That flock should also be serviced last. Infected flocks should be moved to market under controlled conditions as soon as feasible, on a weekend if possible. Trucks carrying infected turkeys spread fecal material and contaminated feathers onto driveways, roads and parking areas in route and at the processing plant. Such material can then be inadvertently carried to susceptible turkeys by footwear, vehicles, etc.

All equipment, clothing, etc. used in catching, loading and hauling TCV–infected turkeys should be isolated, then thoroughly cleaned and disinfected before being used on another farm.

Farms raising turkeys of different ages have the most difficult problem with prevention, but it can and has been done. It is well worth the effort and expense. Each day without the virus gives the poult more resistance against this disease. Growers using good biosecurity in Minnesota could prevent the disease in brooder houses that were separate from grow-out houses. Poults usually stayed in brooder houses until eight weeks of age. With appropriate flock planning, this gave growers at least 30 days to clean up after marketing. Minnesota growers usually could not rid their growout barns of coronavirus after freezing temperatures set in.

After removal of all infected turkeys from the farm (including culls and any other turkeys), the house and outside area contaminated with fecal material during growout and at load out should be quarantined. Leaving the area undisturbed for two weeks (if possible) or even only three to four days is helpful in ridding the area of virus. Adding heat will help to dry any fecal material and kill the virus.

Effective prevention includes preventing unanticipated and "bio-lax" personnel from entering the farm and especially the turkey barns. If possible, doors and entrances should be kept locked.

Effective cleanup is when all coronavirus is either killed or removed from the farm and the potential for its return has been minimized. No infected litter piles may be close by. No fecal-contaminated equipment, etc., may be returned to the farm.

MAJOR CONSIDERATION IN CLEANUP AND PREVENTION INCLUDE:

1. All droppings from all infected turkeys contain the virus 24 - 48 hours before they show signs of the disease. All flocks with suspected exposure should be considered infected.
2. Turkeys, once infected, are lifelong shedders, even though they appear to have recovered. Turkeys can also be infected and never show signs of the disease.
3. TCV–infected feces can be mechanically carried and deposited in a place protected from cleaning and disinfection, or in a place suited for viral preservation (moist and cool) and then carried to or encountered by susceptible turkeys on or off the farm, including those restocked into the house. Personnel (including growers, servicemen, repairmen, live haul crews and visitors) are the most likely carriers on FEET, hands and clothing, particularly at loadout. When the birds are removed from the house,

truck–loading areas, roads, mud puddles and other areas outside the house may become contaminated, and then fecal material can be tracked away from the house or area before or during cleaning or back into the cleaned house. Any equipment contaminated with fecal material can spread the agent. Live haul equipment, including the loaders, are grossly contaminated. In addition, rodents, wild birds, flies and other agents can carry contaminated fecal material and spread the disease.

4. Heat and drying destroy the virus. Cool, moist conditions preserve it. Freezing preserves it indefinitely.
5. Disinfectants approved by the USDA for avian influenza probably destroy the unprotected coronavirus. However, most TCV is protected by fecal material, which tends to inactivate disinfectants and also presents a physical barrier between the virus and the disinfectant. Most disinfectants take 10-20 minutes contact time to destroy organisms. Footbaths and other surfaces, especially those being inactivated by large amounts of organic matter and items with recently applied disinfectant, may still be infective.
6. No host other than the turkey is known. TCV does not cause disease in chickens, pheasants, quail, seagulls, hamsters, or swine. TCV was not recovered from the intestines of any of these species up to three days after oral inoculation. The majority of current evidence does not indicate that removal of other species is necessary for good cleanup and prevention.

Effect of Turkey Coronavirus Infection on Commercial Turkey Flock Performance

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ABSTRACT

Turkey flocks produced by a southeastern US integrator are being monitored by indirect fluorescent antibody test for exposure to turkey coronavirus (TCV). Performance parameters such as daily gain, feed conversion, body weight, and livability are being compared for TCV–positive and –negative flocks. Results from 1997 indicate a 3.86% advantage in livability and a 0.21 feed conversion advantage for TCV–negative light hens. TCV–negative heavy toms showed a 3.35 lb weight advantage. TCV–positive hens and toms cost $0.02 more per lb to produce.

DISCUSSIONS

A serological monitoring program utilizing the indirect fluorescent antibody test (IFAT) began in the fall of 1996. The program calls for tom flocks to be tested at 10 and 18 weeks of age and hens to be tested at 8 and about 13 weeks. All flocks have not been tested exactly according to schedule, but the program has been followed as closely as possible. Over 800 flocks have been tested to date.

Flocks placed in the summer and early fall are more likely to become positive for TCV. Diagram 1 shows number of positive/negative serology tests by month of flock placement.

Diagram 1. Serology results by month of flock placement

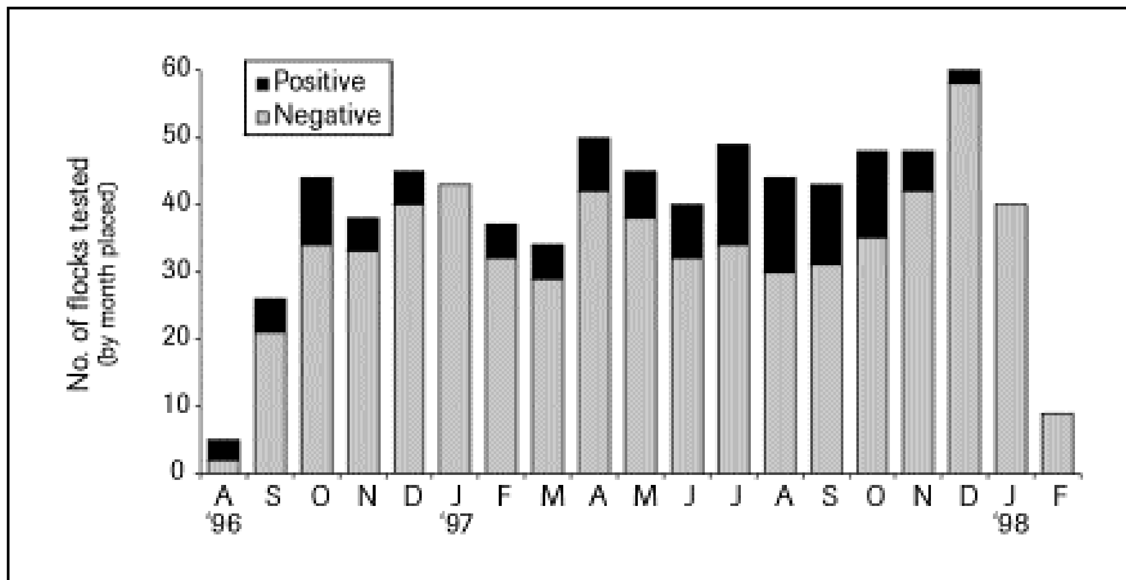
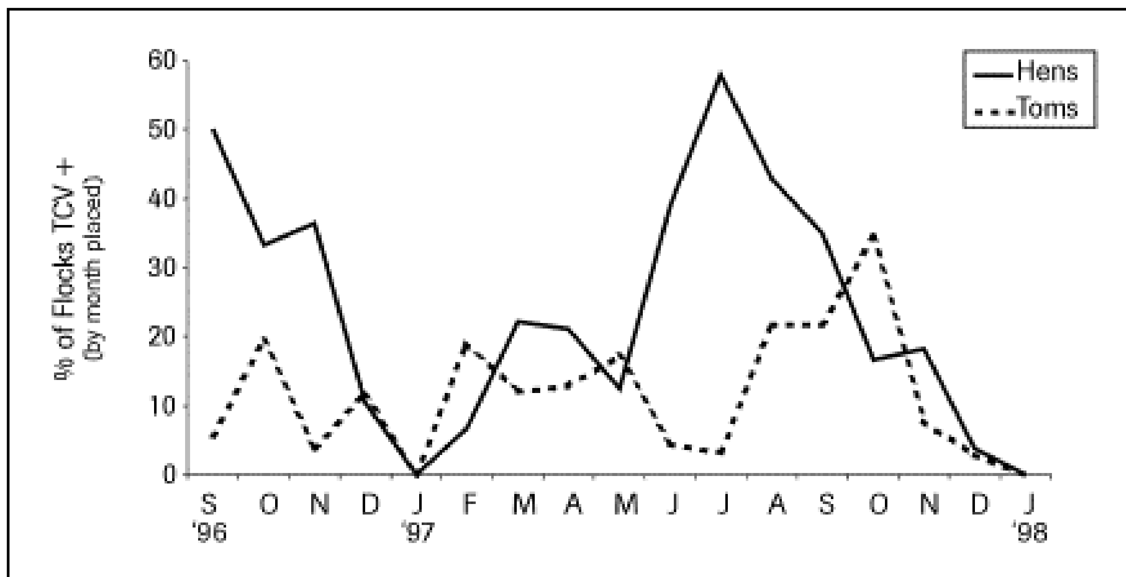


Diagram 2 represents TCV–positive flocks separated by sex. Results are indicated for the flock by the month the flock was placed, not by the month a positive diagnosis was made. It would appear that hens seroconverted earlier in the calendar year than toms. However, this is more of a location (geographic) effect than a sex effect. A geographic region having mostly hen farms went serologically positive first. A different geographical area containing mostly tom farms turned positive later in the year.

Diagram 2. Serology results by month of flock placement, by sex



EFFECT OF TCV ON FLOCK PERFORMANCE

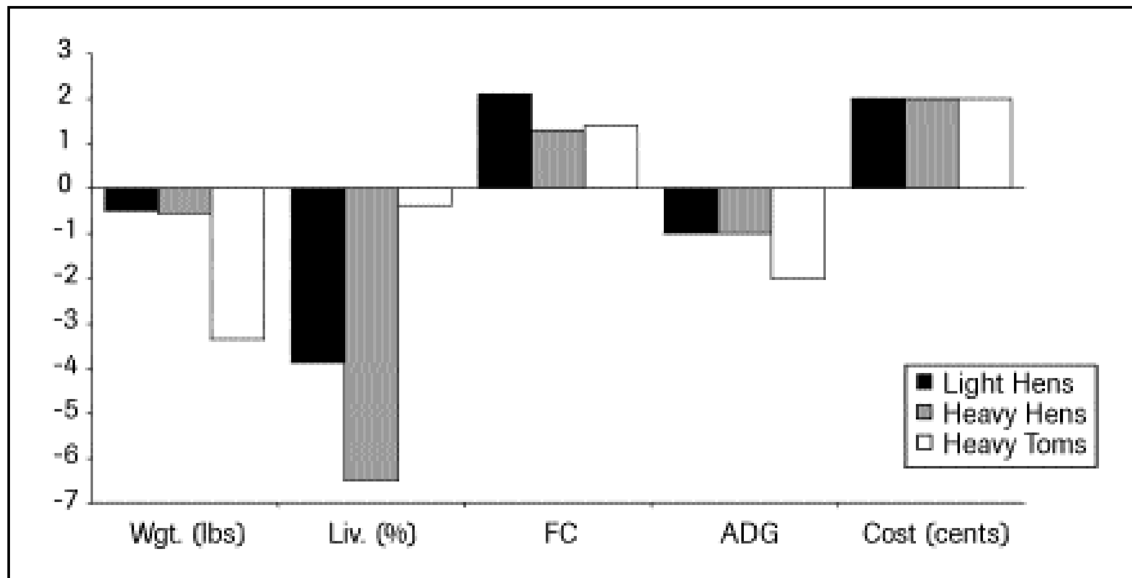
Three categories of turkeys are produced, namely, consumer–size (light) hens (14.0 pounds) marketed at 14 weeks of age, heavy hens (22.0 pounds) marketed at 18 weeks of age and heavy toms (34.0 pounds) marketed at 19 weeks of age. This discussion focuses on the effects of TCV weight (pounds), livability (%), feed conversion (FC, pounds of feed per pound of meat gain), average daily gain (ADG, pounds per day) and cost (dollars).

TCV causes poor flock performance (Table 1). With the heavy hen flock, the large difference in livability is due to one flock with multiple problems. Diagram 3 summarizes the information presented in Table 1. The "0" line equals the average value for each production parameter for negative flocks in each bird category.

Table 1. TCV–positive flock performance, deviation from the company TCV negative flock average

Bird Category	*Light Hens*	*Heavy Hens*	*Heavy Toms*
Target Weight (lbs)	14.0	22.0	34.0
Weight (lbs)	-0.50	-0.56	-3.35
Livability (%)	-3.86	-6.48	-0.37
Feed Conversion	+0.21	+0.13	+0.14
ADG (lbs)	-0.01	-0.01	-0.02
Cost ($)	+0.02	+0.02	+0.02

Diagram 3. TCV–positive flock performance, deviation from the company TCV–negative flock average



EFFECT OF TIME OF SEROCONVERSION TO TCV ON FLOCK PERFORMANCE

If hen flocks seroconverted to TCV early in life (+ at 8 weeks/+ at 13 weeks), compared to a later age (-/+), the effect of TCV on performance is more severe. This is exemplified in Table 2, in which hen flocks TCV–positive at the scheduled 8 weeks of age testing (+/+) are compared to those flocks seroconverting only at the 13 weeks of age testing (-/+). Final flock performance of those light hens seropositive at the first blood sampling (+/+) is $0.023 above cost and 0.78 pounds below average results for negative flocks. But those light hens negative at the first sampling and positive at 13–week testing (-/+) were only $0.004 above cost and 0.47 pounds above weight. Late seroconversion did not depress weight gain for these light hens. Similar results are reported for the heavy hens, but body weights were depressed 0.44 and 1.08 pounds at both the 8– and 13–week sampling, respectively.

Table 2. Effect of time of TCV IFAT seroconversion on hen performance, variation from the company average

Bird Category	*Light Hens*		*Heavy Hens*	
TCV Serology Result at 8/13 Weeks of Age	+/+	-/+	+/+	-/+
Weight (lbs)	-0.78	+0.47	-0.44	-1.08
Livability (%)	-5.11	-0.92	-13.51	-7.00
Feed Conversion	+0.22	+0.06	+0.27	+0.16
ADG (lbs)	-0.013	-0.001	-0.011	-0.005
Cost ($)	+0.023	+0.004	+0.039	+0.024

Body weights were monitored for selected light hen flocks at 8 and 13 weeks of age. Growth rates (Diagram 4) of flocks seroconverting between 8 and 13 weeks (-/+) are very similar to those of negative (-/-) flocks. Flocks seroconverting prior to 8 weeks of age (+/+) grow more slowly during that first 8 weeks and their growth rate for the next 6 weeks is similar to that of TCV–negative flocks. Table 3 compares flock performance from 0 to 8 and from 8 to 13 for light hens that were TCV–positive versus TCV–negative. Note that the difference in average body weight did not change between the early (0 to 8 weeks) and late (8 to 13 weeks) periods.

Diagram 4. Growth rates of light hens seroconverting to TCV at either 8 (+/+) or 13 (-/+) weeks of age versus TCV negative hens

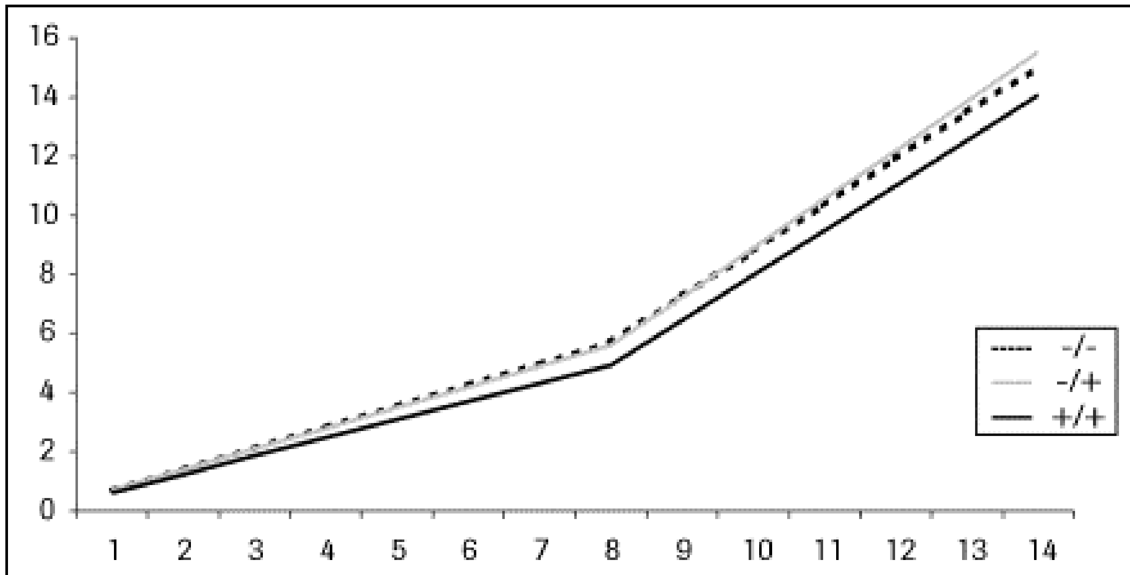


Table 3. Performance of light hens seroconverting to TCV at either 8 or 13 weeks of age, deviation from the company TCV negative average

Grow Period (weeks of age)	*0 to 8*	*8 to 13*
Weight (lbs)	-0.77	-0.78
ADG (lbs)	-0.014	-0.005

When heavy tom flocks seroconvert to TCV early in life (+/+) the effect of TCV on performance is more severe than if the flock seroconverted later (-/+). Tom flocks TCV–positive at the scheduled 10 weeks of age testing (+/+) and those flocks seroconverting at the 18 weeks of age testing (-/+) are compared to TCV–negative flocks (Table 4). Final flock performance of heavy tom flocks seroconverting early (+/+) were 6.74 pounds below weight and $0.03 above cost. Livability was not adversely affected. Flocks converting later (-/+) were only 2.10 pounds below weight and $0.01 above cost.

Table 4. Effect of time of TCV IFAT seroconversion on heavy tom performance, variation from the company average

TCV Serology Result at 10/18 Weeks of Age	+/+	-/+
Weight (lbs)	-6.74	-2.10
Livability (%)	+0.26	-0.66
Feed Conversion	+0.21	+0.12
ADG (lbs)	-0.034	-0.017
Cost ($)	+0.03	+0.01

EFFECT OF TCV EXPOSURE ON INDIVIDUAL FARM PERFORMANCE

Successive flock performance on TCV–positive farms progressively worsen. These farms were TCV–negative for the first flock, and subsequent flocks were positive. The "0" line represents average values for seronegative flocks. Diagrams 5 and 6 depict a light hen farm and a heavy hen farm, respectively. The first flock (Flock 1) was TCV–negative and finished with below average weight and feed conversion. For both hen farms, flocks 2 and 3 were TCV–positive and performance was progressively worse. Diagram 7 depicts results for three successive flocks on a heavy tom farm. Flock 1 was TCV–negative and finished with average feed conversion and cost. Flocks 2 and 3 were positive and performance was progressively worse on each flock. For all flocks, TCV effects on livability were variable.

Diagram 5. Effect of TCV exposure on successive flocks' performance from an individual light hen farm

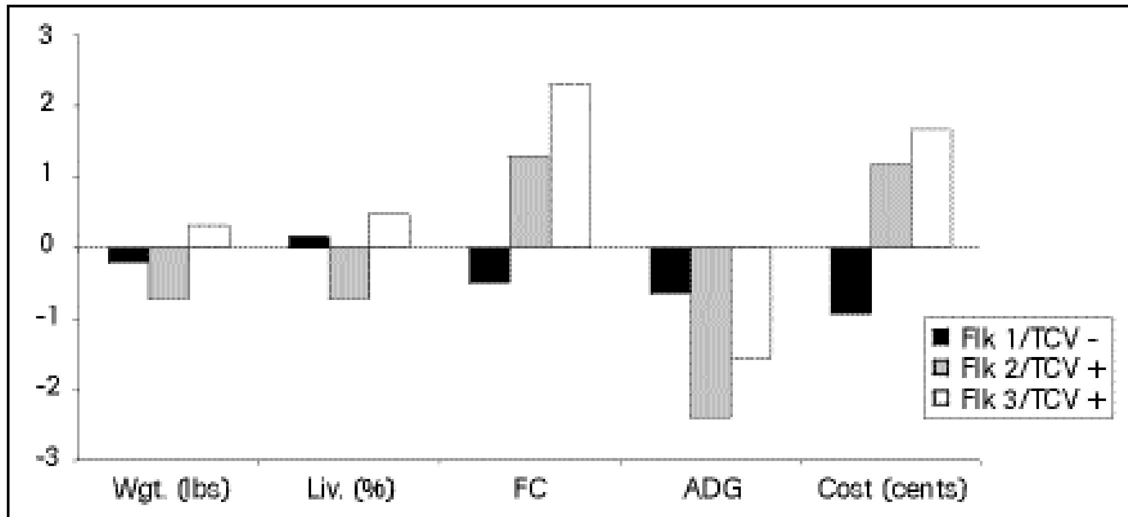


Diagram 6. Effect of TCV exposure on successive flocks' performance from an individual heavy hen farm

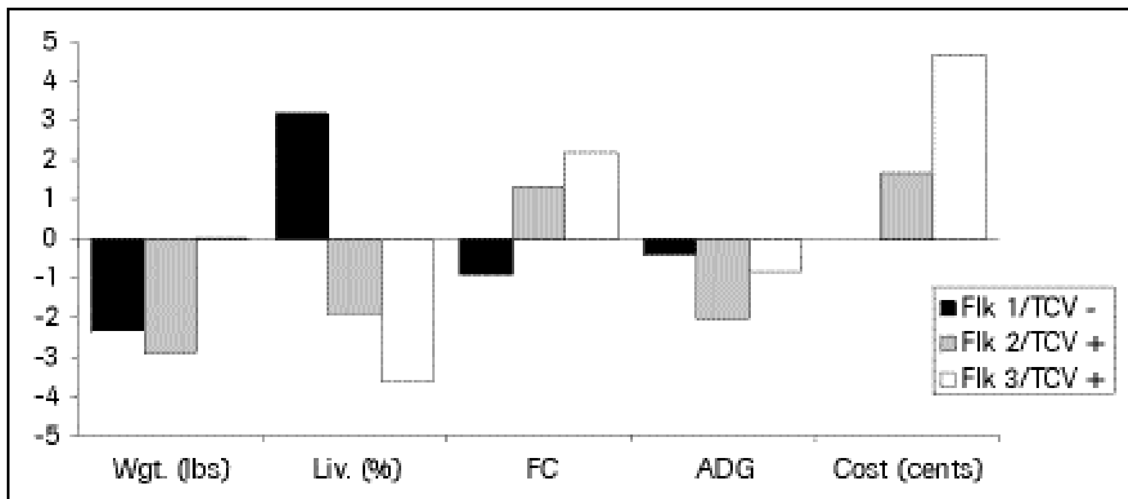
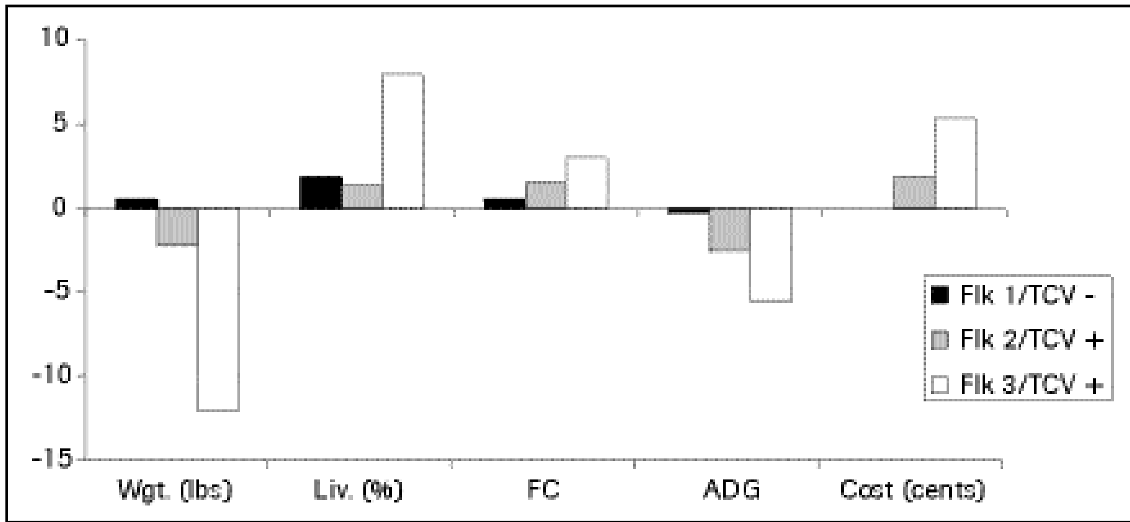
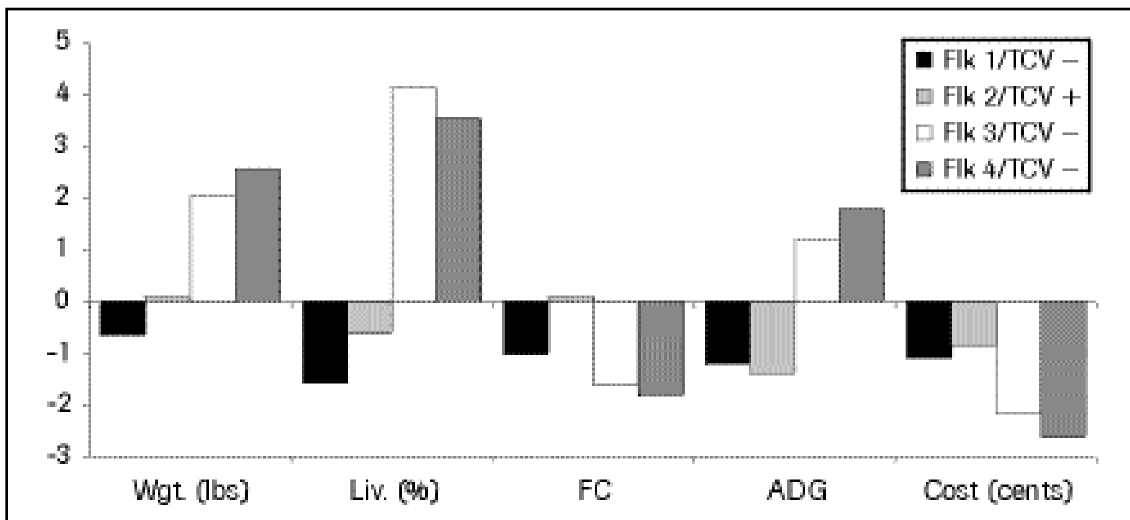


Diagram 7. Effect of TCV exposure on successive flocks' performance from an individual heavy tom farm



Knowing that successive flock performance on TCV–positive farms progressively worsens, it is noted that depopulation dramatically improves performance. Diagram 8 depicts results for four successive flocks on a light hen farm. Flock 1 was TCV–negative and finished with below average feed conversion and cost. Flock 2 was TCV–positive, but performance was still better than average. The decision was made to depopulate the farm after Flock 2. Performance improved dramatically for Flocks 3 and 4.

Diagram 8. Effect of TCV exposure and depopulation on successive flocks' performance from an individual light hen farm



SUMMARY

- Incidence of TCV is seasonal
- Coronavirus costs +$0.02 per pound of meat
- Light hens are most severely affected
- Depopulation can dramatically improve performance of TCV–positive farms

TECHNICAL SUPPLEMENTS

Literature Review: Coronaviral Enteritis of Turkeys and Immunity

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This article was condensed from a research proposal presented to the PEMS Task Force on June 12, 1998.

ABSTRACT

Turkey poult enteritis has contributed to significant economic losses in the turkey industry for the last several years. Turkey poult enteritis, apparently due to turkey coronaviral infection, causes severe diarrhea, decreased weight gain, uneven flock growth, or mortality. In order to effectively control turkey poult enteritis, understanding the immunological responses associated with the interaction between turkey coronavirus (TCV) and turkey poults is crucial.

Turkey flocks recovering from naturally or experimentally induced coronaviral enteritis may develop long term immunity; however, little is known about the specific humoral and cellular immune responses of turkey poults infected with TCV and the roles of turkey coronaviral proteins in eliciting the humoral immune responses. Success in this attempt will provide useful information for diagnosis, prevention, and control of turkey coronaviral enteritis.

CURRENT RESEARCH

Turkey poult enteritis had been successfully reproduced by oral inoculation with sucrose gradient purified TCV or 10% intestinal homogenate from the affected turkey poults. Clinical signs caused by sucrose density gradient purified TCV were similar to those caused by intestinal homogenate. Immunofluorescence assays were positive in the intestines of infected groups. Both groups of turkey poults infected with purified TCV or intestinal homogenate had significantly decreased body weight gain, $P<0.05$ and $P<0.01$, respectively, as compared with the control group. Histopathological changes revealed villous atrophy, particularly at 2 to 5 days PI.

A monoclonal antibody (Mab) was generated using sucrose density gradient purified TCV as inoculum and was applied to the development of antigen-captured ELISA for TCV. The performance of this assay was evaluated with a panel of fecal samples from 47 infected and 34 normal turkey embryos. Sensitivity and specificity of the assay were 60% (28/47) and 85% (29/34), respectively.

LITERATURE REVIEW

An enteric disease of young turkey poults, turkey poult enteritis (also referred to as spiking mortality of turkey poults), has contributed to significant economic losses in the turkey industry for the last several years. As an example of the magnitude of losses due to this disease, the estimated cost (mortality, decreased feed efficiency, stunting and medication cost) in DuBois County, Indiana, alone in 1994 was $19.26 million. A similar disease (poult enteritis and mortality syndrome) has also been identified in eastern and western North Carolina, South Carolina, Georgia, Virginia, and New York. Outbreaks of this disease have resulted in major economic hardships for turkey farmers in North Carolina in 1995, 1996 and 1997.

The clinical signs of turkey poult enteritis usually appear at seven to twenty–eight days of age and have inappetence, wet droppings, ruffled feathers, decreased weight gain and uneven flock growth. The morbidity is usually high and the mortality varies. The intestinal contents analyzed by electron microscopy often revealed turkey coronavirus (TCV). The clinical signs of turkey poults with enteritis and the continuing finding of coronavirus are very similar to the bluecomb disease of turkey (turkey coronaviral enteritis) encountered in Minnesota 20 to 30 years ago.[10] Once the coronaviral enteritis is introduced into areas with high concentrations of turkeys on a year-round basis, it is not easily eliminated and is encountered frequently in turkey poults.

Coronaviruses are enveloped, positive-stranded RNA viruses that infect a wide range of mammalian and avian species. The physicochemical and morphological characteristics of TCV have been shown to be shared by other members of the coronavirus group.[13] The size of coronaviruses vary, ranging from 50 to 150 nm. Coronaviruses have characteristic petal- or pear-shaped surface projections, giving them a morphologic appearance of a solar corona[6,13]. The structural proteins of coronaviruses can be grouped into three major size classes: a predominant phosphorylated nucleocapsid (N) protein with molecular weight (Mr) ranging from 45K to 60K and two major virus–encoded envelope proteins. A peplomeric glycoprotein (spike protein, S), with Mr of 170K to 200K, that makes up the large surface projections of the virion and a transmembrane glycoprotein (matrix protein, M) with an approximate Mr of 20K to 30K. A subset of coronaviruses, TCV, bovine coronavirus (BCV), human respiratory coronavirus, etc., possess a third glycoprotein, the hemagglutinin (HE), on the virion surface.[3,13]

Different isolates of TCV are considered to be antigenically identical or closely related.[4,11] There is no cross reactivity between the prototype TCV-Minnesota strain and coronavirus from other species by immunoelectron microscopy.[12] However, immunoblotting and immunoprecipitation studies using polyclonal antisera showed that the four major structural proteins of TCV crossreacted with the four homologous proteins of bovine

coronavirus (BCV), the nucleocapsid (N) and matrix (M) proteins of mouse hepatitis virus (MHV) serotype 3, and the N protein of avian infectious bronchitis virus (IBV). Bovine coronavirus cDNA probes tested on purified coronaviral preparations and coronavirus-positive fecal samples from turkey poults with diarrhea further confirmed the relatedness of TCV and BCV.[5,15] Nevertheless, the TCV-Minnesota strain could be distinguished from BCV isolates by monoclonal antibodies directed to epitopes of antigenic domain C of spike (S) glycoprotein.[8]

The S protein of many coronaviruses is cleaved into two 90K subunits, S1 and S2. Several epitopes on S1 or S2 of murine hepatitis virus (MHV) can induce neutralizing antibody. An important linear immunodominant domain is localized at residues 846 to 858 within S2 subunit. A domain composed of 330 amino acids at the N terminus of S1 subunit is responsible for both binding receptor and inducing neutralizing antibody. Moreover, two hypervariable regions have been identified at the amino terminus of infectious bronchitis virus (IBV) S protein. The hypervariable region 1 localized around residues 38 to 51 is found to be a neutralizing epitope.[2] The S1 monomeric protein of IBV and the beta-propiolactone inactivated IBV could induce virus neutralization (VN) and hemagglutination inhibition (HI) titer of serum antibody in chicken, but S1-removed IBV could not. Therefore, VN and HI antibodies are induced primarily by S1.[1] Additionally, the immunoaffinity purified S1, N and M proteins of IBV as immunogens induced antibody response, but only S1 protein induced VN titer and protection, as indicated by the absence of challenge virus in tissue. Thus, S protein of coronavirus may be important in inducing the humoral immune responses.

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Coronaviruses

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ABSTRACT

Coronaviruses are generally considered host–specific and cause disease in numerous species, including chickens, turkeys, cows, pigs and cats. Turkey coronavirus (TCV) is highly contagious. It causes severe enteritis with subsequent mortality and stunting in turkeys. Detecting either virus and/or antibodies make a diagnosis of TCV infection by fluorescent antibody tests. By matching the test results with the history of clinical signs and flock mortality, it is possible to estimate when the flock became exposed. This information can be helpful in identifying events that could be associated with how the virus may have gained entrance into the flock.

CORONAVIRUSES

- The virus:
    - Viral surface projections form a crown or wreath
    - Coronaviruses are enveloped RNA virus
        - Part of the envelope is from the host cells
        - Susceptible to organic solvents and detergents
    - Widespread viruses: affecting mammals and birds
    - Generally it is host–specific
    - Young animals are most susceptible
    - Biological vectors are not known
    - Infections of intestinal and respiratory tracts are most common
    - Transmitted by mechanical or fecal - oral or respiratory routes
- Infections in animals and humans:
    - Human coronaviruses: cause the common cold (2 major types)
    - Transmissible Gastroenteritis (TGE) of pigs: causes gastroenteritis
        - Dogs can be inapparent carriers

- Porcine HE (hemagglutinating encephalomyelitis) virus: also called vomiting and wasting disease, which is rare (though the virus is common)
- Porcine Respiratory Coronavirus (PRCV): causes upper respiratory disease
  - Cross reacts with TGE on standard serology tests (must differentiate with ELISA)
- Bovine coronavirus (BCV): causes gastroenteritis, also known as winter dysentery
- Feline Infectious Peritonitis (FIP) of cats: causes systemic chronic infection, also known as wasting disease
- Canine coronavirus: causes enteritis
- Mouse hepatitis virus: affects the liver and intestine, causing central nervous system (CNS) disease
- Also there are coronaviruses infecting rats and rabbits

- Infections in poultry:
  - Infectious bronchitis (IBV) in chickens: respiratory, kidney, reproductive diseases (over 15 types of coronavirus)
  - Transmissible coronaviral enteritis in turkeys: causes enteritis, also known as bluecomb or TCV
  - IBV and TCV are closely related antigentically
  - Reported relationship between bovine coronavirus (BCV) and TCV does not seem to be correct
  - TCV will infect chickens, but does not cause disease
  - IBV is not known to infect turkeys
    - But there are many types of IBV that have not been tested in turkeys
  - Serology tests, from turkeys and chickens, for IBV (a chicken–specific test) and TCV (a turkey–specific test) are often positive for both viruses
    - These tests cross react
  - It is highly likely that there are different types of TCV
    - Some types may not be detected by current diagnostic methods

TCV DIAGNOSIS: CLINICAL DISEASE

- Age resistance is limited just for the first few days
- TCV attacks mature enterocytes only
- Virus is shed in feces as early as 24 - 36 hrs postexposure (PE)
- Initial clinical signs occur between 36 and 48 hrs PE
  - Profuse, watery diarrhea
- Lack of uniformity is evident by day 3 PE
- Mortality increases on day 4 PE
- Peak mortality occurs on days 5, 6, and 7 PE
- Recovery is variable, with some birds remaining affected for long periods
- Reinfection can occur

- Virus shedding is limited to 6 - 12 weeks during cooler periods
    - But during hotter periods (summer) virus shedding probably occurs for the life of the flock
- Two cycles (approximately 6 days) probably are needed for disease to be noted in the flock
- TCV-like infection is noted in flocks from eastern North Carolina

TCV DIAGNOSIS: VIRUS DETECTION

- Two tests used
    - Direct fluorescent antibody (FA)
    - Virus isolation (confirmed by FA)
    - PCR tests under development
- Virus occurs in epithelial cells of intestine and bursa
- Virus is detectable 2 - 10 days PE; days 3 and 4 best
- Usually only 4 - 6 birds in a flock are tested
- Higher detection in sentinels when exposure time is known
- Low sensitivity (false negatives), high specificity (no false positives)
- Estimated time of infection, based on the FA test, is about 7 days earlier
- That is, seroconversion is 7 days PE

TCV DIAGNOSIS: SEROLOGY

- Test used: indirect fluorescent antibody test (IFAT)
    - Test requires considerable experience
    - It is cumbersome, expensive, limited
    - An ELISA test is under development
- First positive between 2 and 3 weeks PE
- Remains positive for life of flock
    - Breeders were positive for over a year
- Usually only 6 birds per flock are tested
- Processing samples considered highly accurate
    - Misses late breaks in a flock
- Best overall test to determine flock status
- There is a period when all tests (IFAT, direct FA, and virus isolation) are negative in an infected flock
- Interpretation of IFAT results to determine how many days prior to bleeding the birds were exposed to TCV:
    - If 1 - 2 out of 6 samples is positive (+): about 14 days
    - If 3 - 4 out of 6 samples are positive (+): about 21 days
    - If 5 - 6 of 6 samples are positive (+): 28 days or longer
    - If IFAT results are negative (-) 4 - 6 weeks after suggested clinical signs, then the flock does not have a (currently recognized) TCV
- Use approximate time of exposure along with clinical findings and mortality increases to pinpoint most likely time of introduction

Laboratory Diagnostic Protocol for Turkey Coronavirus (TCV) and Poult Enteritis and Mortality Syndrome (PEMS)

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ABSTRACT

A suggested protocol is described for collecting samples in the laboratory for the diagnosis of Turkey Coronavirus (TCV) and Poult Enteritis and Mortality Syndrome (PEMS).

DIAGNOSTIC SAMPLING PROTOCOL

I. Record signalment, historical, and clinical observations.

II. Diagnostics:
   - A. Draw blood from 10 birds and submit serum for TCV serology.
   - B. Approximately 3 weeks later, obtain 10 convalescent serum samples. Retest for TCV and save for detection of possible unknown agents.

III. At necropsy:
   - A. Weigh each bird; check for pasty vent
   - B. Collect composite droppings sample
   - C. Culture for Salmonella and Campylobacter
   - D. Do floatation for coccidia and other parasites
   - E. Examine auramine-O stained smears for cryptosporidia
   - F. Swab descending portion of duodenal loop
     1. Streak onto MacConkey agar for *E.coli*
     2. Streak onto MacConkey agar with gentamicin (16 mcg/mL) for resistant *E. coli*
     3. Culture for Campylobacter

IV. Weigh:
   - A. Spleen
   - B. Bursa

C. Thymus
D. Body weight (to calculate thymus:body weight ratio)

V. Submit for histopathology. Place in 10% neutral buffered formalin (NBF).
   A. Midgut (jejunum) with Meckel's diverticulum
   B. Ileo-cecal junction
   C. Duodenal loop (opened) with pancreas
   D. Kidney
   E. Liver
   F. Half of bursa

VI. Scrape adjacent section of jejunum and check for cochlosoma and hexamita (under microscope).

VII. Cut another section of jejunum to submit for direct fluorescent antibody testing (DFAT). Also check for rotavirus, enterovirus, etc.

VIII. Cut sections of jejunum and ileum for touch impression smears of mucosa. Stain with Giemsa and examine for protozoa including coccidia, cryptosporidia, cochlosoma and hexamita.

IX. Bursa:
   A. Submit half for histopathology.
   B. Submit half for DFAT for TCV.

X. Score bird: (1 - 5; minimal to very severe/marked)
   A. Proximal tibial tarsus growth plate (rickets?)
   B. Liver
   C. Intestine (rubbery, thin, etc.)
   D. Ceca
   E. Kidney
   F. Gizzard (ulcers?)
   G. Lungs
   H. Yolk sac
   I. Air sacs
   J. Bursa (cores?)
   K. Foot pads

Field Diagnostic Protocol for Turkey Coronavirus (TCV) and Poult Enteritis and Mortality Syndrome (PEMS)

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ABSTRACT

A suggested protocol is described for collecting diagnostic samples in the field. Samples are submitted to a laboratory for the diagnosis of Turkey Coronavirus (TCV) and Poult Enteritis and Mortality Syndrome (PEMS).

DEFINITIONS

- PEMS: is enteritis associated mortality of > 9.0% over 3 weeks with 3 consecutive days >1.0% (stunting invariably follows the clinical disease).
- TCV (+) positive PEMS: is PEMS with TCV + serology on indirect fluorescent antibody test (IFAT).
- TCV (-) negative PEMS: is PEMS without TCV + serology on IFAT.
- Coronaviral enteritis: is enteritis with or without significant mortality and TCV + serology on IFAT.
- TCV (+) positive flock: is TCV + serology on IFAT with or without clinical signs.

DIAGNOSTIC SAMPLING PROTOCOL

I. At the onset of enteritis (shrill chirping, huddling, diarrhea, litter eating, mortality) submit:
    A. Serum: 10 samples for TCV serology
    B. Histopathology: 3 Birds
        1. Submit samples in 10% neutral buffered formalin (NBF)
            a) Thymus: 1 lobe
            b) Crop wall: ~1.0 square cm
            c) Liver
            d) Duodenum w/pancreas taken from middle of duodenal loop: ~1.0 cm
            e) Jejunum taken at Mekel's diverticulum: ~1.0 cm

            f) Ileum taken at the level of the cecal tip: ~1.0 cm
            g) Cecal tip: ~1.0 cm
            h) Cecal tonsil: ~1.0 cm
            i) Bursa: half
    C. Bacteriology: 3 Birds
        1. Aerobic culturette taken just posterior to Mekel's diverticulum
        2. Aerobic culturette taken from cecum
        3. At the laboratory: culture for coliforms and Salmonella
    D. Parasitology: 3 Birds
        1. Using popsicle stick to scrape, place stick into 10% NBF
            a) Scrape 5.0 cm of duodenal mucosa posterior to site of histopath collection
            b) Scrape 5.0 cm of jejunal mucosa posterior to Mekel's diverticulum
            c) Scrape 5.0 cm of cecal mucosa
            d) 2.0 cm of colorectum with contents
    E. Viral Isolation / Identification: 3 Birds
        1. Freeze remainder of jejunum and ileum
        2. Freeze one entire cecum
        3. Freeze half of Bursa
    F. Record signalment, historical and clinical observations
II. 3 weeks after initial onset of clinical signs, submit:
    A. Serum: 10 samples for TCV serology
III. To monitor for TCV status in previously TCV (+) positive farm, submit:
    A. Serum: 10 samples for TCV serology
    B. Collect at 6, 9, and 12 weeks of age, and at market
IV. To monitor TCV status in all other flocks, submit:
    A. Serum: 10 samples for TCV serology
    B. Collect at 6, 9, and 12 weeks of age, and at market

List of Laboratories Performing Diagnostic Tests for Turkey Coronavirus

T. HOOPER
S. CLARK ***(Roche Vitamins Inc.)***

Purdue University Animal Disease Diagnostic Laboratory
West Lafayette, Indiana, USA

Compiled June 1998

Marion Morgan
Springdale Laboratory
3559 N. Thompson
Springdale, Arkansas 72764
(501) 751-4869

Samples accepted: Sera from in-state only.
Test performed: IFA serology.

Tom Brown
Dept. of Avian Medicine
953 College Station Road
Athens, Georgia 30602-4875
(706) 542-1904

Samples accepted: Sera, Suspect TCV isolates in cell culture, Intestine for primary isolation. All from in and out of state.
Tests performed: IFA serology, Direct FA, Virus isolation, Fecal EM and Immuno EM for TCV, Virus neutralization, and PCR identification of TCV isolates.

Tom Hooper
Animal Disease Diagnostic Laboratory-SIPAC
11367 E. Purdue Farm Road
Dubois, Indiana 47527
(812) 678-3401

Samples accepted: Sera from in and out of state, Intestine in and out of state.
Tests performed: IFA serology, Direct FA, Virus isolation.

Ching-Ching Wu
Animal Disease Diagnostic Laboratory
1175 ADDL, Purdue University
West Lafayette, Indiana 47907
(765) 494-7454 (please call Donna Schrader before submitting samples)

Samples accepted: Sera from in and out of state, Intestine from in and out of state

Tests performed: IFA serology, Direct FA, Virus isolation, Fecal EM. Tests performed per special arrangement with Dr. Wu: PCR and sequencing.

Robert Gaines
Rose Hill Laboratory
North Carolina Diagnostic Laboratory
PO Box 37, Rendering Plant Rd
Rose Hill, North Carolina 28458
(910) 289-2635

Samples accepted: Sera from in-state only.

Test performed: IFA serology.

Bill Pierson
Center for Molecular Medicine and Infectious Diseases
Virginia - Maryland Regional College of Veterinary Medicine
Virginia Tech
Blacksburg, Virginia 24061
(540) 231-4529

Samples accepted: Sera from in and out of state.

Test performed: IFA serology.

Gabriel Meza
Harrisonburg Regional Diagnostic Lab-VDACS
116 Reservoir St
Harrisonburg, Virginia 22801
(540) 434-3897

Samples accepted: Sera from in and out of state.

Test performed: IFA serology.

Jose Linares
TVMDL, Poultry Diagnostic Laboratory
1812 Water St., PO Box 84
Gonzales, Texas 78629
(830) 672-2834

Samples accepted: Sera from in-state only.

Test performed: IFA serology.

Y. M. Saif

Food Animal Health Research Program
Ohio Agricultural Research and Development Center
The Ohio State University
Wooster, Ohio 44691
(330) 263-3743

Samples accepted: Frozen gut contents or unopened guts, acute and convalescent sera.

Test performed: Immune EM, Virus isolation.

Turkey Coronavirus Bibliography

The following is a list of 48 scientific publications, listed in chronological order, related to turkey coronavirus.

PubMed is a service of the National Library of Medicine (NLM). NLM's search service has access to the 9 million citations in MEDLINE and Pre-MEDLINE (with links to participating on-line journals), and other related databases. The PubMed website has detailed abstracts of scientific articles. This site is frequently updated.

Go to the PubMed at http://www.ncbi.nlm.nih.gov/PubMed/ and search for "turkey coronavirus".

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Glossary of Abbreviations

AAAP: American Association of Avian Pathologists
ADDL: Animal Disease Diagnostic Laboratory
ADG: average daily gain
AR: Arkansas
AVG: average
AVMA: American Veterinary Medical Association
BCV: bovine coronavirus
BUTA: British United Turkeys of America
CVM: College of Veterinary Medicine
DFA: direct fluorescent antibody (test)
DFAT: direct fluorescent antibody test
ELISA: enzyme-linked immunosorbent assay
EM: electronmicroscopy
Entero: enterovirus
FA: fluorescent antibody (test)
FC: feed conversion (ratio)
FCR: feed conversion ratio
IBV: infectious bronchitis virus (of chickens)
IEM: immune electronmicroscopy
IFA: indirect fluorescent antibody (test)
IFAT: indirect fluorescent antibody test
Lb: pound
Mil: million
MG: Mycoplasma gallisepticum
MM: Mycoplasma meleagridis
MS: Mycoplasma synoviae
NC: North Carolina
NCSU: North Carolina State University
NDV: newcastle disease virus
OSU: The Ohio State University

PCR: polymerase chain reaction

PI: post inoculation

PEMS: poult enteritis and mortality syndrome

PPM: parts per million

PRCV: porcine respiratory coronavirus

Rota: rotavirus

RT-PCR: reverse-transcriptase polymerase chain reaction

SC: South Carolina

SIPAC-ADDL: Southern Indiana Purdue Agriculture Center, Animal Disease Diagnostic Laboratory

SMT: spiking mortality of turkeys

SPF: specific pathogen free

SS: stunting syndrome

SSA: stunting syndrome agent

STD: standard

TCE: turkey coronaviral enteritis

TCV: turkey coronavirus

TGEV: transmissible gastroenteritis virus (of pigs)

TVMDL: Texas Veterinary Medical Diagnostic Laboratory

TX: Texas

USA: United States of America

USDA: United States Department of Agriculture

VA: Virginia

VI: virus isolation

VN: virus neutralization

WKS: weeks

WT: weight

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