

Case Report—

## High Mortality in Egg Layers as a Result of Necrotic Enteritis

A. S. Dhillon, Parimal Roy, Lloyd Lauerman, Dennis Schaberg, Sylvia Weber, Daina Bandli, and Fonda Wier

Avian Health and Food Safety Laboratory, Department of Microbiology and Pathology, College of Veterinary Medicine, Washington State University, 7613 Pioneer Way East, Puyallup, WA 98371-4998

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**SUMMARY.** A new facility was designed to hold 1.8 million birds in 10 houses; chickens were placed in five of the houses, and the remaining five houses were under construction when this outbreak occurred. An increase in mortality was reported in five houses; however, mortality in house 7 was quite high. Well-fleshed birds were suddenly found dead without a significant drop in egg production. The middle and distal intestines were distended with gas, congested, thin walled, atonic, and bluish or pale in color with sloughed mucosa in some places. Necrotic enteritis was diagnosed as the cause of increased mortality. The ingesta in the crop occasionally contained flies. The 4-wk mortality in house 7 was 6.55% with a loss of 10,898 chickens. The 4-wk mortality rate in the other houses ranged from 0.54% to 1.98%.

The houses affected with necrotic enteritis were treated for coccidiosis with amprolium because low numbers of the oocysts were present in the intestinal specimens of some of the chickens. Household bleach was added to the water at a dilution of one part bleach to 1040 parts water to control bacterial contamination.

The fly (*Musca domestica*) population was out of control. *Clostridium perfringens* was isolated from the alcohol-washed macerated flies caught from houses 4 and 7. Dead flies were often seen in the feed troughs. The chickens may possibly have had *C. perfringens* infection as a result of consumption of dead flies or their secretions/excretions. The alcohol-washed, macerated, clarified fly extract from the affected houses caused death in 11 inoculated mice and paralysis in one mouse. Similarly, illness and mortality were present in four mice inoculated with clarified intestinal contents. The bacterium isolated on anaerobic culture was identified as *C. perfringens* by polymerase chain reaction.

The disease was brought under control after straw was added and mixed in with the litter. As a result, the litter temperature increased, causing a decrease in the fly population. This study suggests that flies in the poultry houses acted as mechanical transmitters of *C. perfringens* and that the development of necrotic enteritis was by ingestion of bacteria present in the flies and their secretions/excretions.

**RESUMEN.** *Reporte de Caso*—Altos niveles de mortalidad en ponedoras comerciales como resultado de brotes de enteritis necrótica.

Se colocaron gallinas ponedoras en 5 de las casetas de una granja nueva diseñada para alojar 1.8 millones de aves en un total de 10 casetas. Las cinco casetas restantes estaban en construcción cuando ocurrió en el brote que se describe. Se reportó un aumento de la mortalidad en las 5 casetas, siendo la mortalidad en la caseta 7 bastante más alta que en las demás. Se encontraron aves muertas en aparente buena condición física, sin que se observaran bajas en la postura. A la necropsia se observó que la parte media y distal de lo intestinos estaban distendidas con gas, congestionadas, con adelgazamiento de las paredes, atónicas y con presencia de fluido azulado o pálido y células de la mucosa descamadas en algunas partes. Se diagnosticó la enteritis necrótica como causa del aumento de la mortalidad. Se observó que la ingesta en el buche de las aves examinadas contenía moscas. El porcentaje de mortalidad observado a las 4 semanas en la caseta 7 fue de un 6.55% para una pérdida total de 10,898

aves. Los porcentajes de mortalidad observados en el resto de las casetas fue de un 0.54% a un 1.98%.

Las casetas afectadas por el brote de enteritis necrótica fueron tratadas contra coccidias mediante el uso de amprolium, debido a que se observó la presencia de oocistos de coccidia en las muestras tomadas de los intestinos de algunas de las aves. El agua de bebida fue tratada con cloro o blanqueador comercial a una dilución de 1:40 para controlar la contaminación bacteriana. La población de moscas (*Musca domestica*) estaba fuera de control en las casetas. Se aisló *Clostridium perfringens* a partir de macerados de moscas lavadas en alcohol, capturadas en las casetas 4 y 7. Se observó la presencia de moscas muertas en las canaletas de alimento en las casetas. Las ponedoras posiblemente contrajeron la infección por *Clostridium perfringens* como resultado de la ingestión de moscas muertas, o sus secreciones. Los extractos clarificados de macerados de moscas lavadas en alcohol causaron parálisis en un ratón y la muerte en 11 ratones inoculados en forma experimental. De forma similar, se observaron signos de enfermedad y mortalidad en 4 ratones inoculados con extractos del contenido intestinal obtenidos a partir de las aves enfermas. La bacteria aislada en medio de cultivo en condiciones de anaerobiosis fue identificada como *Clostridium perfringens* mediante la reacción en cadena por la polimerasa. La enfermedad fue controlada mediante la adición y mezclado de paja en la cama de las casetas. La adición de paja produjo un aumento de la temperatura de la cama, lo cual redujo la población de moscas en las casetas. Este estudio sugiere que las poblaciones de moscas en las casetas actuaron como vector mecánico en la transmisión del *Clostridium perfringens* y que el brote de enteritis necrótica fue el resultado de la ingestión de la bacteria, presente en las moscas o en sus secreciones.

Key words: *Clostridium perfringens*, necrotic enteritis, egg layers, chickens, diagnosis, housefly (*Musca domestica*)

Abbreviations: H&E = hematoxylin and eosin; PCR = polymerase chain reaction

Necrotic enteritis in chickens has been well documented since it was first diagnosed in 1961 (14). The disease is caused by an enteropathogenic bacterium, *Clostridium perfringens*, and is characterized by necrotic lesions in the intestine and high mortality (15) in well-fleshed birds. Predisposing factors such as a high-energy protein diet (8,9,10) and infection with coccidia (1,8,9,10,11,12,13) have been reported to favor the overgrowth of clostridium bacteria in the intestinal tract. Most published reports of necrotic enteritis have involved commercial broiler chickens raised on the floor (8,9,10,11,12,13) in commercial hatcheries (4) or during production and processing (5). One case of necrotic enteritis has been reported from cage-reared commercial layer pullets (2). This paper describes necrotic enteritis in commercial egg laying chickens housed in cages and the possible role of houseflies (*Musca domestica*) in causing necrotic enteritis.

### CASE HISTORY

A new facility was designed to hold 1.8 million birds in 10 houses, each house containing 180,000 birds. The chickens were placed in five houses, and the remaining houses were under construction. An increase in mortality was reported in all houses; however, mortality in house 7 was quite high. Well-fleshed birds were suddenly found dead without a

significant drop in egg production. The 4-wk mortality in house 7 was 6.55%, with a loss of 10,898 chickens (Table 1). The 4-wk mortality rate in the other houses ranged from 0.54% to 1.98%. A normal monthly mortality rate in an egg layer facility should be about 0.5%. Numerous dead flies (*Musca domestica*) were found in the feed troughs of all houses. The fly population was rated 5 on a scale of 1 to 5. A score of 1 was given when a few flies were observed. A score of 5 reflected a fly population so high that the workers could not talk to each other for fear of flies getting into their mouths.

### MATERIALS AND METHODS

**Necropsy findings.** On January 29, 2001, 23 dead birds from two poultry houses were submitted to the Avian Health and Food Safety Laboratory, Washington State University, for postmortem examination. Seventeen birds had lesions of necrotic enteritis. The middle and distal intestines were bluish or pale in color and distended with gas involving some segments of the intestine. Such changes were also present on occasion in the duodenum. Other alterations present included a thin, atonic wall of the intestine and the presence of sloughed mucosa in some segments. In addition, rupture of ovarian follicles resulting in egg yolk peritonitis was often present. Occasionally, dead flies, mixed with feed, were present in the crops. These lesions were of necrotic enteritis,

Table 1. Weekly mortality and 4-wk average mortality from five affected houses.

Week ending	Mortality				
	House 4	House 5	House 6	House 7	House 8
Total number of chickens:	171,613	163,255	158,200	166,434	172,178
Age of chickens:	57 wk	69 wk	76 wk	44 wk	31 wk
1/17/01	275	287	351	73	416
1/24/01	256	292	333	132	464
1/31/01	271	277	625	1,131	403
2/7/01	268	290	616	9,562	514
4-wk mortality	0.62%	0.54%	1.01%	6.55%	1.05%
2/14/01	294	303	553	389	561
2/21/01	292	274	872	160	470
2/28/01	280	266	1,014	118	523
3/7/01	342	264	664	112	327
4-wk mortality	0.71%	0.68%	1.98%	0.50%	1.10%
3/14/01	278	283	538	101	274
3/21/01	304	315	457	118	248
3/28/01	384	297	363	67	521
4/4/01	418	315	394	108	326
4-wk mortality	0.82%	0.75%	1.14%	0.25%	0.81%

possibly of *Clostridium perfringens* etiology. In the first week of February, 22 more dead birds were received from three houses. Ten of these birds had lesions of necrotic enteritis. In the second week of March, dead birds were received twice. Eleven out of a total of 52 chickens had necrotic enteritis. In the second week of April, two out of 59 birds from five houses were diagnosed with necrotic enteritis. Lesions of mild coccidiosis were present. Mucosal scrapings were examined for the presence of *Eimeria* oocysts. The examination of scrapings revealed 2–10 oocysts per 10× microscopic field in the intestines of some chickens. Morphologically, the oocysts resembled those of *Eimeria maxima*. Chickens belonging to three of five affected houses initially submitted contained oocysts. The mortality in the affected houses was not adjudged to be due to coccidiosis. Affected tissues were fixed in 10% neutral buffered formalin. Tissues were embedded in paraffin, sectioned, stained with hematoxylin and eosin (H&E), and observed for microscopic changes.

**Bacteriology.** Bacterial isolates from affected sections of the intestines were composed of moderate to high numbers of gram-positive plump rods and high numbers of *Escherichia coli* by anaerobic techniques (3,7). The bacterium isolated on anaerobic culture was identified as *C. perfringens* by polymerase chain reaction (PCR). *Pasteurella hemolytica* was isolated on occasion in low numbers from livers of the birds received initially. Results of culturing for *Salmonella*, *Campylobacter*, and *Listeria* were negative.

**Culture results of flies.** Both live and dead flies were collected from different areas of some of the affected houses (Table 2), washed three times with ethanol (70%), and rinsed with buffered saline solution. The outer surfaces of these flies were cultured aerobically and anaerobically after the alcohol wash; no bacterial growth was present. The flies were then macerated and again cultured aerobically and anaerobically. The anaerobic cultures revealed the presence of *C. perfringens*. *Escherichia coli* was also isolated from the aerobic and anaerobic cultures. Flies procured from a different company's egg-layer farm, similarly treated before culturing, were negative for *C. perfringens*.

**Mouse inoculation test and results.** The mice used for this study were BKL : Swiss specific-pathogen free, obtained from Animal Technologies, Kent, WA. The mice were inoculated intraperitoneally with a 0.5-ml inoculum. The inoculated mice were observed for 24–48 hr for symptoms of illness, paralysis of legs, or death.

**Testing for toxins in the intestinal contents of affected birds.** The intestinal segments of chickens with lesions of necrotic enteritis were collected, stomached, and centrifuged. The supernatant 0.5 ml was inoculated in four mice intraperitoneally; two mice died and two were moribund 24 hr postinoculation (Table 2). This test indicated the presence of toxins of *C. perfringens* in the intestines of the chickens with lesions of necrotic enteritis.

**Testing toxins in body contents of dead flies.** Dead flies were collected from areas both near the bait stations and far away from the bait stations

Table 2. Mouse inoculation with fly extract from affected houses and other tests.<sup>A</sup>

Source of flies/other material	Location of flies/other material	No. mice inoculated	Results of mouse inoculation
Affected farm H 4 upstairs	Dead flies present not near bait station	3	2 died, 1 partial paralysis
Affected farm H 7 upstairs	Dead flies not near a bait station	3	3/3 dead
Affected farm H 7 upstairs	Dead flies present near a bait station	3	3/3 dead
Affected farm H 7 downstairs	Dead flies present near a bait station	3	3/3 dead
Flies from an unrelated farm	From Pacific Northwest	3	No illness or death
Clarified intestinal contents	Collected from affected farm birds	4	2 died, 2 moribund
Bluestreak™ fly bait <sup>B</sup>	Received from affected farm	3	No illness or death
RAVAP® E.C. insecticide spray <sup>C</sup>	Received from affected farm	1	No illness or death

<sup>A</sup>About 200 flies from each lot were washed three times in alcohol followed each time by a wash in sterile buffered saline solution followed by maceration with mortar and pestle. The macerated material was resuspended in 10 ml of sterile buffered saline. Each suspension was clarified by centrifugation. Mice were inoculated intraperitoneally with 0.5 ml of the inoculum. Intestinal contents of affected chickens were collected in a sterile beaker and clarified by centrifugation. Mice were inoculated intraperitoneally with 0.5 ml of the inoculum.

<sup>B</sup>Bluestreak™ fly bait, manufactured by Farnam Companies, Inc., Phoenix, AZ. The bait was diluted 1:10 in buffered saline. Mice were inoculated intraperitoneally with 0.5 ml of the inoculum.

<sup>C</sup>RAVAP® E.C. insecticide spray manufactured by Boehringer Ingelheim Vetmedica, Inc., St. Joseph, MO. The insecticide spray was diluted 1:10 in buffered saline. Mice were inoculated intraperitoneally with 0.5 ml of the inoculum.

from houses 4 and 7 (Table 2). The flies were tested for the presence of toxins. About 200 flies from each lot were washed three times in alcohol followed each time by a wash in sterile buffered saline solution followed by maceration with mortar and pestle. The macerated material was resuspended in 10 ml of sterile buffered saline. Each suspension was clarified by centrifugation at 10,000 rpm to eliminate bacteria. Four such samples of flies (Table 2) were tested individually. Three mice were inoculated with each sample. All nine mice inoculated with three of the samples died. With the fourth sample, two mice died and one was paralyzed. This test indicated clearly that the toxins were present in the alcohol-washed macerated flies.

**Testing toxins in the body contents of dead flies from a different company egg layer farm.** About 200 dead flies were obtained from a different company's egg layer facility with no history of necrotic enteritis. These flies were similarly treated and three mice were inoculated; no illness or mortality was present in the mice. The result of this test indicated that these flies did not contain any toxins.

**Testing of Bluestreak bait and insecticide spray.** Bluestreak™ fly bait (Farnam Companies, Inc., Phoenix, AZ) was used as bait in the affected houses. It was diluted 1:10 in buffered saline before inoculation in the mice. RAVAP® E.C. insecticide spray (Boehringer Ingelheim Vetmedica, Inc., St. Joseph, MO) was used at this farm. It was diluted 1:100 in buffered saline before inoculation. The above two tests confirmed that the toxic material present in

the fly bait and insecticide spray that caused the death of the flies failed to cause illness or death in the mice.

**PCR.** DNA was extracted from the tissues of the affected intestines, macerated flies, and anaerobic bacterial colonies with a QIA amp DNA mini kit (Qiagen, Valencia, CA). A multiplex PCR was done with primers (Life Technologies, Rockville, MD) for alpha toxin (CPA), beta 1 toxin (CPB1), beta 2 toxin (CPB2), epsilon toxin (CPE), enterotoxin (ET) and iota toxin (IA) genes as described (6,16). The PCR products (amplicons) were analyzed by electrophoresis in a 1.5% agarose gel containing ethidium bromide. All the samples yielded amplicons of approximately 324 bp in size, indicating that they all were positive for a type A toxin. In an earlier study (16), a toxin gene was demonstrated in *C. perfringens* Type A to cause necrotic enteritis in chickens.

**Histopathology.** The microscopic alterations consisted of moderate to severe multifocal necrotizing enteritis with large numbers of gram-negative and intralosomal gram-positive large bacterial rods (H&E stain and Brown and Brenn stain). Alterations in the liver were acute multifocal extensive necrotizing hepatitis. Other alterations were mild multifocal granulomatous serositis with intralosomal eosinophilic material with the presence of bacterial colonization. In addition, egg yolk peritonitis was present. Microscopic examination confirmed the presence of several coccidial oocysts on occasion in the lumen of intestine sections; however, very few organisms were identified in the mucosal epithelium.

**Treatment.** All houses affected with necrotic enteritis were treated with amprolium to control coccidiosis. Household bleach (sodium hypochlorite 6.25%) was added to the drinking water at a dilution of one part bleach to 1040 parts water to eliminate bird-to-bird spread of *C. perfringens*. Straw was added to the litter to increase the litter temperature in order to control the fly population; the litter temperature increased to a level of 125–135 F. Mortality decreased (Table 1) in all houses, including house 7, to normal levels.

## DISCUSSION

The sudden increase in mortality in all houses in the adult flocks was due to *C. perfringens* infection; the bacterium caused severe necrotic enteritis resulting in death. In earlier research findings, coccidial infection in a flock increased the probability of necrotic enteritis due to gut irritation and/or stasis (1,8,13). The same may be true to some degree in this outbreak; however, gross lesions of coccidiosis were minimal. Mucosal scrapings contained very few oocysts and occurred only in three of the five houses affected with necrotic enteritis on initial necropsies. Histopathologic examination revealed several oocysts in the sections of intestine lumen on occasion in some sections, but only a few were invading the epithelial cells. Mortality due to necrotic enteritis continued at the same level for another 9–10 wk posttreatment of the coccidiosis.

The chickens were housed in cages, and there was less of a possibility of soil or poultry litter contamination. Dead birds were picked up every other day. The population of flies in the layer houses was extremely high, and dead flies were often present in the feeding troughs and, on occasion, in the crops of chickens. *Clostridium perfringens* was isolated from the flies as well as from the chicken intestine. The presence of toxins was confirmed by the mouse inoculation test in the affected intestine. The inoculum of macerated and clarified flies caused illness, paralysis, and death in the mouse; this indicates that the flies contained a toxin. The anaerobic bacteria isolated from the intestines of the affected chickens and the alcohol-washed, macerated flies was identified as *C. perfringens* Type A by PCR technique.

This study indicates that *C. perfringens* Type A was responsible for causing necrotic enteritis and high mortality in chickens. The most likely sources of *C. perfringens* in this case were poultry manure, poultry dust, and possibly flies or fly secretions/

excretions deposited on feed in the feed troughs. The likelihood of birds pecking at poultry manure and thus getting infected are less in this outbreak because the birds were on wire in new buildings at this farm. This outbreak was unique; it lasted over 11 wk and correlated chronologically with an enormously high fly population.

If the flies acted as biological carriers as opposed to mechanical carriers, this may be new information in the epizootiology of *C. perfringens* infection in chickens. Future research should be directed to explore if *C. perfringens* multiplies in high numbers and produces enterotoxins in houseflies, and poultry farmers should be advised of the role of flies in causing necrotic enteritis.

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