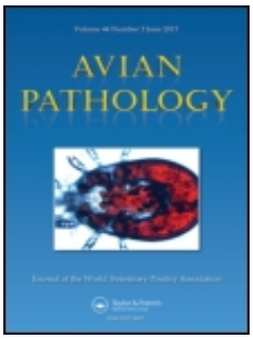

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Case Report

Concurrent occurrence of salmonellosis, colibaccillosis and histomoniasis in a broiler flock fed with antibiotic-free commercial feed

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A flock of 3-week-old broiler chickens fed with antibiotic-free commercial feed developed cyanotic combs, depression, extended abdomens, reddened abdominal skin, faeces-stained vents, and diarrhoea. By the end of week 6, mortality reached 19.3%, and important lesions seen were ascites, airsacculitis, swollen/congested kidneys, fibrinous perihepatitis, fibrinous pericarditis with or without hydropericardium, haemorrhagic enteritis–typhilitis and ballooned caeca. *Salmonella typhmurium* var *Copenhagen* and *Escherichia coli* were isolated from livers, hearts, intestines and caeca. *Histomonas* spp. were detected only in caeca. Thus, concurrent occurrence of salmonellosis, colibacillosis and histomoniasis was diagnosed.

Introduction

Salmonellosis or paratyphoid infection in chickens is caused by *Salmonella* spp. other than *Salmonella pullorum* and *Salmonella gallinarum*. Several salmonellas have been implicated in salmonellosis including *Salmonella typhimurium*, which is of public health concern (Ashton, 1990). Outbreaks of colibacillosis caused by *Escherichia coli* either in single or mixed infection with other chicken pathogens have been well documented (Ashton, 1990; Charlton *et al.*, 1996; Barnes & Gross, 1997). Histomoniasis in commercial chickens has not been reported in tropical regions; however, it has been reported in the US, Canada and Mexico (AAAP Committee on Disease Reporting, 1986; Homer & Butcher, 1991). This paper reports, for the first time, on a concurrent natural infection of *S. typhimurium, E. coli* and *Histomonas* spp. in experimental broiler chickens fed with antibiotic-free commercial feed, and raised under tropical conditions.

Observation

An experimental flock of 750 broiler chickens was raised in an open-sided, slatted-floor chicken house. The birds were fed with commercial feed containing no antibiotics or growth promoters. The daily management of the chickens, housing and environment was as practiced in conventional commercial broiler farming. Live vaccines were used to vaccinate the birds against Newcastle disease (days 7 and 21) and infectious bursal disease (day 14). For prevention of bacterial and mycoplasma diseases, antibiotics were administered in drinking water for the first 5 days. The chickens' health was closely monitored for the entire rearing period of 42 days. This included daily observation for clinical signs and necropsy of dead birds.

For the first 3 weeks, the chickens showed no obvious clinical signs, even though 4.1% mortality was recorded. Necropsy of dead chickens revealed either no significant findings or congestion of lungs and livers.

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Figure 1. *Swollen liver with patches of necrosis and evidence of fibrinous perihepatitis.*

For the second 3 weeks (days 22 to 42), a total of 114 (15.2%) chickens died, with a peak on day 31 (2.4%). Obvious clinical signs of dullness, depression, distended abdomens (filled with fluid), reddened abdominal skin, faeces-stained vent region, and diarrhoea were seen from weeks 4 to 6. Diarrhoea ranged from whitish, yellowish to dark-brownish in colour, and was either watery or semi-solid. Some faeces contained flecks of blood. Panting and gasping for air were also observe, and, on closer examination, a few sick chickens had cyanotic combs.

In total, by day 42, 145/750 (19.3%) of the chickens had died. Out of these, 85 birds were subjected to necropsy. Common and important lesions found were severe ascites (53%), air-sacculitis (49%), swollen/congested kidneys (47%), fibrinous perihepatitis (39%) (Figure 1) and fibrinous pericarditis with or without hydropericardium (39%) (Figure 2). Other prominent and important lesions were severe haemorrhagic enteritis (32%) and typhlitis (5%) ((Figure 3), and ballooned caeca (9%).

Based on the clinical signs and the gross necropsy lesions, complicated chronic respiratory disease, colibacillosis, salmonellosis and coccidiosis were suspected. On three different occasions, five specimens of livers, hearts, intestines and/or caeca were submitted for bacteriological and parasitological examination.

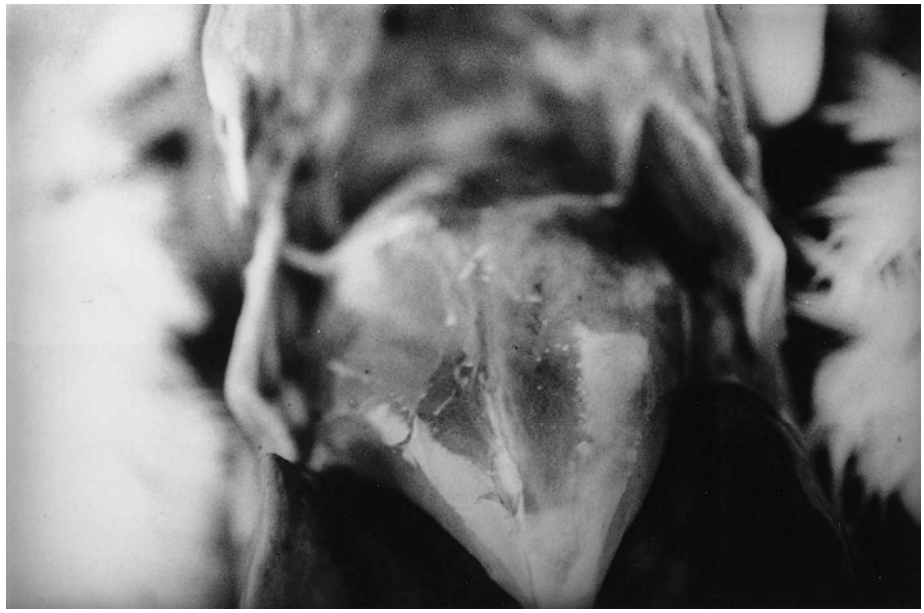

Figure 2. *Pericardial sac containing clear fluid (hydropericardium).*

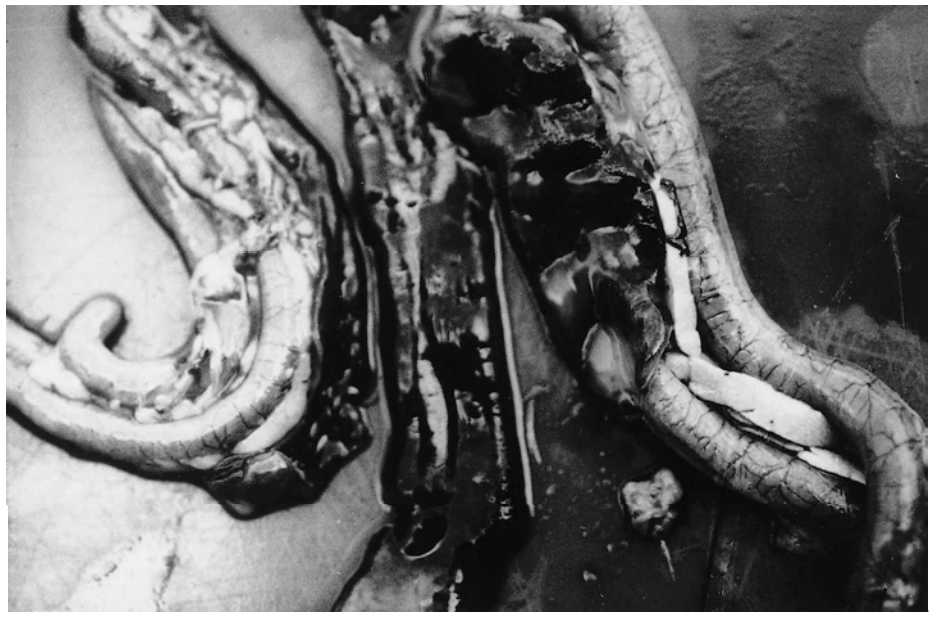

Figure 3. *Haemorrhagic enteritis and typhlitis.*

For bacterial examination, the specimens were inoculated onto blood and McConkey agars, and dipped into selenite broth. For salmonella, the isolation procedure of Waltman *et al.* (1998) was followed. Brilliant Green Agar was used as an enrichment medium, and broths and plates were incubated at 37°C (instead of 42°C), and suspect salmonella colonies were subjected to routine biochemical tests, and agglutination tests against Polyvalent 'O' and Polyvalent 'H' antisera. Serotyping was carried out using Serobact® antisera according to the manufacturer's recommendation (Medvet Science Pty. Ltd., Adelaide, Australia). Out of 15 specimens submitted, *E. coli* was isolated from five hearts, six livers and five intestines/caeca. *Salmonella* spp. were recovered from three hearts, three livers and five intestine/caeca. The salmonellas were later serotyped as *S. typhimurium* var *Copenhagen*. At weeks 3, 4 and 5, tracheal swabs were taken from 20 chickens but no mycoplasma growth was seen; instead, heavy bacterial contamination was found.

For parasitological examination, intestines and caeca with haemorrhage were examined for the presence of parasites. Salt floatation tests were carried out, and direct mucosal smears and Giemsa staining of methanol-fixed mucosal smears were examined (Soulsby, 1986). An invasive type of histomonad was detected in Giemsa-stained mucosal smears of caeca. It measured 12 to 10 μm in diameter, and demonstrated a pink–bluish nucleus but no flagella were seen. Out of 15 caeca examined, four contained mild to severe infestation of histomonads. No other parasites were detected in the intestines/caeca.

Thus, based on the clinical signs, the gross lesions and the laboratory results, the case was diagnosed as concurrent infection of *S. typhimurium, E. coli* and *Histomonas* spp. No attempts were made to isolate viruses.

The chickens appeared to have no obvious health consequences during the starter stage (1 day old to day 21) but a completely different picture developed from week 4 onwards. Most birds showed gastrointestinal and respiratory problems, and the severity of clinical signs was increased on very hot days. At noon, the chickens appeared most depressed with obvious râles, sneezing and panting, and it was recognized that hot and humid weather might have exacerbated the disease severity.

Discussion

It has been reported that *S. typhiumurium* may cause up to 79% mortality in young chicks, mostly in chicks under 1 week old (Ashton, 1990; Mario Padron, 1990; Gast, 1997). In contrast, in this incident, 15.2% mortality was recorded in older chickens. This could have been due to uncontrolled proliferation of the salmonella due to absence of in-feed antibiotics, and co-infection with *E. coli* and histomonads may have exacerbated the disease. It was reported that *S. typhiumurium* causes clinical signs and lesions in young chicks (Mario Padron, 1990; Gast, 1997). In this investigation, clinical signs and lesions suggestive of salmonellosis were found in 4- to 6-week-old broiler chickens. This case has shown that, in the absence of in-feed and in-water antibiotics, environmental and production stress, and the existence of concurrent infections may cause development of clinical salmonellosis and colibacillosis in adult broiler chickens. Occurrences of the bacterial diseases, despite the first 5 days preventive antibiotic administration, indicate that this preventive measure was insufficient to prevent or control the development of disease.

Homer & Butcher (1991) reported an outbreak of histomoniasis in pullets with 2 to 3% mortality, typhilitis, and *Heterakis gallinarum* colonization. In this case, no worms were found in the gastrointestinal tract of necropsied chickens, but signs and lesions suggestive of histomoniasis, such as cynotic combs, yellow faeces and haemorrhagic enteritis–typhlitis (McDougald, 1997), were seen in the adult chickens. McDougald (1997) also stated that the 4- to 6-week-old broilers are most susceptible to histomonas infection. Thus, the age, clinical signs, gross lesions and findings of numerous *Histomonas* spp. in the caeca strongly suggest involvement of the organism.

Colibacillosis was diagnosed based on signs, lesions and isolation of *E. coli* (Ashton, 1990; Charlton *et al.*, 1996; Gast, 1997). Possible sources of *S. typhiumurium, E. coli* and histomonads have been described elsewhere (Ashton 1990; Charlton *et al.*, 1996; Gast, 1997; McDougald, 1997).

In conclusion, this appears to be the first report where *S. typhiumurium, E. coli* and. *Histomonas* spp. were implicated concurrently in adult broiler chickens. The disease picture was exacerbated by the presence of all the three pathogens.

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RÉSUMÉ

Apparition concomitante de salmonellose, colibacillose, histomonose dans un troupeau de poulets de chair nourris avec un aliment commercial sans antibiotique

Des poulets de chair âgés de 3 semaines nourris avec un aliment commercial sans antibiotique étaient abattus et présentaient des crêtes cyanosées, des abdomens distendus avec une peau rougeâtre, des cloaques souillés par les fèces, et de la diarrhée. A la fin de la 6ème semaine, la mortalité a atteint 19,3 % et les lésions importantes étaient de l'ascite, de l'aerosacculite, des reins congestionnés et gonflés, une périhépatite fibrineuse, une péricardite fibrineuse avec ou sans hydropéricarde, une entérite typhlite hémorragique et des caecums ballonnés. *Salmonella typhmurium* var *Copenhagen* et *Escherichia coli* ont été isolés des foies, des cœurs, des intestins et des caecums. *Histomonas* spp n'a été retrouvé que dans les caecums. Ainsi l'apparition concomitante d'une salmonellose, d'une colibacillose et d'une histomonose ont été diagnostiquées.

ZUSAMMENFASSUNG

Gleichzeitiges Auftreten von Salmonellose, Colibacillose und Histomoniasis in einer Broilerherde, die mit einem antibiotika-freien Handelsfuttermittel gefüttert wurde

In einer Herde von 3 Wochen alten Mastküken, die mit einem antibiotika-freien kommerziellen Futter gefüttert wurden, kam es zu zyanotischen Kämmen, Entkräftung, erweiterten Bäuchen, geröteter Bauchhaut, kotverschmierten Kloakenausgängen und Durchfall. Am Ende der 6. Woche erreichte die Mortalität 19,3%, und die wesentlichen pathologischen Veränderungen waren Aszites, Luftsackentzündung, geschwollene/gestaute Nieren, fibrinöse Perihepatitis, fibrinöse Perikarditis mit oder ohne Hydroperikardium, hämorrhagische Enteritis-Typhlitis und aufgeblähte Blinddärme. Salmonella typhimurium var. Copenhagen und Escherichia coli wurden aus Lebern, Herzen, Intestinum und Blinddärmen isoliert. Histomonas spp. wurden nur in Blinddärmen nachgewiesen. Somit wurde das gleichzeitige Auftreten von Salmonellose, Colibacillose und Histomoniasis diagnostiziert.

RESUMEN

Salmonelosis, colibacilosis y histomoniasis concurrentes en un lote de pollos broiler alimentados con un pienso comercial libre de antibióticos

En un lote de pollos broiler de tres semanas de edad alimentado con un pienso libre de antibióticos se observaron animales que presentaban crestas cianóticas, depresión, abdómenes distendidos, enrojecimiento de la piel de la zona abdominal, zona pericloacal teñida de heces y diarrea. Al final de la sexta semana, la mortalidad llegó al 19.3% y se observaron lesiones importantes, tales como ascitis, aerosaculitis, riñones tumefactos y congestivos, perihepatitis fibrinosa, pericarditis fibrinosa asociada en algunos casos a hidropericardio, enteritis-tiflitis hemorrágica y ciegos distendidos. Se aislaron *Salmonella typhimurium* var Copenhagen y *Escherichia coli* a partir de hígado, corazón, intestinos y ciegos. Se detectó *Histomonas* spp únicamente en el ciego. Así pues, en este lote se diagnosticó la aparición concurrente de salmonelosis, colibacilosis e histomoniasis.