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Concurrent outbreak of chlamydiosis and aflatoxicosis among chickens in Himachal Pradesh, India

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ABSTRACT

A severe outbreak of aflatoxicosis and chlamydiosis among chickens in an organised poultry farm, further super-infected by secondary microbial invasion, was investigated. Mortality was 65, 25 and 32% among broiler chicks (1-2 week of age), layer chicks (2-3 weeks of age) and broilers (8-15 weeks of age), respectively. Samples were collected from 20 representative ailing/dead birds included pooled visceral organs (spleen, lung, liver and heart) and intestinal contents in sucrose phosphate glutamate (SPG) for isolation of *Chlamydia psittaci* in 6-8 day-old embryonated chicken eggs; heart blood, tracheal swabs and intestinal contents for isolation of other microbes. Liver, intestinal loop, spleen, kidney and lung for histopathological studies. Feed samples of aflatoxins were also collected for analysis. *C. psittaci* was isolated from 30% (6/20) birds and other microbes, viz. *E. coli, Enterococcus* spp., *Staphylococcus* spp., *Candida albicans*, etc. were also isolated. The aflatoxin level in feed given to different age groups varied from 100 ppb to 500 ppb. Characteristic histopathological alterations corresponding to aflatoxicosis and chlamydiosis, as well as secondary microbial infection, were found in different visceral organs, indicating foetal and complicated disease syndrome.

Key words: aflatoxicosis, chlamydiosis, poultry, microbes, Himachal, India

Introduction

Chlamydiosis among chickens, mostly caused by *Chlamydia psittaci* of avian group occurs as acute and/or sub-clinical infection although various stress factors precipitate the disease (STORZ, 1971). Immunosuppression is

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the major consequence of many poultry diseases, aflatoxicosis being one of them (PIER, 1973; RAO, 1978), leading to increased propensity to various diseases. The present report concerns a field outbreak of aflatoxicosis complicated by chlamydial infection and secondary microbial invasion among chickens in Himachal Pradesh, a sub-Himalayan state of India. Reporting of this outbreak may be very significant, bearing in mind that poultry farming is becoming increasingly intensive and that there is therefore every likelihood that such concurrent infections will emerge, resulting in heavy losses to poultry farmers.

Materials and methods

A total 20 of representative samples, including intestinal swabs and pooled visceral organs (lungs, livers, spleens and heart) from recently dead or moribund birds, were collected in sucrose phosphate glutamate (SPG), pH 7.2 containing antibiotics for isolation of *Chlamydia psittaci*. All samples were processed for isolation in 6-8 day-old embryonated chicken eggs, employing yolk sac route of inoculation, using standard technique (RAKE et al., 1940; PAGE, 1974; GRIMES, 1985). Three blind passages were given before declaring a sample as either positive or negative. All isolates were further confirmed by observing staining reaction with Gimenez, Macchiavello's and Wolbach's Giemsa stains (ANONYMOUS, 1994), embryo mortality pattern and IMIFT on yolk sac membrane impression smears. Samples of different feeds offered to birds of different age groups were collected from feed stores, as well as from feeding troughs, in air-tight polythene sachets for estimation of aflatoxin levels. Representative organs such as livers, kidneys, lungs and intestinal loops were also collected in 10% formal saline for histopathological studies. Blood samples were collected for haematological study from diseased birds in EDTA. Swabs from intestinal contents and heart blood were collected from moribund and recently dead birds, aseptically for microbial isolation on 6% sheep blood agar and Sabouraud's agar; incubated at 37 °C and 25 °C respectively, for bacterial and fungal/yeast isolation. The panorama of microbes were isolated and identified as per standard procedures (BUCHANAN and GIBBONS, 1974).

Results

Different clinical signs exhibited by affected birds included anorexia, depression, prostration, soiled vents, nervous signs such as torticolis, spasm of neck muscles and death with legs extended to the posterior. Post mortem examination revealed generalized paleness of the musculature,

and subcutaneous haemorrhages in thigh and breast regions. In the majority of birds liver was congested with distended gallbladder but some pale discolouration of liver was also discerned. Congestion of lungs, kidneys, intestines, as well as uncoagulated blood in the heart, was also invariably encountered. The duodenal portions of intestine were thickened and contained mucoid exudate.

Table 1. Aflatoxin levels, mortality percentage and *C. psittaci*, as well as other microbes isolated from different categories of chicken in Himachal Pradesh, India

Type of birds	Flock size	Age group (weeks)	Aflatoxin levell (ppb)	Mortality (%)	Microbes isolated
Chicks (broilers)	1300	1-2	100-300	65	**Chlamydia psittaci* (3) ***E. coli, Enterococcus faecalis, Aeromonas hydrophilla, Staphylococcus hyicus* ****Streptococcus pyogenes, E. coli, Enterococcus faecalis, Staphylococcus hyicus* *****Candida albicans*
Chicks (layers)	1367	2-3	100-300	25	**Chlamydia psittaci* (1) ***E. coli, Enterococcus faecalis, Aeromonas hydrophilla, Staphylococcus hyicus, Proteus* spp. ****Streptococcus pyogenes, E. coli, Enterococcus faecalis*
Broilers	419	8-15	300-500	32	**Chlamydia psittaci* (2) ***E.coli, Enterococcus faecalis, Staphylococcus saprophyticus, Proteus* spp. ****Staphylococcus saprophyticus*
Layers	500	52	<30	routine	*Nil ***E. coli, Enterococcus faecalis, Staphylococcus saprophyticus, Proteus* spp. ****Staphylococcus saprophyticus*

*Isolated from pooled visceral organs in 6-8 days embryonated chicken eggs; **Isolations from intestinal contents; ***Isolations from heart swabs; ****Isolations from tracheal swabs

Of 20 representative samples, six birds (30%) yielded *C. psittaci*: four from pooled visceral organs and two from intestinal contents. All isolates were confirmed as *C. psittaci* on the basis of the above-mentioned criteria. Quantitative estimation of aflatoxin in feed samples revealed the presence of aflatoxin reaching toxic levels in chick and broiler feed, i.e. 100 ppb to 500 ppb, but less than 30 ppb in the feed of layers. Mortality percentage by age and group, aflatoxin levels in their feeds and various other bacteria and yeasts isolated from heart blood, tracheal swabs and intestinal contents are presented in Table 1. Characteristic histopathological alterations corresponding to aflatoxicosis (HOERR, 1991; KUMAR et al., 1993) and chlamydiosis (SUWA et al., 1990; CHAHOTA et al., 1997) as well as secondary microbial invasion, were found in different visceral organs. Hepatic parenchyma in almost all cases showed degenerative changes and

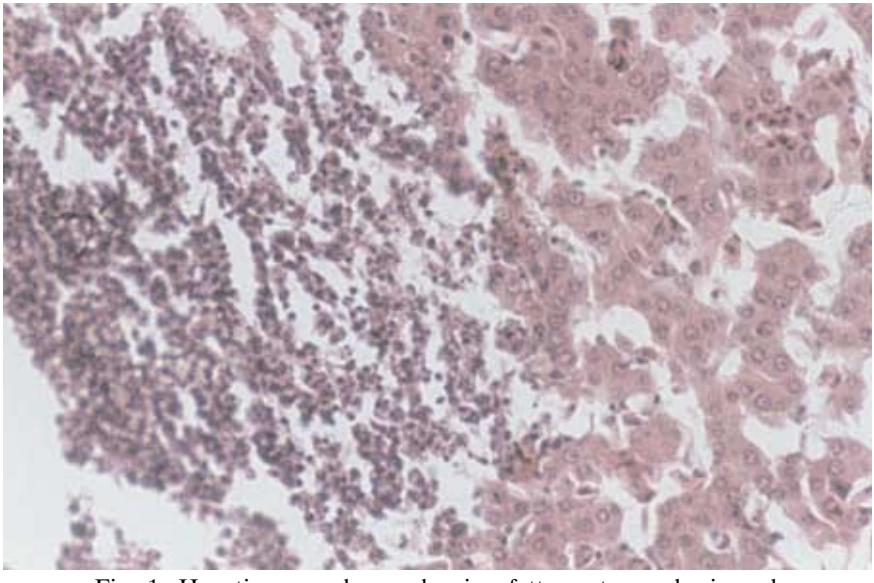

Fig. 1. Hepatic parenchyma showing fatty metamorphosis and focal infiltration of mononuclear cells in a broiler chick; H&E; 45 10.

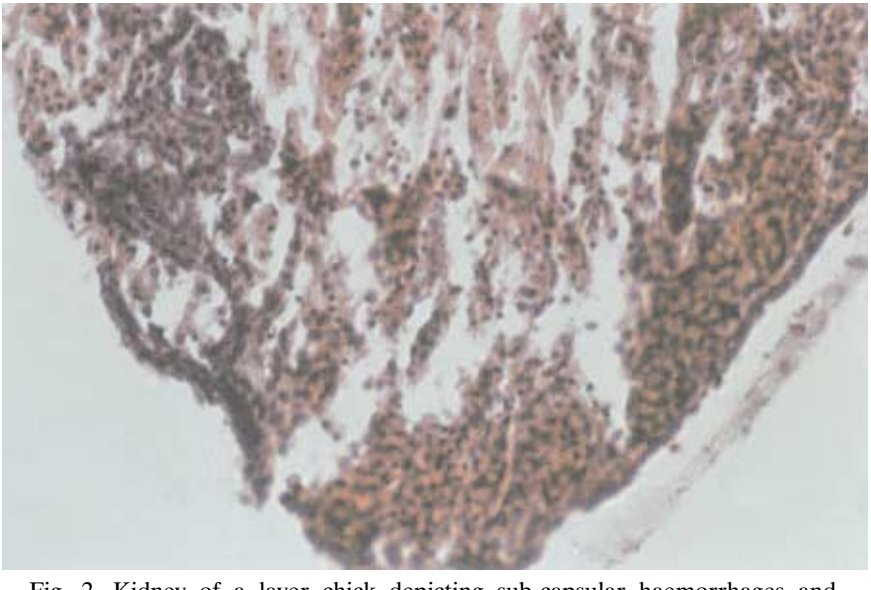

Fig. 2. Kidney of a layer chick depicting sub-capsular haemorrhages and infiltration of inflammatory cells in the cortical region; H&E; 45 10.

fatty metamorphosis, haemorrhages, congestion and aggregates of mononuclear cells (Fig. 1). Focal aggregates of mononuclear cells were also discerned in some cases. Kidneys showed congestion, haemorrhages, nephritic changes, together with sub-scapular haemorrhages (Fig. 2). Severe catarrhal enteritis with sloughing of mucosa was invariably evident in intestines. In lungs the most consistent lesions were parabronchus haemorrhages and mononuclear cell infiltration, coupled with severe generalized congestion. Changes were more acute among broiler chicks, and were chronic or subacute among layer chicks and adult broilers. In general, haematological investigations revealed decreased haemoglobin and packed cell volume. There was an increase in total leukocytic count, together with lymphopenia. The present findings suggest that the effect of aflatoxins is more pronounced in broiler chicks, followed by mature broilers and layer chicks. Interestingly, the mortality was high in those age groups that yielded *C. psittaci*.

Discussion

Chlamydiosis in chicken has been reported from different parts of the world (ARZEY and ARZEY, 1990; HAFEZ and SIEGMAN, 1994; CHAHOTA et al., 1997a). A wide range of conditions exists that are conducive to mycotoxin formation. Where moisture content is more than 15% in feed and temperature is moderately high, *Aspergillus flavus* grows rapidly, producing aflatoxins in feed. Similar climatic conditions prevail in the months of July and August in Himachal Pradesh, especially in areas located at 1300 to 1500 meters above MSL, which where this outbreak occurred. Among chickens, aflatoxin reportedly increases susceptibility to different disease conditions, such as caecal coccidiosis (EDDS et al., 1973), salmonellosis (SMITH et al., 1969), infectious bursal disease (CHANG and HAMILTON, 1982). Earlier, MALKINSON et al. (1987) reported mixed infection of *C. psittaci* and fowl pox as well as *Haemophilus gallinarum* among chickens. However, the present report concerns a rare concurrent chlamydial infection coupled with aflatoxicosis, resulting in heavy mortality. In this outbreak, bacteria due to *E. coli, Enterococcus faecalis*, and some *Staphylococcus* spp. together with respiratory infection (owing to yeasts such as *Candida albicans*) might also have exacerbated losses. From this, it can be inferred that in aflatoxicosis, as well as other diseases, chlamydiosis can also flare up, and which, together with other commensal

microbes, can further compound the disease syndrome, especially among immunocompromised birds, thereby inflicting enormous economic losses.

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SAŽETAK

Istražena je pojava teške aflatoksikoze i klamidioze sa sekundarnom mikrobnom infekcijom u pilića iz organiziranih uzgoja peradi. Pomor je iznosio 65% u tovnih pilića u dobi 1 do 2 tjedna, 25% u pilenki u dobi 2 do 3 tjedna i 32% u tovljenika u dobi 8 do 15 tjedana. Uzorci su bili prikupljeni od 20 slabih ili uginulih životinja. Mješavina tkiva slezene, pluća, jetre i srca te crijevni sadržaj uzeti su u saharoza fosfat glutamatu za izdvajanje vrste *Chlamydia psittaci* u kokošjim zamecima u dobi 6 do 8 dana, a krv izvađena iz srca, bris dušnika i crijevni sadržaj za izdvajanje drugih mikroorganizama. Komadići tkiva jetre, crijeva, slezene, bubrega i pluća bili su uzeti za histopatološku pretragu. Uzorci hrane uzeti su za pretragu na prisutnost aflatoksina. *C. psittaci* je bila izdvojena iz 30% (6/20) pilića, a također su izdvojeni *E. coli, Enterococcus* spp., *Staphylococcus* spp., *Candida albicans* i dr. Razina aflatoksina u hrani iznosila je od 100 ppb do 500 ppb. Histopatološki su nađene promjene osebujne za aflatoksikozu i klamidiozu što upućuju na kompleksni sindrom.

Ključne riječi: aflatoksikoza, klamidioza, perad, mikroorganizmi, Himachal, Indija