CASE REPORT

Big liver and spleen disease in broiler breeders in Italy

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ABSTRACT

For the first time in Italy, we have reported two outbreaks resembling big liver and spleen disease in broiler breeder flocks. The combination of clinical signs and pathological findings and the laboratory investigation results appeared corrisponde to previously recorded outbreaks in other countries.

Key Words: Big liver and spleen disease, Broiler breeders, *Calicivirus*-like virus, Diagnosis.

RIASSUNTO

BIG LIVER AND SPLEEN DISEASE IN RIPRODUTTORI PESANTI IN ITALIA

Per la prima volta in Italia vengono descritti due casi riferibili alla sindrome denominata big liver and spleen disease *in gruppi di polli riproduttori pesanti. I segni clinici e le lesioni anatomo-patologiche osservate nonché i risultati delle indagini di laboratorio condotte sui gruppi colpiti appaiono sovrapponibili a quelle già descritte in passato in altri paesi.*

Parole chiave: *Pollo, Malattia del fegato e della milza grossi, Riproduttori pesanti,* Calicivirus*, Diagnosi.*

Introduction

Big liver and spleen disease (BLS) is an infectious, transmissible disease of uncertain, but probably viral, aetiology. It is characterized by sudden drop in egg production, increased mortality and enlargement of the liver and spleen of mature chickens, especially broiler breeders and, less commonly, egg layers. BLS was first recognized in Australia in 1980. The syndrome is considered economically important to the mayor integrated companies of broiler breeders in that country. Antibody has also been found in the United Kingdom and later in the USA. Moreover infection with the BLS agent has been associated with a condition known as "primary feather and drop syndrome" in the United States (Barnes, 1997). Until now there are no reports of this syndrome in Italy. This paper gives a report of the clinical, pathological and laboratory findings noted in two broiler breeder flocks affected with a disease related to BLS.

Material and methods

Carcasses and blood samples from two broiler breeder farms were submitted for diagnostic examination and laboratory investigation at the Istituto Zooprofilattico Sperimentale of Forlì. Anamnestic data were collected, post-mortem examinations were carried out on the carcasses and appropriate samples were taken for the following laboratory investigation.

Laboratory examinations

For bacteriological examinations samples of liver, spleen and ovary were inoculated on blood agar and Hektoen Enteric Agar and incubated aerobically at 37°C.

For virological examination, samples of liver, spleen, intestine, ovary, trachea, lung and brain were inoculated on Chicken Embryo Liver Cell Cultures (CEL) and Specific Pathogen Free (SPF) embryonated chicken eggs A Polymerase Chain Reaction (PCR) for group 2 avian adenovirus was also performed.

The following serological tests were performed on blood samples: 1) agar-gel diffusion precipitin (AGP) for group 1 avian adenovirus. 2) Enzyme-linked immunosorbent assay (ELISA) for group 2 avian adenovirus (Synbiotics®). 3) ELISA test for Human Epatitis E Virus (Nuclear Laser Medicine®).

For Electron Microscopy (EM) observation, samples of liver and spleen were frozen and thawed twice, the supernatant was harvested and centrifuged at 4,000 *g* for 20 min. and at 9300 *g* for 10 min. for clarification. The second supernatant (85 µl) was then ultracentrifuged in Airfuge Beckman for 15 min. at 21 psi (82,000 g). Grids were then stained using 2% sodium phosphotungstate (NaPt), pH 6.8, for 1.5 min., and observed with a TEM Philips CM10.

For histological examination, representative samples of spleen, liver, heart and kidney were fixed in 10% buffered formalin, dehydrated in ethanol, cleared in xylene and embedded in paraffin, according to standard techniques. Thin sections (4µm) were cut and stained with haematoxylin-eosin.

For genetic identification of avian HEV, reverse transcription polymerase chain reaction (RT-PCR) was run on liver homogenate sample that has been collected from a chicken which has been resulted positive by electron microscopy (EM). Viral RNA was extracted with guanidine/isothiocyanate technology (NucleoSpin RNA II, GTC/silice); total RNA was reverse-trancribed with avian HEV-specific primers FAHEVEpF/RAHEVEpR (Huang *et al.*, 2004) targeting ORF2 region of HEV genome (fragment lenght 372 bp). The parameters for each round of PCR included denaturation at 95°C for 6 min, followed by 35 cycles of denaturation for 1 min at 94°C, annealing for 50 sec at 58°C and extension for 1 min at 72°C, with a final incubation at 72°C for 7 min. Negative control has been included. Finally, PCR product has been analysed by gel-electrophoresis.

Results and discussion

During January 2004, 37 week-old Ross 508 broiler breeders from a farm of 17,000 animals housed in three flocks experienced a slightly increased mortality (+0.2%) in two flocks and a 2% drop in egg production for three weeks. A large number of chickens had a drop of feathers (resembling premature moulting) that persisted for several weeks after recovery from the disease. In the same period a similar syndrome was observed in another farm of 44 week-old Ross 508 broiler breeders of 24,000 animals. At necropsy, all the dead birds were in good condition. The most frequently found lesion was an enlarged spleen. The liver was also enlarged and friable. Other lesions included: cyanosis of the head, hydropericardium, pulmonary congestion and oedema, enteritis, ovarian regression and congestion, swollen kidneys. Microscopically the following lesions were recognized: congestion and oedema of the liver associated with multiple foci of necrosis and an increase of perivascular lymphoid tissue; widespread necrosis of lymphoid tissue and proliferation of macrophages in the spleen; *adipositas cordi* in the heart.; congestion and interstitial infiltration of lymphoid tissue in the kidneys. Bacteriologically a strain of *Escherichia coli* was isolated from the liver. Virological examinations (i.e. embryo egg inoculation, and PCR for group 2 adenovirus) were negative. By negative staining Electron Microscopy few viral isolated particles morphologically resembling calicivirus were observed in the liver and spleen. EM-positive liver sample turned out to be positive for avian HEV by RT-PCR, showing a 372 bp fragment by gel-electrophoresis.

Serological tests demonstrated reacting antibodies with group 1 and group 2 avian adenovirus. Furthermore, 3 of 15 blood samples collected from the first observed outbreak were positive with ELISA test for human hepatitis E virus antibodies.

Conclusions

Clinical signs, macroscopic and microscopic lesions, age and production type of the affected flocks were sufficient for a presumptive diagnosis of BLS. Other diseases that produce splenomegaly were excluded. Seroconversion toward group 2 avian adenovirus wasn't associated to other features of the avian adenovirus group 2 splenomegaly chicken disease (AAS). *Escherichia coli* strain recovered from affected birds is a consequence of BLS, but it is not involved in causing the disease (Barnes, 1997). To confirm the diagnosis of BLS, the electron microscopy observation, of few isolatd calicivirus-like particles in the liver and spleen, was very interesting as well as the RT-PCR results, the histological lesions and the detection of antibodies reacting with human hepatitis E virus. Recently, in the United States, a new virus, designated avian hepatitis E virus (avian HEV) was identified and characterized from chickens with hepatitis-splenomegaly syndrome (HS), a disease very similar to BLS described in Australia (Haqshenas *et al.*, 2001). So far, it is likely that BLS in Australia and the HS syndrome in North America are caused by variant strains of the same virus (Haqshenas *et al.*, 2002). Avian HEV is genetically related to but distinct from human and swine HEV (Huang *et al.*, 2004). The antigenic cross reactivity among avian and human HEVs was confirmed by ELISA (Huang *et al.*, 2002). HEVs (including human, swine and avian strains) were formerly classified as a member of the family *Caliciviridae*. But the lack of common features between HEVs and caliciviruses has led to the recent removal of HEV from this family (Berke *et al.*, 2000). In conclusion field and laboratory data reported in this work lead us to confirm the diagnosis of BLS in the broiler breeder flocks observed.

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