

An unusual outbreak of inclusion body hepatitis on a broiler chicken farm: a case report

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ABSTRACT: This study investigated an outbreak of inclusion body hepatitis in ROSS 308 hybrid broiler type chickens between 19 and 25 days of fattening. For this purpose, clinical observation, ELISA fowl adenovirus and chicken anaemia virus antibody detection in serum at 21 and 42 days, mortality evaluation, epidemiological analysis, histology and genetic identification were performed. The six flocks of the farm consisted of 90,000 chickens. Only one flock of 15,000 chickens was affected on this farm. At 19 days of age, ill chickens showed clinical signs of depression, anorexia, somnolence, ruffled feathers, anaemic comb and wattles and occasionally nervous signs. Based on ELISA titres, the antibody response to fowl adenovirus increased greatly from 21 to 42 days. The antibody response to vaccination against infectious bursal disease virus and chicken anaemia virus was at the expected level in all broiler flocks. Necropsy showed diffuse petechial and ecchymotic haemorrhages in skeletal muscles, liver, pancreas, kidney, together with hepatomegaly, splenomegaly and catarrhal enteritis. Histologically, fatty liver degeneration, multifocal liver necrosis and intranuclear inclusions in hepatocytes, as well as focal necrosis in pancreas and spleen parenchyma were seen. The DNA of AAV-1 (avian adenovirus group 1) was detected using the PCR method in paraffin-embedded liver samples. The results revealed no association of inclusion body hepatitis with infectious bursal disease virus or chicken anaemia virus infection, and suggested primary disease. However, the involvement of only one chicken flock on the farm remains unexplained.

Keywords: poultry; fowl adenovirus; infectious bursal disease virus; chicken anaemia virus; ELISA; PCR; inclusion bodies

Fowl adenoviruses have a worldwide distribution and are reported to be frequently isolated from healthy chickens as well as affected birds (McFerran and Smyth 2000). Clinical presentation can be observed in the form of inclusion body hepatitis, respiratory tract disease, hydropericardium syndrome and gizzard erosion (Ono et al. 2001; Gomis et al. 2006).

Inclusion body hepatitis is caused by fowl adenoviruses of the genus *Aviadenovirus* – group 1 avian adenoviruses (Harrach et al. 2011). Twelve serotypes can be distinguished within fowl adenovirus A–E. Fowl adenovirus causing inclusion body hepatitis (IBH) are predominantly typed as fowl adenovirus D or E (Sun et al. 2004; Ojkic et al. 2008; Marek et al. 2010).

Supported by the Grant Agency for Science of the Slovak Republic VEGA (Grants No. 1/0483/15, 1/0562/16, APVV-0302-11 and APVV-15-0165), by the INFEKTZOON – the Centre of Excellence for Infections of Animals and Zoonoses (ITMS code 26220120002) and the Research and Development operational program financed by the European Regional Development Fund.

The worldwide distribution of the disease illustrates its increasing incidence in many poultry-producing areas (McFerran and Smyth 2000). Historically, inclusion body hepatitis is mostly considered a secondary disease in broilers, associated with immunosuppression due to viral diseases such as infectious bursal disease virus or chicken anaemia virus (Rosenberger et al. 1974) or to environmental factors (Hoerr 2010). However, there is evidence to suggest that avian adenoviruses may be primary pathogens in IBH (Gomis et al. 2006).

The aim of this study was to investigate an outbreak of IBH in broiler chickens between 19 and 25 days of fattening, which lasted for 42 days in total.

Case description

Case history. Fifteen thousand one-day-old chickens of the ROSS 308 hybrid broiler type (from 42-week-old parents) with an average weight of 43 g were kept together in one flock. The stocking rate amounted to 16 chickens/m² (0.69 kg/m²) and on day 19 the net weight was 13.95 kg/m². Bedding for flocks consisted of straw pellets. Bacteriological examination for intestinal presence of *E. coli* was performed at the establishment of the flock. Mycotic examination was also performed and yielded negative results. The chickens' diet was free of animal protein, but contained anticoccidials (Maxiban – starter, grower I, Elancoban – grower II, finisher I). The concentrations of ammonia and CO₂ were measured on the 3rd day (NH₄ – 10 ppm, CO₂ – 2680 ppm), 10th day (NH₄ – 14 ppm, CO₂ – 1820 ppm), and on the 18th day of age (NH₄ – 16 ppm, CO₂ – 1250 ppm) in the flock with inclusion body hepatitis.

Vaccination protocol. During incubation on the 18th day, *in ovo* vaccination was performed against infectious bursal disease virus (strain Winterfield 2512, Cevac Transmune lyophilized). In the hatchery on the 1st day of life, vaccination by aerosol against Newcastle disease (strain PHY.LMV 42, Cevac Vitabron L lyophilised) and infectious bronchitis (variant strain H120+1/96, Cevac I Bird lyophilised) was performed. All vaccines were made in Hungary (Ceva-Phylaxia Co. Ltd., Budapest).

Clinical examination. Clinical signs were observed only a few hours before death and included anaemic combs and wattles, apathy, somnolence, depression, crouched position with droopy head, fuzzy feathers and sporadic nervous signs.

Table 1. Mortality – the number of dead chickens between 19 and 25 days of life

	Age (days)						
	19	20	21	22	23	24	25
No. of chickens	5	35	86	125	66	18	6

A trouble-free course of feeding was observed up to the end of the 3rd week, when mortality was suddenly observed with a peak of clinical signs on the 3rd day and regression after the 4th day (Table 1). Total morbidity between 20 and 24 days of life was 2.23% (341 chickens). The morbidity in the flock was comparatively low, but a high rate of chicken mortality resulting from dehydration and exhaustion was observed. Surviving chickens showed slightly below-average growth by the end of feeding.

Pathology. Autopsies of dead chickens showed petechial haemorrhages to the ecchymoses in skeletal muscles, more visible in the legs than the breasts, in about 20% of birds. Hepatomegaly with pale brownish-to-yellowish colour and fragile consistency was observed, which, macroscopically, revealed parenchymatous and fatty degeneration and diffuse petechial haemorrhages in 100% of autopsied chickens (Figure 1). Visible miliary necrotic foci were spread throughout the pancreas. The spleen exhibited splenomegaly and acute diffuse catarrhal enteritis was observed in small intestine. The kidneys were oedematous with petechial haemorrhages.

Histology. Samples of liver, spleen and pancreas were fixed in 10% neutral buffered formalin, processed using a routine histological procedure and stained with haematoxylin and eosin. The liver samples, which exhibited intensive steatosis, showed



Figure 1. Hepatomegaly, fatty degeneration and haemorrhages in liver

doi: 10.17221/95/2017-VETMED

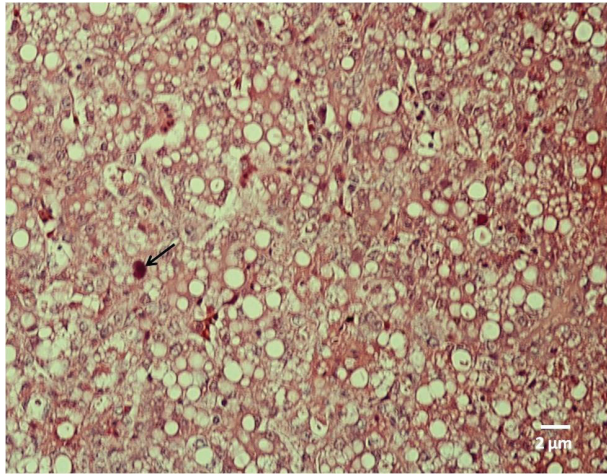


Figure 2. Fatty degeneration and basophilic intranuclear inclusions (arrow) in liver (H&E, bar 2 µm)

the presence of two types of intranuclear inclusions: basophilic, filling almost the whole nucleus (Figure 2), and eosinophilic, with a halo zone at the periphery. Similar intranuclear inclusions were observed also in the pancreas. Moreover, inflammatory mononuclear infiltrations were scattered across this organ (Figure 3).

Serology. An indirect ELISA test for aviadenovirus (Fowl Adenovirus group 1 Antibody test kit, BioChek Netherlands) was used for serological monitoring at day 21 of life to compare the titres in chickens without and with clinical signs. This detection of non-specific serotype common group antigen included 12 serotypes. The ELISA test at the end of feeding on day 42 confirmed the seropositivity (≥ 1071) to aviadenovirus (Table 2).

Serological monitoring of infectious anaemia virus in the breeding flock was also performed using

Table 2. ELISA titres to fowl adenovirus (FAdV) and infectious anaemia virus (CAV) in chickens on day 21 (1–5 without and 6–10 with clinical signs) and day 42 of life

No. of chickens	FAdV		CAV	
	day 21	day 42	day 21	day 42
1	1 : 85	1 : 13577	1 : 68	1 : 845
2	1 : 63	1 : 13091	1 : 56	1 : 1128
3	1 : 36	1 : 13583	1 : 64	1 : 1034
4	1 : 41	1 : 13026	1 : 38	1 : 931
5	1 : 28	1 : 2989	1 : 56	1 : 1278
6	1 : 2	1 : 9169	1 : 38	1 : 1273
7	1 : 41	1 : 1558	1 : 133	1 : 638
8	1 : 26	1 : 2775	1 : 147	1 : 733
9	1 : 333	1 : 11986	1 : 54	1 : 850
10	1 : 856	1 : 14665	1 : 64	1 : 887

the indirect ELISA test at days 21 and 42 of life (seropositivity ≥ 725) as a result of possible predisposition (Chicken Anemia Virus Antibody test kit, BioChek Netherlands). The titres are summarised in Table 2.

The ELISA test for detection of antibodies against infectious bursa disease virus showed titres ranging from 1 : 3015 to 1 : 8966 at the end of the fattening period.

PCR. PCR was performed on DNA isolated from paraffin-embedded liver specimens from infected chickens using the method described by Kolesarova et al. (2012). PCR amplification using primers specific for avian adenovirus group 1 – AAV-1 (Caterina et al. 2004) resulted in detection of a single band of approximately 421 bp (Figure 4).

DISCUSSION AND CONCLUSIONS

Inclusion body hepatitis, as a disease resulting from the immunosuppression caused by viral diseases such as infectious bursal disease virus or chicken anaemia virus, was diagnosed mainly towards the end of the last century (Howell et al. 1970; Rosenberger et al. 1974). Later, several reports described IBH as a primary disease without the association of infectious bursal disease virus or chicken anaemia virus (Gomis et al. 2006; Nakamura et al. 2011). The fact that only one flock in our case exhibited clinical signs of IBH prompted us to examine the possible association of IBH with

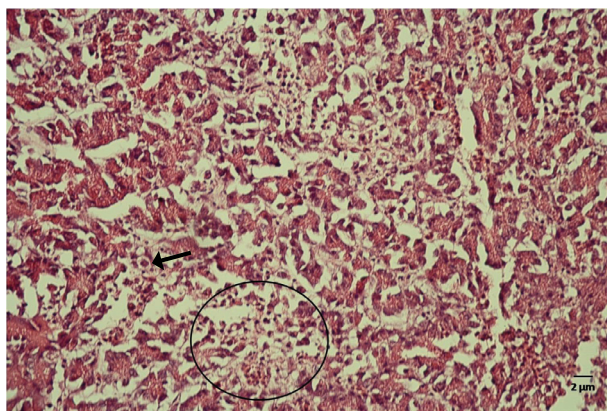


Figure 3. Eosinophilic inclusion body with halo zone at the periphery (arrow) and mononuclear infiltrate in pancreas (H&E, bar 2 µm)



Figure 4. PCR analysis of paraffin-embedded liver samples reveals positivity to AAV-1

infectious bursal disease virus and chicken anaemia virus.

The ELISA test showed that vaccination against infectious bursal disease virus and chicken anaemia virus in our flocks was able to control the immunosuppression. The PCR method demonstrated that the IBH outbreak was initiated by fowl adenovirus group 1. However, fowl adenovirus group 1 is ubiquitous in both healthy and sick poultry flocks (Adair and Fitzgerald 2008). These viruses can act as pathogens causing IBH for reasons which are not completely known. The virus appears to spread via the faecal-oral transmission route, and faeces therefore most likely served as the main source for virus spread within the flock (Yugo et al. 2016). Similarly, the infection of only one of the six flocks on the monitored farm suggests horizontal transmission of the virus. On the other hand, why the neighbouring flocks remained uninfected remains unknown. Ammonia and CO₂ concentrations were low in the environment of the inclusion body hepatitis-infected flock. The nutritional requirements in feed for growth and normal lymphoid organ development were the same for all six flocks kept on the farm.

The mortality rate for chickens in the evaluated flock corresponded to the mortality of chickens in flocks infected with IBH virus. However, Nakamura et al. (2011) observed mortality rates ranging from 1.2% to 17.0% in affected flocks in Japan.

In conclusion, the serological data, PCR analysis, mortality analysis, histological evaluation and epidemiological investigation suggest that IBH can develop as a primary disease. There is currently no vaccine against IBH, and for that reason no specific therapy was used. For prevention of secondary bacterial infection doxycycline and colistin were administered during the first four days. A preparation based on silymarin, L-carnitine and B group vitamins together with amino acids was used as a hepatoprotectant.

Acknowledgement

We thank Andrew Billingham for English language correction.

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doi: 10.17221/95/2017-VETMED

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Received: July 9, 2017

Accepted after corrections: September 15, 2017