Histological and histochemical investigations on hepatic lipidosis in turkeys

Histologische und biochemische Untersuchungen zur Fettleber bei Puten

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Introduction

Some hepatic disorders characterized by fatty change can affect avian species: e.g. fatty liver hemorrhagic syndrome and fatty liver and kidney syndrome are widely investigated hepatoses affecting chickens (LEESEN et al., 1995; HOERR, 1996; CRESPO and SHIVAPRASAD, 2003). On the contrary, in turkeys there is one hepatopathy known as hepatic lipidosis typically characterized by locally extensive areas of hepatocellular lipid accumulation and which has been poorly investigated by pathologists. The cause of this pathological condition is uncertain and only few data exist in literature. GAZDZINSKI et al. (1994) describe this hepatopathy in turkey breeder hens of 12 to 24 weeks of age and speculate about a possible role of nutritional restrictions in the onset of hepatic lipidosis. More recently, GALLAZZI et al. (2007) and AZIZ (2008) pointed out that hepatic lipidosis affects also meat type turkeys whose production is incompatible with diet restrictions. Therefore, the exact cause is still to be clarified and genetic components or exposure to a toxin has to be considered for the pathogenesis (AZIZ, 2008). From a morphopathological point of view, a comparison of hepatic lipidosis with the aforementioned avian hepatoses or other hepatopathies described in mammals could help widening or reducing the range of pathogenetical hypotheses. Characterization of the lipid classes involved in hepatic lipidosis is still lacking. Matter-of-factly, lipidosis is defined as the accumulation of triglycerides, neutral fats and cholesterol (MYERS and MCGAVIN, 2007) and the routine histology cannot differentiate it from phospholipidosis. Accumulation of phosphorylated lipids occurs following administration of Cationic Amphiphilic Drugs (CAD) including gentamicin which is commonly used in poultry production though its toxicity is usually limited to the kidneys (REASOR and KACEW, 2001). Immunohistochemistry could help in assessing the lipid class involved in hepatic lipidosis as recently described in rats (OBERT et al., 2007). Moreover, similarities with Wilson's disease, a copper storage disorder whose features include liver fatty change (MYERS and MCGAVIN, 2007), should be considered as copper salts are empirically recommended for the treatment of candidiasis and prevention of aortic rupture in turkey breeding.

This study describes peculiar cases of hepatic lipidosis and the results of histochemical and immunohistochemical approaches aimed at better detailing this poorly characterized condition.

Materials and Methods

Cases

Over a period of 6 years liver samples or dead birds from 8 cases of suspected hepatic lipidosis were submitted to our laboratory for diagnosis. Material details are reported in Table 1. The birds of 7 cases belonged to two different turkey lines of two different companies. Such information was unavailable for case No. 3. Five to 15 formalin fixed livers were examined for each case.

Post mortem examination

The birds of case No. 4 were submitted for necropsy. Samples of liver from each bird were collected and fixed in 10% buffered formalin.

Histopathology and histochemistry

Formalin fixed samples were routinely embedded in paraffin blocks, sectioned at 4 µm and stained with haematoxylin and eosin for microscopic examination, with James's stain for reticulin fibres (JAMES, 1967) and with rodanine stain for copper (JOHNSON, 1992). Sections of liver from Long-Evans Cinnamon (LEC) rats were included as positive control for rodanine stain.

Immunohistochemistry

Four µm thin sections from liver samples were air-dried and stained using the avidin-biotin complex immunoperoxidase procedure (HSU et al., 1981). Slides underwent deparaffinization, hydration, heat induced epitope retrieval (Sodium citrate at pH 6) in microwave (75% power, 5 min, twice). Then, after normal goat serum was added the slides were incubated at room temperature for 30 minutes.
After washing, the slides were incubated for 20 minutes with Endogenous Biotin Blocking Kit reagent (DAKO, Italy) at room temperature. After rewashing guinea pig anti-adipophilin antibody (Progen, Germany) at a dilution of 1:400 was added and the slides were stored overnight at 4°C. Biotinylated goat anti-guinea pig antibody was used as secondary antibody. Finally 3-amino-9- ethylcarbazol (AEC) (DAKO, Italy) was used as substrate and the sections were counterstained with Mayer's haematoxylin, dehydrated through graded alcohols, cleared in xylene, and mounted with Permount. Liver sections from healthy turkeys were included as negative controls. Sections of lactating mammary glands from mice were included as positive controls.

Results

To describe case history obtained data on mortality are shown in Table 1. In all cases livers appeared enlarged and mottled by pale yellow areas which are intermingling with haemorrhages (Figure 1). In addition, in the flock of case No. 4, both dead and euthanized poults were necropsied but only the dead ones showed hepatic lipidosis. In euthanized birds from the same flock neither gross or microscopic lesions were found in the liver. Virological investigations were also tentatively carried out for the same case with negative results (data not shown).

Microscopic features observed in young breeder hens from cases No. 1 and 2 and in commercial toms of case No. 3 overlapped the findings of cases No. 4 to No. 8 regarding commercial poults up to 26-day old.

The association between hepatocyte fatty change, hepatic cellular necrosis and haemorrhages was constant. The fatty change was characterized by numerous, fine to large intracytoplasmic vacuoles distorting the hepatic cords. Random individual hepatocellular necrosis was associated with haemorrhages which were medium-sized to large and predominantly centred on central veins. Mild to moderate biliary hyperplasia and few, scattered lymphocytes were occasionally detected.

In all cases, the reticular framework was thoroughly maintained as confirmed by James’s stain (Figure 2). Rostenine stain excluded accumulation of copper-containing granules in the liver sections from older birds (cases No. 1 to No. 3) and in a selection of liver samples from poults (cases No. 4 to No. 5).

Discussion

Hepatic lipidosis is an occasional disorder first described by Gazdzinski et al. (1994) in turkeys breeder hens. These birds are normally fed low protein diets, and such alimentary restrictions could lead to amino acids deficiency (particularly methionine and cysteine) which are necessary for the synthesis of the lipoproteins. This hypothesis fails to explain hepatic lipidosis affecting commercial turkeys which are fed without dietary restrictions. More recently, a nutritional approach to hepatic lipidosis was discussed also by Hazell (2009) who suggests deficiency of carnitine or of its precursor amino acids (lysine and methionine) as a likely (but still unproved) cause of accumulation of fatty acids in the hepatocytes. Moreover, five out of the 8 outbreaks of our caseload affected meat-type poults with an age of onset ranging from 5 to 26 days (Table 1). There-
fore, the understanding of hepatic lipidosis requires a more comprehensive re-evaluation. It seems noteworthy to underline that the very name of hepatic lipidosis is insufficient to define this hepatopathy. Among avian species the typical example of liver lipidosis occurs naturally in migratory palmedes during premigration and it is characterized by the sole accumulation of lipid droplets in the hepatocytes. Seemingly, the hepatic disorder in turkeys comprises additional features as haemorrhages and hepatocellular necrosis with uneven distribution of lipid accumulation.

Virological investigations were also tentatively carried out for the same case with negative results (data not shown). The affected flocks belonged to the two prevalent turkey breeds in Italy (ignoring case No. 3 for lack of information). Therefore, it is not possible to argue a genetic hypothesis of abnormal copper storage in hepatic lipidosis pathogenesis can be ruled out for those cases occurring in the first weeks. Anyway, negative rodanine stain excluded abnormal copper storage in all the tested cases.

Conclusions

Here we described 8 cases of hepatic lipidosis occurred over 6 years. Most cases were reported in poults of both sexes within the first month of age (Table 1). This contrasts with the age of occurrence as reported by GAZDZINSKI et al. (1994). Poults dead with hepatic lipidosis were in optimal body condition and mortality due to hepatic lipidosis was very transient in the flocks. No sequel related to hepatic lipidosis.
lipidosis was reported in the following growing phases of the affected flocks. Moreover, case No. 3 was unexpectedly observed at slaughterhouse in apparently healthy toms. Consequently, it is possible to speculate that hepatic lipidosis can be overlooked and underestimated occurring more frequently than reported. As for the involvement of high environmental temperatures in the pathogenesis of hepatic lipidosis hypothesized by Gazdzinski et al. (1994), 6 out of 8 tested cases in the present investigation occurred in summertime. Whether its cause is still to be clarified, it is at least helpful to restrict the range of hypotheses. A better comprehension of hepatic lipidosis could take in account the peculiarities of lipid metabolism in turkeys. Among poultry turkey is recognised as particularly lean with low lipogenic capacity (Kouba et al., 1992). Its plasma lipoprotein profile differs markedly from that found in other avian species with a lower triglyceride content in all lipoprotein classes (Mossab et al., 2001) and its response to dietary fats shows aspects that remain to be explained (Mossab et al., 2002).

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Summary

This study describes peculiar cases of hepatic lipidosis in turkeys and the results of histochemical and immunohistochemical approaches aimed at better detailing this condition. Hepatic lipidosis of the turkey is a syndrome described in turkey and characterized by enlarged livers mottled by pale yellow areas. Histologically, the main feature is fatty degeneration associated with necrosis and haemorrhage. The cause of hepatic lipidosis is uncertain although nutritional and metabolic factors are suspected. Over a period of 6 years, we observed 8 cases of a hepatopathy with gross and microscopic changes consistent with hepatic lipidosis. Two of these cases affected turkey breeder hens of 12th and 21st week of age. Interestingly, another case was observed at slaughterhouse in 20-week old commercial male turkeys, whereas 5 cases involved commercial pouls of 5 to 26 days. Histochemistry revealed the maintenance of the reticulin framework in hepatic lipidosis and excluded a disorder in copper metabolism. Positive adipophilin immunohistochemistry confirmed hepatic lipidosis is characterized by nonphosphorylated lipid accumulations within hepatocytes. Current and alternative pathogenetic hypotheses for hepatic lipidosis are discussed on the basis of these results.

Key words

Turkey, hepatic lipidosis, adipophilin, rodanine, reticulum

Zusammenfassung

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Stichwörter

Pute, Fettleber, Adipophilin, Rodanin, Reticulum

References


