Onderstepoort J. vet. Res., 54, 635–640 (1987)

PATHOLOGICAL FINDINGS IN A NATURAL OUTBREAK OF AFLATOXICOSIS IN DOGS

STELLA S. BASTIANELLO, J. W. NESBIT, M. C. WILLIAMS and A. LUCIA LANGE, Department of Pathology, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, Onderstepoort 0110

ABSTRACT

BASTIANELLO, STELLA S., NESBIT, J. W., WILLIAMS, M. C., LANGE, A. LUCIA, 1987. Pathological findings in a natural outbreak of aflatoxicosis in dogs. *Onderstepoort Journal of Veterinary Research.* 54, 635–640 (1987)

The gross and histopathological lesions of 10 cases in a natural outbreak of aflatoxicosis amongst dogs in the Republic of South Africa are reported.

The 10 cases were classified as acute (1 case), subacute (7 cases) and chronic (2 cases) on the basis of the nature, degree and extent of the following histopathological feactures: hepatocellular fatty degeneration, necrosis or regeneration; proliferation of bile ductules; accumulation of bile within the canaliculi; fibroplasia; and, mucoid degeneration, necrosis or segmental atrophy of the larger intrahepatic bile ducts.

Fatty degeneration was noted grossly in the livers of all 10 cases and bile stasis in 4. Varying degrees of fibrosis were present depending on the stage of the disease. In the 2 chronic cases in which nodular regeneration was also observed fibrosis was pronounced. Other macroscopic findings included icterus, anaemia, ascites, hydrothorax, hydropericardium, anasarca, pulmonary oedema, gastro-enterorrhagia and nephrosis.

INTRODUCTION

The first account of the pathology occurring in dogs as a result of aflatoxicosis was reported from the United States of America (USA) in 1952 (Seibold & Bailey, 1952). At that time the aetiology was unkown and the disease was referred to as hepatitis X. The disease, as reported by these authors, occurred in the form of an outbreak in which most affected dogs were icteric, listless and anorexic and many showed petechiae on the gums, melaena, epistaxis and/or haematemesis. Clinical signs were usually present for a period varying from 1–14 days after which death occurred suddenly (Chaffee, Edds, Himes & Neal, 1969). Some dogs died without developing clinical signs. The following macroscopic findings were reported in 1 or more cases: hepatopathy, icterus, emaciation, ascites, widespread petechiae and ecchymoses, and enterorrhagia. The liver showed either yellow, reddish-yellow or greenish-yellow discouloration, or was shrunken and tan in colour. Histologically they distinguished acute, subacute and chronic cases. In the subacute cases, diffuse hepatocellular fatty changes, hepatocellular necrosis and regeneration, fibrosis, bile ductule proliferation and bile stasis were present in the liver in association with a cholaemic nephrosis. Similar lesions were observed in the chronic cases but were more extensive.

An outbreak of disease similar to hepatitis X occurred amongst dogs during March, April and May, 1984 in Pretoria. Several dogs died suddenly or following a short clinical course. Subsequent investigations indicated that these dogs had all been fed the same commercial brand of dog food. Analysis of various batches of this food yielded c. 100–300 ppb (μg/kg) aflatoxin (T. W. Naude, personal communication, 1986). Most of the affected dogs were icteric and prior to death many exhibited a haemorrhagic diathesis, manifested clinically as haematochezia, epistaxis or petechiation of the visible mucous membranes. Some of the animals died suddenly without showing clinical signs.

The purpose of this report is to describe the macroscopic and histopathological findings in 10 dogs that died during the outbreak.

MATERIALS AND METHODS

Autopsies were performed on all 10 dogs submitted. Samples of liver and other tissues were selected and fixed in 10 % buffered formalin. Sections of these tissues were routinely processed and stained with haematoxylin and eosin (HE) for light microscopic examination. Selected sections of liver, kidney, spleen and lymph node were stained with the long Ziehl Neelsen (ZN) for early lipofuscin, Perls' Berlin blue (BB) for haemosiderin and Hall's bilirubin stains to determine the nature of the pigment granules within macrophages or the monocyte-macrophage systems of the lymphoid tissue and liver, or within the hepatocytes or renal tubular epithelial cells. Sections of the liver and gall-bladder were stained by the periodic acid-Schiff (PAS) method to verify the mucoid nature of the material within the epithelial cells or lumina of the gall-bladder or large intrahepatic bile ducts. Masson trichrome stain was applied to the liver of 1 of the chronic cases to determine the extent of collagenous deposition. Frozen sections of the liver of 1 of the subacute cases were stained with the Oil Red O (Fig. 12) and Sudan IV stains to determine the presence of neutral fats (Bancroft & Stevens, 1977; Pearse, 1985).

RESULTS

Of the 10 cases described in this report, 1 was regarded as acute, 7 as subacute and 2 as chronic. This classification was based on the hepatic histopathology (*vide infra*).

Macroscopic pathology

The majority of the macroscopic findings described below pertain to all 10 cases irrespective of their histopathological characterization.

Moderate to severe icterus was present in 9 cases and anaemia in 7, six of which showed concomitant icterus. Oedema, manifested by ascites, hydrothorax, hydropericardium, anasarca, pulmonary oedema and/or lymph node oedema was observed in 7 dogs. The ascites was moderate to severe and occurred in 6 cases, hydrothorax mild to moderate in 5, hydropericardium mild in 3 and anasarca mild to moderate in 6. Ascites and hydrothorax were present in both chronic cases and anasarca in 1 of them. Oedema, congestion and occasional petechiae occurred in the lymph nodes of 7 dogs. Mild to moderate pulmonary congestion and oedema were present in 6 cases, one of which also revealed pulmonary petechiation. Petechiae and ecchymoses also occurred in the endocardium or epicardium in 3 cases and in the conjunctivae in 1.

The most striking pathology involved the liver. In all 10 cases the liver was either normal in size or slightly enlarged. In 2 cases the surface of the liver had a coarse, nodular appearance. Yellow discoloration and a fatty

Received 28 June 1987—Editor

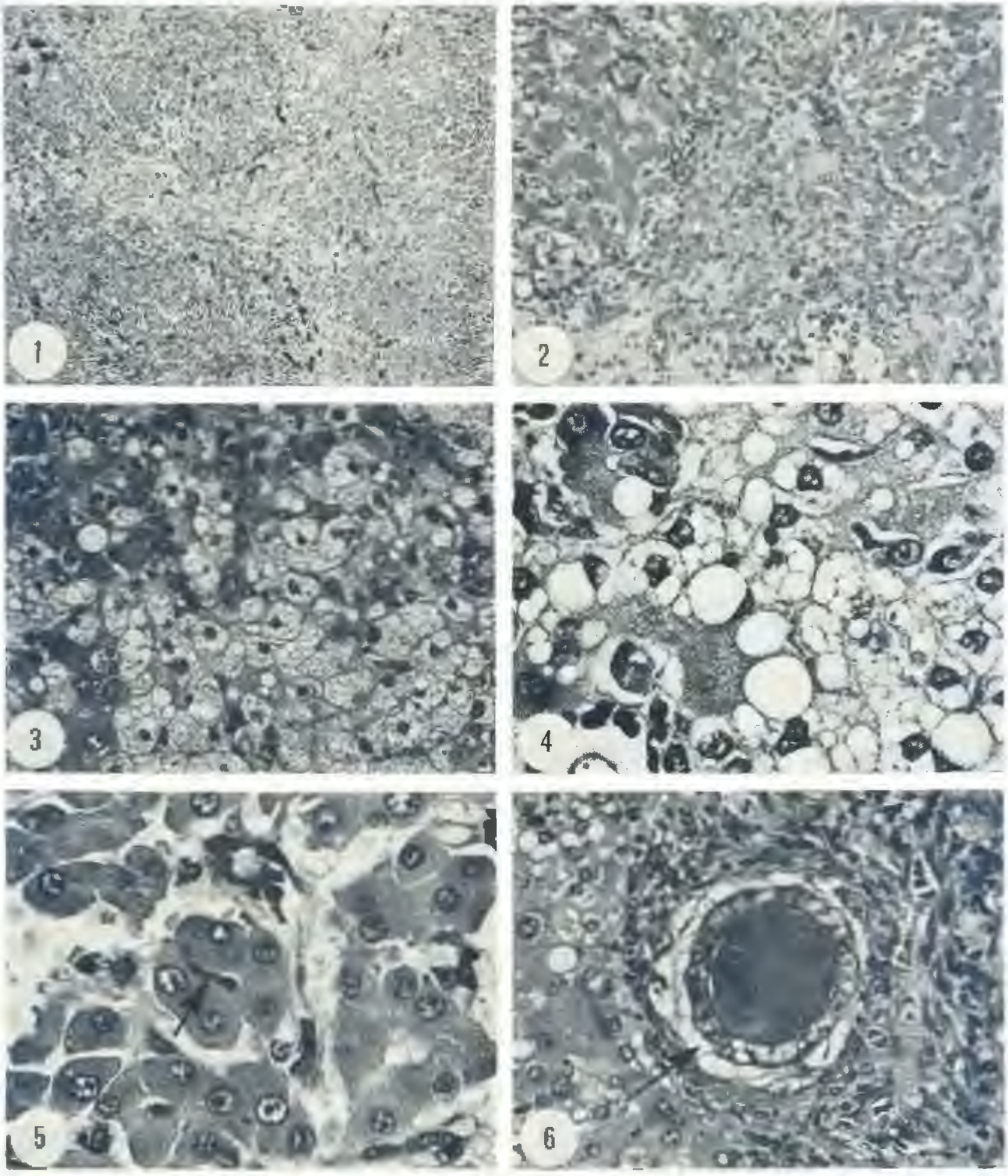


FIG. 1 Subacute aflatoxicosis. Architectural disruption dominated by portal fibrosis resulting in isolation of the hepatic lobules HE × 40

FIG. 2 Subacute aflatoxicosis. Proliferating bile ductules embedded in tracts of fibrous tissue HE × 100

FIG. 3 Subacute aflatoxicosis. Early hepatocellular fatty changes characterised by foamy appearance of the cytoplasm HE × 200

FIG. 4 Subacute aflatoxicosis. Advanced hepatocellular fatty changes characterised by the presence of several vacuoles or a single large vacuole HE × 400

FIG. 5 Subactute aflatoxicosis. Distension of bile canaliculi with accumulated bile (arrow) HE × 400

FIG. 6 Subacute aflatoxisis. Plugged intrahepatic bile duct. Note the debris within the lumen, vacuolar degeneration of the epithelial cells, periductal oedema (arrow), lymphocytic infiltration and fibrosis HE × 200

feel were evidences of the presence of moderate to severe fatty changes in all the livers. In the acute case and subacute cases, the colour of the liver was dull yellow or khaki, whilst in chronic cases it was yellowish-brown. Bile stasis was visible as fine green streaks traversing the parenchyma in 4 of the formalin-fixed livers. The consistency of the liver was increased in 3 cases, including the 2 chronic cases. On the surface of the latter 2 livers, fibrous streaks could be seen coursing throughout the substance. In 1 dog, the mucosa of the gall-bladder was hyperaemic and oedematous. The bile was either viscous or inspissated in nature.

Severe mucosal congestion and oedema of the gastrointestinal tract leading to a moderate to severe gastrorrhagia and/or enterorrhagia was present in 6 cases.

The kidneys were consistently affected and showed a mild to moderate nephrosis. In 9 cases, this nephrosis was regarded to be cholaemic in nature, as the renal cortices and/or medulla were yellow/green to khaki in colour.

Atrophy of the lymph nodes was noted in 1 dog. The spleen was moderately enlarged in 7 cases, 4 of these being due to red pulp hyperplasia and the other 3 as a result of congestion. Hyperplasia of the femoral diaphysial bone marrow was observed in 3 dogs.

Histopathology of the liver

The histopathology of the acute, subacute and chronic cases is described separately. The histopathological classification into acute, subacute or chronic cases was based on the presence, absence or relative degree of the following hepatic features; hepatocellular fatty degeneration, necrosis or regeneration; bile ductule proliferation; accumulation of bile within the canaliculi; fibroplasia; and, mucoid degeneration, necrosis or segmental atrophy of the larger intrahepatic bile ducts.

For ease of description the hepatic lesions are described as those pertaining to: the architecture, the hepatocytes, the biliary system and nature of any pigments present, the vascular system and inflammatory or fibrous reactions.

Acute case

The lobulation was distinct and there was no architectural disruption. Mild dilatation of the centrilobular sinusoids was present.

The hepatocytes revealed severe fatty degeneration particularly around the central veins and extending as wide radiating bands to the portal areas. These fatty changes varied from numerous small vacuoles within the hepatocyte cytoplasm, giving the cells a foamy appearance (Fig. 3) through intermediate stages to cells containing a single large vacuole. The latter cells had a pycnotic nucleus which was displaced to the side and resembled mature adipocytes (Fig. 4). Several cells in the vicinity of the central veins exhibited either coagulative or lytic necrosis.

The canaliculi, especially in the centrilobular area, were distended with bile. Fine to globular, golden-brown pigment granules were present in a samll number of Kupffer cells. Some of the epithelial cells of the larger calibre intrahepatic bile ducts revealed a vacuolar degeneration.

There was a mild congestion of the central and portal veins as well as of the hepatic sinusoids. These vessels also revealed a mild leukostasis involving mostly monocytes and, to a lesser extent, neutrophils.

A mild inflammatory response characterized by moderate infiltration of round cells in the portal areas and around the central veins was present. These included lymphocytes, plasma cells and macrophages. Many of the macrophages contained golden-brown pigment granules. This pigment stained intensely with the long ZN stain and mildly with the Hall's bilirubin and Perls' BB stains.

Subacute cases

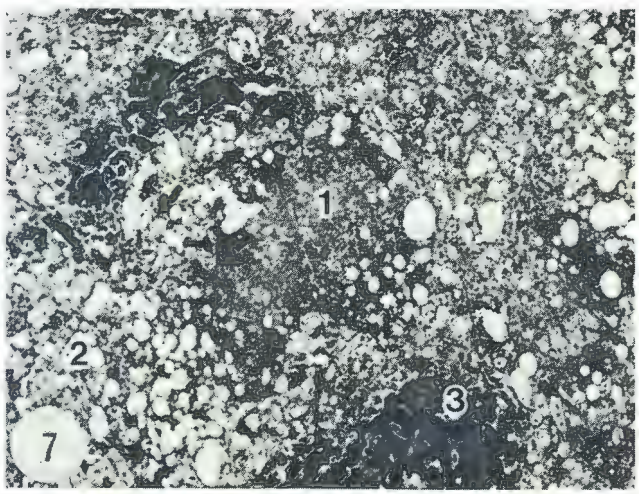
The architecture was either normal or showed various degrees of disruption in the different cases. In mild cases, the lobulation was indistinct, but the portal triads and central veins were still discernible. In intermediate cases, the central veins could not be seen and appeared to be replaced by dilated sinusoids. The most severe cases showed a haphazard arrangement of the hepatocytes resulting in dilatation of some sinusoids and obliteration of others. In the intermediate and severe cases, portal fibrosis of varying extent was evident. In many instances the fibrous tissue extended from one portal tract to the next, leading to encirclement and isolation of the hepatic lobules (Fig. 1).

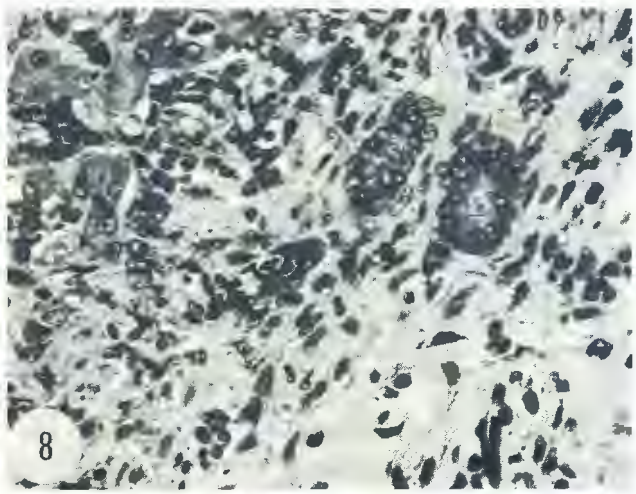
Hepatocellular fatty degeneration was extensive in all 7 cases. The affected hepatocytes had either a foamy (Fig. 3) or adipocytic (Fig. 4) appearance, as was described for the acute case. The fatty changes were evident throughout the lobule but in 3 cases there was a narrow rim of unaffected hepatocytes around the central veins. In the vicinity of the portal triads, the hepatocytes were present as 3 distinct types in various proportions; relatively normal eosinophilic cells, cells in various stages of fatty degeneration and relatively basophilic cells, the latter being representative of regenerating hepatocytes. Many of the regenerative cells were bizarre in shape with prominent nucleoli and revealed anisonucleosis, binucleation or multinucleation (Fig. 10). Mitotic figures were common. In some instances these cells were aggregated together in the form of regenerating nodules (Fig. 7 & 9), whilst in other instances they gave the impression of forming acinar or duct-like structures (Fig. 10). In 2 cases, foci of coagulative or lytic necrosis, involving 20–50 hepatocytes and surrounded by a layer of neutrophils, were disseminated throughout the lobules (Fig. 7). Scattered single hepatocytes undergoing coagulative or lytic necrosis were seen in the other subacute cases.

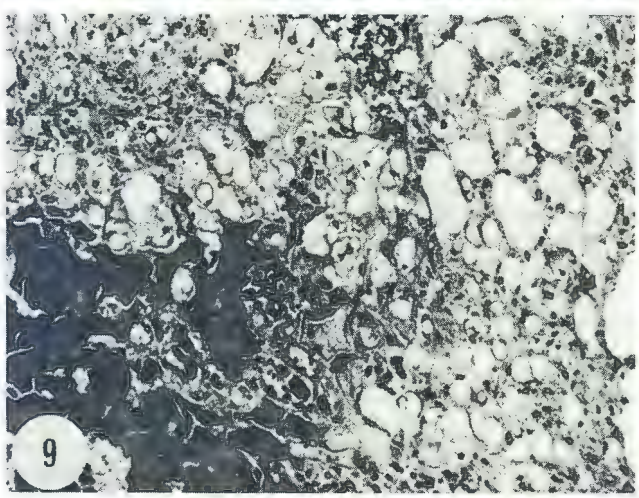
Bile ductule proliferation was a prominent feature in all subacute cases (Fig. 2). In 4 livers, this proliferation was very marked and extended right across the lobule forming bridges between adjacent portal triads. The bile ductule proliferation was associated with a mild, moderate or severe degree of fibrosis (Fig. 8) and intermingled with these ductules there were hepatocytes of the 3 types described above. The majority of the bile ducts within the portal triads had disappeared and been replaced by proliferating bile ductules. In all 7 cases, the larger calibre intrahepatic bile ducts persisted and showed one or more of the following changes: a vacuolar, PAS-positive degeneration of the epithelial cells; necrosis of the mucous membrane with desquamation and erosions; segmental atrophy of the epithelium; the presence of mucoid PAS-positive or proteinaceous necrotic debris within the lumina and, occasionally, an associated periductal oedema and lymphocytic infiltration (Fig. 6). The single gall-bladder examined revealed the same lesions, except that ulcers instead of erosions were present.

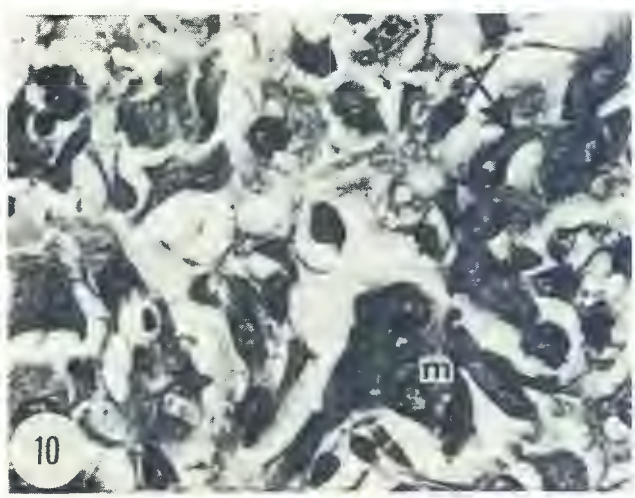
An accumulation of bile was evident within the canaliculi in all 7 cases (Fig. 5). In 5 dogs, this was particularly prominent in the periportal areas, whilst in the other 2 cases, it was widespread throughout the lobule.

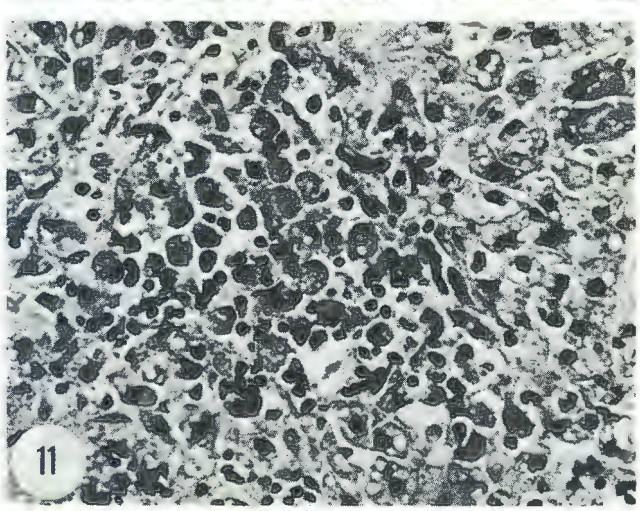
Varying amounts of pigment appearing as fine to globular golden-brown granules with similar staining properties as in the acute case occurred within the cytoplasm of macrophages and the occasional hepatocyte. The macrophages containing the pigment were present either within











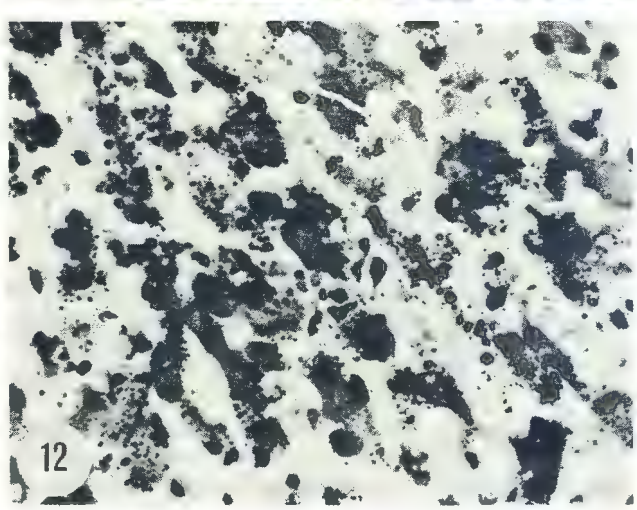


FIG. 7 Subacute aflatoxicosis showing focal necrosis (1), hepatocellular fatty changes (2) and regenerative micronodules (3) HE × 100

FIG. 8 Subacute aflatoxicosis. Extensive portal fibrosis. Note the proliferating bile ductules HE × 200

FIG. 9 Subacute aflatoxicosis. Regenerative micronodule (lower left) comprised of dark-staining hepatocytes HE × 200

FIG. 10 Subacute aflatoxicosis. Regenerating hepatocytes. Note the multinucleated cell (m) and formation of a rudimentary acinus (arrow) HE × 400

FIG. 11 Subacute aflatoxicosis. A focal group of pigment laden macrophages. Long ZN × 200

FIG. 12 Subacute aflatoxicosis. Hepatocytes laden with fat droplets. Oil Red O × 200

the sinusoids, especially centrilobularly, or as focal groups in the portal tracts or around the central veins (Fig. 11).

A fibrosis, varying in degree from mild to severe, (Fig. 8) was present in the portal areas and, together with the proliferating bile ductules, extended between the lobules. An infiltration of lymphocytes, plasma cells and macrophages was associated with the fibrosis. Many of the latter contained pigment. Occasionally mild fibrosis and/or infiltration of macrophages was observed around the central veins.

There was a mild to moderate congestion and leukostasis of the portal and central veins and the sinusoids. These cells were predominantly monocytes together with a few lymphocytes and neutrophils.

Chronic cases

Extensive fibrosis was the most striking feature in both livers. This resulted in architectural disruption, with obliteration of the central veins and their replacement by dilated, tortuous sinusoids embedded within fibrous tissue which formed wide tracts dissecting individual lobules and bridging adjacent lobules. The fibrous tissue was mostly collagenous in nature and associated with severe bile ductule proliferation. Lesions involving the intrahepatic bile ducts were similar to those described for the subacute cases. Macrophages, whether within the sinusoids or occurring singly or as groups in the fibrous tissue, contained golden-brown pigment granules with staining characteristics similar to those in the acute case.

The majority of hepatocytes appeared to be embedded within the collagenous tissue and showed fatty degeneration or lytic necrosis. Hepatocytes outside the fibrous tracts were normal, fatty or regenerative in appearance. Hepatocellular fatty changes were less pronounced than in the acute and subacute cases. Regenerative hepatocytes showed the same characteristics as those in the subacute cases, but the majority were present in groups forming regenerative nodules, many of which were visible grossly.

Histopathology of other tissues

The kidneys examined revealed a mild to moderate nephrosis. Fine, golden-brown pigment granules staining positively with the long ZN method were present within the cytoplasm of some of the renal tubular epithelial cells.

The spleen and lymph nodes were congested. Mild to moderate amounts of golden-brown, BB-positive pigment granules were present within the mononuclear phagocytes.

The intestines were severely hyperaemic and revealed haemorrhage per diapedesis.

No specific abnormalities could be detected in the heart or brain examined.

Discussion

The classification of aflatoxicosis into acute, subacute and chronic cases was based on the histopathology of the liver. The criteria included the nature, degree and extent of the following features: hepatocellular fatty degeneration, necrosis or regeneration; bile ductule proliferation; accumulation of bile in the bile canaliculi; fibroplasia; and, mucoid degeneration, necrosis or segmental atrophy of the larger intrahepatic bile ducts. Based on these criteria the 10 cases were classified as 1 acute, 7 subacute and 2 chronic. Seibold & Bailey (1952) and Newberne, Bailey & Seibold (1955) reported comparable ratios in the original outbreaks of hepatitis X in the USA. Fatty degeneration was present in all 3 stages of the disease and was the principal feature of the acute case. All the above features were present in the subacute cases, but the most characteristic feature of this stage was extensive bile ductule proliferation. For a case to be classified as chronic there had to be extensive fibroplasia.

Extensive fatty degeneration and centrilobular necrosis were characteristic features of the acute disease. Seibold & Bailey (1952) and Chaffee *et al.*, (1969) found similar changes but, in addition, about 50 % of their acute cases showed some bile ductule proliferation. It is our contention that for a case to be classified as acute there should be extensive fatty degeneration frequently accompanied by necrosis, while bile ductule proliferation should be minimal or absent.

The outstanding feature of the subacute cases was the moderate to severe and extensive bile ductule proliferation. Additional features included hepatocellular fatty degeneration, necrosis and attempts at regeneration, accumulation of bile in the canaliculi and fibroplasia. Similar findings have been reported by other workers (Seibold & Bailey, 1952; Newberne *et al.*, 1955; Chaffee *et al.*, 1969).

Chronicity was determined on the basis of advanced fibroplasia. All the features of the subacute cases were present. However, fatty changes were less pronounced, while regenerating hepatocytes were more commonly encountered and frequently formed regenerative nodules.

Lobular architectural disruption did not appear to be an early feature of aflatoxicosis in the dog. Only in subacute cases with extensive fibroplasia and the chronic cases was the disruption severe. The disturbance was most obvious in the central portions of the lobules and was evidenced by obliteration of the central veins and their replacement by tortuous and dilated sinusoidal capillaries embedded in fibrous tissue. In ruminants, veno-occlusive disease has been reported to be a feature of chronic aflatoxicosis (Colvin, Harrison, Gosser & Hall, 1984; Miller, Clark, Hatch & Jain, 1984). Although there was centrilobular fibrosis with obliteration of the central veins in some of our cases, the drainage of the central veins did not appear to be occluded. This lends support to the contention that veno-occlusive hepatic disease is not a feature of aflatoxicosis in species other than cattle.

According to Newberne *et al.* (1955), diffuse fatty degeneration of hepatocyes is a constant finding in aflatoxicosis in dogs. In this series, the fatty changes varied from hepatocytes having a foamy appearance to those resembling mature adipocytes.

Necrosis was evident either as coagulative or lytic necrosis of single hepatocytes or groups of cells in the acute case as well as a 3rd of the subacute cases. The remaining subacute cases and the 2 chronic cases had little evidence of ongoing necrosis, but the loss of hepatocytes and their replacement by fibrosis reflected the previous death of hepatocytes.

Hepatocellular regeneration was noted in all the subacute and both chronic cases, but only in the latter 2 cases was this feature grossly discernible as regenerative nodules. Histologically the regeneration of hepatocytes was evidenced by cytoplasmic basophilia and nuclear changes, such as anisonucleosis, karyomegaly with prominent nucleoli, mitotic figures, binucleation and multinucleation. Anisonucleosis, karyomegaly, binucleation and multinucleation probably represent the direct effects of aflatoxins on the hepatocytes, as aflatoxin B_1 is known to interfere with mitosis (Chaffee *et al.*, 1969). In some subacute cases, the regenerating hepatocytes gave the impression of forming tubules. Although hitherto not described in dogs, this feature may be quite prominent in

subacute or chronic aflatoxicosis in swine (Harding, Done, Lewis & Allcroft, 1963; Gagnè, Dungworth & Moulton, 1968) and is believed to be the forerunner of the development of hepatic neoplasia. It is our opinion that in natural outbreaks of aflatoxicosis in dogs, the toxin levels are too high and administered for too short a period for any hepatic neoplasia to develop.

A constant histopathological feature in the livers of all the subacute and chronic cases was bile ductule proliferation. This finding has been reported by various authors (Seibold & Bailey, 1952; Newberne *et al.*, 1955; Chaffee *et al.*, 1969; Armbrecht, Geleta, Shalkop & Durbin, 1971). The proliferating bile ductules were first noticed in the portal areas and then extended towards the central veins. Although as yet not reported in the dog, Harding *et al.* (1963) noted a similar progression in swine.

Accumulation of bile in the canaliculi was evident in 80 % of the cases in this series and therefore appears to be a relatively constant although not necessarily a characteristic feature of canine aflatoxicosis. It reflects the extensive hepatocellular damage with resultant intrahepatic cholestasis. Seibold & Bailey (1952) reported this finding but offered no comment.

The accumulation of PAS-positive debris in the lumina of the larger intrahepatic bile ducts associated with mucoid degeneration, necrosis, erosions or segmental atrophy of the epithelium was a constant feature observed in all the dogs examined. No reference to such lesions could be found in the litereature reviewed yet it seems that these features can be regarded as relatively characteristic of aflatoxicosis in the dog.

There was gross pathology of the liver in all 10 cases. All were yellow or yellow-brown in colour and bile stasis was evident in 4 cases. Seibold & Bailey (1952) reported that affected livers varied from yellow, orange-yellow, reddish-yellow to greenish-yellow. Macro-nodular regeneration was present in the 2 chronic cases, while in the acute and subacute cases, the surface of the livers was smooth. These findings agree with those reported by Seibold & Bailey (1952). According to Newberne (1973), oedema of the attachments of the gall-bladder is a constant change in chronic aflatoxicosis in dogs and occurs relatively frequently in acute and subacute cases. It was noted in only 1 of the subacute cases in our series. A more constant finding in the present outbreak was the presence of viscous or inspissated bile within the gall-bladder.

Haemorrhagic diathesis evidenced by entrorrhagia or petechiae and ecchymoses in various tissues, especially the subcutis, was found in 60 % of cases. Various workers have reported on the occurrence of haemorrhages at various sites in canine aflatoxicosis (Seibold & Bailey, 1952; Newberne *et al.*, 1955; Newberne, Russo & Wogan, 1966; Chaffee *et al.*, 1969; Greene, Barsanti & Jones, 1977). Chaffee *et al.* (1969) and Greene *et al.* (1977) have propsed 2 hypotheses to explain the development of a haemorrhagic diathesis in canine aflatoxicosis; firstly, decreased levels of prothrombin and fibrinogen due to deficient hepatic production and, secondly, disseminated intravascular coagulation arising from release of tissue thromboplastins from damaged or necrotic hepatocytes leading to the consumption of clotting factors. According to Newberne *et al.*, (1966) and Greene *et al.*, (1977) this haemorrhagic tendency is the immediate cause of death in affected dogs.

Twenty per cent of the dogs in this series were grossly icteric and 70 % appeared to be anaemic. Whereas the former was probably the result of hepatocellular injury and partial obstruction of smaller intrahepatic bile ducts, the latter may be ascribed to the haemorrhagic manifestations of the disease (Seibold & Bailey, 1952; Greene *et al.*, 1977).

The ascites present in 6 out of the 10 cases may be ascribed, firstly, to portal hypertension associated with hepatic fibrosis and, secondly, to the presumed hypoproteinaemia resulting from the severe hepatocellular injury. The latter probably also accounts for the other oedematous changes.

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Printed by the Government Printer, Private Bag X85, Pretoria, 0001