Case Report

A Report of Aflatoxicosis in Hand-fed Ewe Lambs Exhibiting Icterus After Hepatic Failure and Hemoglobinuria

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ABSTRACT

Aflatoxin intoxication has been reported in most species; however, it is infrequently documented in sheep. In this report, three 7-month-old Romane ewe lambs were found with severe icterus, tachypnea, slight fever, diarrhea, anorexia, and lethargy. Two of them were culled, and one recovered after treatment. Necropsy findings showed jaundice, ascites, diffuse hemorrhage on the peritoneum, gall bladder enlargement, and alteration in the color and texture of the liver and kidney. Histopathological evaluation revealed hepatocellular necrosis, megalocytosis, centrilobular to bridging fibrosis in the liver, and hemoglobinuria without nephrotic degeneration in the kidney. The polymerase chain reaction test for leptospirosis as the main differential diagnosis was conducted, and the result was negative. Similarly, the Warthin Starry special staining for spirochetes was negative. Clinical signs and characteristics of microscopic lesions were suggestive of aflatoxicosis in the lambs described in this investigation. As sheep flocks with intensive rearing systems are becoming more common in Iran, the emergence of aflatoxicosis due to fungi-contaminated feedstuff can be a serious health concern in such farms.

Keywords: Aflatoxin, Lamb, Hemoglobinuria, Icterus, Intensive rearing, Hepatic failure and Kidney

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Case History

Aflatoxins are a kind of coumarins produced by *Aspergillus fungi*, including *Aspergillus flavus*, *Aspergillus parasiticus*, and *Aspergillus nomius* (Constable et al., 2017; MGavin &Zachary, 2021). These toxins usually can be found in cereals, especially corn (maize), rice, and cottonseed, either in the field or during storage when environmental conditions are favorable for mold growth (Maxie, 2015b). In tropical regions like Iran, aflatoxicosis poses more threats owing to climatic factors (Gowda et al., 2007)

All animal species are vulnerable to aflatoxicosis, but outbreaks usually occur primarily in pigs, sheep, and cattle (Constable et al., 2017). The clinical signs and disease severity differ in mammals and poultry depending on the species, sex, age, production status, and the duration of intake and level of the toxins in the ration (Gray Allen et al., 2016). Accordingly, lactating, pregnant, and growing animals are most likely seriously affected (Constabl et al., 2017).

While ruminants are comparatively resistant to aflatoxicosis, intensively reared livestock, where a large part of feed consists of stored grain, tend to be more at risk (Bingol et al., 2007). Acute aflatoxicosis is seldom an issue in adult cattle, sheep and goats; however, they will show susceptibility if toxic diets are fed over long periods (Gray Allen et al., 2016) or with very high doses (Edrington et al., 1994).

Animals' exposure to aflatoxin is manifested by chronic and acute hepatocellular damage (Wang et al., 2019). High dosages of aflatoxins give rise to necrosis of hepatocytes, while prolonged low dosages cause reduced growth rate, immunosuppression, and liver enlargement (Ramos et al., 1997). In lambs, consumption of aflatoxins may result in susceptibility to disease due to the suppression of some humoral and cellular immune responses (Fernández et al., 2000).

The diagnosis of aflatoxicosis in sheep is based on observing mortality, gross lesions on the mucosa, cyanosis, and petechial hemorrhage in the liver, associated with weakness and diarrhea (Barbour et al., 2014). The diagnosis is confirmed by detecting aflatoxins in the feed and bloodstream and the characteristic gross and histopathological lesions in the liver and nervous tissue (Constable et al., 2017).

This report describes an incidence of aflatoxicosis in an intensive-rearing Romane sheep flock near Tehran.

Clinical Presentation

In December 2021, a 7-month-old ewe lamb of the Romane breed was found sluggish and lethargic in the barn. The lamb was panting and reluctant to move. A thorough examination was conducted immediately. Slight hyperthermia (39.7°C), severe icterus, tachypnea, and dark brown diarrhea were evident. For welfare considerations, the lamb was culled. At necropsy, the most eye-catching findings were extreme jaundice, ascites (Figure 1A), and diffuse hemorrhage on the peritoneum (Figure 1B). The liver was yellowish-brown and firm, and the gall bladder was heavily swollen (Figure 1C). The kidneys were abnormally large and dark red (Figure 1D). The liver, kidneys, lung, heart, and intestine samples were taken, fixed in a 10% formalin solution, and submitted to the lab for histopathological evaluation. Two other ewe lambs from the same barn were found ill within a week, presenting the exact clinical signs. One of the latter lambs was slaughtered, and the other recovered after receiving supportive treatment for several days. Treatment included fluid therapy and administration of dexamethasone, vitamin B-complex, and phosphorus+B12 components.

Diagnostic Testing

To perform the histopathological examination, 1.5×1.5×1.5 cm liver and kidney samples were placed in 10% neutral buffered formalin. After 48 h, tissue samples were fixed and routinely processed, dehydrated, and embedded in paraffin wax, sectioned at 5-µm thickness (Rotary Microtome RM2 145; Leica) and stained with hematoxylin-eosin (H&E). Additional sections of the liver and kidney were stained histochemically with Masson's trichrome and Prussian blue. Finally, the sections were evaluated via a light microscope and micrographs were taken.

Microscopic examination of the liver section revealed micro and macro vesicles of lipid within some hepatocytes (fatty change), bile retention (Figure 2C), portal/central bridging fibrosis, including necrosis of hepatocytes and replacement with collagen fibers and proliferated fibroblasts and hyperplastic bile ducts (Figure 2E), ductular reaction, cellular and nuclear atypia, infiltration of some neutrophils and mononuclear inflammatory cells (Figure 2B), and some large hepatocytes with giant nuclei (15.06 µm in size) called megalocytosis (Figure 2C).

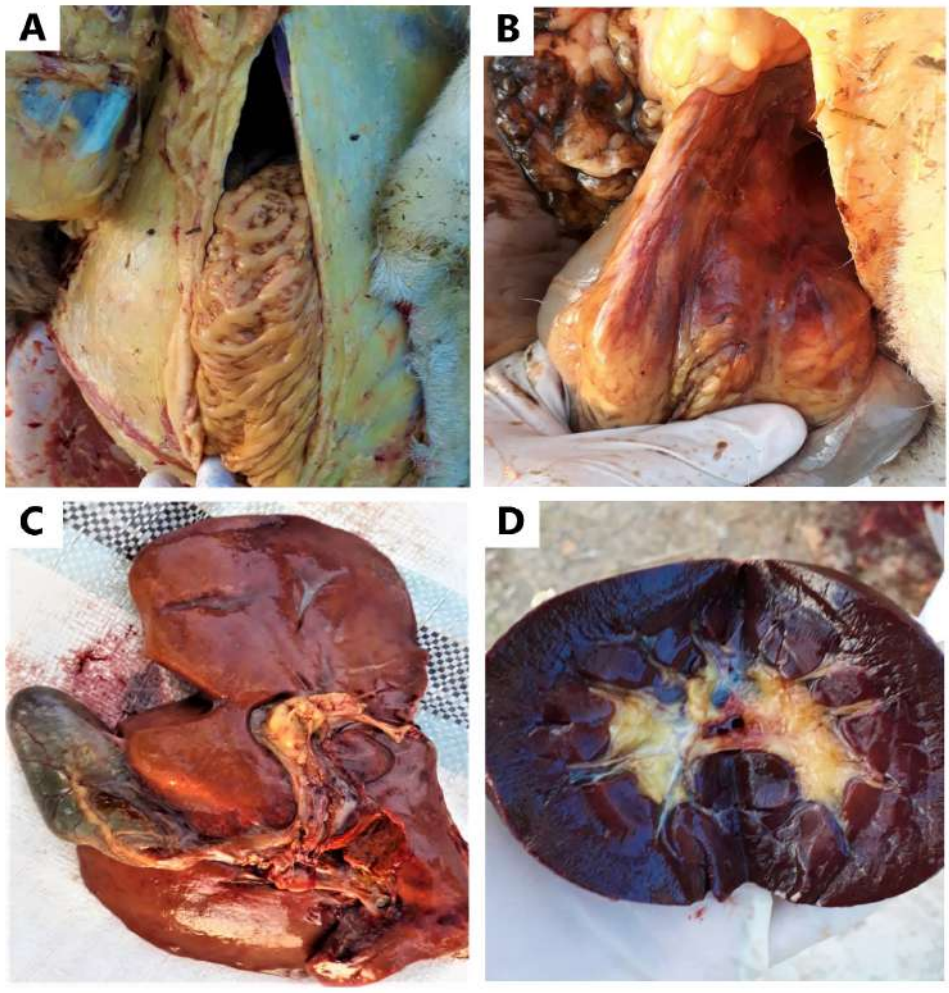

Figure 1. A) Extreme jaundice of the carcass, B) Diffuse hemorrhage on the peritoneum, C) Enlarged yellowish-brown liver with swollen gall bladder, D) Dark red kidney

Histologically, the kidney revealed the existence of numerous casts of hemoglobin (Figure 2F) and red blood cells within renal tubules, proteinaceous materials within urinary spaces and renal tubules, some vacuolated renal tubular epithelial cells, and scant neutrophils and mononuclear inflammatory cells (without remarkable inflammation; Figure 2D). Interestingly, despite the substantial number of tubular casts, no remarkable degenerative alterations were seen in renal tissue.

Assessments

The Food and Agriculture Organization (FAO) presumes that nearly 25% of the world's food commodities are contaminated with mycotoxins, mainly aflatoxin (Cao et al., 2021). Sheep are quite resistant to aflatoxins (Edrington et al., 1994), so aflatoxicosis is uncommon in sheep herds (Maxie, 2015b). However, in the housed flocks, in which animals are hand-fed with stored cereals and hay, the risk of the disease may rise significantly (Constable et al., 2017). The cases described in this report were kept in the barn the whole year, receiving a ration consisting of barley, corn, soybean meal, soybean oil, corn silage, and alfalfa. Field investigation revealed that some parts of the alfalfa used in the feed had been moldy. On the other hand, the farmworkers used a feeder mixer machine to prepare animals' feed, whose container was rarely cleaned. It was observed that a bunch of feedstuffs was trapped under the mixer axis to provide a favorable environment for fungal growth and, ultimately, mycotoxin production.

According to Cammilleri et al. (2019) 18 aflatoxins have been classified, of which aflatoxin B1 is the most potent. The major impacts of perpetuated aflatoxin consumption in livestock are hepatitis and hepatic insufficiency (Wang et al., 2019), leading to poor growth rates, unthriftiness, icterus, and death (Gray Allen et al., 2016). A survey performed by Cao et al. (2021) showed that aflatoxins interrupt the normal microbiota of the ovine

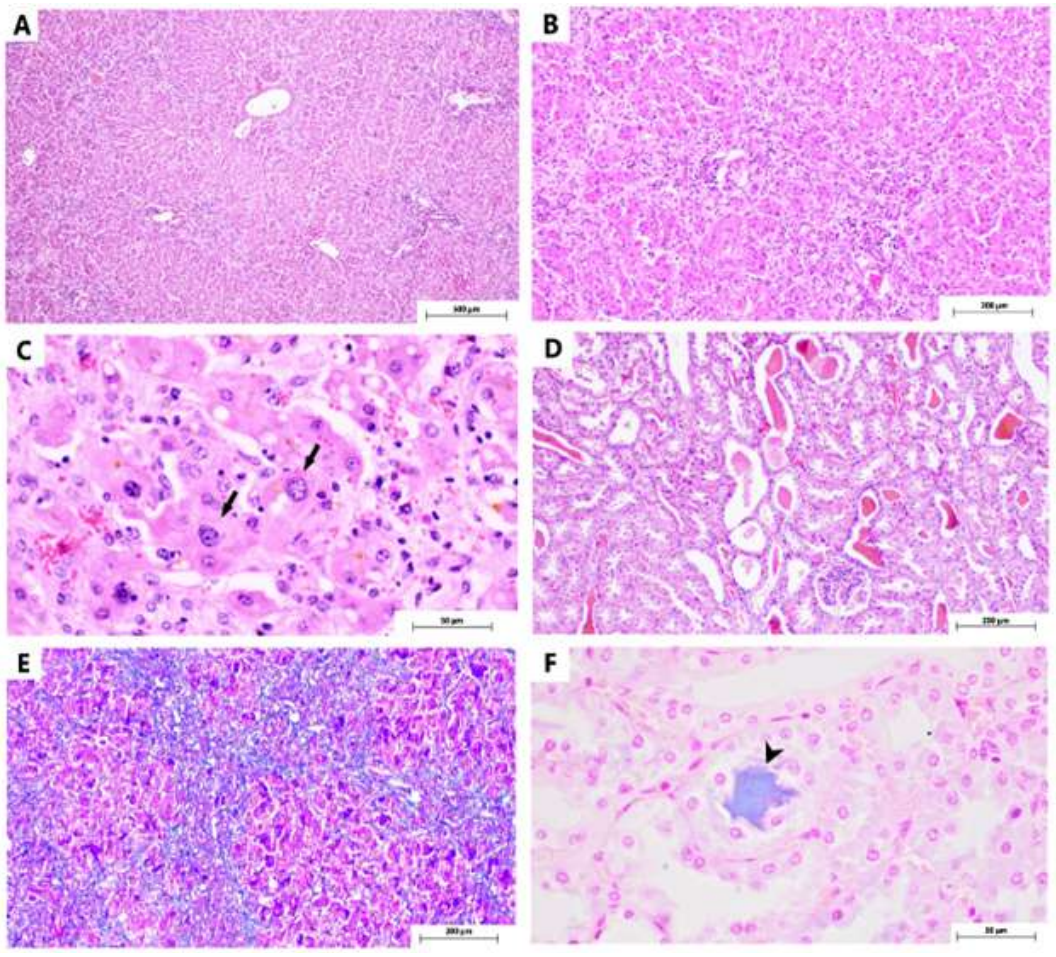

Figure 2. Histopathological micrographs

A) Liver, H&E staining, x4; B) Hepatocellular necrosis, liver, H&E staining, x10; C) Hepatocyte degeneration and necrosis, megalocyte formation (arrows) liver, H&E staining, x40; D) Tubular urinary casts, kidney, H&E staining, x10; E) Blue areas of collagen deposition indicating bridging fibrosis, liver, masson's trichrome staining, x10; F) Hemoglobin cast (arrowhead), Prussian blue staining, x40

intestine. All three animals in this study exhibited poor performance, depression, and jaundice at the onset of the disease.

Some disorders share identical clinical signs with aflatoxicosis in sheep. Given the farm condition, leptospirosis was considered the principal differential diagnosis in this case. Thus, a polymerase chain reaction test for leptospirosis from the fresh kidney specimen was performed, and the result was negative. Therefore, the characteristic hepatic and renal histopathologic changes can be confirmatory for the diagnosis of aflatoxicosis in this survey.

All three ill lambs underwent treatment with the following prescription for three consecutive days: Vitaforte B (Razak Co., 5 mL IM), multivitamin (Abureihan Co., 5 mL SC), Vetacoid (Abureihan Co., 4 ml IV), Cobaject (Royandaru Co., 5 mL IV), Duphalyte (Zoetis Co., 25 mL IV), Oxivet 20% (Razak Co., 5 mL IV, one day only) and 500 mL of sterile normal saline infusion. Eventually, one lamb recovered after treatment, while two others were culled due to poor prognosis.

Conclusion

In the literature, sheep are considered resistant to aflatoxin intoxication, and reports on aflatoxicosis in sheep are scarce. Nevertheless, as ovine-housed flocks with the hand-fed system are becoming increasingly common in Iran, the emergence of aflatoxicosis can emerge as a serious problem in such farms if it is not adequately addressed.

Ethical Considerations

Compliance with ethical guidelines

All ethical principles are considered in this article and the necropsy was done with the consent of the farm owner.

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Authors' contributions

Conceptualization, methodology and validation: All authors; Investigation, resources and data curation: Mohammadreza Mokhtari Hooyeh, Mahya Lalehpoor, Nazanin Samiee, Seyedeh Fatemeh Safavi; Writing, visualization and supervision: Hossein Aminianfar, Aghil Sharifzadeh, Mohammadreza Mokhtari Hooyeh; Project administration and funding acquisition: Hossein Aminianfar.

Conflict of interest

The authors declared no conflict of interest.

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گزارش موردی

مسمومیت با آفلاتوکسین در بره میش‌های یک مزرعه صنعتی پرورش گوسفند

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چکیده

مسمومیت با آفلاتوکسین در اکثر گونه‌های حیوانی مشاهده شده است. باوجوداین، وقوع آفلاتوکسیکوز به‌ندرت در گوسفند ثبت شده است. در این گزارش، ۳ رأس بره میش ۷ ماهه نژاد رومن مبتلابه زردی شدید، تاکی پنه، تب خفیف، اسهال، بی‌اشتهایی و بی‌حالی مشاهده شد. ۲ رأس از آن‌ها به‌علت عدم پاسخ به درمان ذبح شدند و یکی پس از درمان بهبود یافت. یافته‌های کالبدگشایی شامل زردی، آسیت، خونریزی منتشر در چادرینه، بزرگ شدن کیسه صفرا و تغییر رنگ و قوام کبد و کلیه بود. در ارزیابی هیستوپاتولوژیک، نکروز سلول‌های کبدی، مگالوسیتوز و فیبروز سانتریلوبولار در کبد و هموگلوبینوری بدون دژنراسیون نفروتیک در کلیه مشاهده شد. برای لپتوسپیروز به‌عنوان تشخیص افتراقی اصلی، آزمون PCR انجام شد که منفی بود. به‌طور مشابه، رنگ‌آمیزی نقره (رنگ‌آمیزی اختصاصی وارتین استاری) برای اسپیروکت‌ها منفی بود. علائم بالینی و ضایعات میکروسکوپی مشخصه، حاکی از آفلاتوکسیکوزیس در بره‌هایی بود که در این بررسی توضیح داده شد.

کلیدواژه‌ها: آفلاتوکسین، گوسفند، هموگلوبینوری، ایکتروس، پرورش صنعتی، نارسایی کبدی و کلیه

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