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Protothecosis and chlorellosis in sheep and goats: a review

Franklin Riet-Correa,¹  Priscila Maria Silva do Carmo, Francisco A. Uzal

Abstract. Protothecosis and chlorellosis are sporadic algal diseases that can affect small ruminants. In goats, protothecosis is primarily associated with lesions in the nose and should be included in the differential diagnosis of causes of rhinitis. In sheep, chlorellosis causes typical green granulomatous lesions in various organs. Outbreaks of chlorellosis have been reported in sheep consuming stagnant water, grass from sewage-contaminated areas, and pastures watered by irrigation canals or by effluents from poultry-processing plants. *Prototheca* and *Chlorella* are widespread in the environment, and environmental and climatic changes promoted by anthropogenic activities may have increased the frequency of diseases produced by them. The diagnosis of these diseases must be based on gross, microscopic, and ultrastructural lesions, coupled with detection of the agent by immunohistochemical-, molecular-, and/or culture-based methods.

Key words: chlorellosis; goats; protothecosis; sheep.

Introduction

Prototheca spp. (achlorophyllous algae) and *Chlorella* spp. (chlorophyll-containing algae) are ubiquitous algae that reproduce asexually by internal septation (endosporulation) in which a parent cell (sporangium) divides into 2–20 sporangiospores. After maturation, the wall of the sporangium ruptures and the sporangiospores are released to complete the cycle.^{2,12,22} Algal infections have been reported in domestic animals, including goats and sheep.^{3,14,15,19,22} *Prototheca* spp. and *Chlorella* spp. are widespread in the environment, including stagnant water, sewage-contaminated pastures, and areas contaminated by mud, soil, or feces.^{2,10,14,15,20,22} The recent increase in the number of cases in goats and sheep may therefore be associated with environmental and/or climatic changes promoted by anthropogenic activities.^{2,3,11,14,15,19,22} It is also possible that because of the sporadic characteristic of the diseases, which usually affect only a few animals at a time, at least some cases in goats and sheep go undiagnosed, especially in areas of extensive goat and sheep production. We review here the main features of protothecosis and chlorellosis in goats and sheep, which may contribute to the diagnosis of these diseases in these species.

Protothecosis

Etiology and epidemiology

Protothecosis is a rare disease of humans and animals that is caused by achlorophyllous yeast-like microalgae of the genus *Prototheca*.^{2,12,18,22} Seven species of *Prototheca* (family *Chlorellaceae*) have been reported, but until 2008 only *P. wickerhamii* and *P. zopfii* genotypes 1 and 2 were considered

pathogenic for animals and humans.^{1,2,12,26} Genotype 2 is involved more frequently than genotype 1 in protothecosis of animals and human.^{1,2,12,16,26} Since 2008, 2 more species have been recognized as pathogens: *P. blaschkeae* was isolated from the mammary gland of cows with mastitis,¹⁶ and *P. miyazii* was isolated from a human patient with systemic disease.¹⁷

P. zopfii is an important cause of mastitis in cows.⁵ In dogs, *P. zopfii* and *P. wickerhamii* cause cutaneous,²⁶ intestinal,⁸ or systemic infections affecting various internal organs, including the eyes and brain.²⁴ In cats, protothecosis caused by *P. zopfii* genotype 2 is manifested by cutaneous infections, affecting mainly the skin of the face and nose.⁹ In horses, the same microorganism causes rhinitis.²⁵ In humans, protothecosis, caused mainly by *P. wickerhamii*, occurs in 3 forms: cutaneous, olecranon bursitis, and disseminated.^{2,12,18} The cutaneous lesion is usually localized in sites of continuous minor trauma in immunocompetent individuals; however, in immunocompromised patients, it can become widespread.^{2,12,18}

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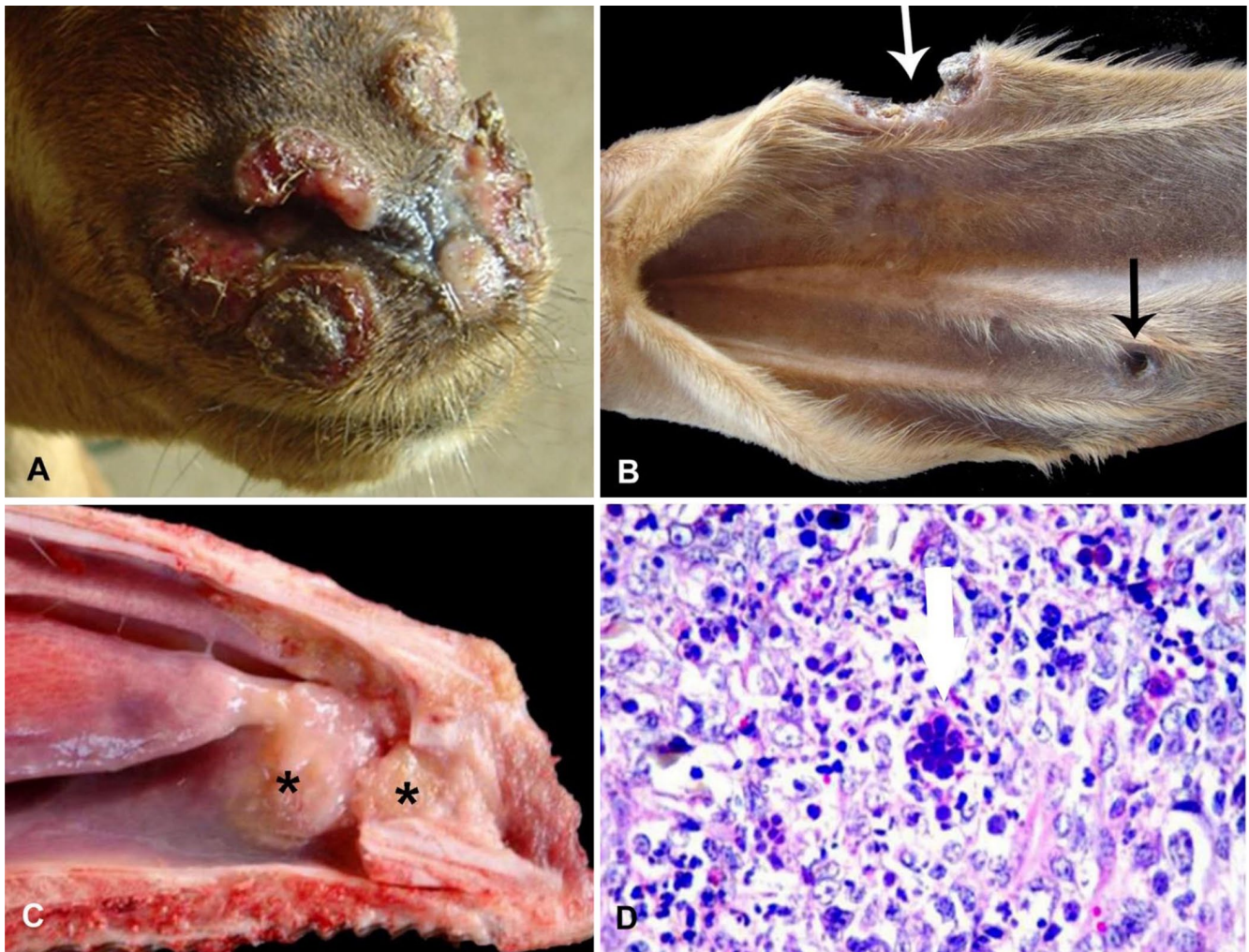


Figure 1. Protothecosis in goats. **A.** Ulcerative dermatitis of the nose. Reprinted with permission of Vet Pathol.¹⁵ **B.** Ulcerative dermatitis in the edge and center of the pinna (arrows). **C.** Sagittal section of the head; granulomatous rhinitis extends from the nostrils to the rostral portion of the turbinates (asterisks). Reprinted with permission of Mycoses.³ **D.** Pyogranulomatous cellulitis with non-walled sporangia within the inflammatory exudate. A typical daisy-shaped sporangium is present in the center of the lesion (arrow).

Sporadic cases of cutaneous and nasal protothecosis caused by *P. wickerhamii* have been reported in adult immunocompetent goats in northeastern Brazil. These cases were characterized by chronic, slowly progressive pyogranulomatous and necrotizing lesions of the nasal vestibule, mucocutaneous junctions of the nostrils, and subcutaneous tissues and skin of the face and head.^{3,15} It is thought that the infection probably started by contact of the nostril mucosa or skin with water contaminated by *P. wickerhamii*.^{3,15} Protothecosis occurs with very low frequency, or not at all, in sheep. Because *Prototheca* spp. are widespread in the environment, they can be ingested by domestic and wild animals and be eliminated in their feces.^{5,20}

Clinical signs

Clinical signs of protothecosis in goats include mucopurulent nasal discharge, inspiratory dyspnea and stertor, inflation of

the cheeks during expiration, and frequent sneezing. Proliferative nodules, up to 3 cm diameter, sometimes ulcerated, are observed on the nasal mucosa, mucocutaneous junction at the nasal vestibule, and skin of the lips, muzzle, and pinna (Fig. 1A, B).^{3,15} The disease has a long clinical course and, after several months of respiratory difficulty, the animals develop an orthopedic position, with neck extension, abduction of the thoracic limbs, and open-mouth breathing, as well as tongue protrusion and sinking of the intercostal spaces during inspiration.^{3,15} No effective treatment against this disease has been reported in goats, to our knowledge.

Gross lesions

At autopsy, coalescing yellow nodules, up to 3 cm diameter, are observed in the subcutaneous tissue of the skin over the nasal bone, lips, and muzzle. Soft yellow masses with an irregular, ulcerated, and fibrinonecrotic surface are observed

on the nasal mucosa, extending from the nostrils to the rostral portion of the turbinate bones (Fig. 1C).^{3,15}

Microscopic lesions

Microscopically, protothecosis is characterized by necrotizing, pyogranulomatous dermatitis, rhinitis, and occasionally osteomyelitis. Fibrosis may be prominent around the lesions. Myriad 3–15 µm wide, non-budding sporangia are observed extracellularly within the inflammatory exudate and in the cytoplasm of macrophages and giant cells. Some sporangia are internally septate and contain variable numbers of sporangiospores, which form a 10–15 µm diameter morula-like structure. In cases of *P. wickerhamii* infection, some of these morula-like structures have a daisy-like appearance, with a central round endospore surrounded by a crown of endospores (Fig. 1D). Dissemination of the infection to other organs has not been reported.^{3,15}

Diagnosis

A presumptive diagnosis of protothecosis in goats can be established based on gross and microscopic findings. *Prototheca* spp. are morphologically different from other non-sporulated fungi because of their larger sporangia, which helps in establishing a presumptive diagnosis. Final confirmation of the diagnosis, however, should rely on isolation of *P. wickerhamii* on blood agar or selective fungal media (Sabouraud dextrose agar, with or without chloramphenicol). *Prototheca* spp. can be further identified by biochemical tests, PCR, and/or DNA sequencing.² In cases of *P. zopfii* infection, the genotype should be identified by rDNA amplification by PCR and restriction-fragment length polymorphism (RFLP) analysis.^{1,26}

The clinical signs of protothecosis in goats are similar to those of nasal aspergillosis caused by *Aspergillus niger*, therefore the observation and identification of the causal agent is important for the differential diagnosis. Conidiobolomycosis, caused by *Conidiobolus* spp., is a rare disease in goats characterized by rhinitis and skin lesions similar to protothecosis, and should be differentiated by histology, PCR, and/or culture-based methods.

Chlorellosis (green algae infections)

Etiology and epidemiology

Chlorellosis is caused by members of the genus *Chlorella*, which are unicellular, chlorophyll-containing green algae (order Chlorellales, family Chlorellaceae).⁶ *Chlorella* spp. have been reported to cause focal cutaneous lesions secondary to wounds in humans¹⁰ and in a gazelle,⁶ lymphadenitis and peritonitis in cattle,^{7,23} enteric infection in a dromedary,¹³ and disseminated infection in a dog.²¹ In sheep, chlorellosis has been reported as an incidental finding in slaughterhouses

affecting the liver and lymph nodes^{4,27,28} or in outbreaks affecting several animals, causing systemic infection associated with progressive weight loss,^{11,14,19,22} diarrhea,¹⁹ or ascites.^{11,14,19} To date, no cases of chlorellosis have been reported in goats; chlorellosis may occur in very low frequency in goats, or may be of no clinical significance.

Although little information is available about the pathogenesis of chlorellosis, it is thought that the infection occurs as a result of the consumption of stagnant water, grass from areas contaminated by sewage,¹⁴ pastures irrigated via canals containing algae,¹¹ or pastures irrigated with treated effluent from poultry-processing plants.¹⁹ The occurrence of lesions in the gut, lymphatic and blood vessels, and mesenteric lymph nodes suggests an oral route of infection followed by dissemination to other organs.

Clinical signs

In sheep, chlorellosis can cause progressive weight loss,^{11,14,19,22} diarrhea,¹⁹ or ascites.^{11,14,19} Subclinical cases can also occur.^{27,28}

Gross lesions

At autopsy, several lymph nodes may be enlarged, edematous, and diffusely green; mesenteric lymph nodes are usually the most severely affected (Fig. 2A). Mesenteric lymphatics are distended and green. Green multifocal-to-coalescent nodules are observed in liver, lungs, kidneys, and intestine (Fig. 2B, 2C). The wall of the gut is thickened, and the mucosa is multifocally ulcerated. The characteristic green color of the lesions, as a result of the presence of chlorophyll, remains obvious even after formalin fixation, but is removed by histologic tissue processing.^{6,10,14,22}

Microscopic lesions

Histologically, pyogranulomas are observed, with myriad intralesional, 8–30 µm diameter, oval-to-round algae (Fig. 2D), with a thick periodic acid–Schiff (PAS)-positive capsule.^{11,14,19,22} Unlike *Prototheca* spp., *Chlorella* spp. organisms contain chloroplasts and starch bodies, which appear as abundant PAS-positive cytoplasmic granules that can also be visualized by electron microscopy.⁶ As with *Prototheca* spp., *Chlorella* spp. reproduce by endosporulation, and each sporangium may contain 2–20 sporangiospores.⁶

Diagnosis

Chlorellosis may cause intestinal or systemic infections in sheep, which should be differentiated from several other diseases, including gastrointestinal parasitic infections. Because *Chlorella* spp. are difficult to isolate and cultural methods are inconclusive,¹³ the diagnosis of chlorellosis is made by observing the typical green gross lesions, coupled with microscopic observation of the lesions. The characteristic

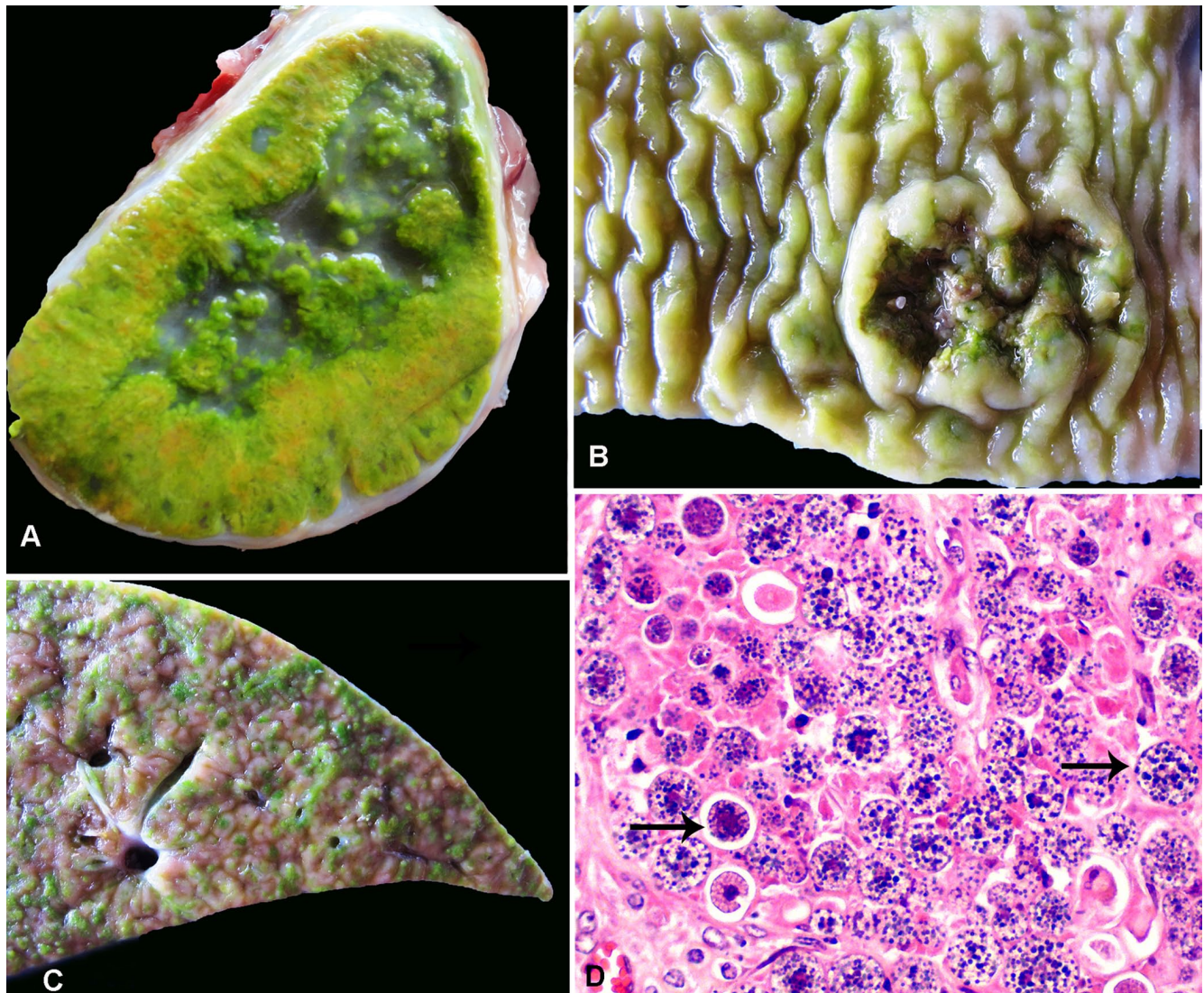


Figure 2. Chlorellosis in sheep. **A.** Lymph node, **B.** intestine, and **C.** liver, with typical green discoloration. **D.** Pyogranuloma with myriad intralesional, 8–30 µm diameter, oval-to-round algae (arrows). H&E.

chloroplasts and starch granules can also be visualized by electron microscopy.^{13,22} In a report of cutaneous green algae infection of a gazelle, the ultrastructural study suggested that the lesion was caused by the genus *Bracteacoccus*.¹³ In another report of green algal peritonitis of cows, the PCR assay confirmed that the green algae involved was *Scenedesmus* sp.⁷ These results indicate that green algal infections can be caused by different species of the order Chlorellales.¹³


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