15-month-old, Limousin heifer with multifocal to coalescing hyperkeratosis, lichenification and alopecia on head, neck, mammary region and perineal and perivulvar areas

Which of the following is the most likely diagnosis?

1. Dermatophylosis
2. Besnoitiosis CORRECT
3. Dermatophytosis
4. Lumpy skin disease
5. Sarcocystosis

**Signalment and history:** In a herd of 130 Limousin cattle, two heifers, ranging from 13 to 15 months of age, presented with multifocal to coalescing skin lesions, characterized by hyperkeratosis, lichenification and alopecia. Lesions were particularly prominent on the head (especially in the periocular – Fig. 1 – and perilabial area as well as around the nares), on the neck (Fig. 2), on the mammary region and on the perineal and perivulvar area. On the epibulbar conjunctiva and on the nictitating membrane, numerous roundish and elevated whitish lesions were evident of about 0.5 mm in diameter. The same lesions were evident in the trachea (Fig. 3) and on the nasal mucosa (Fig. 4). One of the heifers was euthanized, because it presented severe ocular and nasal discharge, lymphadenomegaly of prescapular and precrural lymph nodes, weight loss and reluctance to move.

**Histopathologic Description:** Multifocally to coalescing, expanding the superficial dermis and deep dermis and occasionally extending into the subcutis, are numerous, round to ovoid, 250-400 μm diameter protozoal cysts that compress adjacent dermal collagen and adnexal structures (Fig. 5). The overlying epidermis is diffusely and irregularly hyperplastic with formation of rete ridges and moderate orthokeratotic hyperkeratosis, and focal hemorrhage. Cysts have a 10-30 μm thick, hyaline capsule that surrounds a 5-10 μm thick rim of host cell cytoplasm with multiple enlarged but flattened nuclei. In the center of the cysts are numerous, densely packed, crescentic, 3-5 μm bradyzoites (Fig. 6 and Fig. 7). Surrounding the cysts, there are multifocally, mild to moderate numbers of macrophages, lymphocytes, plasma cells and fewer eosinophils (Fig. 8). Some cysts are also surrounded by a mild to moderate amount of mature dermal collagen (fibrosis) (Fig. 8). Rarely, cysts are ruptured or collapsed and devoid of bradyzoites, but filled instead with inflammatory cells, in particular eosinophils (Fig. 9).

**Morphologic diagnosis:** Dermatitis, lymphohistiocytic and eosinophilic, multifocal to coalescing, severe, with epidermal hyperplasia and intradermal and intracytoplasmic protozoal cysts, etiology consistent with Besnoitia besnoiti

**Name of the disease:** Bovine Besnoitiosis

**Comment:** Bovine besnoitiosis is a severe parasitic disease due to Besnoitia besnoiti, with a worldwide distribution and recently classified as “re-emerging” disease by EFSA (2010). Its European diffusion in last decade, its effect on cattle, lack of effective vaccines and therapies, lack of knowledge on many aspects of its etiology and pathogenesis made of it a newsworthy topic of study. Besnoitia is a protozoal parasite in the phylum Apicomplexa with a two-host life cycle: the definitive host is a carnivore and becomes infected by ingesting tissue containing cysts; the bradyzoites released by the cysts infects enterocytes and endothelial cells and, via merogony and gametogony, develop oocysts which are released in the environment through feces. In the environment, the oocysts sporulate and are infective to the intermediate host. The intermediate host (an herbivore) ingests the sporulated oocyst from which the sporozoites are released and penetrate into host tissues through the gastrointestinal tract. In the intermediate host’s tissues, the tachyzoites proliferate in the macrophages, fibroblasts and endothelial cells
with possible vasculitis and thrombosis and then develop into bradyzoite cysts within fibroblasts. Arthropod transmission of bradyzoites may occur between intermediate hosts. The exact biological cycle is only known for three species (B. darlingi, B. wallacei, B. oryctofelis), although it is likely that this cycle model is valid for all species of the genus Besnoitia (Fig. 10).

![Diagram of Besnoitia life cycle](image)

**Fig. 10.** Suspected life cycle and transmission of *Besnoitia besnoiti* in cattle (from Alvarez-García G, 2013)

In the bovine, the disease can be divided into acute, subacute, and chronic stages. The acute stage is characterized by tachyzoite proliferation within vascular endothelial cells. Bradyzoites proliferate during the subacute and chronic stages within mesenchymal cells resulting in the cyst formation. Cysts develop in connective tissue (fibroblasts and histiocytes) throughout the body, especially in the skin, conjunctiva, mesentery, and scrotum. In some cases, the disease appears to be much more widespread and severe with ulceration of the skin, cysts in the nasal mucosa, visceral serosa, tendons, tendon sheaths, hoofs and testes. Cysts in the intima of blood vessels can cause vasculitis and thrombosis, which result in reduced blood flow; this can lead to male sterility if it occurs in the testis.

Histologically, the lesions are characterized by the presence of very large cysts in the dermis having 4 typical layers, which are, from the outside to the inside: 1. A layer of compressed dermal collagen with or without fibrosis; 2. A variably thick, hyalinized extracellular capsule; 3. The host cell with its cytoplasm present as a thin rim at the margin of the enlarged cell and the elongated host cell nuclei that can be seen as an inner cyst membrane; 4. A large parasitophorous vacuole filled with bradyzoites. Cysts may be accompanied, especially if degenerated, by inflammatory and fibrotic processes. Inflammatory cells are characterized by macrophages, lymphocytes, plasma cells and eosinophils.
References:


Contributor: Drs. Daniele Francia, Elvio Lepri and Chiara Brachelente, Department of Veterinary Medicine, University of Perugia, Italy.
Figures legend:

Figure 1. 15-month-old, Limousin heifer. The skin around the eye is severely and irregularly hyperplastic and hyperkeratotic, with formation of thickened skin folds. The lesions are alopecic/hypotrichotic and partially eroded and ulcerated.

Figure 2. 15-month-old, Limousin heifer. The same lesions as depicted in Fig. 01 are present on the ventral part of the neck.
Figure 3. 15-month-old, Limousin heifer. On the mucosa of the trachea and of the epiglottis numerous small, whitish, mildly elevated and round lesions, of about half a millimeter in diameter, are present.

Figure 4. 15-month-old, Limousin heifer. On the mucosa of the nasal cavity numerous small, whitish, mildly elevated and round lesions, of about half a millimeter in diameter, are present (stereoscopic microscope image).
Figure 5. 15-month-old, Limousin heifer. The dermis is thickened due to the presence of numerous, round to ovoid, 250-400 um diameter protozoal cysts compressing adjacent dermal collagen and adnexal structures.
Figure 6. 15-month-old, Limousin heifer. Cysts are characterized by an external, 10-30 um thick, hyaline capsule that surrounds a 5-10 um thick rim of host cell cytoplasm with multiple enlarged but flattened nuclei. The cyst contains numerous, densely packed, crescentic, 3-5 um bradyzoites.

Figure 7. 15-month-old, Limousin heifer. The host cell cytoplasm is present as a thin rim at the margin of the enlarged cell. Elongated host cell nuclei may be seen as an inner cyst membrane.
**Figure 8.** 15-month-old, Limousin heifer. Surrounding the cysts, there are multifocally, mild to moderate numbers of macrophages, lymphocytes, plasma cells and fewer eosinophils. There is also mild to moderate fibrosis.

**Figure 9.** 15-month-old, Limousin heifer. Some cysts are devoid of bradyzoites, but filled instead with inflammatory cells, in particular eosinophils.