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Redescription of *Besnoitia bennetti* (Protozoa: Apicomplexa) from the donkey (*Equus asinus*)☆

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Abstract

Besnoitia bennetti tissue cysts were found in four naturally-infected donkeys (*Equus asinus*) from the USA. Infectivity of its bradyzoites and tachyzoites to animals and cell culture was studied. The bradyzoites were not infectious to out-bred Swiss Webster mice, rabbits or gerbils. When fed tissue cysts, cats did not excrete oocysts. However, the parasite was infectious to interferon-gamma gene knock out mice. The parasite from tissues of two donkeys was grown successfully in bovine monocyte monolayers for the first time. Non-dividing, uninucleate tachyzoites were approximately 6×1.5 μm in size. Longitudinally-cut bradyzoites in tissue sections measured 8.7×1.9 μm. Ultrastructurally, tachyzoites and bradyzoites were similar to those in other *Besnoitia* species, and in particular to parasites described from cattle (*Besnoitia besnoiti*) and reindeer (*Besnoitia tarandi*), in that their bradyzoites lacked enigmatic bodies. Based on comparative analysis of three portions of nuclear ribosomal DNA (the small and large subunits and the first internal transcribed spacer) *B. bennetti* was found to be more closely related to the other congeners described from ungulates. The parasite was formally redescribed and specimens deposited in the US National Parasite Collections.
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Keywords: *Besnoitia bennetti*; Donkeys; *Equus asinus*; USA; Mice; Gerbils; Cell culture; Ultrastructure; Sequence analysis; Taxonomy

1. Introduction

Species of the coccidian genus *Besnoitia* parasitise cattle, goats, equids, reindeer, caribou, opossums, rabbits, rodents, and lizards (Dubey et al., 2003a). There are several recognised and unrecognised species in the genus but the life cycles of only three (*Besnoitia darlingi, Besnoitia wallacei*, and *Besnoitia oryctofelisi*) are known, and morphological differences among the species are poorly defined (Dubey et al., 2003a). Although parasites isolated from different intermediate host species have traditionally been presumed to represent distinct parasite taxa, it will be difficult to test their actual host specificity until natural definitive hosts are identified.

Besnoitia bennetti was first reported by Bennett (1927, 1933) in four horses from Sudan. He believed this parasite was a species of *Sarcocystis* or *Globidium* and that it infected both cattle and horses. Babudieri (1932) transferred this parasite to the genus *Besnoitia*. Schulz and Thorburn (1955) found *Besnoitia* sp. tissue cysts in a horse from South Africa. Pols (1960) listed other unpublished reports of cutaneous besnoitiosis in horses in South Africa and indicated that he could not infect two horses with *Besnoitia besnoiti* of cattle. Bigalke (1970) found *Besnoitia* sp. in donkeys, mules, and horses in South Africa; he was unable to transmit the *Besnoitia* species from equids to cattle or

☆ Nucleotide sequence data reported in this paper are available in the GenBank, EMBL and DDBJ databases under the accession numbers: AY827838, 827839.

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from cattle to equids, indicating that equine besnoitiosis may result from a parasite specific to equids. Furthermore, equid *Besnoitia* sp. was not infective to rabbits, hamsters, white mice, or guinea pigs. van Heerden et al. (1993) provided the first ultrastructural description of *B. bennetti* from a horse from South Africa. Lane et al. (1986) reported *B. bennetti*-like tissue cysts associated with laryngeal papillomatosis in a horse imported to England from Argentina. However, the photomicrographs lack details that permit unambiguous diagnosis.

The only reports of equine besnoitiosis out side of Africa are from the USA. Terrell and Stookey (1973) found *Besnoitia* in two of 15 Mexican burrows in a closed experimental herd maintained at the US Army Research Institute, Frederick, Maryland. Davis et al. (1997) found *Besnoitia* sp. in one of eight donkeys on a farm in Montana, USA. Recently clinical besnoitiosis was reported in 14 of 38 miniature donkeys from a herd in Michigan, USA (Elsheikha et al., 2005). To our knowledge, besnoitiosis in horses has not been reported in the USA.

The life cycle of *B. bennetti* is not yet completely known. Only the tissue cyst and bradyzoite stages are known, and their structures are not fully described. Thus, little basis exists to evaluate whether the same etiological agent establishes infection, and induces disease, in horses and donkeys and possibly in other hosts. Here, we report the successful isolation of *B. bennetti* in culture and in immunodeficient mice, describe the structure of its bradyzoites and tachyzoites, and define portions of its ribosomal DNA to aid its diagnosis and comparison to related coccidia. Specimens are also deposited in museum collections to aid future comparison.

2. Materials and methods

2.1. Naturally-infected donkeys

2.1.1. Donkey no. 1

A two year old female (Fig. 1E) and her sibling were purchased when seven months old by the present owner from a commercial breeder in Texas. This donkey developed severe pruritus and loss of hair at about 18 months of age, whereas its sibling and two other donkeys (nos. 2–4),

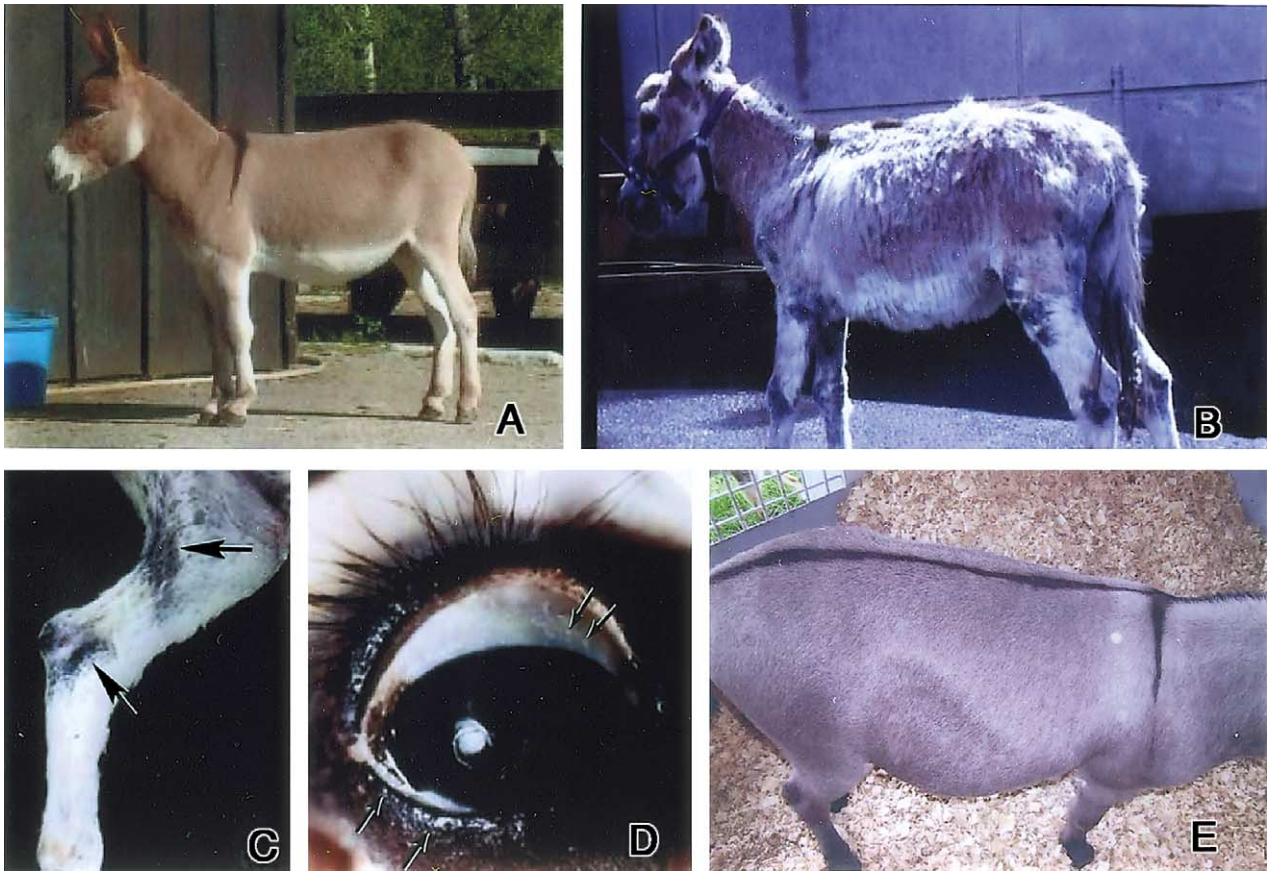

Fig. 1. Lesions and *Besnoitia bennetti* tissue cysts in donkey no. 5. (A) Before onset of clinical signs at approximately one year of age. (B) Before euthanasia, eight months after onset of clinical signs. Note marked cachexia, poor hair coat, and skin lesions (alopecia, hypotrichosis, hyper pigmentation, thickening and crusting) over lateral neck, shoulders, carpi, stifle, hock, periocular region, around ears and muzzle. (C) Lateral aspect of hind limb. Note alopecia and hypotrichosis, with thickened, irregular crusty hyper pigmented skin over caudal aspect of stifle and lateral hock (arrows). (D) Multifocal white pinpoint granular structures (*Besnoitia* tissue cysts) within the sclera and conjunctiva (arrows) of the eye. (E) Lateral view of donkey no. 1 after treatment with SMZ and TM. Note improvement in hair coat.