Natural \textit{Besnoitia} sp. infection in domestic rabbits from Argentina

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Received 17 January 2002; received in revised form 27 May 2002; accepted 28 May 2002

Abstract

\textit{Besnoitia} sp. are apicomplexan coccidian parasites affecting several species of mammals and cold-blooded animals in several countries. \textit{Besnoitia} sp. tissue cysts were seen in several tissues of five rabbits from a rabbit breeder in La Plata, Argentina. Bradyzoites released from macroscopic tissue cysts were inoculated onto bovine monocytes, and into interferon gamma gene knockout (KO) mice. \textit{Besnoitia} sp. tachyzoites were seen in the peritoneal exudate of KO mice on day 10 pi and these tachyzoites were infective to other KO mice. Tachyzoites grown in cell culture were infective to gerbils (\textit{Meriones unguiculatus}). This is the first report of \textit{Besnoitia} sp. infection in any host in Argentina.

Published by Elsevier Science B.V.

Keywords: \textit{Besnoitia} sp.; Argentina; Rabbits; Cell culture; Bradyzoites; Gerbils

1. Introduction

\textit{Besnoitia} sp. are apicomplexan coccidian parasites affecting cattle, goats, equids, reindeer, caribou, opossums, rodents, and lizards (Dubey, 1993; Paperna and Lainson, 2001). There are several named (\textit{B. jellisoni}, \textit{B. wallacei}, \textit{B. darlingi}, \textit{B. bennetti}, \textit{B. besnoiti}, \textit{B. caprae}, \textit{B. tarandi}) and unnamed species of \textit{Besnoitia} (Frenkel, 1977; Leighton and...
Gajadhar, 2001). Of the Besnoitia species, life cycles of only B. darlingi and B. wallacei are known and cats are their definitive hosts (Frenkel, 1977; Smith and Frenkel, 1977, 1984). Although certain species of Besnoitia can be experimentally transmitted to rabbits (Frenkel, 1965; Bigalke, 1967, 1968; Basson et al., 1970; Ito et al., 1978), there is only one report of natural Besnoitia infection in a rabbit from Kenya (Mbuthia et al., 1993). To our knowledge, there is no report of Besnoitia infection in any animal from Argentina. We report Besnoitia sp. infections in naturally infected rabbits from Argentina.

2. Materials and methods

Five (four live and one dead) of 16 rabbits from a small family farm near La Plata, Argentina were submitted for etiologic diagnosis because some had died earlier and were not necropsied. Rabbits were euthanized and at necropsy, tissues were fixed in 10% buffered-neutral formalin for histologic examination. In addition, protozoan tissue cysts were collected unfixed for identification. Small pieces of serosal layers from the abdomen of two rabbits were shipped unrefrigerated in antibiotic solution by air mail from Argentina and they arrived in the United States Department of Agriculture laboratory in Beltsville, Maryland 6 days later. Macroscopic tissue cysts (Fig. 1) were teased from host tissue, washed several times in antibiotic saline (0.85% NaCl) containing 1000 units of penicillin and 100 μg of streptomycin per milliliter of saline. Tissue cysts were pooled from both rabbits, homogenized in a mortar with pestle, and the zoites released were inoculated onto bovine monocytes as described by Dubey et al. (1999), and into six interferon gamma gene knockout (KO) mice (Dubey and Lindsay, 1998). Tachyzoites grown in cell culture and peritoneal exudate of KO mice were inoculated subcutaneously (s.c.) into seven gerbils (Meriones unguiculatus) obtained from Charles River, Lake View, Neufield, New Jersey.

3. Results

Pinhead-sized tissue cysts were found in the fascia and serosal layers of the thorax, abdomen and subcutaneous tissues of rabbits (Fig. 1A). In the rabbit that died, there were macroscopical tissue cysts only in the uterus. Histologically, tissue cysts were mostly on the surface of tissues. Tissue cysts had a thick cyst wall enclosing multiple host cell nuclei (Fig. 1B). Tissue cysts lacked septa and contained numerous bradyzoites.

Besnoitia sp. was cultivated in vitro cells inoculated with bradyzoites from naturally infected rabbits. Live intracellular and extracellular tachyzoites were seen in cell culture 6 days post-inoculation (pi). Most cells in the original flask were destroyed by 21 days pi and organisms were subpassaged successfully into a new flask. Organisms from the original flask were removed from the medium and inoculated into gerbils.

Bradyzoites released from tissue cysts from rabbits were infective to KO mice, both by the intraperitoneal (ip) and s.c. routes. The KO mice died of hepatic necrosis associated with intracellular growth of tachyzoites within 2 weeks pi (Fig. 2A). Tachyzoites were found in the peritoneal exudate of mice inoculated ip and in impression smears of liver and lungs of the mice inoculated s.c.
Fig. 1. *Besnoitia* sp. tissue cysts in rabbits from Argentina. (A) Mesentery with numerous glistening white tissue cysts (arrows). Unstained. (B) Section of lung with a tissue cyst. Note cyst wall enclosing several host cell nuclei (arrowheads) and numerous bradyzoites (arrow) (H and E stain).
Fig. 2. Besnoitia sp. stages in sections of tissues of KO mice and gerbils (H and E stain). (A) Liver of KO mouse, 10 days pi. Note necrosis of hepatocytes (double arrowheads), single tachyzoites (arrowheads), dividing tachyzoites (small arrow), and a large group of tachyzoites (bigger arrow). (B) Lung of gerbil, 12 days pi. Note Besnoitia zoites (arrow) in a vacuole and host cell nucleus (arrowhead). (C) Skeletal muscle of a gerbil, 33 day pi. Note granulomatous inflammation around a tissue cyst (arrow).
Besnoitia sp. tachyzoites were found in histologic sections of several tissues of four gerbils examined 12–20 days pi. Tissue cysts were seen in the heart, lungs, intestinal mucosa, kidneys and skeletal muscle of three gerbils examined 33 or 47 days pi. Granulomatous inflammation accompanied intact and degenerating tissue cysts both in rabbits and in experimentally infected gerbils (Fig. 2C).

4. Discussion

Macroscopic tissue cysts in rabbits were identified as Besnoitia sp. based on morphology of the parasite. At present, there are no definitive morphologic criteria to distinguish Besnoitia species. Therefore, the parasite in the rabbits was not identified further. Whether Besnoitia sp. in rabbits caused clinical disease in rabbits was not known. The granulomatous lesions were associated with degenerating tissue cysts, were localized and no other cause was established.

Although the species of Besnoitia in the naturally infected rabbit from Kenya (Mbuthia et al., 1993) was unknown, B. besnoiti of naturally infected cattle can be experimentally transferred to rabbits; B. besnoiti is the most pathogenic species of Besnoitia (Bigalke, 1968). Rabbits inoculated with tissue homogenates of naturally infected cattle developed clinical disease simulating the disease in naturally infected cattle (Bigalke, 1967). The parasite from rabbits was successfully transferred to cattle (Bigalke, 1967, 1968). Besnoitia wallacei of cats (Ito et al., 1978) and Besnoitia sp. of blue wildebeest (Connochaetes taurinus) and impala (Aepyceros melampus) (Basson et al., 1970) can also be transmitted to rabbits.

The infectivity of different species of Besnoitia to rodents is variable (Frenkel, 1977). For example, B. jellisoni and B. darlingi are infective to outbred albino mice, whereas B. wallacei is not infective to mice. Besnoitia jellisoni is also infective to hamsters (Frenkel, 1955). The species of Besnoitia from rabbits was infective to KO mice (which are immunosuppressed) and gerbils. The gerbils inoculated with Besnoitia tachyzoites developed tachyzoites and tissue cysts and thus may be a good animal model to maintain Besnoitia. Besnoitia besnoiti can cause clinical besnoitiosis in another species of gerbil, Meriones tristrami (Shkap et al., 1987). Attempts are now underway to determine the life cycle of the Besnoitia from the rabbits in Argentina.

References


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