Pneumocystis carinii Infection in a Domestic Goat (Capra hircus domesticus) with Multibacillary Paratuberculosis

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NOTE: Pneumocystis carinii is an opportunistic fungal pathogen commonly found in many mammalian host species, but rarely in goats. A 3-year-old, female, Tokara-native-goat (Capra hircus domesticus) died of apparent malnutrition caused by multibacillary paratuberculosis. While inflammatory response was slightly observed in the respiratory organs, P. carinii trophozoites and cysts were immunohistochemically observed in the pulmonary alveoli of the infected animal. P. carinii specific DNA was amplified from the formalin fixed and paraffin embedded lung samples. Molecular phylogenetic analyses of the mitochondrial large subunit ribosomal RNA region of P. carinii revealed genetic divergence from previously described P. carinii isolates from other mammalian host species. This is the first description of concurrent infection with P. carinii and the Mycobacterium avium subspecies paratuberculosis in a domestic goat.

KEYWORDS: goat, Mycobacterium avium paratuberculosis, Pneumocystis carinii.


Pneumocystis carinii (P. carinii) is an opportunistic fungal pathogen that can cause severe and sometimes fatal pneumonia in immunosuppressed hosts. It affects a variety of mammalian host species including humans, monkeys, rats, mice, ferrets, sloths, dogs, cats, sheeps, marmosets and voles [1]. In goats, P. carinii infection is very rare with only a few clinical cases reported thus far [9, 15]. The basic biology and genomic signature of goat-derived P. carinii have not yet been verified.

Mycobacterium avium, subspecies paratuberculosis (MAP), is the causative bacterial agent of ‘paratuberculosis’ (or ‘Johne’s disease’) in ruminant species. The resulting infection will either be cleared by the host or develop into subclinical and in some cases clinical disease. Classical clinical signs of paratuberculosis in cattle are chronic, profuse and watery diarrhea coupled with chronic emaciation, despite adequate food intake [16]. In goats, the signs are less specific, and weight loss is the only obvious sign [16]. In Japan, paratuberculosis in goats occurs sporadically, and, despite nationwide eradication strategies, new cases of paratuberculosis are often identified. The purpose of the current report is to describe the histopathological and molecular features of P. carinii infection in a MAP infected goat.

A 3-year-old female Tokara-native-goat (Capra hircus domesticus) with a 6-month history of systemic hair loss, intermittent ‘muddy’ diarrhea and marked emaciation died and was submitted to the Ishikari Livestock Hygiene Service Center for postmortem examination. The necropsy revealed that the cecal mucosa was dark red in color and heavily thickened. The jejunum, ileum and colon had a similar hue. There were annular thickenings of the wall or scattered, firm elevations on the mucosal surface of the jejunum and ileum, with smaller nodules sparsely distributed on the mucosal surface in the colon. The jejunal, ileal, cecal, colonic and mesenteric lymph nodes were highly enlarged, and scattered small white foci were observed on section. Cultures of rectal feces and mesenteric lymph nodes were performed as described previously [8] and MAP isolated from both samples.

Representative tissues were fixed in 10% neutral buffered formalin, embedded in paraffin, processed routinely, sectioned at 4 µm and stained with hematoxylin/eosin (HE), Ziehl-Neelsen and Giemsa for light microscopy. Immunohistochemistry was completed on sections of lung. Three mouse anti-P. carinii monoclonal antibodies (product number 0921, ViroStat, Portland, OR, U.S.A.; Clone 3F6, BioGenex, San Ramon, CA, U.S.A.; product number M778, Clone 3F6, Dako, Glostrup, Denmark) were used as primary antibodies. Granulomas were present in the small and large intestines. The ileum was most severely affected, with the majority of the wall composed of granulomatous tissue, extending into the mesentery. Focal necrosis was frequently seen. Tissues contained epithelioid macrophages harbouring numerous acid-fast bacteria (Fig. 1A), and an admixture of smaller numbers of neutrophils and lymphocytes. Granulomatous tissue, with areas of necrosis, was observed in the macroscopically enlarged lymph nodes. Epithelioid macrophages containing bacteria were rare in the lamina propria of the rumen and abomasum, hepatic interlobular connective tissues and alveolar spaces. These results indicate that multibacillary paratuberculosis is the primary cause of death. Although small numbers of reactive inflammatory cells were found in the lungs, foamy eosinophilic material adherent to the alveolar and bronchiolar surfaces was often seen, composed of...
globular cysts containing spores revealed in Giemsa-stained sections (Fig. 1B). The cyst wall stained positively with anti-\textit{P. carinii} antibody, clone 0921 (Fig. 1C).

For further characterization of \textit{P. carinii} in goat, molecular phylogenetic analysis was performed. Total DNA was extracted from paraffin-embedded sections of lung by means of proteinase K digestion and phenol-chloroform-isoamyl alcohol extraction. Polymerase chain reaction (PCR) assays were carried out using standard \textit{P. carinii} primers for the 5’ end of the large subunit of the mitochondrial ribosomal RNA gene (mtLSU rRNA; primers pAZ102-E and pAZ102-H) [2, 20]. Amplicons were visualized by electrophoretic separation on a 2.0% agarose gel. Direct sequencing of purified PCR products was performed, and the nucleotide sequences were deposited in DDBJ under accession number AB602435. Sequence analysis included a BLASTn search (http://blast.ncbi.nlm.nih.gov/) of the National Center for Biotechnology Information GenBank database and phylogenetic analysis. \textit{P. carinii} specific DNA (358 base pair) was amplified from the lung tissue samples in which cysts were detected. The phylogenetic analysis inferred from the mtLSU rRNA sequence comparison demonstrated that \textit{P. carinii} from goat was clearly different from all previously published \textit{P. carinii} sequences (Fig. 2). By pair-wise comparison of the nucleotide sequences, there was an 8.0% sequence divergence with \textit{P. carinii} from \textit{Equus caballus}, 10.5% sequence divergence with \textit{P. carinii} from \textit{Sus scrofa domesticus}, 11.0% sequence divergence with \textit{P. carinii} from \textit{Oryctolagus cuniculus}, 11.2% sequence divergence with \textit{P. carinii} from \textit{Saguinus midas} and 11.7% sequence divergence with \textit{P. carinii} from \textit{Homo sapiens}, indicating that goat-derived \textit{P. carinii} is most closely related to \textit{P. carinii} from \textit{Equis caballus} (Table 1). While \textit{P. carinii} organisms from goat are microscopically similar to those observed in other mammalian hosts in their shape, these genetic differences suggest that infection may be host specific.

To characterize antigenic differences between \textit{P. carinii} subspecies, monoclonal antibodies (mAb) are generally used. Although most mAb react only with the subspecies used for the immunization [10, 12, 13], some mAb react with \textit{P. carinii} from different host species. In the present study, goat derived \textit{P. carinii} showed positive staining with one mAb (clone, 0921), but not with another (clone, 3F6) made against \textit{Pneumocystis jirovecii} (\textit{P. jirovecii}), human-specific pneumocystis species, as also observed in pigs [11]. In contrast, equine \textit{P. carinii} stains positive with the latter [7]. Deduced from both mAb recognition sites, goat \textit{P. carinii} is antigenically similar to swine \textit{P. carinii}, but different to \textit{P. jirovecii} and equine \textit{P. carinii}.

\textit{P. jirovecii} infection in humans is usually found in children and, most frequently, in premature babies or immuno-

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**Table 1.** Matrix of mtLSU rRNA sequence divergence of \textit{P. carinii} derived from various mammalian hosts

<table>
<thead>
<tr>
<th>Source of rRNA</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 goat (Capra hircus)</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>2 foal (Equis caballus)</td>
<td>90.0</td>
<td>90.0</td>
<td>90.0</td>
<td>90.0</td>
<td>90.0</td>
<td>90.0</td>
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<tr>
<td>3 Garden Dormouse (Eliomys quercinus)</td>
<td>11.0</td>
<td>11.0</td>
<td>11.0</td>
<td>11.0</td>
<td>11.0</td>
<td>11.0</td>
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<tr>
<td>4 pig (Sus scrofa domesticus)</td>
<td>12.0</td>
<td>12.0</td>
<td>12.0</td>
<td>12.0</td>
<td>12.0</td>
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<tr>
<td>5 rabbit (Oryctolagus cuniculus)</td>
<td>13.5</td>
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<td>13.5</td>
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</tr>
<tr>
<td>6 red-handed tamarin (Saguinus midas)</td>
<td>15.0</td>
<td>15.0</td>
<td>15.0</td>
<td>15.0</td>
<td>15.0</td>
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</tr>
<tr>
<td>7 human (Homo sapiens)</td>
<td>18.5</td>
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<td>18.5</td>
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</tr>
</tbody>
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a) Numbers 1 to 7 refer to numbered sources of rRNA.
compromised infants 1.5 to 4 months of age with diseases, such as leukemia, primary agammaglobulinemia and acquired immune deficiency syndrome (AIDS) [4]. Diarrhetic conditions, resulting in a decreased absorption of essential nutrient factors and a loss of protein, may also allow unrestrained growth of *P. jirovecii* in the lungs due to impairment of the immune system [5]. A similar pathogenesis has been observed in animals, e.g. in rats given a protein deficient or

Fig. 2. Phylogenetic tree constructed with mtLSU rRNA sequences of *P. carinii* from goat and 26 representative human and animal species. The number on the scale bar shows the percentage occurrence in 1,000 bootstrap replicates. The isolate reported in this study is indicated in bold. The DDBJ accession numbers of *P. carinii* species used in this study are as follows: AF257179 (*P. carinii* from *Mus musculus*), AF279099 (*P. carinii* from *Microtus agrestis*), AF308809 (*P. carinii* f. sp. *rattus-quart*), AF362455 (*P. carinii* from *Saguinus midas midas*), AF362456 (*P. carinii* from *Callithrix geoffroyi*), AF362459 (*P. carinii* from *Hapalemur griseus*), AF362462 (*P. carinii* from *Saguinus fuscicollis*), AF362463 (*P. carinii* from *Maki macaco*), AF362467 (*P. carinii* from *Macaca mulatta*), AF362469 (*P. carinii* from *Macaca fascicularis*), AF362463 (*P. carinii* from *Saguinus fuscicollis*), AF362467 (*P. carinii* from *Macaca mulatta*), AF362469 (*P. carinii* from *Macaca fascicularis*), AF461781 (*P. carinii* f. sp. *macacae*), AY265383 (*P. carinii* from *Macaca nemestrina*), AY279098 (*P. carinii* from *Eliomys quercinus*), AY279099 (*P. carinii* from *Sorex araneus*), AY279100 (*P. carinii* from *Talpa europaea*), AY279103 (*P. carinii* from *Sorex araneus*), DQ452954 (*P. jirovecii* from *Homo sapiens*), FJ357851 (*P. jirovecii* from *Homo sapiens*), M58605 (*P. carinii* from *Homo sapiens*), S42915 (*P. carinii* from *Oryctolagus cuniculus*), S42921 (*P. carinii* from *Mustela putorius furo*), S42923 (*P. carinii* from *Homo sapiens*), S69692 (*P. carinii* from *Oryctolagus cuniculus*), S79766 (*P. carinii* from *Homo sapiens*) and U20169 (*P. carinii* from *Rattus norvegicus*). *P. c.* = *P. carinii*. 

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Fig. 2.
protein-free diet, hares with coccidial enteritis and a roe deer with seriously impeded feeding due to a shotgun lesion in the jaw bone [6, 18, 19]. Paratuberculosis is a chronic wasting disease leading to severe emaciation, and the presence of P. carinii in the lungs is common in case of such condition. Severe pneumonia associated with opportunistic P. carinii infection has been reported in a 4-month-old female Boer goat with abomasal and intestinal hemorrhage coupled with immune dysfunction of unknown etiology [15]. Pneumocystis pneumonia (PCP) diagnoses should thus be considered early in cases of immunocompromised goats of any age.

In young, adult, human patients, opportunistic infections usually occur in immunocompromised patients with conditions, such as AIDS. CD4+ T cells, dendritic cells and macrophages are depleted in AIDS patients, and thus intracellular bacteria and other pathogens normally controlled by these immune cells are common [3]. PCP in AIDS patients is characterized by the absence of inflammatory cells within alveoli [21]. Gross changes in goats with paratuberculosis are often difficult to detect [16], but in the current case, distinct macroscopic lesions were noted with large numbers of macrophages in the intestinal lesions. This increase in intestinal macrophages may have resulted in a reduction in macrophages mobilized to alveolar spaces and, since macrophages play an important role in host defense against the fungi [14, 17], active growth of P. carinii organisms.

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REFERENCES