Theileria buffeli infection of a Michigan cow confirmed by small subunit ribosomal RNA gene analysis

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Abstract

Theileria buffeli, generally a benign parasite of cattle, has been reported in animals from Texas, Missouri and North Carolina. To date, there have been no reports of the parasite in cattle residing in northern portions of the US. An 8-year-old cow (Maine Anjou × Angus cross-bred) in Michigan presented with hemoglobinuria and a packed cell volume of 9. Blood films stained with Giemsa showed numerous intraerythrocytic parasites morphologically consistent with T. buffeli. The parasite was confirmed to be T. buffeli by SSU rRNA gene sequence analysis (SSU rRNA sequence, Type A). This represents the first report of this parasite in an animal in Michigan. Crown Copyright © 2002 Published by Elsevier Science B.V. All rights reserved.

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Theileria mutans, a benign hemoprotozoan parasite of cattle, was first reported in the United States in Kansas in 1950 (Splitter, 1950). Twenty five years later, Kuttler and Craig reported the parasite in cattle in Texas (Kuttler and Craig, 1975). The Texas isolate was recently shown to be Theileria buffeli rather than T. mutans based on SSU rRNA gene sequence analysis (Chae et al., 1999a). Although considered a nonpathogenic parasite of cattle, T. buffeli infections have been confirmed in cattle exhibiting clinical signs consistent

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with theileriosis in Texas, Missouri and North Carolina (Chae et al., 1999a,b). Directly as a result of these clinical cases, the known geographic range of the parasite was extended. Positive confirmation of the vector tick(s) involved would establish the potential range of *T. buffeli*.

To date, the parasite has not been known to occur in the northern US. This report confirms the presence of *T. buffeli* in a cow residing in Michigan and extends the known range of the parasite.

An 8-year-old cow (Maine Anjou × Angus cross-bred) from Eaton County, MI, presented to Michigan State University Veterinary Teaching Hospital, East Lansing, MI, with a temperature of 99.7 °F, pulse rate of 88 and respiration rate of 24. Depression, icterus, anemia, dehydration (estimated 8–10%), hemoglobinuria and a profuse clear, odorless nasal discharge were noted. The mucus membranes were very yellow and tacky with a capillary refill time of 1.5 s. The lungs and trachea sounded normal and rumenations were present, but weak and incomplete. A scant amount of feces was found on rectal examination. The animal aborted an autolyzed 7-month-old fetus the next day.

A complete blood count revealed a regenerative anemia (packed cell volume 9%) and a leukocytosis (49,000 μL−1) with a severe lymphocytosis (46,000 μL−1). The animal subsequently tested positive for bovine leukemia virus. The serum chemistry profile showed elevated blood urea nitrogen, creatinine, liver enzymes and total protein (9 g/dl). Urine collected free-catch was red in color from hemolyzed blood and had a specific gravity of 1.015 and 1 + protein.

During a routine laboratory evaluation, intraerythrocytic hemoprotozoan parasites were found in blood films. A tentative diagnosis of babesiosis was made and the animal was treated with a whole blood transfusion, intravenous fluids and oxytetracycline. Although the animal’s condition remained stable, inappetance persisted. The animal was humanely euthanatized 9 days after admission.

Blood collected in EDTA was shipped to the National Veterinary Services Laboratory (APHIS-NVSL/USDA), Ames, IA, where the parasite was identified as *Theileria* sp. The blood was stored refrigerated until shipped by overnight express to Texas A&M University, College Station, TX, for molecular identification of the parasite. The sample arrived at Texas A&M University 7 days after collection. Giemsa-stained blood films revealed the presence of numerous *Theileria*-like organisms as shown in Fig. 1. The parasitemia was determined to be 3% (3000 erythrocytes counted).

A parasite SSU rRNA gene fragment (Fig. 2A) was amplified from genomic DNA (gDNA) (Fig. 2B) purified by a standard phenol–chloroform extraction method (Allsopp et al., 1989). The amplification reactions (Advantage 2 PCR Enzyme System, CLONTECH Laboratories, Palo Alto, CA) contained 1 μL of gDNA and 0.1 μM forward and reverse strand primers 989 and 990 (5′-GGTAGGGTATGGCCTACGGT-3′ and 5′-AAAGCTCCCCTCTAAGAAGC-3′, respectively, specific for piroplasm SSU rRNA genes) (Allsopp et al., 1993) in a 25 μL reaction volume. The cycling profile was initial denaturation at 94 °C for 5 min, 30 cycles of denaturation at 94 °C for 10 s, annealing at 60 °C for 10 s, and extension at 72 °C for 3 min, final extension at 72 °C for 7 min and hold at 4 °C.

 Appropriately sized amplicons of approximately 1000 bp (Fig. 2A) were directly ligated within 24 h of PCR into the plasmid vector pCR®4-TOPO and TOP 10 One Shot® Chemically Competent *Escherichia coli* cells transformed following manufacturer’s
Fig. 1. Giemsa-stained blood smears from the Michigan cow showing representative morphological forms of *Theileria* sp. organisms. Bar = 5 μm.

instructions (TOPO TA Cloning® Kit for Sequencing, Invitrogen®, San Diego, CA, USA). Plasmid DNA was purified from overnight cultures of four selected clones by a modified alkaline lysis minipreparation (QIAprep® Spin Miniprep Kit; Qiagen, Valencia, CA, USA) and used in sequencing reactions (BigDye® Termination Cycle Sequencing Ready

Fig. 2. (A) SSU rRNA gene fragment amplicon obtained from *Theileria* sp. gDNA using primers 989 and 990 (lane 2) electrophoresed through an agarose gel and stained with ethidium bromide (lane 1, 100bp DNA ladder). (B) *Theileria* sp. gDNA (1 μl) (lane 2) electrophoresed through an agarose gel and stained with ethidium bromide (lane 1, high mass marker (10 KB band = 50 ng)).
Reaction; PE Applied Biosystems, Norwalk, CT, USA) with the SSU rDNA internal primer 528F (Elwood et al., 1985) in order to sequence through the SSU rRNA V4 region (Chae et al., 1998). Automated sequencing was carried out in either an ABI PRISM Model 373A or ABA Model 377 automated sequencer with Version 1.2.2 or 2.1.1 software, respectively. Both sequencing reactions and sequencing were done by the Gene Technologies Laboratory (Institute of Developmental and Molecular Biology, Department of Biology, Texas A&M University, College Station, TX, USA). The resulting sequences were submitted to the GenBank database (National Center for Biotechnology Information, National Institute of Health) for BLAST homology searches (Altschul et al., 1990).

An alignment of the sequences from the four clones shows that the bovine *Theileria* sp. SSU rRNA gene sequences were identical to each other with a single exception at position 177 in clone 3, where a cytosine replaced a thymidine (Fig. 3). This substitution may be the result of a sequencing or amplification error since this is a conserved position. The consensus sequence of the four plasmid clones matched the *T. buffeli* (Type A) SSU rRNA gene sequence (GenBank Accession Nos. Z15106 and U97047, respectively) in the GenBank database (Fig. 3).

Three cases of bovine theileriosis associated with *T. buffeli* in the US were previously documented (Chae et al., 1998, 1999a,b; Stockham et al., 2000). In each of these cases, only a single animal was clinically affected, despite the presence of cohort cattle exposed to the same risk factors as the index animal. In the current case, this cow was also the sole animal in the herd to develop clinical signs. It is not unlikely that clinical theileriosis may have been exacerbated by other factors affecting the immune competence of the animal, such as the concurrent bovine leukemia virus infection.

The source of *T. buffeli* infection for the Michigan cow remains unclear. Since *T. buffeli* is generally not pathogenic in cattle (Kuttler and Craig, 1975), it is possible that this animal

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\begin{align*}
T. \textit{buffeli} & \quad \text{AGTGCCTAAA GCAGGCTTTT GCCTTGAAAT GTTTAGCATG GAAATATAAA} \\
\text{M1 clone 1} & \quad \text{AGTGCCTAAA GCAGGCTTTT GCCTTGAAAT GTTTAGCATG GAAATATAAA} \\
\text{M2 clone 1} & \quad \text{AGTGCCTAAA GCAGGCTTTT GCCTTGAAAT GTTTAGCATG GAAATATAAA} \\
\text{M1 clone 3} & \quad \text{AGTGCCTAAA GCAGGCTTTT GCCTTGAAAT GTTTAGCATG GAAATATAAA} \\
\text{M2 clone 3} & \quad \text{AGTGCCTAAA GCAGGCTTTT GCCTTGAAAT GTTTAGCATG GAAATATAAA} \\
\text{M1 clone 4} & \quad \text{AGTGCCTAAA GCAGGCTTTT GCCTTGAAAT GTTTAGCATG GAAATATAAA} \\
\text{M2 clone 4} & \quad \text{AGTGCCTAAA GCAGGCTTTT GCCTTGAAAT GTTTAGCATG GAAATATAAA} \\
T. \textit{buffeli} & \quad \text{GTAGGACTTT GTTCTGATTT TGTGGTATTT AGTTACAAA GTAATGTTTA 200} \\
\text{M1 clone 1} & \quad \text{GTAGGACTTT GTTCTGATTT TGTGGTATTT AGTTACAAA GTAATGTTTA} \\
\text{M1 clone 2} & \quad \text{GTAGGACTTT GTTCTGATTT TGTGGTATTT AGTTACAAA GTAATGTTTA} \\
\text{M1 clone 3} & \quad \text{GTAGGACTTT GTTCTGATTT TGTGGTATTT AGTTACAAA GTAATGTTTA} \\
\text{M1 clone 4} & \quad \text{GTAGGACTTT GTTCTGATTT TGTGGTATTT AGTTACAAA GTAATGTTTA}
\end{align*}
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Fig. 3. Michigan bovine *Theileria* sp. SSU rRNA gene V4 region sequences from plasmid clones aligned with the corresponding sequence from *T. buffeli* (GenBank Accession No. Z15106). A single base substitution, a cytosine in place of a thymidine at position 177 (asterisk), was found in gDNA clone 3 is indicated in bold.
was carrying the undetected parasite prior to the onset of clinical illness. The complete prior history of the animal is unknown. An ear-tag indicated the cow was vaccinated against brucella in South Dakota. The current owner purchased the animal 3–4 years ago from a herd dispersal sale in Michigan and was of the opinion that the cow had resided in Michigan for at least 6 years.

While on the current premises in Eaton County, the animal was kept in a closed breeding herd of approximately 30 cows and was on pasture supplemented with dry hay. In close proximity was an open commercial herd comprised of cattle that had been purchased from Pennsylvania, Colorado and Oklahoma, but no new animals had been introduced within the last 6–12 months. Previously a *T. buffeli* infected, clinically normal steer was identified in Oklahoma by SSU rRNA sequence analysis (P.J. Holman, unpublished data). It is possible that one or more of the animals introduced from Oklahoma might be reservoirs of the parasite. Also, some of the cattle in the commercial herd were part of a show string and were taken to competitions out of state as far away as Louisville, KY. Therefore it is possible that one or more of these animals might have acquired *T. buffeli* from out of state, and then served as a reservoir of infection for the index animal. Finally, the numerous white-tailed deer that populate the area may harbor ticks that carry *T. buffeli* and may come into contact with the cattle. Screening of the cohort animals for *T. buffeli* is needed to identify or eliminate possible sources of infection. Screening ticks collected in the area for *T. buffeli* also would be helpful, since the vector for *T. buffeli* in the US is unknown. Identification of the tick vector(s) for *T. buffeli* is critical to determine the source of infection in this animal, since the geographic range of the origin of infection is clearly limited by the geographic range of the vector tick.

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References


