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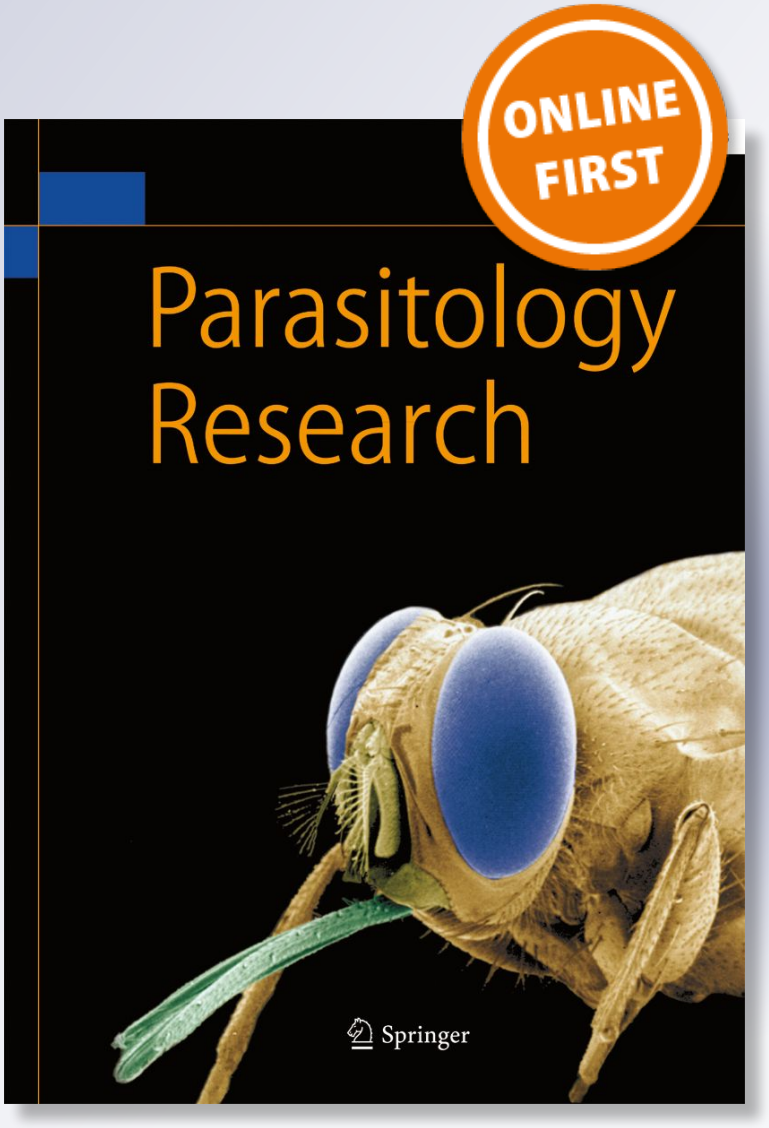
Parasitology Research
Founded as Zeitschrift für Parasitenkunde

ISSN 0932-0113

Parasitol Res
DOI 10.1007/s00436-018-6007-3



Springer

Parasitology Research
https://doi.org/10.1007/s00436-018-6007-3

SHORT COMMUNICATION

Molecular verification of transplacental transmission of *Theileria lestoquardi* in goat

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Received: 4 January 2018 / Accepted: 4 July 2018

Abstract

Ovine and caprine malignant theileriosis (OCMT), a critical condition in small ruminant production, causes lethal infections. In September 2016, a total number of 400 goats of Marghoz breed (the largest population of goat breed in Iran), in northwest of Iran, were examined for approximately 25 pre-partum abortions and 7 goats' mortality for a period of 3 days. A dead goat and her aborted fetus were brought into the Urmia Veterinary Hospital for further diagnostic investigations. The microbiological assessments including direct microscopical examination of the vaginal discharges and placentome with respect to the differential staining (Gram's staining method), conventional pure culturing, fetal abomasal contents, and the liver were negative. Microscopic examinations of blood smears of the goat and the fetus revealed characteristic of *Theileria* spp. piroplasms and impression smear samples from goat liver and fetal spleen were positive for *Theileria* Koch blue bodies. DNA analysis was performed using PCR technique and specific primers derived from the nucleotide sequences of 18S rRNA gene of *T. lestoquardi*; following extraction from blood samples, placentome, goat liver, and spleen of the fetus. The amplified DNA was sequenced afterwards and the corresponding sequence was registered under GenBank accession number MG208059. The sequence alignment showed that the products of PCR had a homology of 99% to known *T. lestoquardi* sequence registered under accession numbers of KY352037.1, KC778786.1, and JQ917458.1 in the GenBank. To our knowledge, this is a report demonstrating molecular verification of *T. lestoquardi* transplacental transmission in a neonatal kid of Marghoz breed of goats, its feasible role in induction of perinatal deaths and abortion in goat flocks.

Keywords *Theileria lestoquardi* · Transplacental transmission · Goat · Abortion · PCR

Introduction

Ovine and caprine malignant theileriosis (OCMT) are important protozoan diseases transmitted by vectors that causing an adverse effect on health and productivity of tropical and subtropical ruminants by reducing the body condition of breeding ewes and wide range of reproductive wastage such as decreased conception rate, increased number of abortions, and decreased survival rate and body weights of the newly born lambs (Mukasa-Mugerwa et al. 2002; Musa et al. 2005). OCMT is frequently reported from various regions parts of Iran that is caused by an apicomplexan parasite *Theileria lestoquardi* and carried by ixodid ticks such as *Hyalomma anatolicum* (Hooshmand-Rad and Hawa 1973). There are reports on transplacental transmission of *Theileria* agents in cattle (Baek et al. 2003) as well as in horses (Allsopp et al. 2007; Sudan et al. 2015a) that has resulted in abortion, stillbirth, or the birth of live foal with neonatal piroplasmosis in horse (Phipps and Otter 2004; Allsopp et al. 2007). Polymerase chain reaction (PCR) can confirm the transplacental transmission of *Theileria sergenti*, *T. annulata*, and *T. lestoquardi* in cattle and sheep (Baek et al. 2003; Zakian et al. 2014; Sudan et al. 2015b). The present study described the first molecular evidence regarding the detection of *T. lestoquardi* from goat in the aborted yeanling.

Section Editor: Leonhard Schnittger

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Published online: 13 July 2018

Materials and method

The study was carried out in Khoy district of West Azerbaijan province, where it was located in an important livestock production region in northwest of Iran. Easy to manage, high prolificacy, less rearing cost, broad climatic adaptation, minimum rearing space, and high need for goat products made goat rearing rising as a very important economical occupations in this province. In September 2016, a total number of 400 goats of Marghoz breed (Iran's largest population of goat breed), were examined for approximately 25 pre-partum abortions and 7 goats' mortality for a period of 3 days. A case of recently aborted fetus with the relative goat was admitted to the Veterinary Teaching Hospital, Urmia University, for further diagnostic investigations. Sudden fall of appetite, fever (41.6 °C), dullness, anorexia, pale mucosa, and tick presence on the body of animal were the prominent clinical signs. The subscapular lymph node enlargement, weakness, increased respiration, and pulse rates were also observed. Peripheral blood smears were prepared from maternal jugular vein and also made from fetus semi-clotted blood, fixated in methanol and finally stained in 10% Giemsa solution in phosphate buffer solution (PBS), pH 7.2, for 20 min and examined under an oil-immersion objective of a magnification of × 1000 for the presence of intracellular forms of the parasite with morphology compatible with *Theileria* spp. Also, goat blood sample was assigned to routine hematological parameters, including red blood cell count (RBC), the values of packed cell volume (PCV), and hemoglobin concentration by automated hematology analyzer (Autolyser AL 820, Switzerland) (Schalm et al. 1986). Furthermore, the microbiological assessments including direct microscopical examination of the vaginal discharges and placentome with respect to the differential staining (Gram's staining method), conventional pure culturing, fetal abomasal contents, and the liver were accomplished.

After abortion and fatal disease, samples were prepared from the alive and dead goats. Blood samples were collected from both goat and fetus. Small pieces of placentome, goat liver and spleen of aborted fetus were cut and transferred into the tubes containing 70% ethanol and direct impression smears were also prepared from abovementioned organs. The ethanol-fixed samples were subjected to molecular analysis and the impression smears were stained in 10% Geimsa solution. DNA was extracted from the placentome, liver, and spleen using DNA extraction kit (MBST, Iran).

A pair of primers, sense 5′-CAC AGG GAG GTA GTG ACAAG-3′ and antisense 5′-CTA AGA ATT TCA CCT CTG ACA-3′ were used to amplify a 428-bp fragment of 18S rRNA gene from *T. lestoquardi* according to method described by Shayan et al. (2011). The PCR products were isolated from the agarose gel using the gel extraction Kit (MBST, Iran); DNA bands were cut briefly under UV illumination and dissolved in the binding buffer at 60 °C. The dissolved agarose was transferred into the MBST column, washed twice with washing buffer and eluted using 20 μL Tris–EDTA buffers. The PCR products, extracted from the agarose gel were sent for sequencing (SinaClon Company, Tehran, Iran). Advanced BLAST similarity search option was used to search the obtained 18S rRNA sequences at GenBank (National Centre for Biotechnology Information, Rockville Pike, Bethesda, USA) and compared to the 18S rRNA sequences of *T. lestoquardi* strains.

Buparvaquone 5% (Butonix®, Razak, Tehran, Iran) were prescribed as anti-*Theileria* drug in the present clinical case.

Results

Microscopic examination of peripheral blood smear revealed characteristic "round to oval and ring-shaped" of *Theileria* in the red blood cell (RBC) of blood smears from goat and the fetus (Fig. 1a, d). All of these forms were classified as *Theileria* spp. Although there were no schizonts in the samples, schizonts-like structures were identified in the impression smears of the liver and spleen (Fig. 1b, c). Hematologic tests indicated very low packed cell volume (18%), RBC count (7.79×10^6/μL), and hemoglobin (6.14 g/dL) (Blood 1997). However, the microbial cultures from the goat vaginal discharges and fetal abomasal contents and the liver were negative.

PCR analysis showed an expected 428-bp fragment of 18S rRNA gene of *T. lestoquardi* was amplified from samples prepared from placentome, the goat liver, and the respective fetus spleen that were tentatively scored as positive for *T. lestoquardi* (Fig. 2). Negative control reactions of each PCR run did not yield amplification products. The PCR product was purified, sequenced, and registered under accession number MG208059 in GenBank. The sequences of 18S rRNA gene of *T. lestoquardi* obtained in the present study were compared with other ribosomal DNA (rDNA) sequences retrieved from GenBank. Sequence analysis showed 99% similarity between obtained sequences with registered sequence of GenBank *T. lestoquardi* under accession numbers of KY352037.1, KC778786.1, and JQ917458.1. Anti-*Theileria* treatment was also relatively efficient in decrease of mortality and abortion.

Discussion

Previous report of the region confirmed *T. lestoquardi* as the main *Theileria* infection in goat (Mohammadi et al. 2017). Although previous studies (Riaz and Tasawar 2017; Ullah et al. 2018) seem to agree on goat's breeds including Beetal and Kajli play an important role in breed specific susceptibility to caprine theileriosis, current study is the first report

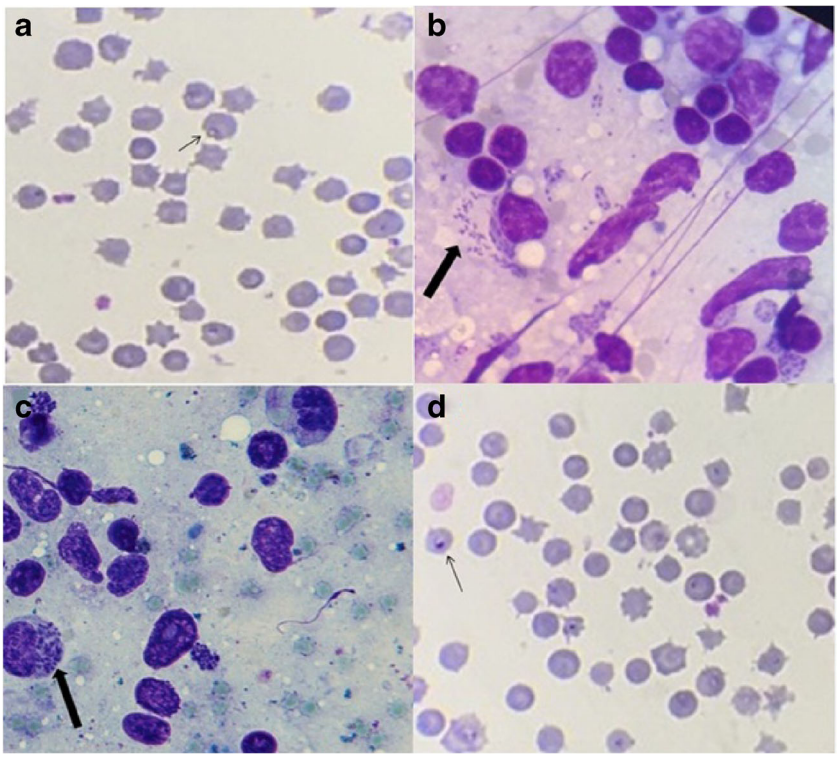

Fig. 1 Shows peripheral blood smears of (**a**, **d**) with *T. lestoquardi* piroplasms (↑) in the red blood cells, and impression smears (**b**, **c**) of goat liver and the respective fetus spleen (⬆); stained with Giemsa. Fetus (**a**, **b**); goat (**c**, **d**)

demonstrating the transplacental transmission of *T. lestoquardi* under field conditions in Marghoz breed of goats.

Using the NCBI tool and excluding PCR assay, the sequence data of the amplified product was applied for molecular confirmation through database search. Through analysis, the nucleotide sequence of *T. lestoquardi* in database revealed a 99% similarity to the obtained sequence. High mortality, fever, autoimmune hemolysis, and poor bone-marrow response as well as toxic shock were caused by induction of cytokine production such as TNF-α, IL-1, and IL-6 in infected cells that may take part in the occurrence of abortion (McGuire et al. 2014) could to be the most economically well-known impacts of the malignant theileriosis in small ruminant.

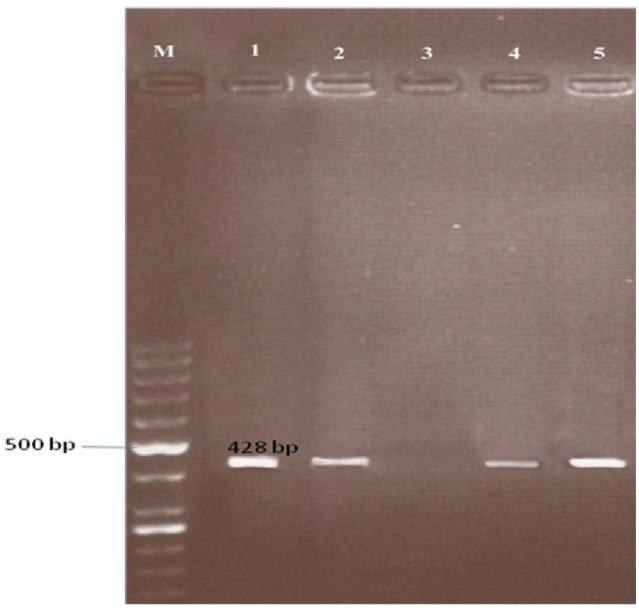

Fig. 2 Agarose gel electrophoresis of *T. lestoquardi* 18S rRNA gene products by PCR, isolated from the infected goat and respective aborted fetus. Lane 1: positive control, Lane 2: placentome; Lane 3: negative control; Lane 4: the goat liver; Lane 5: the respective fetus spleen

Despite several reports on intrauterine transmission of various *Theileria* species in the literature (Phipps and Otter 2004; Sudan et al. 2015a, b; Zakian et al. 2014; Baek et al. 2003; Swilks et al. 2017), there was no valid documentation on the transplacental transmission of caprine malignant theileriosis. It appears that *Theileria* may block and/or traverse the placental barrier and subsequently can affect fetal growth, development, and contribute in abortion. However, further reliable and detailed methodologies are needed to precisely elucidate the exact of *Theileria* spp. mechanisms involved in prenatal infection.

In conclusion, we demonstrated that transplacental transmission of *T. lestoquardi* can occur in a neonatal Marghoz breed yeanling, and its possible potential role to induce perinatal death and abortion storms in goat flocks. However, Buparvaquone 5% might have an effect on cease of *T. lestoquardi*-induced mortality and abortions.

Acknowledgments The authors would like to thank the staff at Parasitology Division of Faculty of Veterinary Medicine, Urmia University, Iran for their helps.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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