Annals of Parasitology 2016, 62(1), 39–45
doi: 10.17420/ap6201.30

Original papers

The pulmonary involvement in *Theileria lestoquardi* naturally infected sheep

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ABSTRACT. Malignant Ovine Theileriosis (MOT) caused by *Theileria lestoquardi* is considered a major constraint for sheep production in many areas of the world including Sudan. Pulmonary oedema is thought to be the main cause of animal death, but the mechanism, the cell types involved and/or the probable cause of this pneumonia has yet to be defined. The present study was carried out to investigate the pulmonary involvement post *T. lestoquardi* infection and to identify the cell types involved in pneumonia. Apparently healthy sheep were exposed to ticks challenge in *T. lestoquardi* endemic area. Lungs impression smears and tissue sections for histopathology were processed. At necropsy, fifteen infected sheep revealed severe pneumonia associated with oedema and accumulation of creamy-grayish frothy exudates. The microscopic findings of examined lungs showed emphysema, congestion, collapse and proliferation of immense amount of different kinds of cells. The current study indicates that *T. lestoquardi* infections are accompanied with remarkable pulmonary involvements and may lead to respiratory failure and death.

Key words: Malignant Ovine Theileriosis**,** pulmonary involvement**,** sheep, *Theileria lestoquardi*

Introduction

Theileria lestoquardi [1] earlier known as *T. hirci*, is a tick-borne protozoan parasite, transmitted by *Hyalomma anatolicum* [2,3] and causes a disease known as Malignant Ovine Theileriosis (MOT) and Malignant Small Ruminant Theileriosis [4]. MOT infects Ovine [5–7], Caprine [8–10] and Bovine [11]. The disease is usually acute and highly fatal to small ruminants [7,12] and manifested by fever and pneumonia [13,14]. Despite an immense amount of small ruminant research, MOT remains an important disease of sheep and goats. To date, little is known about the mechanisms involved in its pathogenesis [14–16], but the pronounced pathology and high mortality [7] are likely to be linked to the ability of its schizonts to stimulate uncontrolled proliferation of the infected leukocyte inducing a phenotype typical of tumour cells [17]. Consequently, severe tissue destruction and pulmonary oedema leading to respiratory failure are thought to be the main cause of death [7,18–20]. Although, it is clear that pulmonary manifestations in *T. lestoquardi* infections could induce pneumonia [7,18], but the mechanism, the cell types and/or the probable causes have not been clearly described. To investigate how *T. lestoquardi* affects host pulmonary organs sheep were naturally exposed to *T. lestoquardi* infection, and the cell types that occur in pneumonia have yet to be defined.

Materials and Methods

Experiments were conducted according to the animal ethics guidelines which approved by the Postgraduate Studies Advisory Committee, Faculty of Veterinary Medicine, University of Khartoum, Sudan and it guarantee that animals do not suffer unnecessarily. A total of 45, males, four to five months old, apparently healthy sheep were purchased from known disease free districts [21], transferred to the known *T. lestoquardi* endemic

focus in Atbara area, Northern Sudan [11] and were maintained for three months under natural tick challenge. Selection and maintenance of experimental sheep and clinical observations were described elsewhere [20]. Treatment was initiated for recumbent or progressively emaciated animals using Buparvaquone at a dose rate of 2.5mg/kg and some end stage animals were euthanized using barbiturate (Sodium pentobarbital) 100 mg/kg IV.

The necropsy usually performer as soon as possible on 15 infected and 7 non-infected sheep. Lungs impression smears and histopathological sections were made on microscope slides. Three to four impression smears from each lung were made and were stained with 10% Giemsa and Indirect fluorescent antibody (IFA) test stains. The details of the latter staining procedure were described elsewhere [25].

For histopathology, 120 lung tissues specimens were sampled, fixed and were processed using standard methods [22]. Briefly, 1 cm^3 specimen cut from different individual animal lungs were kept in tissue containers, fixed in 10% neutral buffered formalin, labelled and were kept until used.

The materials and methods for blood sample, DNA extraction and reaction conditions of conventional Polymerase chain reaction (PCR) were also described elsewhere [20].

Results

The lung post-mortem lesions of *T. lestoquardi* in naturally infected sheep showed prominent and remarkable macroscopic lesions during the different courses of the disease. All the infected animals (numbers=15) revealed severe pneumonia. Pneumonia was associated with oedema, prominent interstitial emphysema and accumulation of creamy-grayish frothy exudates filling the interlobular septae, pulmonary bed, bronchi, trachea bifurcation and trachea were constantly seen. In 12 infected sheep, lungs lobules were non-collapsed with rubbery texture (interstitial pneumonia) and multiple haemorrhagic foci were diffusely scattered. In addition, ten pulmonary lymph nodes were markedly enlarged and oedematus.

The results of impression smears and histopathological changes from different examined lungs during the course of the disease are shown below.

The results of microscopic examinations of lungs impression smears post *T. lestoquardi* infections revealed variable cell types. Relatively uniform, large parasitized lymphocytes were detected in nine examined lungs. Approximately 30–300 merozoites were seen in the cytoplasm of these cells (Fig. 1). In addition, numerous enlarged cells with a circular outlet cytoplasm were occasionally noticed in impression smears made from five infected lungs. Also massive numbers of schizonts parasitized cells were predominantly detected in all lungs (Fig. 2). In six lungs, lymphocytes containing schizonts were closely encircled with eight to nine monocytes were predominately noticed in rosette shapes (Fig. 3). Some of these parasitized lymphocytes were seen ruptured leaving empty circles (Fig. 4). Four lung impression smears showed ubiquitous neutrophils

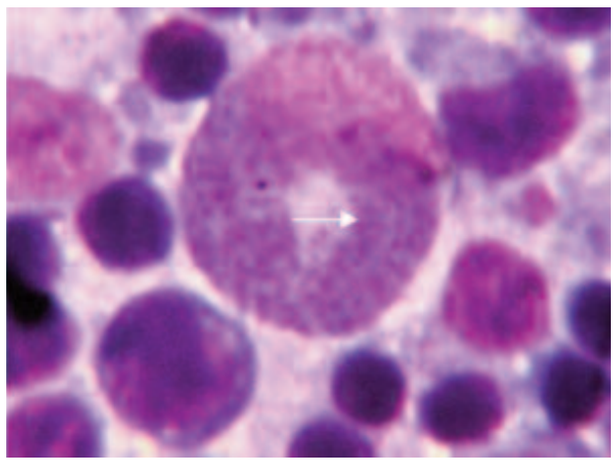

Fig. 1. Photomicrograph of lung impression smear showing large lymphocyte filled with 300 *T. lestoquardi* merozoits (arrow) (Giemsa stain ×100)

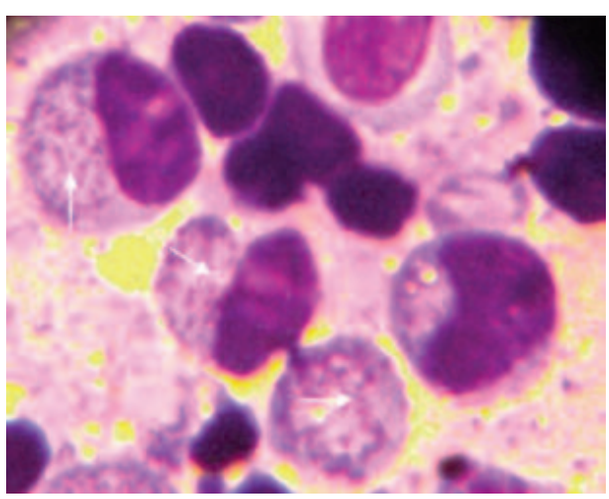

Fig. 2. Photomicrograph of lung impression smear showing massive proliferation (arrow) of *T. lestoquardi* schizonts infecting lymphocytes (Giemsa stain ×100)

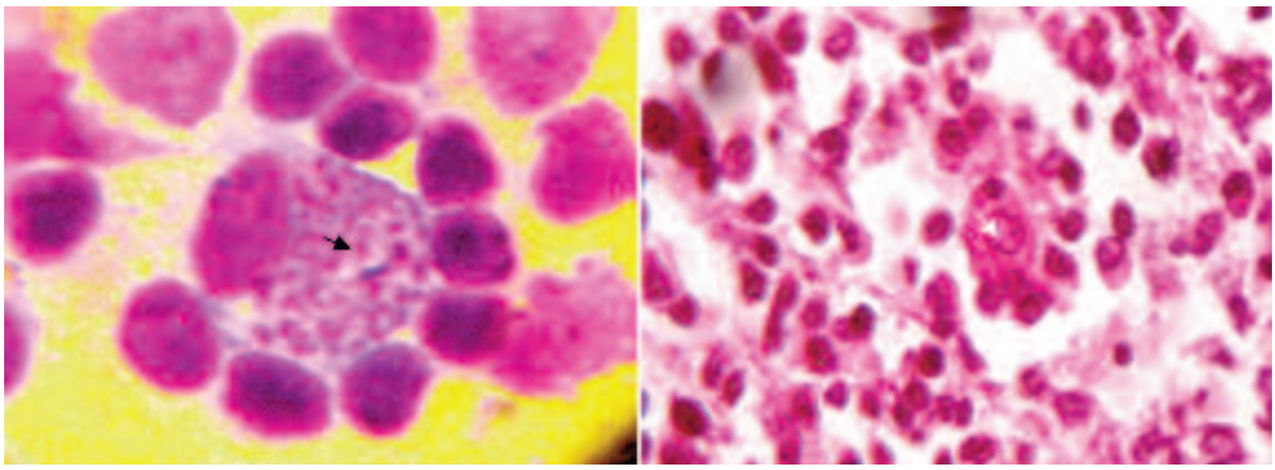

Fig. 3. Photomicrograph of lung impression smear (right, Giemsa stain ×100) and histological section (left, H & E stain ×100), showing *T. lestoquardi* schizont (arrows) encircled by 9 monocytes

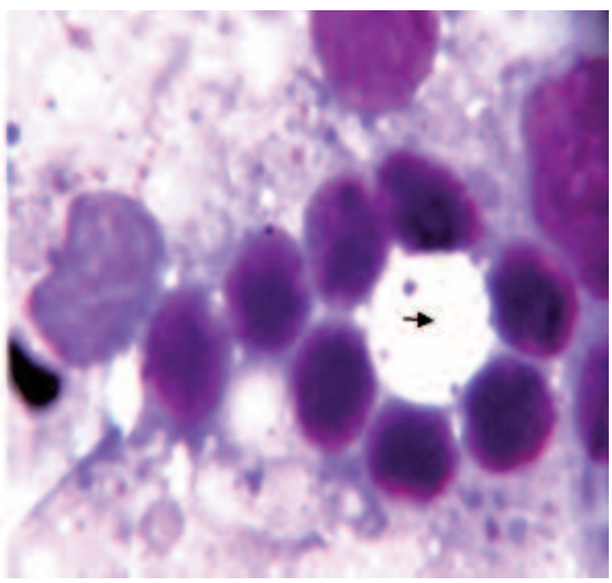

Fig. 4. Photomicrograph of lung impression smears showing *T. lestoquardi* schizonts encircled with monocytes (Giemsa stain ×100) showing empty space (arrow) surrounded by 9–8 monocytes

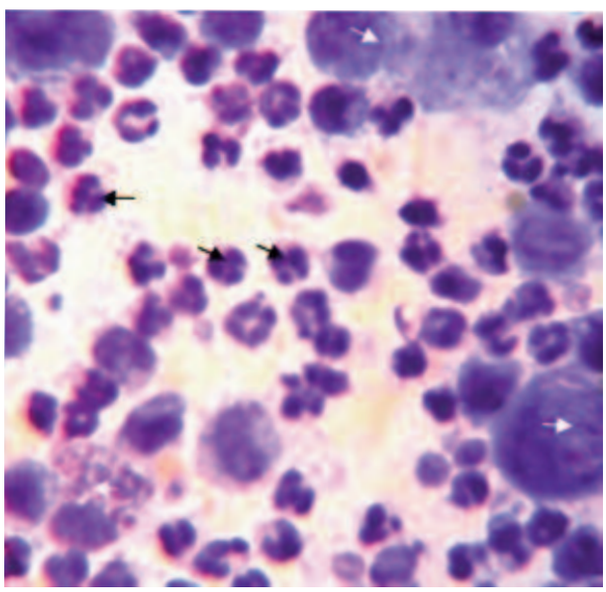

Fig. 5. Photomicrograph of lung impression smear showing massive neutrophil infiltrations (black arrows) and *T. lestoquardi* schizonts (white arrows) (Giemsa stain ×100)

which disseminated throughout the pulmonary bed (Fig. 5), also an eccentric E cells were also seen (Fig. 6). These heterogeneous E cells resemble the typical lymphocyte but with some disparities. They have abundant basophilic cytoplasm with round to oval nuclei and reddish-blue and fine to slightly condensed chromatins. Five to six condensed reddish-blue inclusion body-like structures were clumped and/or splintered in their cytoplasm. These cells approximately represented 6% of the lung lymphocytes. Notably, the same cells were noticed in different mitotic division stages (Fig. 7). The result of lung impression smears stained with IFA test showed massive *T. lestoquardi* schizonts infected cells arrested in the pulmonary bed (Fig. 8).

The histological alterations in *T. lestoquardi* infected lungs showed emphysema, congestion, collapse and proliferation of large mononuclear cells (mainly lymphocytes and macrophages) in alveoli and in blood vessels (Fig. 9). Alveolar wall appeared thickened and pneumocytes looked cuboidal with distinct nuclei and infiltrated with round giant cells. In only one section suppurative bronchopneumonia was diagnosed, whereas no changes seen in non-infected control lungs.

The PCR confirmatory test proved that all impression smears and tissue sections were sampled from *T. lestoquardi* infected animals.

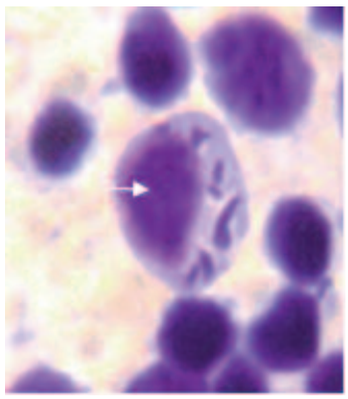

Fig. 6. Photomicrograph of lung impression smears showing E cells (arrow) (Giemsa stain ×100)

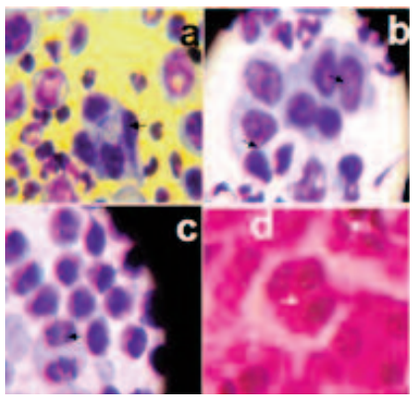

Fig. 7. Photomicrograph of lung impression smears (a, b, c) (Giemsa stain ×100) and lung section (d) (H & E stain ×100) showing E cells in different stages of mitotic divisions (arrows)

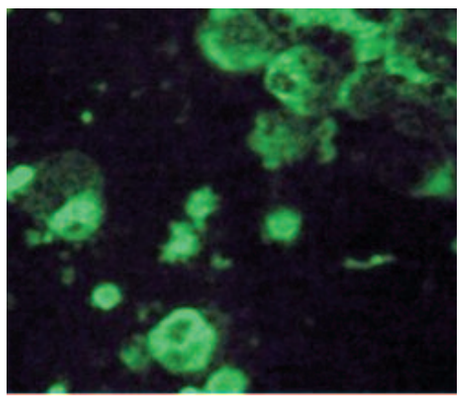

Fig. 8. Photomicrograph of a lung impression smear showing massive *T. lestoquardi* schizonts sequestration in the pulmonary bed (IFA test stain ×100)

Discussion

This experimental study provides details of macroscopic and microscopic findings of sheep lungs naturally infected with *T. lestoquardi*. Hepatization and rubbery texture of the infected lungs were previously reported [7]. In the present study, the different types of schizont infected

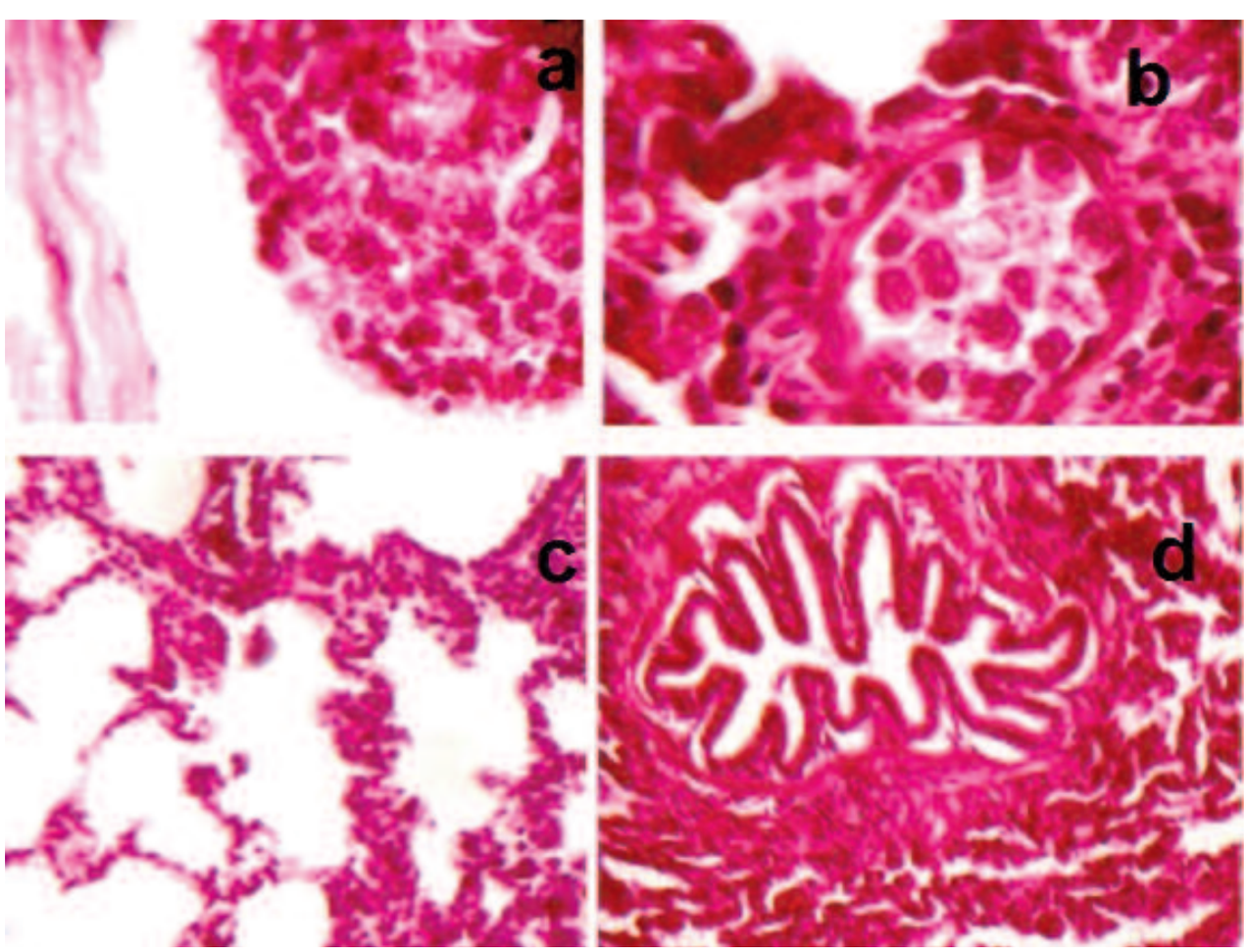

Fig. 9. Photomicrograph of lung section showing (a) congestion, (b) proliferation of large mononuclear cells in alveoli and in blood vessels, (c) emphysema and (d) collapse (H & E stain ×100)

lymphocytes were detected in impression smears taken from infected lungs; this could indicate that *Theileria* schizogony take place in the lungs [19]. Infection of lymphocytes may be extremely important in the pathogenesis of *T. lestoquardi* infections. The present study represents the first report of schizont parasitized cells which were closely encircled with more than eight to nine monocytes in a rosette shape. Some of these rosette cells were seen around empty circles could indicate their role in restriction and destruction of the schizont infected cells. These detected monocytes could presumably initiate the animals' adaptive defence mechanisms and may found in direct contact with the antigens. In fact, lung is populated by large numbers of memory T cells [23] than in peripheral circulation [24]. The functions of these cells are thought to be important in host defence [25,26] and the inflammatory response [27,28].

The activated neutrophils reported in the current examined lungs have been previously implicated in the pathogenesis of the Respiratory Distress Syndrome (RDS) [29] and in lungs endothelial and epithelial injury [30–37]. Therefore, may results in increased wet-to-dry ratios, an increase in bronchoalveolar fluid that is implicated in the pathogenesis of RDS [38]. The current study indicates that *T. lestoquardi* infections are accompanied by severe pulmonary involvements. The complications of pulmonary oedema in RDS in case of *Theileria* infected animals are not studied and the magnitudes of these clinical complications are not undertaken [7]. Presumably, *Theileria* and *Plasmodium falciparum* exhibit astonishing similarities with regard to parasite infectivity and pathological changes that involve the lungs. Therefore, pulmonary oedema as a result of increased vascular permeability is a cardinal feature of the impairment of lung function and RDS in severe *P. falciparum* malaria [39] and in *Plasmodium vivax* infection [40]. The severe tissue destruction and pulmonary oedema may involve in respiratory failure in *lestoquardi* infected animals [18,19] and in human malaria [41–44].

The phenotypes of ovine cell lines infected with *T. lestoquardi* are monocytes/macrophages and B cells, diffusely scattered in lungs and constitutes the critical mainstay in elimination of infected cells [16]. The E cells detected in the present study are probably macrophages, transformed into larger macrophages and may capable of substantial phagocytosis. The condense inclusion body-like splintered in the E cell's cytoplasm may be fragments of schizont infected cell. In fact, schizont-infected cells undergo a wide range of phenotypic alterations and may enhance the production of a number of cytokines, surface receptors and adhesion molecules [45,46]. The remarkable lungs inflammatory reactions and the metastases of schizont infected cells and large phagocytic cells reported in the present study are coinciding with previous reports [7,19,47,48] and could stimulate a severe proliferation of uninfected T lymphocytes [49]. Obviously, the detailed macroscopic and microscopic findings of infected lungs and the different cells proliferation could explain the pulmonary involvement.

Conclusions

Hitherto, the cells types and the clinical complications of pulmonary oedema in RDS in *Theileria* infected animals are not studied. The current study indicates that *T. lestoquardi* infected lymphocytes tends to metastasize and sequester in the pulmonary tissues and proliferation of other kinds of cells that may result in severe pulmonary involvements. Consequently, we could state that *T. lestoquardi* is a respiratory disease.

Acknowledgements

We are grateful to the University of Elimam Elmahadi, Ministry of Higher Education and Scientific Research, Sudan for financial support and Atbara Regional Veterinary Research Laboratories for technical assistance.

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Received 16 November 2015
Accepted 2 February 2016