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Macroscopic and Microscopic Findings in *Theileria lestoquardi* Naturally Infecting Sudanese Sheep

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Abstract

Malignant Ovine Theileriosis (MOT), caused by *Theileria lestoquardi*, is a major constraint for sheep production in many areas of the world including Sudan. Export sheep and sheep products are a major component of Sudan national economy and foreign income. Despite the importance of the disease, there is a considerable lack of detailed information regarding the postmortem and histological manifestations in the infected sheep. To specify the macroscopic and microscopic findings in sheep infected with MOT, 45 Sudanese sheep, *T. lestoquardi* negative were selected and were maintained for 3 months under natural ticks challenge. Necropsy was performed on 15 dead infected and 4 euthanized non-infected control, all pathological lesions were recorded. Kidneys, liver, lungs, spleen, heart, lymph nodes, stomach, intestine, pancreas and brain from test and control groups were sampled, fixed and were processed for histopathological examinations. The 15 infected sheep displayed severe enteritis with scattered areas of petechial hemorrhages on the serosal and mucosal surface along the small and large intestines. In most animals (n = 7-14), their superficial lymph nodes, liver and spleen were enlarged and their gall bladder were distended. Heart showed petechial hemorrhages and kidneys were congested. All infected animals (100%) revealed sever pneumonia associated with edema and frothy exudates. Comparatively, the most remarkable microscopic lesions in infected sheep were obviously seen in the lungs which exhibited emphysema, congestion, collapse and proliferation of large mononuclear cells. The present study indicates that *T. lestoquardi* infections are accompanied by severe pulmonary involvements, suggesting that emphysema and interstitial pneumonia may lead to respiratory failure and could provide evidence for death. Our findings may assist our knowledge about the microscopic and macroscopic lesions caused by *T. lestoquardi* and could contribute to raise awareness among veterinary authorities regarding the pathognomonic lesions for early and/or differential diagnosis.

Keywords: Sheep, Sudan, Theileria lestoquardi, macroscopic and microscopic lesions, Pneumonia.

1. Introduction

Theileria lestoquardi (Morel and Uilenberg, 1981) is a tick-borne protozoan parasite of sheep, transmitted by *Hyalomma anatolicum* (Tageldin *et al*., 2005; Taha and El Hussein, 2010) and causes a disease known as Malignant Ovine Theileriosis (MOT). The disease was first described in Egypt in exported Sudanese sheep and was subsequently reported in Sudan (Tageldin *et al*., 1992; Latif *et al*., 1994; El Ghali *et al*., 1994), Saudi Arabia (El-Metenawy, 1999; El-Azazy *et al*., 2001) and in Sultanate of Oman (Tageldin *et al*., 2005). Sudan is endowed with large livestock wealth, 36% of which is sheep (Sulieman *et al*., 1990) and it is of particular importance (Abualazayium, 2004). Accordingly, export sheep and their products are a major component of the national economy and foreign income. So far, the disease prevalent in different parts of Sudan, where up to 23% sero-prevalence rate (Salih *et al*., 2003) and 100% mortality in outbreaks were reported (Latif *et al*., 1994; El Ghali and El Hussein, 1995). Therefore, the improvement of the sheep production in the country is hampered.

Sheep are considered as very receptive host for natural *T. lestoquardi* infection that evolves as sub-acute and acute theileriosis (Tageldin *et al*., 1992, 2005, El Hussein *et al*., 1998, El Imam *et al*., 2015). The objective of the present study was to specify the macroscopic and microscopic findings in sheep infected with MOT under natural conditions.

2. Materials and Methods

The experiment was designed to study the macroscopic and microscopic alterations of naturally infected sheep with *T. lestoquardi* and was conducted

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according to the animal ethics guidelines which guarantee that animals do not unnecessarily suffer. A total of 45 apparently healthy male sheep, 4 to 5 months old, were purchased from known disease free districts (Hassan and Salih, 2009); they were introduced to the known *T. lestoquardi* endemic focus in Atbara, Northern Sudan (Taha *et al*., 2013) and maintained for 3 months under natural ticks challenge. Maintenance of the experimental sheep and the clinical observations are described elsewhere (El Imam *et al*., 2015). Treatment was initiated for recumbent or progressively emaciated animals using buparvaquone at a dose of 2.5 mg/kg body weight and *in the extremis* some animals were euthanized using barbiturate (Sodium pentobarbital)100 mg/kg IV, (D Special, Shering-Plough Animal Health, Germany). The animals were considered infected (n = 15) if they were PCR-positive for *T. lestoquardi* and showed schizonts in lymph node biopsy and/or piroplasms in peripheral blood smear and 4 out of 11 PCR-negative sheep were subsequently used as a non-infected control group (n = 4).

2.1. Macroscopic Findings

Within 30 minutes after death, necropsy was performed on dead and/or euthanized sheep. All pathological lesions were recorded in *T. lestoquardi* naturally infected and non-infected control animals.

2.2. Microscopic Findings

For histopathology, 200 tissues specimens (kidneys, liver, lungs, spleen, heart, lymph nodes, stomach, intestine, pancreas and brain) from infected and control animals were sampled, fixed and processed using standard methods (Bncroft *et al*., 1996).

2.3. Confirmatory Test for T. lestoquardi Infection

The materials and methods for blood sample, DNA extraction and reaction conditions for conventional PCR to confirm the disease infection were also described elsewhere (El Imam *et al*., 2015).

3. Results

T. lestoquardi schizonts and piroplasms (6.3-14.6/10^2 cells parasitaemia) were detected in 28 animals (6 suddenly died prior showing any significant clinical signs, 7 recovered and 15 were sampled) while the other 17 proved negative.

3.1. Macroscopic Findings

At necropsy, the 15 infected dead sheep displayed severe enteritis of all intestine and congestion of the digestive system with scattered areas of petechial hemorrhages on the serosal and mucosal surface of small and large intestine. Icterus (n = 12) was evident by the diffuse yellowish discoloration of the body fat and fluids (Plate 1). The superficial and mesenteric lymph nodes (n = 14) were variably enlarged (congestion, hemorrhage and/or edema) (Plate 2). The livers (n = 10) were relatively enlarged, congested and showed evidence of fatty change (n = 4) and the gall bladders (n = 9) were distended with viscid greenish bile (Plate 3). The spleens (n = 11) were congested and extremely enlarged with prominent splenic pulp (Plate 4) and contained scattered foci of capsular hemorrhage. The hearts (n = 10) were flabby and showed a petechial hemorrhage in both edocardial and epicardial surfaces. The kidneys (n = 7) were severely congested and the fat around the kidneys was relatively depleted and gelatinous. In addition, the lymph nodes near the hilus were markedly enlarged.

Comparatively, the most prominent and remarkable macroscopic lesions during the different courses of the disease were obviously seen in the lungs. All the infected examined animals (n = 15) revealed severe pneumonia associated with edema and accumulations of creamy-grayish frothy exudate. In 8 infected animals, the lungs lobules were non-collapsed with rubbery texture (interstitial pneumonia) and multiple hemorrhagic foci were diffusely scattered. In addition, the pulmonary lymph nodes (n = 14) were markedly enlarged and edematous.

Plat1 . Photograph of heart showing yellow fat (arrow), indicating jaundice in *T. lestoquardi* infected sheep.

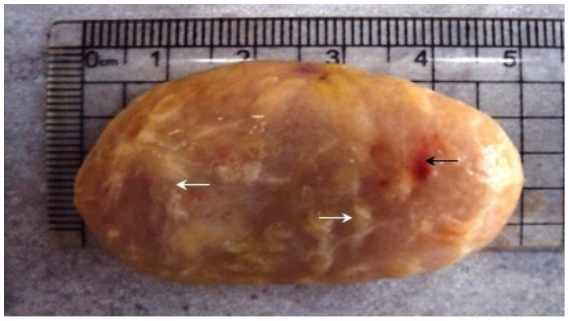

Plate2. Photograph of prescabuler lymph node showing remarkable nodular masses (white arrows) and hemorrhagic foci (black arrow) in *T. lestoquardi* infected sheep.

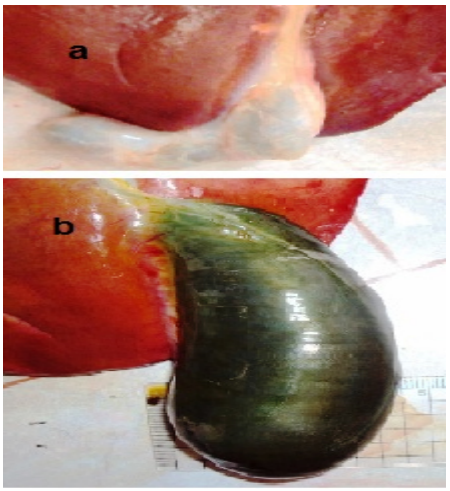

Plate3. Photograph of gall bladder in non-infected control (a) and *T. lestoquardi* infected sheep (b).

Plate4. Photograph of spleen showing splenomegaly and prominent splenic pulps in T. lestoquardi infected sheep.

3.2. Microscopic Findings

The most important histological alterations in *T. lestoquardi* infected sheep were also seen in the lungs and exhibited emphysema, congestion and collapse (Plate 5). 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.

The spleen section showed lymphoid hyperplasia with a prominent white pulp and a periarterial lymphocytic sheath, hemosiderin deposition and mononuclear cells proliferations (Plate 6).

The microscopic lesions of the lymph nodes in many sections (n = 14) showed lymphoid hyperplasia (Plate 7). Lymphoid follicles were distinct but sometimes appeared with proliferating lymphocytes. Medullary sinuses contained large lymphocytes, and the macrophages and the medullary cord were thickened.

In some sections from the heart (n = 6), the muscle cells were widely separated, or closely packed with each other. In few sections, a focal proliferation of interstitial cells was seen with the presence of prominently large mononuclear cells.

The liver in some sections (n = 3) exhibited a marked sinusoidal congestion with dilated central veins and infiltration of portal trials with mononuclear cells. In the congested sinusoid, large mononuclear cells were seen. Many sections (n = 8) showed widened sinusoid, thickened hepatic cord and large monocyte cells in sinusoid. Some of these cells appeared to have more than one nucleus or appear to show cytoplasmic granules. In two cases, capsule was markedly thickened and other two showed distinct cytoplasmic vaculations indicative of fatty change (Plate 8).

Most sections (n = 10) of the kidneys appeared normal, though, in all glomerular tuft appears cellular. In few cases (n = 3), the glomerular tuft was either highly cellular or with lobulated tuft or shrunken tuft and widened Bowman's capsule. In two cases, there were few focal areas of interstitial mononuclear cells infiltration. In a number of sections, the tubular epithelial cells, particularly in medulla, sloughed into the lumen.

The sections of the stomach appeared normal but a mucosal edema in four cases and in some mononuclear cells infiltration at the base of gastric glands or in submucosa was seen.

The section of the pancreas, rumen and the intestine appeared normal, but hypercellular of lamina propria was commonly observed in the small intestine.

Vacuolations with gliosis and satillitiosis were the prominent histological changes noticed in the brain sections. In addition only one small area of hemorrhage was seen. No macroscopic and microscopic changes seen in non-infected control animals.

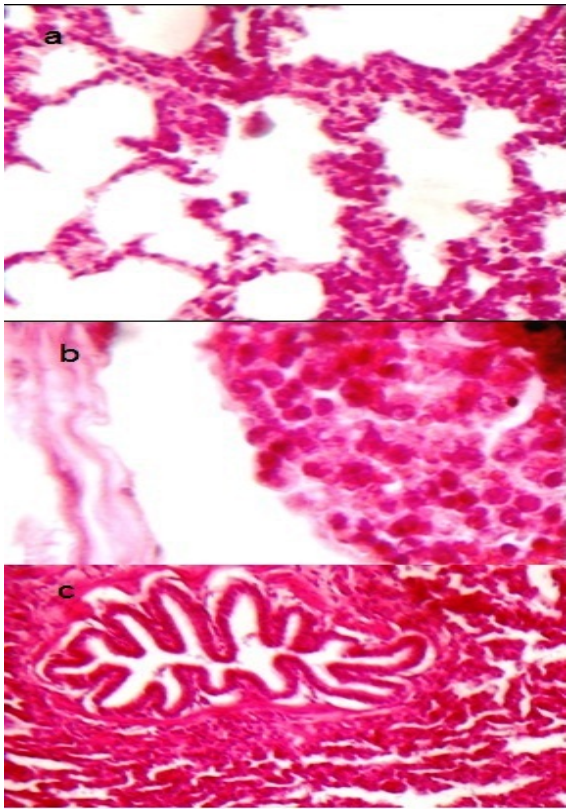

Plate5. Photomicrograph of lung showing (a) emphysema, (b) congestion and (c) collapse in *T. lestoquardi* infected sheep (H&E stain X100).

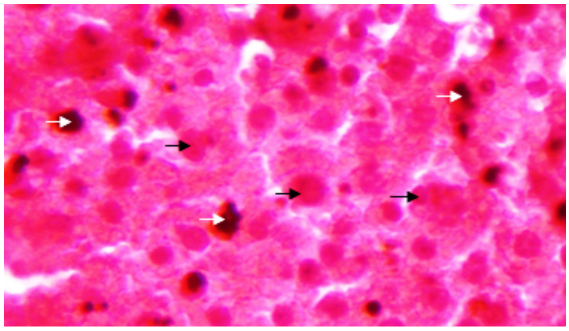

Plate6. Photomicrograph of spleen showing haemosiderin deposit (white arrows) and macrophages (black arrows) in T. lestoquardi infected sheep (H&E stain 100).

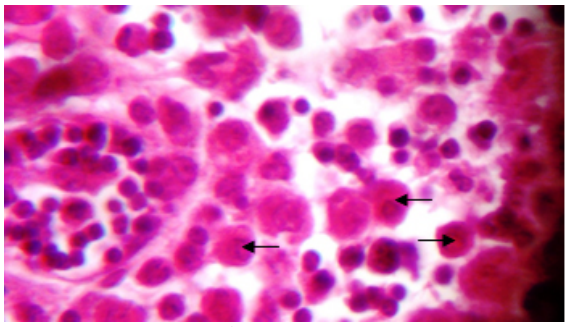

Plate7. Photomicrograph of lymph node showing hyperplasia and large mononuclear cells (arrows) in the medullary sinuses in *T. lestoquardi* infected sheep (H&E stain X100).

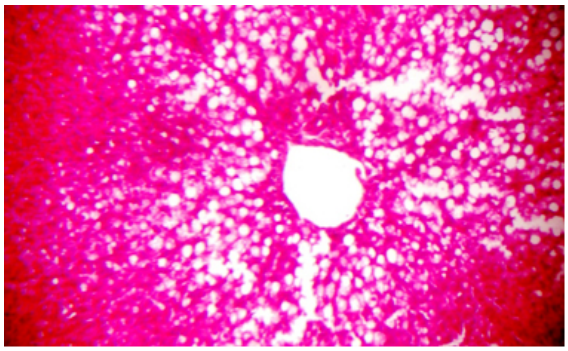

Plate 8. Photomicrograph of liver showing cytoplasmic vaculations indicative for fatty change in *T. lestoquardi* infected sheep (H&E stain X100).

3.3. The Confirmatory Tests for T. lestoquardi Infection

The PCR documentations of *T. lestoquardi* infections are shown in (Figure 1). The PCR confirmatory tests proved all the infected (n = 28) and the non-infected (n = 17) animals were positive or negative for the infection with *T. lestoquardi*.

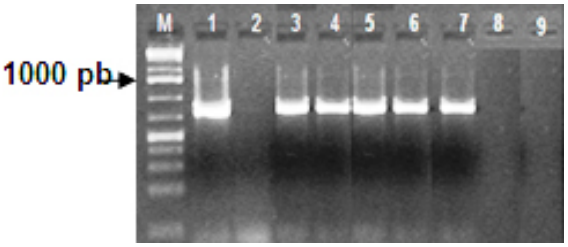

Figure 1. PCR confirmation of *T. lestoquardi* infected and control sheep.Lane M, standard size marker, L_1 positive control, L_2 negative control, L_{3-9} test samples.

4. Discussion

In Sudan, up to 100% sheep losses during *T. lestoquardi* outbreak were reported (Tageldin *et al*., 1992). Therefore, *T. lestoquardi* is a lethal disease that causes high morbidity and mortality among naive sheep population if they are exposed to *T. lestoquardi* infected ticks in endemic areas such as Northern Sudan. However, this location is currently considered a suitable region for raising sheep for commercial and export purposes to the neighboring countries.

The pronounced pathology and high mortality are likely to be linked to the ability of *T. lestoquardi* schizonts to stimulate uncontrolled proliferation of the infected leukocyte inducing a phenotype typical of tumor cells (von Schubert *et al*., 2010). The severe enteritis and congestion of the digestive system noticed in the present study could be explained by the fact that sheep are important receptive host for *T. lestoquardi*, as infection usually evolves into sub-acute and acute theileriosis (Tageldin *et al*., 1992;El Hussein *et al*., 1998; Tageldin *et al*., 2005; El Imam *et al*., 2015). The remarkable distension of gall bladder with green bile may be attributed to the heavy destruction of infected RBCs. *T. lestoquardi* infected sheep manifested severe erythrocytes destructions (El Imam *et al*., 2015). Many studies tried to clarify these mechanisms (Shiono *et al*., 2004), where, as observed, morphological changes occur in RBCs surface and increase in its osmotic fragility (Yagi*et al*., 1989), changes in membrane glycolipid components (Watarai *et al*., 1995), oxidative injuries (Shiono *et al*., 2001, 2003; Yagi *et al*., 2002), binding of IgG (Shiono *et al*., 2004) and cytokine tumor necrosis factor (Ahmed, 2002) may play a role in severe RBCs destruction and later result in destructive jaundice.

The disease severity and their pathological changes were similar to the previous reports in Sudan (Tageldin *et al*., 1992; Osman, 1999). The hepatization and the rubbery texture of the infected lungs, observed in addition to the accumulations of excessive fluid and exudate in the chest cavity, were previously reported (Uilenberg, 1981; Irvin and Morrison, 1987; Tageldin *et al*., 2005). Comparatively, lungs exhibited the most prominent microscopic findings of examined organs and proliferation of large mononuclear cells. Macrophages are tissue cells that derive from circulating blood monocytes. Usually, they are diffusely scattered and greatly found in organs such as lungs (alveolar macrophages) and may act as a filter for particular agent. These cells constitute the critical mainstay during the antigenic infection leading to eliminations of the infected cells. Serious tissue destructions and pulmonary edema suggest that emphysema and interstitial pneumonia may lead to a respiratory failure and could provide direct evidence for death (Uilenberg 1981; Irvin and Morrison, 1987; Tageldin *et al*., 2005; El Imam, 2015; El Imam and Taha, 2015). Consequently, we may speculate that *T. lestoquardi* is a respiratory disease.

The results of the PCR confirmation certainly proved that all animals that showed macroscopic and microscopic alterations were infected with *T. lestoquardi* and no postmortem and histopathological changes were reported in the non-infected control animals. The present study may represent a precise study on macroscopic and microscopic findings of pathogenic *Theileria* infecting small ruminant.

5. Conclusion

The prominent and remarkable macroscopic and microscopic lesions during the different courses of the disease were seen in the lungs. The serious pulmonary tissues destructions suggest that emphysema and interstitial pneumonia may lead to a respiratory failure and could provide evidence for death. The present

investigation gives clear evidence that native Sudanese sheep are highly susceptible to *T. lestoquardi* infections. Therefore, the disease will be lethal to sheep population if they are exposed to infected ticks in endemic area, such as Atbara, warranting more attention to ticks control strategy and vaccine production.

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