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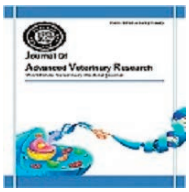

Pathological Observations in Horses Naturally Infected with *Trypanosoma equiperdum* in Western Arsi Zone, Ethiopia

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ABSTRACT

Dourine, a venereal transmitted trypanosomosis is endemic in Ethiopia and it is the major health problem threatening equines. Until recently only few studies were conducted on pathological tissue changes associated with *T. equiperdum* infection in horses. A cross-sectional study design and purposive sampling were used from November 2014 to June 2015 to identify and select dourine infected horses. Out of 480 (201 mares and 279 stallions) totally examined horses, only twelve mares were positive. Despite attempts made to isolate the parasite using Woo test, no trypanosomes were detected in all of examined blood samples. From the twelve positive mares, two severely affected mares (M1 and M2) with history of sexual infection and suggestive clinical signs as well as serologically positive by CATT/RoTat 1.2 test were purchased and euthanized for postmortem examination. Gross lesions observed in the two euthanized infected mares include, swollen vulva with visible areas of depigmentation, congestion of the mucosa of vagina, thickened and congested mucosa of uterus, ovarian follicular cysts, slightly enlarged and congested spleen, enlarged and swollen liver with multiple necrotic foci. Microscopically, mononuclear cell infiltration mainly of lymphocytes and plasma cells and periglandular inflammation were observed in the vulva, vagina, cervix and uterus. In addition, interstitial mastitis, haemosidrin deposition in the spleen and liver and lymphocytes depletion in the spleen were observed. The gross and histological findings indicated the presence of various organs involvement with severe degree of lesions. Therefore, experimental infections of natural hosts, and unnatural hosts with trypanosome obtained direct from the natural host is recommended in order to study the pathology of dourine in detail in the future.

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Introduction

The world equine population is estimated at 44 million donkeys, 11 million mules and 59 million horses (FAOSTAT, 2012). More than 97% of the world's donkey and mule populations, and over 72% of the world's horse population is found in developing countries specially kept for draft purpose (Swann, 2006). Ethiopia has more than 6 million donkeys, the second largest donkey population in the world next to China, 1.9 million horses and over 350,000 mules (FAOSTAT, 2012) specifically kept for work.

Throughout the world, the one common factor leading to the ill health, suffering and early demise of equines is the protozoan parasite, *Trypanosoma equiperdum* (*T. equiperdum*) (Stephen, 1986). Dourine is a contagious disease of equids caused by the protozoan parasite *T. equiperdum* (Sidney *et al.*, 2013). It is the only trypanosomosis that is not transmitted by blood-feeding vectors. Unlike other trypanosomal infections, dourine is transmitted almost exclusively during coitus (Claes *et al.*, 2003).

T. equiperdum differs from other trypanosomes in that it is primarily a tissue parasite that rarely invades the blood. The trypanosomes, which are present in the seminal fluid and mucous membranes of the genitalia of the infected donor animal, are transferred to the recipient during sexual intercourse. Parasites then may pass into the blood, where they are carried to other parts of the body. In typical cases, this metastatic invasion gives rise to characteristic cutaneous plaques (Hoare, 1976; Stephen, 1986).

The constant antigenic variations of the parasite results in the release of large amount of biological active products and the formation of immune complexes, which are certainly major factors in triggering a variety of clinical and pathological changes (Zwart, 1989). During the course of trypanosomosis infection, trypanosomes cause specific and non-specific damage to some of the organs involved in the reproductive process as well as the fetus. The organs include the pituitary gland, testis, epididymis, ovary and uterus. Lesions in the gonads lead to infertility while those in the fetus lead to fetal death, and/or neonatal death. Superimposed on these changes is damage to the pituitary gland (Ikede *et al.*, 1988).

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Hence, the parasite is a tissue parasite, it stays only for a brief period of time in circulation. Because of this nature of the parasite, attempt for isolation of *T. equiperdum* is difficult, as demonstrated by the low number of isolates in the past decades (OIE, 2008; Hagos *et al*., 2010a). Despite this difficulty, Fikru *et al*. (2010) and Suganuma *et al*. (2016) isolated *T. equiperdum*, directly from the blood of infected mares and the urethral tract of an infected stallion respectively. Moreover, dourine is a chronic disease whose signs are not constantly present and whose pathogenicity can vary, depending on the strain concerned. Though dourine still occurs in many parts of the world, since its eradication from North America and northern Europe, published research on the pathology, pathogenesis, immunology and chemotherapy of the disease has been neglected. Pascucci *et al*. (2013) and Ahmed *et al*. (2019) reported, congestion of vaginal and uterine mucosa with widespread hemorrhage, are characteristics gross lesion of dourine in the reproductive organs of infected mares. Plasma cell infiltration in many organs is characteristic microscopic lesion of dourine (Ahmed *et al*., 2019). Until recently few studies were conducted on tissue changes associated with the disease and so far the literature on pathological lesions caused by *T. equiperdum* in horses is scarce. Therefore, the present study is designed with the aim of characterizing the pathological lesions of dourine.

Materials and methods

Study Area

The present study was carried out in two dourine prevalent or endemic foci districts namely Asasa and Dodola located in the Western Arsi highlands of Oromia regional State, Ethiopia (Fig 1). Asasa is located in West Arsi Zone, about 300 km South from Addis Ababa with geographical coordinates of 7° 05' 60.00" N Latitude and 39° 11' 60.00"E Longitude with an elevation 2,600 to 2,650 m above sea level. Dodola is also located in the West Arsi Zone, 320 km away from the capital Addis Ababa at 6° 58' 59.99" N Latitude and 39° 10' 60.00" E Longitude with an elevation ranging from 2,362 to 2,493 m above sea level (ABZARDO, 2009). Equine population is the highest in Oromia region mainly of the Arsi-Bale highlands (ABZPO, 2009).

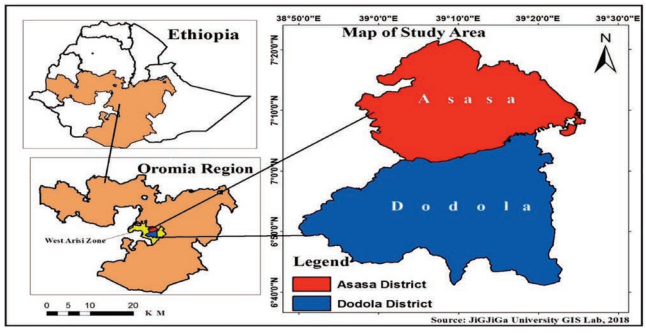

Fig. 1. Map of Ethiopia showing Asasa and Dodola districts in West Arsi Zone

Study Population

The animals included in this study were sexually mature adult horses, suspected of *T. equiperdum* natural infection, presenting typical signs of dourine natural infection, and, living under a traditional management system of free grazing. Four hundred eighty (480) sexually matured horses of both sexes (including 279 stallions and 201 mares) were observed during the study period.

Study Design and Sampling Method

A cross-sectional study design and purposive sampling method were used to identify horses showing typical clinical signs of dourine from November 2014 to June 2015. Horses present in the study area were examined clinically for the presence of typical signs of dourine and were then followed by sample collection and laboratory examination.

Clinical Examination

Careful and systematic clinical examinations of sexually matured horses in the study area were done. Special attentions were given to the body condition, skin, external reproductive genitalia and nervous system. During field observation detailed records about history, sex, age, history of sexual infection, observed signs and body condition of the animals were recorded. In addition, a detailed physical examination of horses including the measurement of the vital parameters (temperature, heart and respiratory rate) was conducted. Infected animals showing typical signs of dourine were tested using buffy coat for parasitological examination and serum for Card Agglutination Test for Trypanosomosis (CATT/*T. evansi*) obtained from the Institute of Tropical Medicine (Antwerp, Belgium) (Claes *et al*., 2003).

Blood Collection

Blood samples were collected from the jugular vein of horses showing sign of dourine twice using plain for serological and heparinized vacutainer tubes and needles for parasitological examination, after the site had been wiped with cotton wool soaked in alcohol.

Parasitological Examination

Hematocrit centrifugation technique (mHCT) was used to isolate the parasite from blood owing to the low numbers of parasites (low level of parasitemia) in the blood or tissue fluids. Microhematocrit Centrifugation Technique (mHCT) is a blood concentration technique (also called the capillary tube centrifugation technique or the Woo test) which was developed 40 years ago and is still the most frequently applied concentration technique with better sensitivity than direct microscopic examination. In this test, two capillary tubes with an internal diameter of 1 mm were filled with blood up to three-fourth (50 µl) of the capacity and centrifuged for 5 minutes in micro-centrifuges at maximum 12,000 rpm. The capillary tubes are then mounted in a viewing slide and the buffy coat plasma interface layers were examined at low magnification of 10x under microscope as described by Reid *et al*. (2001) to look for live parasites.

Serological Examination

The CATT/RoTat 1.2 serological test was performed at field level, which is considered as a rapid field screening tests in accordance with Claes *et al*. (2003). The antigen and buffer of CATT/*T. evansi* test was obtained from Ethio-Belgium VLIR-OUS dourine team project laboratory supplied from Institute of Tropical Medicine (Antwerp, Belgium). It is a rapid direct agglutination test, which uses formaldehyde fixed, Coomassie stained, freeze-dried trypanosomes of *T. evansi* VAT RoTat 1.2. In the CATT/ *T. evansi* test, 30 µl of sera diluted two-fold with PBS were mixed with 30 µl of reagent on a test card. The reagent and test serum were mixed, spread over approximately 1.5 cm. The tests were then checked with positive and negative controls before all the samples were tested. The pres-

ence of antibodies was revealed by macroscopic agglutination at a dilution of 1:8 (Verloo *et al*., 2001).

Case Diagnosis

This study was conducted in two dourine endemic foci areas of Western Arsi Zone, Oromia region, Ethiopia namely in Asasa and Dodola Districts. Previous studies in Ethiopia indicated that dourine is prevalent in these areas (Alemu *et al*., 1997; Hagos *et al*., 2010b; Clausen *et al*., 1999). Likewise, the area is free from *T. evansi* infection (Alemu *et al*., 1997). Thus, in this study, diagnosis of infection with *T. equiperdum* were made based on the typical clinical signs of dourine, history of sexual infection, epidemiology of the disease, parasitological and CATT test results. According to Büscher *et al*. (2019), diagnosis of dourine heavily relies on the combination of clinical signs, serological evidence of infection and epidemiological context.

Necropsy and tissue sample collection

Two mares (M1 and M2) with history of sexual infection, suggestive clinical signs as well as serologically positive by CATT/RoTat 1.2 test were purchased and euthanized using sodium pentobarbital at a dose of 100 mg/kg through intravenous route. Necropsy of euthanized mares was performed according to the procedure by Dennnis and Joanna (2006). Euthanized mares were then examined thoroughly for gross pathological lesions in various reproductive organs (udder, vulva, clitoris, vagina, uterus, oviduct, ovary and genital mucosa) as well as the skin, iliac, supramammary, popliteal lymph nodes, spleen, liver, kidney and lung according to VMTD (2009). Tissues with lesions were sampled for histopathological examination. The lesion part of the tissue including the normal part were taken and preserved by 10% buffered formalin according to Talkuder (2007). The sampled tissues were then transported to the National Animal Health Diagnostic and Investigation Center (NAHDIC), Sebeta, Ethiopia for histopathological processing.

Histopathological Examination

Tissue specimens were processed using standard methods of tissue processing procedure described by Talkuder (2007). Tissues were trimmed, fixed in 10% buffered neutral formalin, dehydrated in ascending grades of alcohol, cleared with xylene and impregnated with molten paraffin wax. Then tissues were sectioned at a thickness of five micrometers, spread on warm water and mounted to frosted glass slides. Stained slides were examined under microscope using 4x, 10 x, 40x magnification and photomicrographs were taken for documentation (Talkuder, 2007).

Ethical Statement

For the collection of blood specimens from horses and humane killing of horses (Euthanasia) for postmortem examination, ethical approval was obtained from the Ethical Committee of Addis Ababa University, College of Veterinary Medicine and Agriculture (CVMA) (Permit No.VM/ERC/004/03/07/2015).

Results

Clinical Cases

Out of 480 examined horses, twelve mares (12/201) and 0/279 stallions were found showing typical signs of dourine and were serologically positive. These twelve infected mares were local breed and aged between five to ten years managed under extensive production system form both study districts. Two mares, M1 and M2 showing typical clinical signs of dourine, which included genital signs (edema of the external genitalia, depigmentation of vulval skin) as shown in Fig. (2a-b), neurological signs (difficulty in walking with marked ataxia of hindquarters and spreading of the limbs) and emaciation were selected purposively, purchased and euthanized for the pathology study.

Fig. 2. Typical clinical findings of dourine observed in both mares; a) swelling of the vulva and depigmentation of the skin around the vulva in infected mare (M1); b) ulcerative lesion and depigmentation of the skin around the vulva in infected mare (M2)

Demonstration of the Parasite and Serology

Out of 480 examined horse, a total of twenty (20) clinically suspected horses were identified. From these twenty clinically suspected horses, twelve (12) mares were found positive serologically using CATT/*T. evansi* test. Eventhough, several attempts were made to isolate the parasite in the buffy coat examination using Woo test of blood samples from clinically and serologically (CATT/*T. evansi*) positive horses, no trypanosomes were detected in the examined blood samples.

Gross and Microscopic Lesions

Lesions encountered in reproductive organs

Mammary gland lesions

Grossly, the udder was slightly swollen and hard. Up on incising of the udder in the second mare (M2), calcified area of white chalky soft lesion was observed (Fig. 3a). Serum-like fluid was oozing from the base of udder up on incision in both euthanized mares. Microscopically, the mammary glands showed interstitial mastitis marked by infiltration of the interstitial space and periglandular regions with mononuclear cells mainly of lymphocytes and plasma cells (Fig. 3b). There were large aggregates of lymphocytic nodules scattered in the mammary glands (Fig. 3c). In addition, udder skin hyperplasia and areas of necrosis characterized by the loss of glands were observed (Fig. 3d).

Vulval lesions

Grossly, the vulva was swollen and with visible wide spread areas of depigmentation on the labia and thickening of the skin (Fig. 4a). In addition to these, ulceration of vulva was also seen in one mare (M2). The mucosa of the vulva was normal grossly. Microscopically, the depigmented area of the vulval skin, was characterized by severe necrosis of kerationcytes that

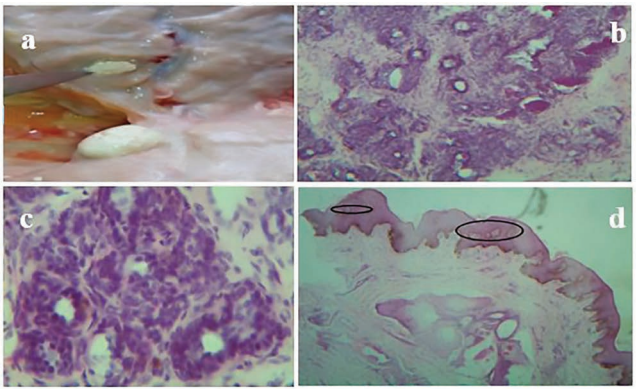

Fig. 3. Gross and histopathological findings in the mammary gland of mares. a) soft, white, chalky calcified substance within the mammary gland (gross); b) interstitial and periglandular infiltration with lymphocytes and plasma cells (10x); c) periglandular infiltration (40 x); d) hyperplasia of epidermis of skin part of the mammary gland (circled areas) (4x).

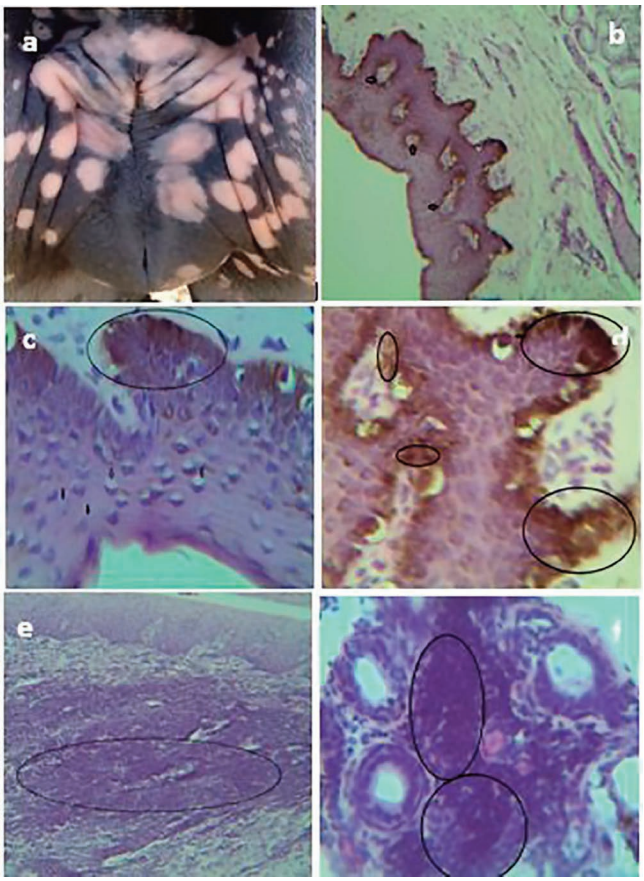

Fig. 4. Gross and histopathological findings in the vulva. a) depigmentation of the vulva skin (gross); b) necrotized areas in the Stratum spinosum living cavity like large vacuoles (arrows); c) vacuolar degeneration of the cells (lighter arrows) and necrotized cell (darker arrow) in the Stratum spinosum, degeneration and necrosis of the basal cells with melanin pigment were evident (circled areas); d) excess free melanin in the Stratum spinosum (small circles) and within basal layer (large circles); e) sever dermatitis with infiltration of lymphocytes and plasma cells in the epidermis and dermis (circled area); f) periglandular infiltration within the vulva with lymphocytes and plasma cells.

resulting in several cavities like structures in the Stratum spinosum (Fig. 4b), vacuolar degeneration of keratinocytes, presence of excessive free melanin in the Stratum spinosum and basal layer. Similarly, degeneration of the basal cell layer including the melanocytes was frequently observed (Fig. 4c-d). A histologic feature of dermatitis marked by accumulation of mononuclear cells mainly of lymphocytes and plasma cells was evident in the dermis part of vulval skin (Fig. 4e). In addition, periglandular inflammation marked by infiltration of lymphocytes was also seen within the vulva (Fig. 4f). Figure 4 is reproduced from Yonas *et al.* (2017) under the terms of the Creative Commons Attribution 4.0 International License (http:// creativecommons.org/licenses/by/4.0/).

Vaginal lesions

Grossly, vaginal lesions were congestion and presence of frothy mucus on mucosal surface in one of the mares (M1) (Fig. 5a). However, there was no visible gross vaginal lesion in the other mare (M2). The microscopic vaginal lesion includes vaginitis with infiltration by mononuclear cell mainly of lymphocytes and plasma cells forming large aggregates or follicles in the mucosa and sub mucosa of vagina (Fig. 5b). The vaginal submucosal blood vessels were hyperemic, distended and appeared full of RBC were observed (Fig. 5c). Periglandular and glandular (mural) inflammation characterized by infiltration of mononuclear cells mostly of lymphocytes were another vaginal lesion frequently observed.

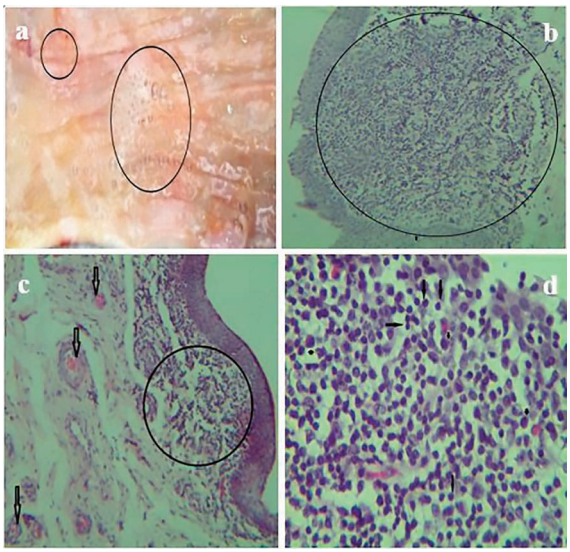

Fig. 5. Gross and histopathological findings in the vagina. a) congestion (small circle) and frothy mucus on the mucosal surface of vagina (large circle) (gross); b) aggregations of mononuclear cells mostly of lymphocytes and plasma cells in the mucosa and sub mucosa (circled area); c) infiltration of the sub mucosa (circled area) and congestion with evident of capillaries full of RBC (arrows); d) mononuclear cells in the mucosa of vagina showing lymphocytes (longer arrows) and plasma cells (shorter arrows).

Uterine Cervical lesions

Grossly, there were no visible cervical lesions observed. However, microscopically infiltration with lymphocytes and plasma cells in the mucosa and submucosa layer of cervix and periglandular inflammation was observed (Fig. 6a-b).

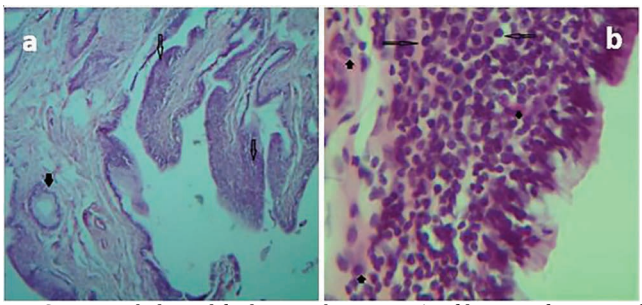

Fig. 6. Histopathological findings in the cervix. a) infiltration of mucosa of the cervix with lymphocytes and plasma cells (lighter arrows) and periglandular infiltration with lymphocytes and plasma cells (darker arrow); b) shows lymphocytes (lighter arrows) and plasma cells (darker arrows) in the mucosa of cervix.

Uterine lesions

Grossly, the entire mucosa of uterus was thickened and hyperemic in both mares (Fig. 7a). Microscopically, there were severe endometritis that were characterized by infiltration of mononuclear cells in the endometrium. Periglandular inflammations marked by aggregations of lymphocytes and plasma cells with hyperplasia of endometrial glands were also evident (Fig. 7b, d-e). In the sub-epithelial region, there were lymphocytic peri-vascular cuffing and blood vessels were hyperemic, dilated and full of RBCs (Fig. 7c). The myometrium was also infiltrated by lymphocytes (Fig. 7f).

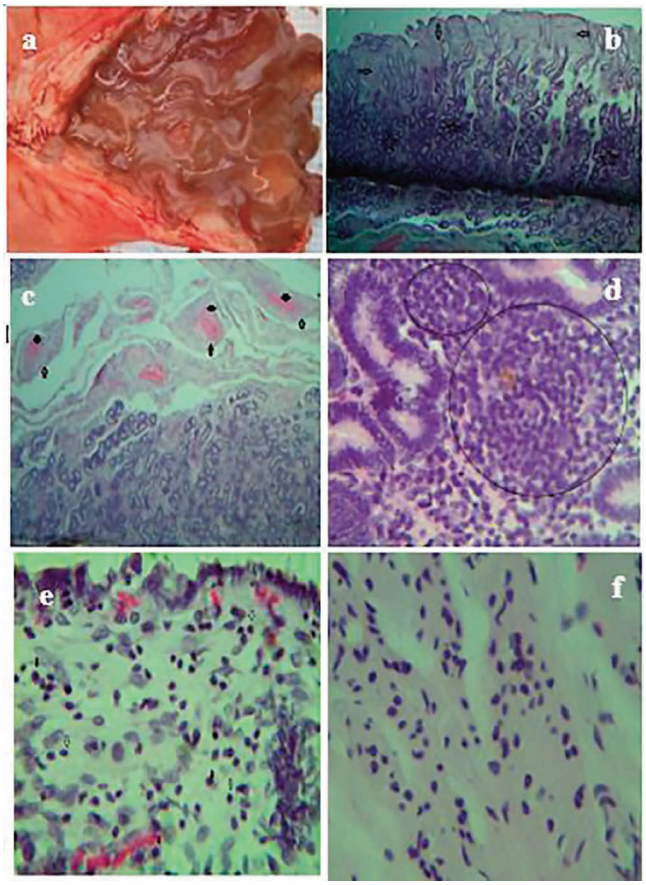

Fig. 7. Gross and histopathological findings in the uterus. a) thickened and hyperemic uterus (gross); b) infiltration of endometrium with mononuclear cells (arrows) and hyperplasia of endometrial glands (stars); c) congestion (darker arrows) and perivascular cuffing of lymphocytes (lighter arrows); d) periglandular aggregation with lymphocytes and plasma cells (circled areas); e) infiltration of endometrium with lymphocytes (lighter arrows) and plasma cells (darker arrows); f) mononuclear cells in the myometrium mostly of lymphocytes.

Ovarian and oviduct lesions

There were no gross and microscopic lesions within the ovary and oviduct in both mares (M1 and M2).

Ovarian follicular cysts

Ovarian follicular cysts were detected in both right and left ovaries of both mares. The cysts were multiple in number and the right ovaries contains more cyst compared to the left ovaries in both cases. Four follicular cysts were found in the right ovary of one mare (M1) and three in the other mare (M2) while left ovaries have two follicular cysts in both mares. These cysts contained clear light yellowish fluid, enclosed in a thin wall. Externally the cysts were transparent and vascularized with visible capillaries on their surface (Fig. 8a). Microscopically, these cysts were lined by thin layer of granulosa cells, theca interna and externally theca externa and were fluid filled at the center (Fig. 8b).

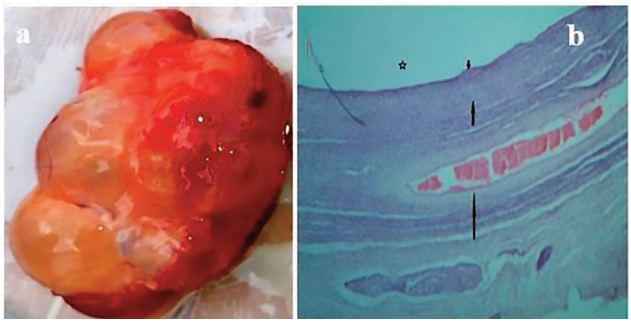

Fig. 8. Gross and histopathological findings on the ovary of both infected mares. a) follicular cyst in the ovary (Gross); b) microscopic features of follicular cyst (H&E stain) (Star=cyst cavity, short arrow=thin layer of granulosa cell, medium arrow=theca enterna, long arrow=theca externa and congested blood vessel)

Lesions encountered in non-reproductive organs

Spleen

Grossly, the spleens of both mares were slightly enlarged and congested (Fig. 9a). Microscopically, there was depletion of lymphocytes in the white pulp and at the germinal centers, multifocal congestions marked by dilated and distended sinusoids with blood. Heamosiderin pigment deposition was evident in both the red and white pulp (Fig. 9b-c). Heamosiderin laden macrophages was also evident (Fig. 9d).

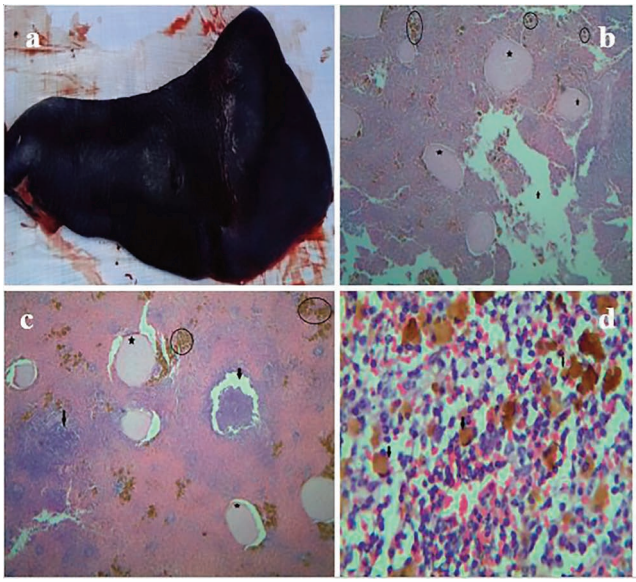

Fig. 9. Gross and histopathological findings in the spleen of infected mares. a) Enlarged and congested spleen (gross); b-c) Histopathologic features of spleen with depletion of lymphocytes (arrows), congestion (stars), heamosidren diposition (circled areas); d) heamosidren containing macrophages (arrows).

Liver

Grossly, the liver was enlarged and swollen with multiple necrotic foci and patchy fibrinous material (Fig. 10a). Accentuated lobular pattern and slight pale liver indicating cellular swelling was also clearly seen grossly (Fig. 10b). Microscopically, in the liver there were periportal mononuclear cell infiltrations specifically lymphocytes, plasma cells and some macrophages. The portal areas were severely congested with widening of sinusoids and blood vessels (Fig. 10c). Hemo-

siderin deposits were evident as golden-yellow globules. Areas of necrotic foci marked by degeneration (swelling of hepatocytes) and necrosis of hepatocyte were also observed microscopically (Fig. 10d).

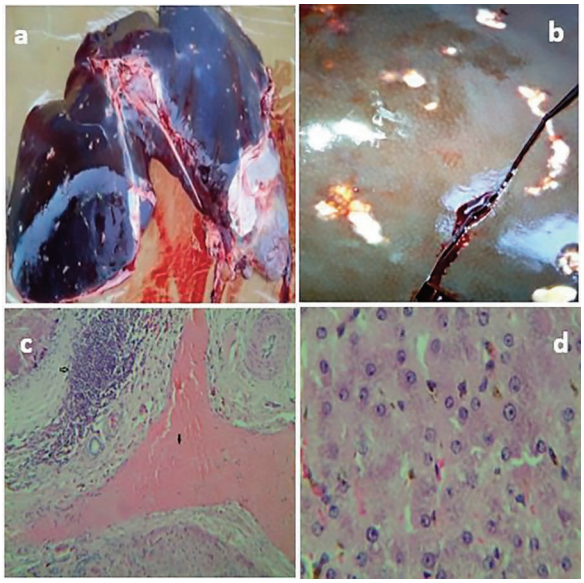

Fig. 10. Gross and histopathological findings in the liver of infected mares a) swollen liver with multiple necrotic foci and patchy fibrinous material (gross); b) accentuated lobular pattern and slight pallor liver (gross); c) periportal infiltration with mononuclear cells lighter arrow) and congestion of blood vessel (darker arrow); d) degeneration (swelling of hepatocytes) were the nucleus of hepatocytes are to the periphery and necrosis of hepatocyte, heamosidren pigment were also evident.

Abdominal cavity

In one mare (M2), there was increased peritoneal fluid within the abdominal cavity but no visible lesions were observed in the mesothelium (Fig. 11).

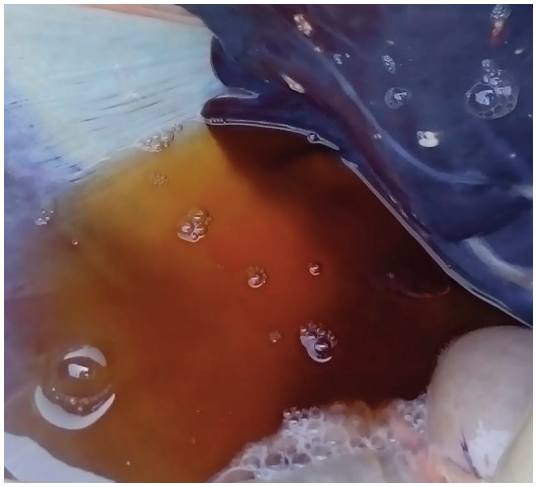

Fig. 11. Increased peritoneal fluid within the abdominal cavity (gross)

Discussion

In this study, we encountered stallions showing only neurological signs of dourine including difficulty in walking with marked ataxia of hindquarters and spreading of the limbs. However, all the typical signs of dourine were not observed in the suspected stallions. This might be due to stallions were in the late stage of the disease when signs became mild and or difference in susceptibility to infection between male and female. This is supported with the observation of Vulpiani *et al.* (2013), who reported that infected stallions showed mild signs compared to infected mares. In the experiment, Vulpiani *et al.* (2013) observed stallions for six months after infection, the stallions were almost asymptomatic. However, the possibility of difference in susceptibility to infection with regard to sex was not described in the study due to small sample size used and yet not known.

Despite the several attempts made, it was not possible to isolate the parasite by buffy coat examination from the blood of infected horses, which showed clinical evidence of infection with *T. equiperdum*. This could be due to the low number of parasites normally present in infected tissues and the mild, short-lasting parasitaemia (Hoare, 1976; Stephen, 1986; Vulpiani *et al*., 2013; OIE, 2013). *T. equiperdum* is considered primarily a tissue parasite in nature and rarely found in the blood (Hoare, 1976; Stephen, 1986). According to literature, these diagnostic difficulties are typically due to disease caused by *T. equiperdum* (Zablotskij *et al*., 2003). Despite these difficulties and failure to isolate *T. equiperdum* from the blood of infected horses, Fikru *et al*. (2010) isolated the parasite from the blood of two clinically sick horses in Dodola district of Western Arsi Zone, Ethiopia. In addition, recently Suganuma *et al*. (2016) and Ahmed *et al*. (2019) isolated *T. equiperdum*, directly from the urethral tract and blood of infected horses respectively.

The gross reproductive pathological lesions such as lesion within the mammary gland, congestion and mucus on the surface of vagina and follicular cysts encountered in this study in infected mares were not reported in the available literature, Ahmed *et al.* (2019) reported absence of macroscopic lesions of the genital tract except congestion of uterine mucosa with widespread hemorrhages. However, congestion of the uterine mucosa observed in the present study agrees with the report of Pascucci *et al*. (2013) and Ahmed *et al*. (2019).

Ovarian follicular cysts, which were observed in both infected mares in this study were not reported in literatures with infection of *T. equiperdum*. However, Vohradsky (1971) reported the presence of cystic ovaries and endometritis in cattle infected with *T. vivax*. Isoun and Anosa (1974), also observed numerous ovarian cysts containing trypanosomes in two sheep experimentally infected with *T. vivax*.

Ovarian cysts occur due to ovulation failure. The cause of ovulation failure in mares has been suggested to be endocrine in nature, either from a lack of sufficient pituitary gonadotropin stimulation to induce ovulation or from insufficient estrogen production from the follicle itself (McCue, 1998). Based on this, the occurrence of follicular cysts in the present study in infected mares might be suggested to occur due to damage to pituitary gland caused by infection with *T. equiperdum*. This is supported with researches done on other trypanosome species. Focal coagulative necrosis and interstitial mononuclear infiltration in pituitaries of sheep infected with *T. brucei* were reported by (Ikede and Losos, 1975). Ikede *et al.* (1973) reported mononuclear infiltration of the pars nervosa and surrounding meninges of horse infected with *T. brucei*. Similarly, necrotizing adenohypophysitis characterized by widespread necrosis and disruption of the architecture of the adenohypophysis were observed in *T. brucei* infected dwarf does (Leigh *et al*., 2015). Degenerative changes in the secretory cells of the adenohypophyseal region were also reported in cattle experimentally infected with *T. congolense* (Abebe *et al*., 1993).

Gross lesions in the non-reproductive organs, which include swollen liver and patchy fibrinous material in the surface of the liver with necrotic foci and accumulation of fluid in the abdominal cavity observed in this study are inconsistent with the report of Pascucci *et al.* (2013), who observed no lesions in the parenchymatous organs except congestion of spleen. However, congestion of spleen observed in the present study

was consistent with the finding of Pascucci *et al*. (2013). The accumulation of fluid in the abdominal cavity observed in this study, is in line with the finding of Ahmed *et al*. (2019), who reported that gross lesions in non-reproductive organs were not common except for abdominal fluid accumulation. The presence of increased amount of fluid in the abdominal cavity observed in one of the mare (M2) might occurred due to low albumin level. Allam *et al*. (2011) indicated that albumin level usually drop in trypanosome infections. The edema reported in the dependent parts of the body during the chronic stage of trypanosomosis could be due to a significant decrease in the albumin level that possibly indicates great liver damage (Orhue *et al*., 2005).

Microscopic lesions observed in the present study such as severe interstitial mastitis with infiltration of mononuclear cells, periglandular inflammations with in vulva, vagina and uterus, infiltration of mononuclear cells within the kidney, heamosidren deposition within the spleen and periportal infiltration are in line with the observation of Pascucci *et al*. (2013) and Ahmed *et al*. (2019), who reported similar findings in naturally infected mares with *T. equiperdum*. However, according to the available literatures, hyperplasia of skin part of the udder, severe infiltration of mucosa of vagina and cervix with lymphocytes and plasma cells, endometritis (infiltration of the endometrium with mono nuclear cells) with hyperplasia of endometrial glands and infiltration of myometrium with lymphocytes observed in this study were not reported previously. This difference might be due to giving less attention to these lesions in previous studies or difference in the host response, strain of the parasite and stage of disease.

Although, depigmentation around the perineum often described as characteristic of clinical cases of dourine (Stephen, 1986; Claes *et al*., 2003; Vulpiani *et al*., 2013; Hagos *et al*., 2010b), no microscopic description of such lesions were cited in the previous available literatures. Sever dermatitis with hydropic degeneration and necrosis of the keratinocytes of stratum spinosum, necrosis of basal cells including the melanocytes with excess free melanin pigment within the epidermis observed in this study were not characterized in the previous available literatures. The probable cause of depigmentation around the vulval skin of infected mares could be due to severe necrosis of melanocytes, as the depigmented areas were microscopically characterized by sever necrosis of cells, excess free melanin and formation of cavity like structures in the epidermis. McGavin and James (2008) stated; melanin is stored in melanosomes in the cytoplasm of melanocytes. However, damage to cells which contain melanin (e.g., damage melanocytes and basal cells of the skin), causes loss of melanin pigment in the epidermis (leukoderma) resulting in depigmentation.

The depletion of lymphocytes observed in the germinal centers of the spleen in the present study could be due to the body requirement of this cell to combat the parasites in circulating blood and this agrees with the findings of Chaudhary and Iqbal (2000) in camels infected with *T. evansi*.

Haemosidren deposition in the spleen and liver of both mares observed in this study was in agreement with the finding of Pascucci *et al*. (2013) and Ahmed *et al*. (2019), who reported haemosidren deposition in the spleen of mare naturally infected with *T. equiperdum*. The presence of increased haemosiderosis might be an indication of the major role the spleen plays in the destruction of red blood cells during trypanosomosis (Taylor and Authie, 2004).

Infiltration of tissues with mononuclear inflammatory cells especially lymphocytes, plasma cells and few macrophages is a hallmark of chronic inflammation (Jones *et al*., 1997). The microscopic findings of the present study, which were shown majority of mononuclear infiltration especially of lymphocytes, indicated the presence of chronic inflammatory process in several tissues (Morrison *et al*., 1981; Rodrigues *et al*., 2009).

Conclusion

The gross and microscopic findings in the present study indicate that there is severe organ involvement with infiltration of many organs with lymphocyte, plasma cells and few macrophages. The microscopic lesions in the reproductive organs of infected mares indicate massive infiltration of mononuclear cells mainly of lymphocytes, plasma cells and few activated macrophages. The tissue changes observed indicate that damage in tissues caused by *T. equiperdum* might occur due to direct damage by the parasite itself and or immune response by the host. Further pathological studies on the disease should be conducted in naturally infected horses (mare and stallions) by increasing the sample size by considering early/acute and chronic/advanced clinical cases. Experimental infections of natural hosts, experimental infections of unnatural hosts-all with trypanosome obtained direct from the natural host should be done in order to study pathology of dourine in detail in the future.

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Conflict of interest

The authors declare no conflict of interest exist.

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