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Full Length Research Paper

Pathological Changes in the Nervous System of Horse Naturally Affected By Dourine in Arsi-Bale Highlands, Ethiopia

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A cross-sectional study was done in three selected districts of horse-breeding in the Arsi–Bale highlands of Ethiopia. The present study was aimed at investigating the effect of natural infection by *Trypanosoma equipper dum* (dourine) in horses, determining the pathological lesions the nervous system, and to reveal the presence of the parasite in the nervous tissue or cerebrospinal fluid or in both. For this purpose, we used two horses having clinically positive and strong seropositive result in Card Agglutination Test for Trypanosomosis (CATT) test and a post-mortem examination and followed by a histopathological test. Pathological lesions in the nervous system of infected animals included degenerative changes and neuronal necrosis, which were more pronounced in the lumbar and sacral parts of spinal cord than cervical and thoracic parts and brain tissues, and cellular infiltration and degeneration in the left sciatic nerve than the right sciatic nerve. The pathological lesions were associated with the absence of the parasite (in Giemsa stain) and viral infection (in cell culture) related to increased immunological reactions.

Keywords: Arsi–Bale highlands, Dourine, Ethiopia, Histopathology, Horses.

INTRODUCTION

In Ethiopia, there are two regions (South West and North West) with a high prevalence of Trypanosomosis. In addition, six species of the disease recorded, from which *T. congolense*, *T. vivax,* and *T. brucei* are the important species causing major economic loss in domestic animals. The other species of major economic importance are *Trypanosoma equipper dum* which affect horses and *Trypanosoma evansi* affecting camel (Alemu *et al*., 1997).

Trypanosoma equipper dum is known to cause dourine, which is with characteristics of genital edema, incoordination, ocular lesions, anemia, progressive emaciation, fever, cutaneous eruptions and facial paralysis (Luckins, 1994). Equines are the only natural host of *T. equipper dum* (Claes *et al*., 2005). Horses are highly susceptible to *T. equipper dum* and they die after lasting 1-2 years with chronic disease. Donkeys and mules, even if are susceptible to the infection, they may remain asymptomatic or show mild syndrome(Taylor and Authié, 2004).

The main lesions of the disease appear in genital organs because of the transmission through the act of coition. It is known that trypanosomes cannot be found either in the peripheral or central nervous system, therefore the effect on nervous system is can be caused

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by toxins released by the organism. Detail diagnosis to demonstrate the parasite, biochemical, serological and molecular tests should be used in addition to the use of clinical signs (Robert, 1919).

There are few studies conducted in Ethiopia with regard to Dourine like based on clinical signs (Dagnachew *et al*., 1980), serologically by ELISA and CFT (Alemu *et al*., 1997), by ELISA, CATT/*T. evansi* and Latex/ *T. evansi* (Hagos *et al*., 2010) and molecular by PCR (Clausen *et al*., 1999). Therefore the objectives of the present study was to describe the neurologic signs and the pathologic findings in the nervous system of horses naturally affected by dourine, and to reveal the presence of the parasite in the nervous tissue or cerebrospinal fluid or in both.

MATERIALS AND METHODS

Study Area

The study was conducted in three districts of the Arsi-Bale highlands of Ethiopia, namely Assassa, Adaba and Dodola. The Arsi-Bale highlands are found in the Oromiya Regional State with the altitude of 500 to 4,130 meters above sea level and a bimodal rainfall occurring from July to October and April to May. Average annual temperature of 20–25°C and rainfall of 200 mm in the lowlands as well as 10–15°C and a rainfall of 400 mm in the highlands are recorded (Arsi-Bale Zone Agricultural and Rural Development Office, 2009).

Study Population and Design

The study animals include sexually mature horses suspected with *T. equipper dum* infection under natural condition. The study was cross-sectional (observational) study. A total of 20 local breeds of horses, which were kept under traditional management system of free grazing and for dourine, weer considered as study animals and out of them two highly seropositive clinically positive mares purchased for detail and thorough post mortem examination and histopathology. Castrated animals and animals with trypanocidal treatment recently were excluded purposively from the sample.

Clinical Examination

Clinical examination is done by observation based on symptoms and signs mentioned by Claes *et al.* (2003) as a pathognomonic sign like typical cutaneous plaques, with sizes ranging from extremely small to hand sized. In addition, the characteristics of the disease such as damage to the genitalia, edema , and tumefaction were used as a diagnosis tool (Claes *et al*., 2005). The last stage of dourine is characterized by progressive anemia, disorders of the nervous system mainly paralysis of the hind legs and paraplegia and, finally, death. The clinical examination was done on affected horses that were coming to the clinics of the three selected horse-breeding districts of the Arsi–Bale Highlands. Based on the clinical signs, horses that showed the neurologic signs with hind leg paralysis and pathognomonic signs in the genitalia were selected for further diagnosis and histopathology.

Parasitological Tests

The parasitological test was done as described in Woo, (1969); Reid *et al*. (2001):

From 12 horses suspected clinically, about 7.5 ml of blood samples were collected from jugular vein using venoject needles and heparinized vacutainer tubes. The blood collection site was wiped with cotton wool soaked in alcohol. The capillary tube was filled up to three-fourth volumes and centrifuged for 5 minutes in micro-centrifuges at 12,500 rpm, for the parasitological test. Then, the capillary tubes were placed in a holder known as viewing chamber and microscopically examined at 10X magnification to look parasites on the buffy coat. The tests were conducted at field level in Arsi-Bale highlands in Assassa, Dodola and Adaba veterinary clinic.

Serological Test

Whole blood samples collected for serological testing were allowed to clot overnight at room temperature. Separated serum was filled with serum cryogenic vials and stored at -20°C until testing. CATT for *Trypanosoma evansi*, which is a rapid direct agglutination test, that uses formaldehyde fixed, Coomassie stained, freeze-dried trypanosomes of *T. evansi* VAT RoTat 1.2 was employed to demonstrate the presence of antibodies in serum samples according to Claes *et al.* (2005). The tests were conducted at Dodola veterinary clinics. Positive results were determined at cut-off point dilutions 1:4 (Verloo *et al*., 2000).

Postmortem Examination

Out of twelve horses thoroughly examined, two mares suffering from classical nervous signs and having strong positive for CATT were euthanized with sodium pentobarbital at 100 mg/kg dose through intravenous injection for postmortem and histopathological examination. The CSF (cerebrospinal fluid) was collected from the atlanto-occipital space as described

by Furr and Andrew 2008 and stored at -20 °C until processing. Samples of CSF were centrifuged at 3000 rpm for 15 min, where trypanosomes are detected at the bottom layer.

Thick smears were prepared and stained with Giemsa solution for morphological examination under oil immersion. Gross lesions in the different parts of the nervous system were recorded. Histopathological samples were taken at a thickness of 5-10mm from brain (cerebral cortex and cerebellum), spinal cord (cervical, thoracic, lumbar and sacral parts), and peripheral nerve (sciatic nerves). Tissue samples were fixed with buffered formalin, and transported to National Animal Health Diagnostic and Investigation Center (NAHDIC), for histopathology.

Histopathological Examination

Formalin-fixed nerve tissues were trimmed at an appropriate thickness. Tissues were dehydrated using ascending concentration of alcohols, cleansed by xylene and impregnated with molted paraffin wax. Following embedding with paraffin, tissues were sectioned at 5-µm thickness (Lillie and Fulmer, 1976) and, stained with hematoxylin and eosin stains. Duplicate sections were stained with Giemsa for parasitological examination for the detection of the parasite in the nerve tissues. Slides were then examined under a microscope.

Detection of Viral Growth in Cell Cultures

This has been performed to exclude viral diseases having similar neurological clinical signs such as equine infectious anemia and equine herpes virus 1 infection. A total of four spinal cord and sciatic nerve samples were collected from two mares and preserved in phosphate buffered saline (PBS) solution containing gelatin and antimicrobial agents and kept in refrigerator (+4^{o}C) until and during transport in ice box to National Animal Health Diagnostic and Investigation Center (NAHDIC), for viral growth detection in cell culture.

Viral culturing techniques: Tissue samples were homogenized using mortar and pestle, centrifuged at 3400 rpm for 10 min and 0.5 ml of the supernatant was inoculated on the confluent BHK-21 cells and incubated at 37°C. Viral growth was recognized by the cytopathic effect (CPE), characterized by a change in shape, cell detachment, fusion leading to syncytium formation, the presence of inclusion bodies and cell death (Gelagay *et al*., 2013).

Ethical Clearance

The study animals, horses, especially the two mares are euthanized based on the permission obtained from the Animal Research Ethical Review Committee, that provided the author an ethical clearance certificate with Ref. No. VM/ERC/004/03/07/2015, and date of review: VM/ERC/004/07/015, 17/04/2015.

RESULTS

Parasitological and Clinical Observations

A total of 20 naturally infected mares were studied. No rise in body temperature was observed in any of the infected animals. Based on the characteristic dourine signs, clinically sick horses were observed (Figure 1). In 4 mare animals, external genitalia edema, mucopurulent vaginal discharge with a foul odor and depigmented scars over the external genitalia were the prominent signs observed in the genital form of the disease (Figure 1B and C).

In 8 mares, a frequent ulceration was observed, and there were ulcers on the clitoris and the labia. Lameness in one or both legs, restlessness, partial dragging or stiffness of the hind legs, asymmetrical posture, incoordination, and shifting weight from one leg to another were the dominant signs observed in the nervous signs of the disease. The left hind legs of the two slaughtered mares were often dragged on the ground. As the disease progressed, movement difficulties became prominent and the affected animals were not capable of moving.

Both veterinarians and farmers observed that some horses with the nervous forms of the disease become paraplegic with marked muscular atrophy in the gluteal region followed by paralysis and finally death. In addition to all these clinical signs, dourine-suspected horses were frequently emaciated, weak and poor in body condition (Figure 1A). The cutaneous form of the disease, which is mainly characterized by ‘urticarial plaques` marked by distinct, raised round- or oval-shaped patchy eruptions were not observed in the present study. There was also no loss of appetite.

Serological and Histopathological Examination

Out of the 12 sera tested with CATT, 2 mares showed high positivity while 8 mares showed moderate seropositivity. Mares with high seropositive result were purchased for post-mortem examination. There were no remarkable gross lesions in the brain, spinal cord, and sciatic nerves in both mares slaughtered. No trypanosomes were detected in all Giemsa-stained smears (CSF) and tissue sections, and in blood samples under haematocrit centrifugation technique “WOO’s technique”.

Figure 1. Weight loss (A) and edema of the external genitalia (B) and depigmented scar (C)

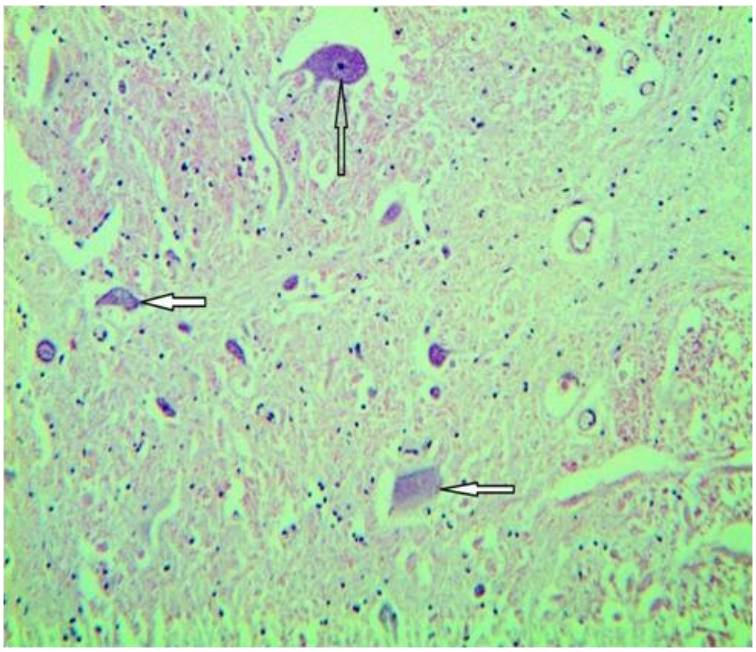

Figure 2. The lumbar region of the spinal cord, Horse No. 2. Central chromatolysis(open arrows); shrunken, necrotic neuron (closed arrows). H&E stain 10x.

Nervous tissue using haematoxylin-eosin stained tissue sections for histopathological examinations showed microscopic lesions primarily in the sacral and lumbar regions the left sciatic nerve and of the spinal cord. The primary lesions were degenerative changes (central chromatolysis) (figures 2, 3, 4, and 5) and neuronal necrosis (figure 2 and 4) of the spinal cord, and cellular infiltration and Wallerian degeneration of the sciatic nerve (figure 6).

Detection of Viral Growth

Nervous tissues were cultured in the monolayer of BHK-21 cell line to detect the presence of viral growth; however, no cytopathogenic effects (CPE) were observed.

DISCUSSION

Data collected in this study demonstrate that most of the signs observed coincide with those reported in previous studies. The clinical picture of the disease corresponds closely to that described in earlier reports (Claes *et al*., 2005; Laveran and Mesnil, 1907; Walker, 1918).

Weight loss was observed in all animals, especially in 12 of the mares selected. There was no any appetite

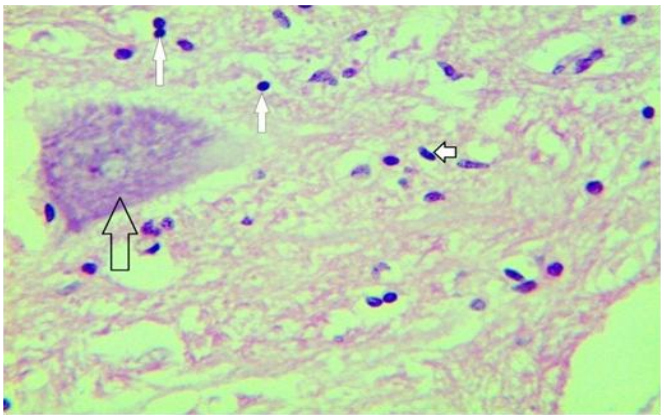

Figure 3. The lumbar region of the spinal cord, Horse No. 1. Central chromatolysis(open arrow); Perineuronal satellite oligodendroglia (closed arrows) surround a degenerate neuron with condensed chromatin and little cytoplasm. H&E stain 40x.

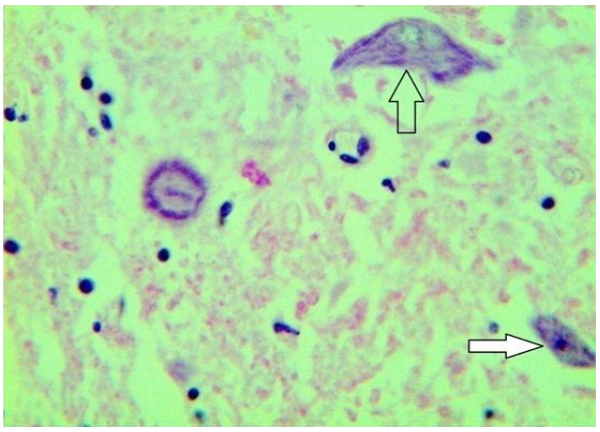

Figure 4. The sacral region of the spinal cord, Horse No. 2. Central chromatolysis(open arrows); shrunken, necrotic neuron (closed arrows). H&E stain 40x.

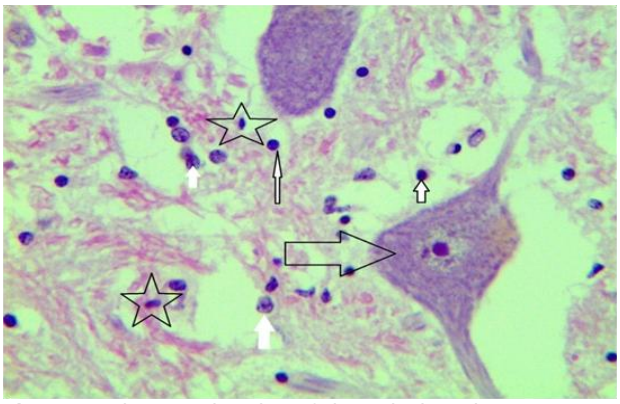

Figure 5. The sacral region of the spinal cord, Horse No. 1. Central chromatolysis(open arrow); Perineuronal satellite oligodendroglia (closed arrows) surround a degenerate neuron with condensed chromatin and little cytoplasm; white arrow (Astrocytes) have larger nuclei (condensed chromatin) and the cell membrane and cytoplasm are rarely seen in no diseased conditions. Microglial cells with small, dense elongated nuclei *(inside a star*). H&E stain 40x.

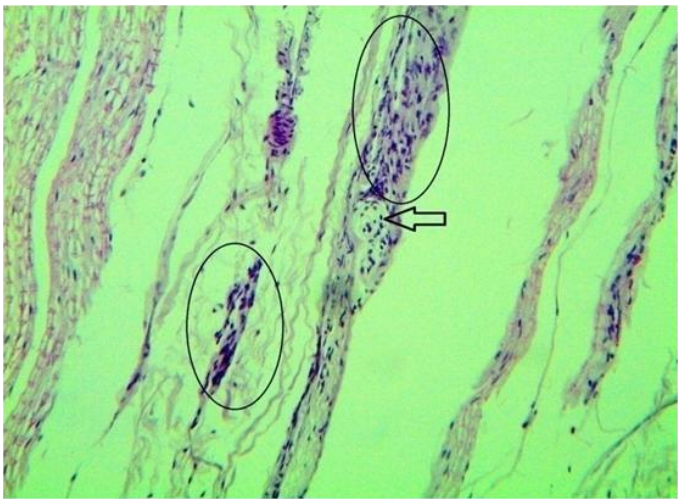

Figure 6: Left sciatic nerve, Horse No. 1. Wallerian degeneration with "digestion chamber" (open arrow) and cellular infiltrations (circled area). H&E stain 10x.

loss. It should be underlined that weight loss is one of the early signs that could lead the veterinarian or owner to suspect Dourine. No rise in body temperature was observed in any of the infected animals, which is in agreement with other studies (Coetzer, 2004) but it is often reported in the literature (Claes *et al*., 2005; Brun *et al*., 1998).

Ventral edema was not seen in all observed mares and this may be attributed to the stage of the disease as most of them were in the third stage, but genital edemas were observed in few of animals. This could be explained because the horses were from natural infection and already showed signs of active disease on arrival at the veterinary clinic.

Caporale suggested that the first stage of the disease was not reported or undetected at the holding (Caporale *et al*., 2005). In addition, the route used for experimental infection was not the natural route of infection which is the sexual transmission. Oedema of the mammary glands was not seen in all mares in the present study; however, Vulpiani *et al*. (2012) reported its persistence until the animal was euthanized when present.

The classic "silver dollar" plaque was not seen in the present study. According to Barrowman (Barrowman, 1976), these lesions rarely occur and transient in nature. These lesions are also difficult to differentiate from wheals triggered by other causes (allergic reactions to blood transfusion or insect bites) (Vulpiani *et al*., 2012), but they are considered pathognomonic by some authors (Claes *et al*., 2005; Brun *et al*., 1998), although their presence seems to be inconstant.

Most authors report only the sporadic presence of this sign (Claes *et al*., 2005; Walker, 1918; Watson, 1920), although it has been reported more frequently in outbreaks in Europe and North Africa (Coetzer, 2004). Some attribute this difference to the *T. equipper dum* strain involved and others to the immune response. In any case, presence of wheals should lead to suspicion of Dourine infection (Vulpiani *et al*., 2012). Thus, although the plaque lesion may be pathognomonic, its rare occurrence, transient nature and possible confusion with other skin eruptions limit its diagnostic value.

Woo test showed no parasite in the blood samples taken from long-standing cases of unknown duration. This is supported by Barrowman (Barrowman,1976), in his report stated that an attempts to demonstrate the parasite in the blood of long-standing cases of unknown duration were unsuccessful; however, it has been possible to demonstrate the more prolonged presence of the trypanosomes in circulating plasma in horses whose actual infectious service date was known.

Except Rouget (Rouget, 1986) and Gari *et al*. (2010) who reported the parasite in blood from horses suspected of dourine, most authors have experienced difficulty in demonstrating the parasite in naturally infected horses (Laveran and Mesnil, 1907; Khalilik, 1973) and have had only occasional success at the onset of the disease. Parkin recorded the more consistent finding of the parasite during the disease early stages in horses infected by blood at sub-inoculation (Parkin, 1948).

In the present study, despite the absence of the parasite in the blood by parasitological (Woo test) test, out of 12 horses examined by serological test (CATT test) two mares' revealed strong seropositive results, but there was no any gross pathological lesions observed on the different part of spinal cord, brain and on the sciatic nerves.

There are very limited information on the pathology

of dourine either experimental or natural infection in horses, especially on the nervous system of horses. In the current study, the presence of neurological signs confirms the tropism of *T. equipper dum* for the peripheral nervous system and the lack of involvement of the central nervous system, in contrast with other groups of trypanozoon (Barrowman, 1976; Berlin *et al*., 2009).

In contrast with *T. equipper dum*, McCully and Neitz reported that the most remarkable lesion in horses experimentally infected with *T. brucei* were found in the brain, and consisted of a diffuse meningoencephalitis characterized by massive perivascular cuffs of large lymphocytes, plasma cells, and a few typical cells of Mott (McCully and Neitz, 1971).

In the current study, degenerative changes and neuronal necrosis were present in the spinal cord, and cellular infiltration and degeneration in the sciatic nerves. As a result, from both slaughtered mares, the microscopic lesions of the nervous system were found to involve primarily the lumbar and sacral regions of the spinal cord and the left sciatic nerve.

Histopathological changes in these tissues have been recorded by Barrowman who reported radiculitis and polyneuritis, involving mononuclear cellular infiltration (MCI) and degenerative changes of the spinal nerves and spinal ganglia, extending along the larger sciatic nerves as the primary lesions (Barrowman, 1976). In contrast to the involvement of the central nervous system in *T. gambiense* infections in man (Van Bogaert and Janssen, 1976) and experimentally prolonged *T. brucei* infections in horses (McCully and Neitz, 1971), histopathological changes have not been reported in the central nervous system of under natural *T. equipper dum* infections, except that Matt1906, 1907 found the lesion in the spinal nerves extending into the posterior columns of the spinal cord (Laveran and Mesnil, 1907).

According to Rodrigues *et al*. (2009), *T. evansi* infection of horses, unlike to *T. equipper dum,* the pathological lesions involving the brain and characterized by moderate to severe perivascular lymphoplasma cytic meningoencephalitis, necrosis, edema and hemorrhage.

In this study, an attempt has been made to demonstrate whether the parasite presents in the cerebrospinal fluid or in the nervous tissue, or in both samples, but the trypanosome was not seen in either of the samples. This is contrary to Barrowman, who reported that trypanosomes were shown to be present in the CSF of horses dying from the nervous form of the disease and in live, naturally-infected horses with nervous manifestations, whereas they were not found in CSF from horses without these signs (Barrowman, 1976).

No growth of virus was seen in the present study complementing that the slaughtered mares were not infected with viruses, which potentially confuse with the disease and thus helps the author to exclude viral diseases having similar neurological signs with dourine such as equine infectious anemia and equine herpes virus 1 infection.

CONCLUSION

Dourine is endemic in the study area in Arsi-Bale highlands, Oromia region, Ethiopia. The current study revealed that even though the parasite is not found in the CSF and nervous tissues, it causes degenerative changes and neuronal necrosis in the affected parts of a spinal cord, MCI and degeneration of sciatic nerve. In nutshell, the disease revealed the moderate degree of microscopic pathological lesions on the nervous tissues at the third stages of the disease.

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Conflict of Interest

No conflict of interest declared.

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