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# Prevalence and characterization of *Trypanosoma* species from livestock of Cholistan desert of Pakistan

Sobia, M.[1], Mirza, I.S.[2*], Nuzhat, S.[3], Sonia, T.[4*], Abul, H.[5], Hafiz, M.A.[2], Muhammad, D.[6] and Muhammad, F.Q.[7]

[1]Department of Zoology, Government Sadiq College Women University of Bahawalpur, Pakistan
[2]University College of Veterinary and Animal Sciences, The Islamia University of Bahawalpur, Pakistan
[3]Department of Life Sciences, The Islamia University of Bahawalpur
[4]Department of Zoology, Govt. Syed Chiragh Shah Degree College, Kasur
[5]Department of Zoology, University of Peshawar, Pakistan
[6]Department of Statistics, The Islamia University of Bahawalpur, Pakistan
[7]College of Veterinary and Animal Sciences, Jhang, sub-campus, UVAS. Lahore
[*]Corresponding author e-mail: mirza.imran@iub.edu.pk, soniaasghar@live.com
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**Abstract.** Trypanosomiasis is one of the most pathogenic infection of livestock caused by several *Trypanosoma* species. The current study aims to determine the current prevalence of trypanosomiasis in livestock of Cholistan desert, Pakistan by molecular characterization. A total 272 animals, 61 camels, 59 cattle, 50 goats, 50 sheep and 52 donkeys that were bled and processed for thin smear microscopy, packed cell volume (PCV), DNA extraction, PCR, formol gel test and for cryo preservation. For diagnosis and molecular characterization, three sets of primers including TBR, RoTat 1.2, TRYP4 were used which detect their targets including repeated satellite DNA region, variant surface glycoprotein (VSG) gene and the ITS ribosomal DNA sequence of microbe. According to results, 6.2% animals were positive by microscopy, 51.1% by formol gel, 54.7% by PCV and 36.7% by PCR. The results of this study support the idea that PCR is a sensitive, robustic and reliable technique to diagnose trypanosomiasis and it should be added in conventional setup along with microscopy to avoid false negative and positive results. The PCR based order of prevalence of trypanosomiasis in Cholistani livestock was sheeps>camels>donkeys>goats>cattle. Similary, on the basis of thin smear microscopy the prevalence of trypanosomiasis was donkeys>camels>sheep>goats>cattle. It was observed that *Trypanosoma evansi* is the most prevalent specie involved in trypanosomiasis in Cholistani livestock.

## INTRODUCTION

Trypanosomiasis is the most widely pathogenic protozoan infection which is caused by different *Trypanosoma* species including *Trypanosoma brucei*, *T. evansi*, *T. congolense*, *T. equiperdum* and *T. vivax* etc. This disease affects number of animals including, camels, cattle, buffaloes, donkeys, horses, dogs, elephants, pigs, cats, tapirs, capybaras and deer (Hamilton *et al.*, 2007). In camels (*Camelus dromedarius* and *C. bactrianus*) symptoms of the disease include soaring fever, anemia, weakness and mortality (Parsani & Momin, 2008). In cattle it is characterized by reduced milk production, fever and abortion (Pholpark *et al.*, 1999; Kashiwazaki *et al.*, 1998). Furthermore, this disease reduces calving levels and animal labor, while calf death is also common in endemic regions (Swallow, 2000). In horses, the symptoms appear after one to two or eight weeks of incubation period along with fever, anemia, weight loss, weakness and laziness depending upon the severity of disease (Stephen, 1986).

Mechanically, it is transmitted by a variety of blood-sucking flies (Gill, 1977). *T. evansi* (Surra) is very common in the North-Africa, Middle East, South-East Asia and Latin America (Parsani and Momin, 2008). Economic losses due to Surra are frequently under estimated due to low infection exposure rates. Diagnostic tool such as PCR has proved to be very effective and has played important role in differentiating various *Trypanosoma* species (Wuyts *et al.*, 1994). Over the last few years, various diagnostic techniques have been developed thereby improving diagnosis of the disease. In poor resourced labs, *T. evansi* detection mostly depends on blood microscopic examination or by the micro hematocrit centrifugation technique (mHCT) (Herrera *et al.*, 2004; Baticados *et al.*, 2011). Different studies have reported the presence of surra from Pakistan. Tehseen *et al.* (2015) have reported parasitological, serological and molecular survey of *T. evansi* infection in camels of Cholistan Desert of Pakistan. Similar, work has been carried out by Abbasi *et al*. (2014) from Karachi, around Lyari Town and its surrounding, Nadeem *et al*. (2011), Aslam *et al.* (2010) and Tehseen *et al*. (2017) reported trypanosomiasis in horses from district Gujranwala, Lahore and Bahawalpur, respectively. Similalry, Mirshekar *et al.* (2017) has studied *T. evansi* infection and major risk factors for one-humped camels of Iran and found similar results as that of Pakistani studies.

Cholistan is the largest hub of livestock in Pakistan. Being a desert and remote area of country, the accurate measures of trypanosomal infection in livestock is not possible. It is very hard to estimate the subsequent economic losses, morbidity and mortality in animals in the field. The present study was designed by keeping in view the importance of Cholistani livestock in nomadic form of life and gross domestic product (GDP) of the country. This study aimed to determine the overall prevalence of trypanosomiasis in livestock from different localities of Cholistan desert and to characterize the parasite species associated with infections by using different sets of primers such as genus specific (TBR and TRYP4 ) or specie specific (RoTat 1.2) primers.

## MATERIALS AND METHODS

### Collection of samples

Sampling was done through different sites in and around the Cholistan belt and Bahawalpur region. These sites include Jugait Peer, Yazman, Hasilpur, Khairpur, Bahawalpur, Derawar, Chak 29 BC and Samma Satta area. Total 272 blood samples were collected between January and August 2014. Out of total, 61 samples were taken from camel, 59 from cattle and 50 each belong to goat and sheep and 52 from donkey. All animals were bled through jugular vein. The collected blood was divided into three parts. The first part was taken in EDTA coated tubes; second part in serum separating tubes and third part in cryopreservative tubes for cryopreservation at -80ºC.

### Thin smear microscopy

Thin smear slides were prepared and stained with Geimsa stain (3.8 g Geimsa powder, 250 mL methanol and 250 mL glycerine). The slides were studied at 800X under simple microscope (Abbasi *et al.*, 2014).

### Formol gel test

The formol gel test was performed as specified by OIE, (2012), to evaluate overall elevation of immunoglobulins in serum sample of each animal.

### Packed cell volume (PCV)

Packed cell volume of each blood sample was determined by using micro haematocrit centrifugation method (mHCM) (Hamid *et al.*, 2012). This test was performed to differentiate between sick (anemic) and healthy animals. The samples that were equal or less than 20 PCV value were considered as sick or positive and the samples that were equal or more than 25 PCV values were considered as healthy or negative (Farougou *et al.*, 2012 & Hamid *et al.*, 2012). Same parameters were used for blood samples of each species.

**Polymerase chain reaction (PCR)**

DNA was extracted from each 1mL blood sample by using kit provided by Bio Basic Inc (cat # BS684, Canada) and subjected to PCR test. Three sets of primers i.e. TBR 1/2 (F: 5'GAATATTAAACAATGCGCAG3'; R: 5'CCATTTATTAGCTTTGTTGC3'), RoTat 1.2 (F: GCGGGGTGTTTAAAGCAATA; R:ATTAGTGCTGCGTGTGTTCG), TRYP4 (F:5'CGTCCCTGCCATTTGTACACAC3'; R: 5'GGAAGCCAAGTCATCCATCG') were used (Claes, *et al.*, 2014; Salim *et al.*, 2011; Pruvot *et al.*, 2010; Gari *et al.*, 2010 & Tran *et al.*, 2009). TBR primer was genus specific but RoTat1.2 and TRYP4 were species specific primers. Almost same PCR profile was used for each primer with slight variation in annealing temperature. Denaturation was done at 94°C for 1 min and primer annealing at 54 to 58°C from 1 min to 1.5 min. Elongation was done at 72 for 1.5 min. Total no. of cycles were 35 in each case.

**Prevalence (%)**

The formula reported by (Abbasi *et al.*, 2014) was used to calculate prevalence after each test.

$$\text{Prevalence (\%)} = \frac{\text{Total no. of positive samples under specific technique at particular time}}{\text{Total no. of samples taken from whole population of an area at particular time}} \times 100$$

**Statistical analysis**

The data was properly tabulated by using Microsoft excel. Statistical analysis was performed using SPSS version 16. Chi square ($\chi^2$) and Kappa values were used to determine the level of agreement between diagnostic tests with keeping 0.05 significance level.

## RESULTS

**Thin smear microscopy**

From this technique only 6.0% samples of sheep, 6.5% samples of camel, 11.5% samples of donkeys, 4.0% samples of goat and 3.3% sample of cattle were found positive. More than one different morphological strain of *Trypanosoma* species were observed through microscopy (Table 1, Figure 1). Overall 6.25% of samples were found positive with microscopy.

**Formol gel (FG) test**

According to formol gel test, 60.0% samples of sheep, 55.7% samples of camel, 50.0% samples of donkeys, 68.0% samples of goat and 25.4% samples of cattle were positive (Table 1). Overall 51.1% of samples were positive with FG test.

**Packed cell volume (PCV)**

According to this test 90.0% sample of sheep, 18.0% samples of camel, 17.3% samples of donkeys,74.0% samples of goat and 79.6% samples of cattle were positive (Table 1). Overall 54.7% of samples were positive with PCV.

**Polymerase Chain Reaction (PCR)**

According to this test more samples were positive with TBR as compared to RoTat 1.2 and TRYP4 primers. The exact size of PCR product by TBR primer was 164bp, from RoTat 205bp and by TRYP4 was 150 to 476bp (Figure 2 a, b, c, d). In camels overall 39.3% samples were confirmed positive through PCR. Out of these, 11 were positive with TBR primer, 6 with RoTAT 1.2 and 7 with Tryp 4 primers. In case of sheep, 48.0% samples were positive. Out of these, 13 were positive with TBR, 9 with RoTat1.2 and 2 with Tryp4 primer. In case of donkeys, 38.4% samples were positive. Out of these, 10 were positive with TBR, 3 with RoTat 1.2 and 7 with Tryp4 primers. In case of goats, 36.6% samples were positive through PCR. Out of these, 15 were positive with TBR, 2 with RoTat 1.2 and 5 with Tryp4 primer. In case of cattle total 16.9% samples were confirmed through PCR. Out of these, 5 samples were confirmed with TBR, 3 with RoTat 1.2 and only 2 with Tryp4 primers (Table 2). The Overall 36.7% samples were positive through PCR.

Statistically the degree of agreement was performed through Chi square and Kappa test by taking camel samples. The samples were analyzed microscopically, with FG, PCV and also with PCR. Microscopic results were revealed that the statistical data is non-

Table 1. Confirmation of *Trypanosoma* spp. in Cholistani livestock by different diagnostic techniques

| Animals | Thin Smear Microscopy | | Formol Gel | | PCV | |
|---|---|---|---|---|---|---|
| | Infected | Preval (%) | Infected | Preval (%) | Infected | Preval (%) |
| Camel | 4/61 | 6.55 | 34/61 | 55.7 | 11/61 | 18.03 |
| Cattle | 2/59 | 3.38 | 15/59 | 25. 4 | 47/59 | 79. 6 |
| Goat | 2/50 | 4.0 | 34/50 | 68.0 | 37/50 | 74.0 |
| Sheep | 3/50 | 6.0 | 30/50 | 60.0 | 45/50 | 90.0 |
| Donkey | 6/52 | 11.5 | 26/52 | 50.0 | 9/52 | 17.3 |
| Total | 17/272 | 6.25 | 139/272 | 51.10 | 149/272 | 54.77 |

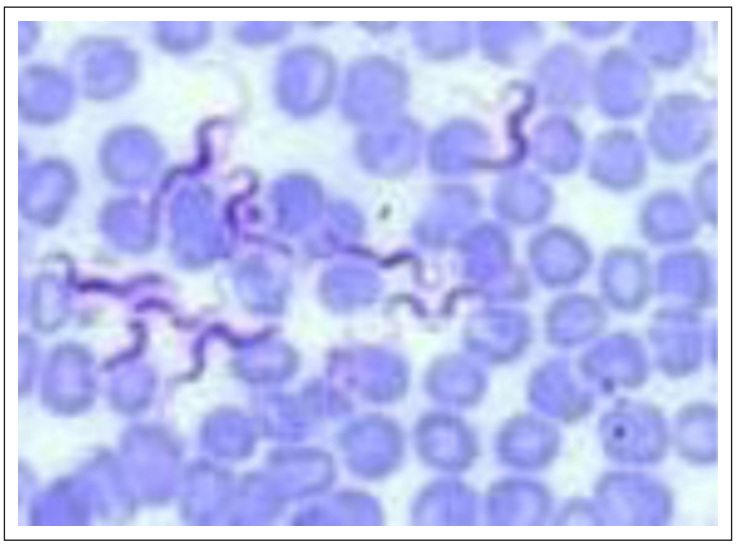

Figure 1. Giemsa stained thin smear microscopy. The slide is visualized at 800X oil emersion lens.

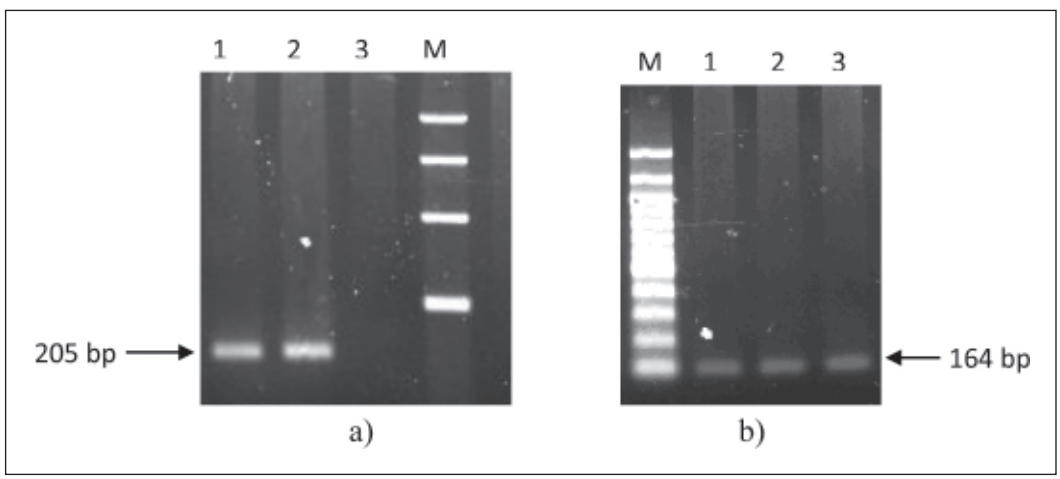


Figure 2. PCR Amplification by different genus and specie specific primers. (a) PCR by sequence specific primer RoTat 1.2, Lane 1-3 PCR from sheep blood samples; Lane M = 1kb marker (NORGEN: cat # 11700). (b) PCR by sequence specific primer TBR, Lane 1-3 PCR from sheep blood samples. Lane M = 100 bp DNA marker (Cat # N3231L, New England Biolabs, USA).

Table 2. PCR based diagnosis of *Trypanosoma* spp. from Cholistani livestock

| Animal | Primers | | | Total prevalence |
|---|---|---|---|---|
| | TBR-ve : +ve | RoTat-ve : +ve | TRYP4-ve : +ve | |
| Camel | 50 : 11 | 55 : 6 | 54 : 7 | 24/61 = 39.3% |
| Cattle | 54 : 5 | 57 : 2 | 56 : 3 | 10/59 = 16.9% |
| Goat | 35 : 15 | 48 : 2 | 45 : 5 | 22/50 = 36.6% |
| Sheep | 37 : 13 | 41 : 9 | 48 : 2 | 24/50 = 48.0% |
| Donkey | 42 : 10 | 49 : 3 | 45 : 7 | 20/52 = 38.4% |

Table 3. Statistical analysis of different diagnostic techniques in comparison to Microscopy and PCR

| Animals | Cross tests | Pearson chi square | Significance | Kappa value | Significance |
|---|---|---|---|---|---|
| Camel | Formol gel | 3.399 | 0.065 | 0.106 | 0.065 |
| | PCV | 11.501 | 0.001*** | 0.326 | 0.001*** |
| | PCR | 6.599 | 0.010*** | 0.195 | 0.010*** |
| | Microscopy | 6.599 | 0.010*** | 0.195 | 0.010*** |
| Cattle | Formol gel | 6.073 | 0.014*** | 0.187 | 0.014*** |
| | PCV | 0.529 | 0.467 | 0.018 | 0.467 |
| | PCR | 10.144 | 0.001*** | 0.293 | 0.001*** |
| | Microscopy | 10.144 | 0.001*** | 0.293 | 0.001*** |
| Goat | Formol gel | 0.980 | 0.322 | 0.038 | 0.322 |
| | PCV | 0.732 | 0.392 | 0.029 | 0.392 |
| | PCR | 2.652 | 0.103 | 0.101 | 0.103 |
| | Microscopy | 2.652 | 0.103 | 0.101 | 0.103 |
| Sheep | Formol gel | 2.128 | 0.145 | 0.082 | 0.145 |
| | PCV | .355 | 0.552 | 0.014 | 0.552 |
| | PCR | 3.457 | 0.063 | 0.129 | 0.063 |
| | Microscopy | 3.457 | 0.063 | 0.129 | 0.063 |
| Donkey | Formol gel | 6.783 | 0.009* | 0.231 | 0.009* |
| | PCV | 32.406 | 0.000*** | 0.768 | 0.000*** |
| | PCR | 10.852 | 0.001*** | 0.345 | 0.001*** |
| | Microscopy | 10.852 | 0.001*** | 0.345 | 0.001*** |

significant with 0.065 values as compared to the samples treated with FG method. On the other hand, it was found significant than PCV and PCR with 0.001 and 0.010 values. In case of cattle samples, microscopic results were non-significant with 0.467 values as compared to the samples treated with PCV. On the other hand, it was significant than FG and PCR with 0.014 and 0.001 values. Similarly, in case of goat and sheep samples, microscopic results were non-significant than FG, PCV and PCR with 0.322, 0.392, 0.103, 0.145, 0.552 and 0.063 values respectively. But in case of donkey samples, microscopic results were significant with FG, PCV and PCR with 0.009, 0.000 and 0.001 significance values respectively (Table 3).

Statistically, PCR was compared with other diagnostic tests and degrees of agreement were also obtained through Chi square and Kappa test. In case of sheep and goat samples, PCR was non-significant with 0.103 and 0.063 values as compared to the samples treated with microscopy. In contrast, it was significant than FG and PCV with 0.000, 0.000, 0.000 and 0.024 values. In case of camel samples, PCR test was more significant with 0.010, 0.000 and 0.000 values as compared to the samples treated with microscopy, FG and PCV methods. In case of

donkey samples, PCR was significant with 0.000, 0.000 and 0.001 values as compared to the samples treated with microscopy, FG and PCV procedures. In case of cattle samples, PCR was non-significant with 0.080 value as compared to the samples treated with PCV. On the other hand, it was significant than microscopy and FG with 0.001 and 0.000 significant values respectively (Table 3).

## DISCUSSION

The present study was performed with 272 blood samples obtained from Cholistani livestock including camels, cattle, goats, sheep and donkeys. Sampling was done through different localities in and around the Cholistan belt. To confirm whether animal was sick or not, different tests were applied including thin blood smears microscopy, formol gel (FG) test, packed cell volume (PCV) and PCR. Microscopy and PCR were considered as specific tests for *Trypanosoma* species detection while FG and PCV were used to confirm whether animal was sick or not. FG test confirms all types of immunoglobulins in serum, no specificity for anti typanosomal antibodies, though this test has its own importance in initial screening to make differences between sick and healthy animals. Similarly, PCV is also a non-specific test and is used to check whether animal was anemic or not. Several studies support the use of this test especially against parasite like *Trypanosoma* species (Gadahi *et al.*, 2014). PCR and microscopy are considered as reference tests for *Trypanosoma* species especially PCR which is used as the test of choice after its comparison with other tests. It not only confirms the presence of microbe but also characterizes it at subgenus, species or even type or strain level (Gadahi *et al.*, 2014). In current study, TBR 1/2, RoTat 1.2 and TRYP4 sets of primers were used to evaluate trypanosome from different animal species and TBR was found to be most sensitive. Similar results were reported by Pruvot *et al.* (2010), in which six set of primers including TBR1/2, ESAG6/7, TEPAN1/2, (pMUTEC F/R, TRYP1 R/S and TRYP4 R/S were used to confirm different dilutions of *T. evansi* genome from infected rats and Thai dairy cattle. TBR1/2 set of primer was most sensitive and it has detected 0.01 pg of DNA of *T. evansi* from samples.

In the present study trypanosoma prevalence through microscopy from sheep, camels, donkeys, goats and cattle samples were 6.0, 6.5, 11.5, 4.0 and 3.3% respectively. The overall rate of detection was 6.2%. The same technique was used to report trypanosomiasis in camels of Sindh, Pakistan and the overall prevalence was 13.7% (Shah *et al.*, 2004). The high rate of prevalence in Sindh area could be due to poor veterinary practices and difference in environmental conditions than Cholistan. This technique has been successfully used in detection of *T. evansi* prevalence from Nili-Ravi buffalo (*Bubalus bubalis*) with almost same findings i.e 5.5% prevalence from Okara district of Pakistan (Shahzad *et al.*, 2010). Similarly from Lyari, Karachi, Pakistan 8.4% cases of trypanosomiais in donkeys has been recorded by electron microscope (Abbasi *et al.*, 2014).

A number of internationals studies has highlighted the effective use of microscopy in detection of *Trypanosoma* spp. from Algeria, it showed 14.0% prevalence in camels (Bennoune *et al.*, 2013) from Egypt 4.14% (Abdel-Rady, 2008), and from Sudan 1.7% (Croof *et al.*, 2012). Other studies include 43% prevalence of *Trypanosoma* spp. in cattle of Sudan (Salim *et al.*, 2011), 9.1% in cattle from Brazil (Pimentel *et al.*, 2012), 67% cases of *Trypanosoma* infection in cattle and 3.8% in sheep on the basis of thin smear microscopy from West Atacora (Farougou *et al.*, 2012). From Eastern Zambia the rate of infection in cattle, goats and pigs was recorded as 13.5, 0 and 0.9 respectively (Simukokoa *et al.*, 2007), and while from India 3.27% samples of camel, 0% samples from donkey and dogs were found positive through microscopy (Ravindran *et al.*, 2008).

Although formol gel test is non-specific but researchers have tried to report the prevalence of *Trypanosoma* spp. on the basis of this test like Sow (Sow *et al.*, 2013) from Burkina Faso have reported the prevalence of *Trypanosoma* spp. in cattle 34.2%, in sheep 20.9%, in goats 8.5% and in donkeys 5.8%. Similarly from Lahore, Pakistan 120 out of

500 (24.0%) equines were found positive through formol gel test (Aslam *et al*., 2010 & Tehseen *et al*., 2015).

On an average, the prevalence of disease through PCV was 37.2%. These results are in accordance with other studies published at worldwide. A total 3.7% of trypanosomiasis in camels from Gedariff and Kordofan states of Sudan was recorded (Abdel-Rady, 2008), and from Eastern Zambia PCV was used to monitor the prevalence of trypanosome in cattle, pigs and goats (Simukokoa *et al.*, 2007). Similarly from Western Ethiopia the mean parasitaemic and aparasitaemic values through PCV in donkey were 31.3% and 33% respectively and overall 6.2% donkey samples were positive through this test (Mesele and Leta, 2010).

In PCR, all primers were effective in diagnosing *Trypanosoma* spp. but TBR was most sensitive primers. Diagnosis through more than one primer confirms the presence of *T. evansi* in Cholistani livestock. Similar results were reported by different researchers from all over the world like using PCR technique to diagnose *Trypanosoma* spp. ranging from high prevalent areas to low prevalent areas of Sudan where the prevalence was 57.1% and 6.0% respectively (Salim *et al.*, 2011). From Egypt 56.9% prevalence of *Trypanosoma* spp. in camels was reported by using NRP1 and NRP2 primers (Bennoune *et al*., 2013), and from Sudan 90.0% detection rate in camels by PCR was reported by using TBR1 and TBR2 primers (Abdel-Rady, 2008).

It is concluded that trypanosomiasis is commonly prevalent in the Cholistan desert of Pakistan including small ruminants like goats and sheep. PCR is the best technique to be used in diagnosis of *Trypanosoma* species and according to results of current study more than one *Trypanosoma* species are involved in causing trypanosomiasis in this area.

Although more than 20 species of Trypanosma are present worldwide but in the South East Asia, 2 to 3 species including *T. evansi*, *T. lewisi* and *T. epinepheli* are present (Pumhom *et al*., 2013 & Su *et al*., 2014). Owing to the absence of biological vectors in cholisatni livestock, existence of *T. evansi* basically depends on the availability of biting flies such as Tabanids *Stomoxys* spp. etc (Tehseen *et al*., 2015).

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## REFERENCES

Abbasi, I.H.R., Sahito, H.A., Sanjrani, M.I., Abbasi, F., Memon, M.A., Menghwar, D.R., Kaka, N.A., Shah, M.N. & Meena, M. (2014). A disease complex pathogen "*Trypanosome congolense*" transmitted by tsetse fly in donkeys. *Herald Journal of Agriculure & Food Science Research* **3**: 044-048.

Abdel-Rady, A. (2008). Epidemiological studies (parasitological, serological and molecular techniques) of *Trypanosoma evansi* infection in camels (*Camelus dromedarius*) in Egypt. *Veterinary World* **1**: 325-328.

Aslam, A., Chaudhary, Z.I., Rehman, H., Ashraf, K., Ahmad, N., Yaqub, T., Maqbool, A. & Shakoori, A.R. (2010). Comparative evaluation of parasitological, serological and DNA amplification methods for diagnosis of natural trypanosomal infection in equines. *Pakistan Journal of Zoology* **42**: 371-376.

Baticados, W.N., Castro, D.L. & Baticados, A.M. (2011). Parasitological and PCR detection of *Trypanosoma evansi* in buffaloes from Luzon, Philippines. *Ceylon Journal of Science (Biological Science)* **40**(2): 141-146.

Bennoune, O., Adili, N., Amri, K., Bennecib, L. & Ayachi, A. (2013). Trypanosomiasis of camels (*Camelus dromedarius*) in Algeria: First report. *Veterinary Research Forum* **4**: 273-275.

Claes, F., Radwanska, M., Urakawa, T., Majiwa, P., Goddeeris, B. & Büscher, P. (2004). Variable surface glycoprotein RoTat 1.2 PCR as a specific diagnostic tool for the detection of *T. evansi* infections. *Kinetoplastid Biology Diseases* **3**: 1-6.

Farougou, S., Allou, S.D., Sankamaho, I. & Codjia, V. (2012). Prevalence of Trypanosome Infections in Cattle and Sheep in the Benin's West Atacora Agro-ecological zone. *Tropicultura* **30**: 141-6.

Gadahi, J.A., Bhutto, B., Javaid, S.B., Kashif, J. & Duchoan, T. (2014). Diagnostic approach towards Camel Trypanosomiasis. *International Journal of Livestock Research* **4**(1): 41-45.

Gari, F.R., Ashenafi, H., Tola, A., Goddeeris, B.M. & Claes, F. (2010). Comparative diagnosis of parasitological, serological, and molecular tests in dourine-suspected horses. *Tropical Animal Health Product* **42**: 1649-1654.

Gill, B. (1977). *Trypanosomes and Trypanosomiasis of Indian Livestock*, ICAR: Indian Council of Agricultural Research, New Delhi, India, 1st edition.

Hamid, I.M.N.C., Hamid, S.A. & Nahla, O.M.A. (2012). Assessment of *Trypanosoma evansi* infection in camels herd from gedariff and kordofan states. *Journal of Veterinary Medicine & Animal Production* **3**: 3-27.

Hamilton, P.B., Gibson, W.C. & Stevens, J.R. (2007). Patterns of co-evolution between trypanosomes and their hosts deduced from ribosomal RNA and protein-coding gene phylogenies. *Molecular Phylogenetics Evolution* **44**: 15–25.

Herrera, H.M., Davila, A.M.R., Norek, A., Abreu, U.G., Souza, S.S., Iandrea, P.S. & Jansen, A.M. (2004). Enzootiology of *Trypanosoma evansi* in Pan-tanal. Brazil. *Veterinary Parasitology* **125**: 263-275.

Kashiwazaki, Y., Pholpark, M., Polsar, C. & Pholpark S. (1998). Haemoparasite infections in newly introduced dairy cattle in Loei Province, Thailand: *Trypanosoma evansi* antigen levels by ELISA referring to abortion. *Veterinary Parasitology* **80**(2): 99-109.

Mesele, F. & Leta, S. (2010). Prevalence of tse tse transmitted donkey trypanosomosis in Dale Wabera District, Western Ethiopia. *Global Veterinaria* **5**(3): 180-183.

Mirshekar, F., Yakhchali, M. & Shariati-Sharifi, F. 2017. *Trypanosoma evansi* infection and major risk factors for Iranian one-humped camels (*Camelus dromedarius*). *Journal of Parasitic Diseases* **41**(3): 854-858.

Nadeem, A., Aslam, A., Chaudhary, Z.I., Ashraf, K., Saeed, K., Ahmad, N.I. & Rehman, H. (2011). Indirect fluorescent antibody technique based prevalence of surra in equines. *Pakistan Veterinary Journal* **31**(2): 169-170.

OIE. Manual of diagnostic tests and vaccines for terrestrial animals. Chapter 2.1.17. Trypanosoma evansi infection (surra). (2012). http://web.oie.int/eng/normes/MMANUAL/2008/\pdf/2.01.17_TRYPANO.pdf. Accessed 17-6-2017.

Parsani, H.R. & Momin, R.R. (2008). Common parasitic diseases of camel. *Veterinary World* **1**(10): 317-318.

Pholpark, S., Pholpark, M., Polsar, C., Charoenchai, A., Paengpassa, Y. & Kashiwazaki, Y. (1999). Influence of *Trypanosoma evansi* infection on milk yield of dairy cattle in northeast Thailand. *Preventive Veterinary Medicine* **42**(1): 39-44.

Pimentel, D.S., Ramos, C.A.N., Ramos, R.A.N., Araújo, F.R., Borba, M.L., Faustino, M.A.G. & Alves, L.C. (2012). First report and molecular characterization of *Trypanosoma vivax* in cattle from state of Pernambuco, Brazil. *Veterinary Parasitology* **185**: 286-289.

Pruvot, M., Kamyingkird, K., Desquesnes, M., Sarataphan, N. & Jittapalapong, A. (2010). Comparison of six primer sets for detection of *Trypanosoma evansi* by polymerase chain reaction in rodents and Thai livestock. *Veterinary Parasitology* **171**: 185-193.

Pumhom, P., Pognon, D., Yangtara, S., Thapratrorn, N., Milocco, C., Douangboupha, B., Herder, S., Chaval, Y., Morand, S., Jittapalapong, S. & Desquesnes, M. 2013. Molecular prevalence of *Trypanosoma* spp. in wild rodents of Southeast Asia: influence of human settlement habitat. *Epidemiology and Infection* **1**: 1-10.

Ravindran, R., Rao, J.R., Mishra, A.K., Pathak, K.M.L., Babu, N., Satheesh, C.C. & Rahul, S. (2008). *Trypanosoma evansi* in camels, donkeys and dogs in India: Comparison of PCR and light microscopy for detection. *Veterinarski Arhive* **78**(1): 89-94.

Salim, B., Bakheit, M.A., Kamau, J., Nakamura, I. & Sugimoto, C. (2011). Molecular epidemiology of camel trypanosomiasis based on ITS1 rDNA and RoTat 1.2 VSG genes in the Sudan. *Parasites & Vectors* **4**: 31-36.

Salim, B., Bakheit, M.A., Salih, S.E., Kamau, J., Nakamura, I., Nakao, R. & Sugimoto, C. (2011). An outbreak of bovine trypanosomiasis in the Blue Nile State, Sudan. *Parasites & Vectors* **4**: 74-79.

Shah, S.R., Phulan, M.S., Memon, M.A., Rind, R. & Bhatti, W.M. (2004). Trypanosomes Infection in Camels. *Pakistan Veterinary Journal* **24**: 4-7.

Shahzad, W., Munir, R., Khan, M.S., Ahmad, M.D., Ijaz, M., Ahmad, A. & Iqbal, M. (2010). Prevalence and molecular diagnosis of trypanosoma evansi in Nili-Ravi buffalo (Bubalus bubalis) in different districts of Punjab (Pakistan). *Tropical Animal Health Production* **42**: 1597-1599.

Simukokoa, H., Marcottyb, T., Phiria, I., Geysenb, D., Vercruyssec, J. & Van Den Bossche, P. (2007). The comparative role of cattle, goats and pigs in the epidemiology of livestock trypanosomiasis on the plateau of eastern Zambia. *Veterinary Parasitology* **147**: 231-238.

Tehseen, S., Nusrat, J., Muhammad, F.Q., Marc, D., Mirza, I.S., Stij, D. & Philippe, B. (2015). Parasitological, serological and molecular survey of *Trypanosoma evansi* infection in dromedary camels from Cholistan Desert, Pakistan. *Parasites & Vectors* **8**: 415-419.

Sow, A., Ganaba, R., Percoma, L., Sidibe, I., Bengaly, Z., Adam, Y., Kone, P., Sawadogo, G.J., Van Den, Abbeelef, J., Marcotty, T. & Delespaux, V. (2013). Baseline survey of animal trypanosomosis in the region of the Boucle du Mouhoun, Burkina Faso. *Research in Veterinary Science* **94**: 573-578.

Stephen, L.E. (1986). Trypanosomiasis: A Veterinary Perspective. Pergamon Press, Oxford, New York, NY, USA.

Su, Y., Feng, J., Jiang, J., Guo, Z., Liu, G. & Xu, L. 2014. Trypanosoma epinepheli n. sp. (Kinetoplastida) from a farmed marine fish in China, the brown-marbled grouper (Epinephelus fuscoguttatus). *Parasitology Research* **113(1)**: 11-8.

Swallow, B.M. (2000). Impacts of trypanosomiasis on African agriculture. In: *PAAT Technical and Scientific Series*. FAO, Animal Production and Health Division, International Livestock Research Institute, Nairobi, Kenya.

Tran, T., Claes, F., Verloo, D., Greve, H. & Buscher, P. (2009). Towards a new reference test for Surra in Camels. *Clinical and Vaccine Immunology* **16**(7): 999-1002.

Wuyts, N., Chokesajjawatee, N., Sarataphan, N., Panyim, S., Wuyts, C.N., Sarataphan, N. & Panyim, S.A. (1994). Simplified and highly sensitive detection of *Trypanosoma evansi* by DNA amplification. *Southeast Asian Journal of Tropical Medicine & Public Health* **25**: 266-271.