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Iatrogenic transmission of *Trypanosoma evansi* infection in camels and its consequences

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Trypanosoma evansi **infection has started to become a wide spread phenomena around the camel-rearing areas of North Africa and the Middle East. The disease caused by trypanosomes is locally known as "Surra" and it can seriously impact not only the health of domestic animals but the local economy as well. After taking over the management of a farm containing approximately 700 camels, it was found that a large number were suffering from trypanosome infection and it was of the utmost importance to find the source of this infection. An extensive dive into the records and observations were initially made to identify the infected population. Under closer inspection it was found that the infection was limited mostly to female individuals that had undergone extended reproductive analysis or treatment. Blood samples were taken from each of the individuals for buffy coat test and blood smears. Among the total number of tested camels (n = 590), almost 40% were infected with trypanosomes. The number and percentage of infection correlate with the number of fertility and pregnancy treatments that the camels had undergone. The most severely infected group, underwent between 17 and 20 instances of treatment or tests, had an infection rate of almost 90%. The devastating effect of trypanosomiasis on camel pregnancy and birth were also verified with 61% of all abortions and 82% of all neonatal deaths coming from trypanosome infected individuals. These results clearly demonstrate how damaging iatrogenic infections of** ***T. evansi*** **can be and how simply they could have been prevented.**

Trypanosoma evansi is a parasitic protozoan that belongs to the genus *Trypanosoma* subgenus *Trypanozoon*[1]. As the causative agent of "Surra," it has a large diversity of mammalian hosts, most notably horses, camels, cattle, buffalo and dogs[2] and it is generally mechanically transmitted through the bites of Tabanid and Stomoxes species and vampire bats[3]. Most mammalian species are susceptible to *T. evansi* infection but its susceptibility varies greatly between one species to another[4].

Trypanosoma evansi is generally present through the tropical and subtropical regions of Northern Africa, Southeast Asia as well as Central and South America[5]. T. evansi is thought to be derived from *Trypanosoma brucei brucei* following mutations allowing it to be transmitted by biting flies[1]. Originating in Northern Africa, *T. evansi* can be found in essentially all the countries of North Africa such as Mauritania, Morocco, Algeria, Tunisia, Libya, Egypt, as well as Sudan, Eritrea and Ethiopia[6]. It is thought to have further spread East into the arid desert regions of the Arabian Peninsula (Saudi Arabia, United Arab Emirates, Oman, Jordan, Israel, Lebanon, Syria)[7–10]. From there it seems to have spread further east into Asia, from Iran and Pakistan all the way to South East Asia (Vietnam, Thailand, Malaysia, Indonesia and the Philippines[11,12]. It is even present in the New World with *T. evansi* infections recorded all the way from Panama in the north to Paraguay[1] and most recently it seems to be creeping up north in Europe with identified cases in the Canary Islands first[13] and further up to Spain[3] and France[14]. This disease has a large economic impact in those areas as well as the dangerous potential as a zoonotic disease[15].

Trypanosoma evansi infection is usually regarded as a serious disease of horses and camel, causing high mortality, whereas the disease is typically mild in other domestic animals[11,16]. General pathology of *T. evansi* infection are similar with other mammalian trypanosomes such as fever, anemia, weight and appetite loss, lethargy, nervousness, abortions, cachexia and death[4]. However, the expression and intensity of the pathology can differ

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between host animals and region. In the case of camels, Surra may present acutely with high fever, anemia, weakness, and death but it is most often presented as a chronic disease that can last ~ 2 to 3 years. *T. evansi* is not only an intravascular parasite but it has also been reported to infiltrate into the central nervous system (CNS) which is potentially fatal. *T. evansi* is difficult to diagnose and it is also a very resilient parasite with widespread drug resistance. It has a characteristic mechanism where it can escape the host immune response by constantly changing its Variant Surface Glycoprotein (VSG)[17] as well as inducing immunosuppression which, not only prevents the immunopathology that injures the host and causes the traditional symptoms of Surra, but also reduces host immune responses to other diseases and greatly lowers the protective potential of vaccines[1]. Mammalian hosts can also appear asymptomatic for long periods of time[1] and potentially spread the infection to other animals.

Considering the large economic impact that *T. evansi* has in various agriculture-oriented countries, preventive strategies to control its spread have been severely lacking[8,18,19]. Not much is known about the full situation of Surra in the UAE as it seems likely that a considerable amount of cases are under reported. *T. evansi* has been reported to be prevalent in Pakistan and this could be a potential reservoir for the infection of domestic animals in the UAE. Various preventive strategies have been loosely implemented such as vector control, screening of imported animals and movement restrictions but these strategies have not greatly impacted curving the spread of *T. evansi*. Another worrisome trend is the increase in cases of iatrogenic transmission of the pathogen due to the lack of knowledge or will of the caretakers and antiquated veterinarian practices. For example, reusing needles or syringes for the treatment of multiple animals continues to be practiced by many veterinarians, despite the low cost of syringes. Here we present a case where general managerial incompetence and carelessness has led to an infection rate of nearly 90% and considerable economic loss.

Methods

Study location and animals

All the camels in this case study were in the Emirate of Fujairah, situated on the east coast of the UAE by the Gulf of Oman (25° 16′ N/56° 20′ E). Among the affected camel herd which was comprised of a total of 786 camels, the study was carried out in 590 dromedary camels (486 Female, 104 male) which were tested routinely. All the camels were raised in semi-desert condition in outdoor facilities. The camels were fed with rhodes grass and water ad libitum and, depending on the condition of the camel, they were given between 0 and 5 kg of alfalfa, 0–5 kg of fattening mix, 0–2 kg of dairy mix and 0–2 kg of bran. All the dromedary camels in the study were tested for *Trypanosoma evansi* infections between July 2022 and March 2023. Most of the tested animals were part of a breeding cycle between October 2021 and April 2022. Initial tested animals and correlations made in animals of reproductive age > 5 years. The gestational period of the camels, was calculated based on their mating date and date of birth or fetal loss.

Blood sampling and diagnosis of *Trypanosoma evansi*

Blood samples were collected through jugular venipuncture. Around ~ 3 to 5 ml of blood was collected in Vacutainers with EDTA and kept on ice, while in the field and at 4 °C in the laboratory, until they were ready for diagnostics. Samples were used to verify the presence of the parasite through the buffy coat test and blood smears. Another ~ 3 to 5 ml of blood was collected in vacutainers without anticoagulants. The samples were placed on ice and immediately brought to the Central Veterinary Research Laboratory (CVRL, Dubai, UAE) for Ab ELISA verification of *Trypanosoma evansi* infection. The ELISA procedures were carried out by CVRL through their previously published[20] in-house tests. Their cut-off value was calculated as the mean plus one standard deviation (SD) of the OD value of 50 surra negative sera. The cutoff values were > 1.5: Strong Positive, 0.5–1.5: Positive, and < 0.5: Negative. A micro-capillary tube was filled with EDTA blood and sealed on one end. The samples were centrifuged at 10,000 rpm for 5 min in a microhematocrit centrifuge. The capillary tube was then placed above a glass slide and fixed in place with tape. The buffy coat area was checked under a microscope with 400× magnification to check for motile *T. evansi*. Additionally, the buffy coat area of the capillary tube was transferred onto an objective glass slide and spread out into a thin film. After briefly air drying, the sample was fixed in pure methanol for 3 min and allowed to dry. The sample was then stained with a few drops of Giemsa for 20 min. The slide was washed with ddH_20 and allowed to dry. The blood smears of the camels were visualized using a Nikon Eclipse Ni (Nikon Corporation, Tokio, Japan) microscope with an attached Nikon Intenslight E-HGFI fluorescence illuminator. Images have been edited with the NIS Elements Imaging Software v. 5.2 (Nikon Corporation, Tokio, Japan).

Statistical analysis

The chi-squared test was performed using GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego California USA, www.graphpad.com). *P*-values below 5% were considered significant.

Ethics approval

All procedures and protocols were approved by the internal committee at Fujairah Genetics Center and in accordance to standard veterinary practice. All methods are reported in accordance with the ARRIVE guidelines.

Results

A total of 590 camels were examined for *T. evansi* infection through ELISA and select samples were visualized through Nikon Eclipse Ni microscope (Fig. 1). ELISA testing was conducted first and the overall infection rate was 37.97%. Among the ELISA tested camels, 26.61% tested as strong positive, 11.36% tested positive while the remaining 62.03% tested negative (Table 1). With such a high prevalence of *T. evansi* infection the different possibilities that could have led to this outcome were considered. Interestingly, trypanosome infection appeared

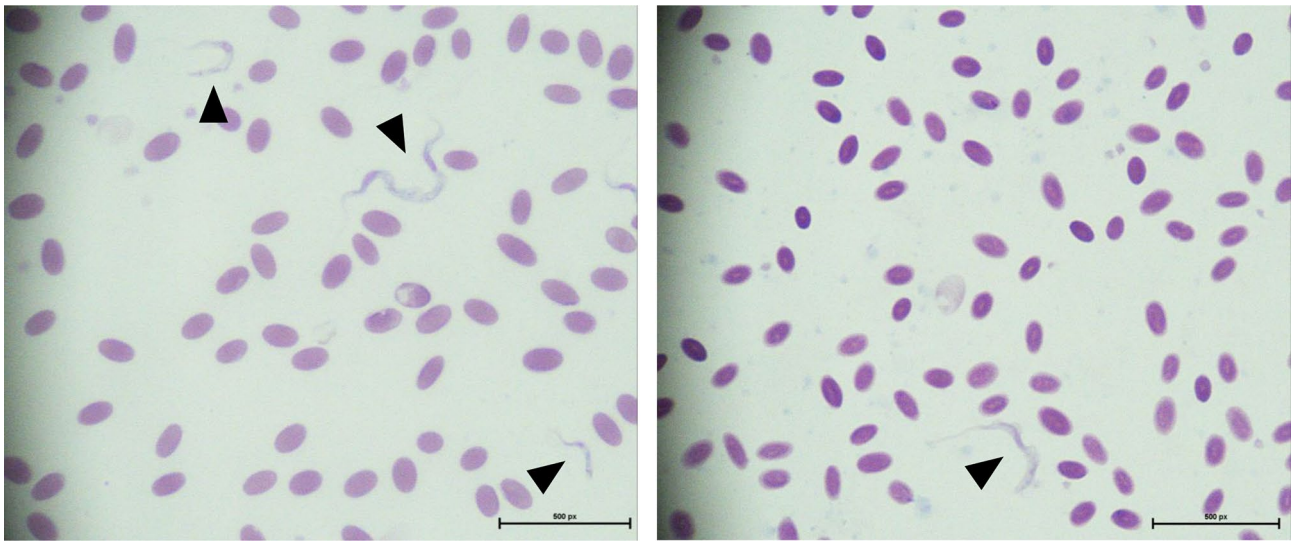

Figure 1. *Trypanosoma evansi* (black triangle) visualization in representative blood smears.

ELISA Results	Female		Male		Grand Total		X^2 (*df*)
	N	(%)	N	(%)	N	(%)	
Negative	262	53.91	104	100	366	62.03	77.27 (2)
Positive	67	13.79		0	67	11.36	
Strong positive	157	32.30		0	157	26.61	

Table 1. Sero-positivity of *Trypanosoma evansi* in camels by sex in the Fujairah Emirate. Chi-squared test (X^2 (df)), groups are significantly different ($p < 0.0001$).

to be limited to females (Fig. 2). Although female camels do outnumber the males almost 5 to 1, the infections were limited to the female camels and no male camels were infected (Table 1). As males and females were kept in the same locations, flies as a potential vector for infection were ruled out. Furthermore, flies could be excluded as the vector source as the differential infection rates was present within animals that were housed in the same location and were clearly split based on sex of the animals (Table 2).

We discovered that infected females had previously undergone extensive reproductive and fertility treatments. It became clear that the treatments were being administered through outdated techniques that did not take into consideration the possibility for cross contamination and infection. Among the 486 female camels, 201 of them did not receive any sort of treatment during the season of 2021–2022. We observed an infection rate of 71.93% on the camels that were treated compared to an infection rate of only 9.45% on camels that did not receive any treatment (Table 2). There is a clear correlation between the infection rate and the number of treatments received. 42.22% of the camels that received ~ 1 to 4 instances of treatment were infected, 61.54% of the camels that received ~ 5 to 8 instances of treatment were infected and it kept on gradually increasing until it peaked at 88.24% infection for camels that received ~ 17 to20 treatments (Table 3). The marked increase of

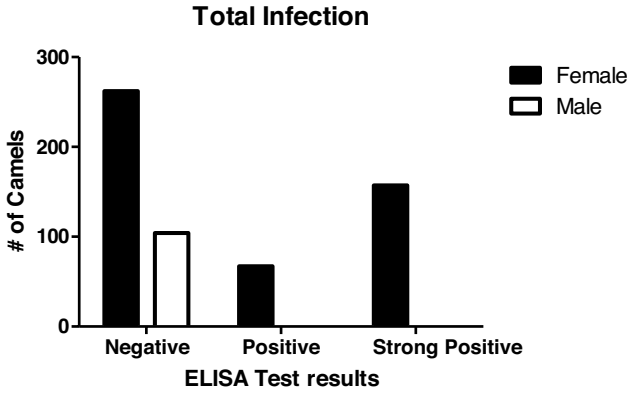

Figure 2. *Trypanosoma evansi* infection between male and female camels.

ELISA Results	Treated		Untreated		X^2 (*df*)
	N	(%)	N	(%)	
Negative	80	28.07	182	90.55	185.2 (2)
Positive	61	21.40	6	2.99	
Strong positive	144	50.53	13	6.47	
Total	285		201		

Table 2. Sero-positivity of *Trypanosoma evansi* based treatment for pregnancy in female camels. Chi-squared test (X^2 (df)), groups are significantly different ($p < 0.0001$).

# of treatments	Total	Negative		Positive		X^2 (*df*)
		N	(%)	N	(%)	
0	201	182	90.55	19	9.45	214.0 (6)
1–4	45	26	57.78	19	42.22	
5–8	65	25	38.46	40	61.54	
9–12	70	14	20.00	56	80.00	
13–16	40	6	15.00	34	85.00	
17–20	34	4	11.76	30	88.24	
≥21	31	5	16.13	26	83.87	

Table 3. Sero-positivity of *Trypanosoma evansi* based on the number of treatment for pregnancy in female camels. Chi-squared test (X^2 (df)), groups are significantly different ($p < 0.0001$).

infections after only ~ 1 to 4 instances of treatment demonstrate how easy and devastating iatrogenic transmission can be. The likely source of the high rate of infection was the re-using of syringes or needles when treating many camels at the same time.

It has been reported numerous times how *T. evansi* infection can induce abortions[21,22]. Considering that most of the infections were from camels receiving reproductive/fertility treatment, we took this opportunity to observe the potential effects of trypanosome infection on the pregnancy and delivery of the camels. The pregnancies verified were from camels that had been mated between 2021 and 2022. There was a total of 145 camels that had been mated during the 2021 to 2022 mating season and 2 failed to be successfully impregnated. Among all the pregnancies, there were a total of 106 births and 37 abortions and 64.87% of the abortions were from camels that were infected with trypanosomes while 70.75% of the births came from camels that were infected with trypanosome (Table 4). However, 31.13% of births from trypanosome positive mothers did not survive the first week, several died within one day (33/106). When considering the total number of post-natal deaths, 81.81% came from trypanosome infected camels. Interestingly, only 18.18% of the births from healthy camels resulted in the death of the calf after birth (Table 5). Although it could be said that trypanosome infection contributes to the abortion rate in camels there was no significant difference (p-value 0.2411) in their role on post-natal death. However, there is a distinctive trend towards the negative impact of *T. evansi* in overall post-natal mortality of the calves. Further analysis with a higher population will be necessary to conclusively verify the detrimental effects of *T. evansi* infection on camel reproduction.

Discussion

Trypanosoma evansi poses a potential threat to the wellbeing of camel livestock in the UAE and there has been an increasing trend for cases of iatrogenic transmission of the pathogen due to the lack of knowledge or will of the caretakers and the antiquated veterinarian practices. In this study we show how the infection rate was limited

ELISA results	Abortion		Birth		X^2 (*df*)
	N	(%)	N	(%)	
Negative	13	35.14	31	29.25	6.372 (2)
Positive	3	8.11	30	28.30	
Strong positive	21	56.76	45	42.45	
Total	37		106		

Table 4. Sero-positivity of *Trypanosoma evansi* in camel calves by birth or abortion. Chi-squared test (X^2 (df)), groups are significantly different ($p < 0.0413$).

ELISA results	Surviving		Post-natal death		
	N	(%)	N	(%)	X^2 (*df*)
Negative	25	34.25	6	18.18	2.845 (2)
Positive	19	26.03	11	33.33	
Strong positive	29	39.73	16	48.48	
Total	73		33		

Table 5. Sero-positivity of *Trypanosoma evansi* by post-natal survival of calves. Chi-squared test (X^2 (df)), groups are not significantly different ($p < 0.2411$).

mostly to female individuals that had undergone extended reproductive analysis or treatment, suggesting that iatrogenic transmission was the root cause. Among the total number of female camels that were examined (n = 486), almost 38% were infected with trypanosome, and the number of infections correlated with the number of exams and treatments that the camels had undergone, gradually increasing from 42.22% infection rate in camels that received between 1 and 4 instances of medical intervention up to 83.87% infection rate in camels that received 21 or more. The study also showed a potential trypanosomiasis effect on camel pregnancy and birth, with 65% of all abortions and 82% of all neonatal deaths coming from trypanosome-infected individuals. Although the abortion and post-birth mortality calves were not further analyzed, it is possible that trypanosome infection impacted the healthy development of the fetus, leading to premature abortions and high calf mortality[10]. Further research into the mechanisms of *T. evansi* on camel pregnancies might help prevent further losses.

There have been numerous studies conducted on the pathology and epidemiology of "Surra" in the region but, as far as we know, this is the first reported case of iatrogenic transmission of *T. evansi* in the region. We can naturally presume that the reason for iatrogenic transmission cases going unreported is due to the implications that the managers, veterinarians and/or caretakers have made a mistake. Generally, they would rather not mention the case or attribute the infections to other classical methods of transmission. It is clearly important to point out this avenue of transmission as under representing such cases could undercut the dangers that it represents. However, there have been instances of iatrogenic transmission being reported in other countries in South America such as Brazil, Argentina and Colombia[23] and in countries of Australasia such as Australia, Papua New Guinea and Indonesia[12]. Both cases mentioned that the case for iatrogenic transmission could be due to mass vaccination programs, implying that they reused syringes during these mass vaccination programs. Although it might be more comfortable and less of a hassle to treat numerous animals using the same syringe, the same number of camels can be treated equally as fast and comfortably by preparing the necessary number of syringes for each animal beforehand. Even with the relatively higher cost of using different syringes, the cheap price of syringes and the possibility to greatly reduce the spread of *T. evansi* and mitigate the economic losses that they incur more than makes up for the cost. It is difficult to track the different instances and what outdated practices are being carried out for such wide spread iatrogenic transmission. The most likely explanation for this widespread spread of trypanosomiasis seems to be the result of the constant reuse of needles and syringes on multiple animals. Not only that but there was also a potential that this sub- optimal treatment of trypanosomiasis could lead to the development of resistant *T. evansi* strain. This environment also brings the terrible potential of increasing the population of asymptomatic individuals that can act as carriers or vector reservoirs that can prevent the eradication of *T. evansi* in a particular region.

This study shows that preventive strategies to control the spread of the disease are severely lacking in camels and that iatrogenic transmission of the pathogen due to the lack of knowledge or will of the caretakers and antiquated veterinarian practices is a worrisome trend. The establishment of proper veterinary practices and preventive strategies in controlling the spread of diseases in domestic animals is an urgent necessity to curb the continuous spread of trypanosomiasis. The iatrogenic outbreak of trypanosome infection in camels shows the devastating consequences of poor management and carelessness. The article calls for increased awareness and knowledge of veterinary practices and preventive strategies in countries that heavily rely on agriculture and animal husbandry.

Data availability

The datasets used and/or analyzed during the current study available from the corresponding author on reasonable request.

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Author contributions

M.N. collected and prepared the samples as well as collated the data. H.D.K. performed the statistical and data analysis, and wrote the manuscript. M.F.P. prepared the samples. J.Y.K. reviewed the statistical analysis and prepared the Tables. P.O.O. Managed and organized the project and study and edited and revised the manuscript. All authors reviewed the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

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