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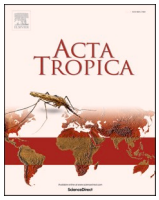

Surveillance and control of *Trypanosoma evansi* in the canary Islands: A descriptive analysis

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ABSTRACT

This study examines the occurrence of Surra, a disease caused by *Trypanosoma evansi*, in camels in the Canary Islands. The 1997 detection of *T. evansi* in camels in the Canary Islands led to the implementation of an initial control program, resulting in a decrease in prevalence. Following an outbreak in 2014, and due to the impossibility of eradicating it using the conventional measures, a lazaret was set up to separate positive and suspicious animals, in addition to the control measures previously implemented. *Stomoxys calcitrans* was the only vector captured, and no other animals tested were found to be positive for *T. evansi.* In November 2019, the last camels that tested serologically positive were detected; however, since February 2018, no camels that tested positive for PCR have been found in the farms were the outbreak was detected, suggesting that the sanitary measures implemented are adequate. The duration of the outbreak control and potential eradication for the disease has yet to be established. This study provides evidence to facilitate the control of African Animal Trypanosomosis in endemic areas of the world, which may contribute to revise the World Organization for Animal Health (WOAH) protocol to implement recommendations of surveillance and control strategies for animal Trypanosomosis in camels.

1. Introduction

Trypanosoma evansi (*T. evansi*) is a unicellular parasite belonging to the sub-genus *Trypanozoon*, which is the causative agent of the disease Surra (Desquesnes et al., 2013b). The disease is primarily diagnosed in camels, horses, and cattle, and is typically transmitted by biting flies, such as tabanids and *Stomoxys* (Desquesnes et al., 2013a). Additionally, transmission by vampire bats has been reported in South America (Austen and Barbosa, 2021; Queiroz et al., 2000). *T. evansi* has the ability to infect carnivorous animals that consume uncooked meat sourced from an animal infected with the parasite (Calvo-Méndez et al., 1994). Given its modes of transmission, *T. evansi* has the potential to become a global issue, necessitating control and surveillance measures (Austen and Barbosa, 2021; Dorny et al., 2009).

Trypanosoma evansi, which originated in Africa, has been reported to have spread to Asia and South America, and a few outbreaks have also been reported in the Canary Islands, Spain (Gutierrez et al., 2010, 2005, 2000; Molina et al., 2000; Rodríguez et al., 2013, 2012). Outbreaks in France and mainland Spain have been attributed to the importation of camels from the Canary Islands (Desquesnes et al., 2008; Gutierrez et al., 2010; Tamarit et al., 2010). Given this, *T. evansi* is considered a potential emerging disease in Europe and resources have been allocated for its

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prevention, control, and eradication (Boulangé et al., 2022; Dorny et al., 2009).

For years, the sanitary criteria for exporting live camels have not been clearly defined. *T. evansi* infection was listed as a notifiable disease for horses, but not for camels. Nowadays, the World Organisation for Animal Health (WOAH) includes all mammals in its "case definition" for infection with *T. evansi* ("OIE Case Definition for Infection with T. evansi (Surra) - WOAH - World Organisation for Animal Health," 2023).

The Canary Islands are located in the North Atlantic Ocean off the northeast coast of the African continent, about 100 km away. They are a region of Spain with a population of 2.261,654 people (latest statistics from 2022) and a 35% tourism-dependant economy (Gobierno de Canarias, 2023). This archipelago received a total of 12.6 million tourists in 2022. Dromedary camels are exclusively linked to this tourist activity as they are no longer used for agriculture as in the past.

The dromedary camel (*Camelus dromedarius*) in the Canary Islands represents the most important indigenous European camel population (Schulz et al., 2010) and currently there are about 1400 animals registered. Therefore, the Canary Islands differ from other European regions in terms of the high camel population on the islands, making Surra eradication challenging. Several cases have been described in dromedary camels on the Islands. In 1997, the first reports of the disease within this species were recorded (Gutierrez et al., 1998). Subsequent zoosanitary and therapeutic measures have not been completely successful to eliminate the disease. Several cases diagnosed in camels have been described from time to time in two farms located in Gran Canaria Island (Gutierrez et al., 2005; Molina et al., 2000; Rodríguez et al., 2013). In 2014, an outbreak of *T. evansi* was detected on these two farms in Gran Canaria. Since 2017, the official animal health services of Canary Islands Government have implemented a surveillance and control programme of *T. evansi* in camels with the goal of eradicating this disease in the archipelago.

The transmission routes of *T. evansi* in the Canary Islands have not been reported in the literature. *Stomoxys calcitrans* is the only haematophagous insect that has been described in the Canary Islands as a possible vector of the disease (Rodríguez et al., 2014). Within the framework of the COMBAT project (Boulangé et al., 2022), a study is being conducted on the presence of haematophagous flies that could mechanically transmit the parasite. In the Canary Islands there are no vampire bats or wild carnivores that could become infected after ingesting infected meat.

The objective of this article is to delineate the methodology and outcomes of a Surra control program carried out in the Canary Islands with the intention of minimizing the infection of *T. evansi*/Surra.

2. Material and methods

2.1. Animals and farms

In 2014, *T. evansi* infection was diagnosed in dromedaries in the Canary Islands. This last outbreak was detected in two farms belonging to the same owner on Gran Canaria Island. The movement of animals between these two farms was common.

Following the official detection of the outbreak, all PCR-positive animals on the affected farms were moved to a neighbouring farm, also belonging to the same owner, which was used as a lazaret. One of the positive animals must has been euthanized because of the severity of the symptoms, and the remaining ones were treated with Tripanoquin® (a combination of quinapyramine sulphate and quinapyramine chloride) in July 2017. Since January 2018, official surveillance and control measures of *T. evansi* have been implemented for all camels in the Canary Islands Archipelago. Consequently, all camels from the camel farms in Canary Islands underwent testing.

In the Canary Islands, the Department of Agriculture, Livestock and Fisheries of the Government of the Canary Islands allocates an ear tag to each dromedary camels (*Camelus dromedarius*) held in farms, to ensure the correct registration and traceability of their movement. This is part of the annual sanitation campaigns conducted for all livestock animals. Over the years, the census of these animals has varied, mainly due to births and deaths. In the year 2018, a total of 950 camels underwent testing for the detection of *T. evansi* infection though the analysis of blood samples, 925 in 2019, 868 in 2020, and 814 in 2022. The COVID-19 pandemic prevented the inspection of all animals and farms in 2021 due to technical, operational and economic reasons.

The sampling protocol in the farms where the outbreak was identified was executed at a higher frequency compared to the other farms situated on the islands. The investigation on farms where positive animal cases were observed was conducted multiple times within a year due to the necessity of more frequent veterinary examinations.

2.2. CATT/ T. evansi

The Card Agglutination Trypanosomosis Test (CATT/*T. evansi)*, developed by the Institute of Tropical Medicine, Antwerp, Belgium, is a serological technique for the detection of antibodies against the predominant variable antigen RoTat 1.2 of *T. evansi* (Bajyana Songa and Hamers, 1988). The CATT/ *T. evansi* is an effective and reliable tool for the early identification of *T. evansi* infection for a control program in camels (Desquesnes et al., 2022a, 2022b; Selim et al., 2022). All serum samples from all censused animals were tested using CATT/ *T. evansi*, with results indicated by scoring the Ag-Ab visible reaction on the card (-, +/-, +, ++, +++). Positive samples and those with a doubtful reaction (+/-) were investigated via PCR.

2.3. Polymerase chain reaction (PCR)

Polymerase Chain Reaction (PCR) was performed on all CATT/ *T. evansi* samples that tested positive. Genomic DNA was extracted from blood samples of animals that showed positive results using CATT/ *T. evansi* serology, employing a commercially available QIAamp DNA mini kit (Qiagen, Hilden, Germany). The purified DNA was stored at −20 °C for later use. Two PCRs were carried out, one for the detection of the subgenus *Trypanozoon* and another for species-specific identification of *T. evansi*. Primers, namely pMURTec.F and pMURTec.R, were designed to target a highly repetitive region of DNA within the *Trypanozoon* subgenus, as described by Wuyts et al. (1994). The primers, designated as pMURTec.F and pMURTec.R, amplified a 227 bp fragment from the genomic DNA, as reported by Njiru et al. (2004) (Details on Table 1).

A 25 μl PCR reaction mixtures consisting of 10x reaction buffer (Bioline), 2.5 mM $MgCl_2$, 300 μM each of four deoxynucleoside triphosphates (dNTPs; Amersham), 100 ng of primers and 0.75 U of Taq DNA polymerase (Bioline) was used. 2 μl of DNA template was added to each reaction. The PCR cycles included an initial step at 94 °C for 4 min, followed by 30 cycles of denaturing at 94 °C for 30 s, annealing at 60 °C for 30 s and extension at 72 °C for 30 s, with elongation continued at 72 °C for 5 min.

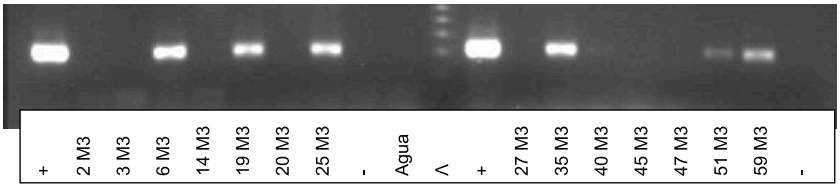
Additionally, a 205-bp fragment of the RoTat 1.2 gene was amplified from the extracted genomic DNA. A primer specific to the targeted region of this gene, following the method described by Deborggraeve et al. (2008), was used. The PCR reaction had an annealing temperature of 59 °C. This amplification served as an assay for the presence of *T. evansi.*

The resulting amplification products (seven microliters) were then separated using 2% molecular grade agarose gel electrophoresis (Roche Diagnostic, Barcelona, Spain) (Fig. 1). A 100 base pair marker was included as size reference. To validate the experiment, blood sample from a dromedary camel that had been confirmed positive for *T. evansi* both serologically and parasitologically was used as a positive control in the PCR assay.

Table 1
Applied PCR protocols for parasite identification.

Target group	Target gen	Primer	Primer sequences	Ta (°C)	Amplicon (bp)	References
Trypanozoon	pMURTec	pMURTec.F	5′-TGCAGACGACCTGACGCTACT-3′	60	227	(Njiru et al., 2004)
		pMURTec.R	5′-CTCCTAGAAGCTTCGGTGTCCT- 3′			
T. evansi	RoTat 1.2	RoTat 1.2-F	5′-GCGGGGTGTTTAAAGCAATA-3′	59	205	(Claes et al., 2004)
		RoTat 1.2-R	5′-ATTAGTGCTGCGTGTGTTCG-3′			

Ta = annealing temperature; bp = base pairs.



The amplified RoTat 1.2 gene was utilized as an assay to detect the presence of *T. evansi*.

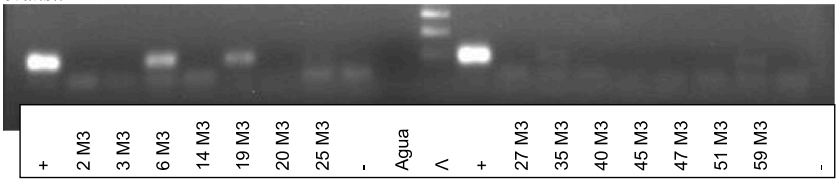


Fig. 1. depicts an illustrative gel image showcasing the PCR amplification.

3. Results

The first data on this outbreak date back to October 2014, when 133 samples of dromedary camels from affected farms were tested by CATT/*T evansi*. Of those, 23 were positive and sent to the Central Laboratory for Animal Health (LCSA) for further testing. At the LCSA, serum agglutination test was repeated and, according to the World Organisation for Animal Health (WOAH, former OIE) Manual Chapter 3.01.21 published in 2018 (*Terrestrial Code*, 2018), the positive samples were analysed by PCR. The results showed 21 samples as CATT positive. On the two PCR performed in this study (one PCR assay detected the presence of the subgenus *Trypanozoon*, while the other PCR assay provided species-specific identification of *T. evansi*), two samples were compatible with *T. evansi* and 10 were compatible with subgenus *Trypanozoon* (which does not exclude the presence of *T. evansi*).

In March 2015, 122 samples from the same farms were analysed at the LCSA, yielding 31 CATT-positive results, five of which were confirmed as being Subgenus *Trypanozoon* by PCR.

The pMURTec.F and pMURTec.R primers were designed to target a DNA region that is highly repetitive within the *Trypanozoon* subgenus.

In October 2016, 119 samples were taken and analysed at the LCSA, of which 42 were positive for CATT. Subsequent, PCR testing revealed 9 to be compatible with *T. evansi* and 6 to be compatible with Subgenus *Trypanozoon*.

In December 2016, all the animals were identified with microchips due to suspicion of ear tag dancing and were subsequently bled and analysed at the LCSA. A total of 104 camels were tested using CATT, of which 10 were found to be positive. These animals were the PCR tested, with negative results with the exception of one sample, which was inconclusive.

The results of the camel census from 2018 onwards, excluding the farms where the outbreak was detected, are presented in Table 2. The two variants of Polymerase Chain Reaction (PCR) described in the Materials and Methods section were conducted on every blood sample under investigation.

Table 2
Data from all the camel census of Canary Islands. *Not including the farms where the outbreak was detected.

Island	Year	Number of animals analysed	CATT (positive)	CATT (doubtful)	PCR (+)
Gran Canaria*	2018	71	3	6	0
	2019	73	32	4	0
	2020	64	0	0	NT
	2021	33	0	0	NT
	2022	62	0	1	0
Lanzarote	2018	325	36	26	0
	2019	326	6	4	0
	2020	278	0	0	NT
	2021	38	0	0	NT
	2022	262	1	10	0
Fuerteventura	2018	382	1	11	0
	2019	358	13	0	0
	2020	325	0	0	NT
	2021	31	0	0	NT
	2022	314	0	4	0
Tenerife	2018	43	0	0	NT
	2019	45	1	0	0
	2020	42	0	0	NT
	2021	39	0	0	NT
	2022	43	2	2	0
La Palma	2018	6	0	0	NT
	2019	2	0	0	NT
	2020	2	0	0	NT
	2021	2	0	0	NT
	2022	2	1	1	0

NT = Not tested.
Table 3 presents the data collected from 2017 onwards from the farms where the outbreak was detected.

4. Discussion

Surra is a disease that causes a reduction in camel production and animal welfare. In 1997, the first detection of *T. evansi* in camels in the Canary Islands was documented, and seroprevalence values of 6.2% (Gran Canaria), 7.1% (Lanzarote) and 1.7% (Fuerteventura) were

Table 3
Data of the farms where the outbreak was detected.

Date	Number of animals analysed / Not analysed	CATT (positive)	CATT (doubtful)	PCR (+)
April 2017	123/11	22	0	5*
July 2017	111/21	21	0	2**
February 2018	126/0	15	0	0
March 2018	123/0	1	17	0
April 2018	123/0	18	6	0
July 2018	121/0	45	0	0
October 2018	125/0	37	12	0
November 2019	125/0	6	0	0
November 2020	125/0	0	0	NT
July 2021	0/115	NT	NT	NT
March 2022	131/0	0	0	NT

* 4 Profiles compatible with *T. evansi*. 1 not concluding profile.
** 1 profile compatible with *T. evansi* and 1 profile compatible with subgenus *Trypanozoon*.

determined in camel samples collected from 1997 to 1999 using CATT/*T. evansi* test (Gutierrez et al., 2000). In response to these data, an initial control program was implemented and all parasitologically and/or serologically positive animals were treated. Consequently, prevalence decreased progressively, and since 2003, *T. evansi* has only been confirmed by PCR in camels on Gran Canaria, specifically on two camel farms on the island, where clinical cases of surra have been also observed. In the other islands of the Archipelago, CATT positive and doubtful results have been found from time to time, also in a decreasing number, but neither clinical signs in animals nor PCR positive results have been observed.

To explain the sustained presence of *T. evansi* in the two farms, our research group evaluated the role of other animals in the surrounding ecosystems (wild rodents, domestic ruminants, and equines) as possible reservoirs of infection (Rodríguez et al., 2013, 2012, 2010); however, no animals tested were found to be positive for *T. evansi*. Additionally, potential vectors of *T. evansi* in the area were also surveyed (Rodríguez et al., 2014) with *Stomoxys calcitrans* being the only one captured.

Following the last outbreak of the disease, that started in 2014 in the referred farms, the Department of Agriculture, Livestock and Fisheries of the Government of the Canary Islands initiated a comprehensive campaign to officially control the disease.

In 2017, a lazaret was set up to separate animals from the rest, in addition to the control measures previously implemented. Sumba et al. (1998) found that viable *T. evansi* can remain in the proboscis of *Stomoxys* spp. for up to 5–7 min after feeding. Desquesnes et al. (2013a) report that the transmission of *T. evansi* by *Stomoxys* is efficient only when the time interval between two interrupted blood meals is short, implying that this could occur when animals are in close proximity (intra-herd transmission). Therefore, the establishment of a lazaret for positive camels, at a sufficient distance, obstructs vector-borne transmission of the parasite.

In November 2019, the last camels that tested serologically positive were detected in the farms were the outbreak occurred in the past; however, since February 2018, no camels that tested positive for PCR have been found within those farms.

Based on the results presented in this paper, it appears that the sanitary measures implemented were adequate as it is evidenced that the CATT/*T. evansi* and PCR positive results have been reduced. The duration of the outbreak control and potential eradication for the disease has yet to be established because the disease still cannot be considered eradicated. Particularly, a protocol (Woo, CATT and PCR for example), a rate of sampling (100% for example), a frequency (quarterly for example) and a duration (2 years for example) could be proposed to demonstrate the absence of suspicion on an animal that was previously positive to a trypanosome diagnosis method.

In the farms where any animal with clinical sings has been observed since March 1999 (Gutierrez et al., 2000), 4 CATT/*T. evansi* positive results were detected out of 683 animals tested in 2022. These animals should be considered as suspect but could also be considered false positive which may vary depending on the host (Verloo et al., 2000). Extravascular foci may serve as refuge for the parasite, while the infected animal remains asymptomatic, so does not present any laboratory or clinical sign of surra (Brun et al., 2001). For this reason, in an area where some positive have been found in the past, it is necessary to maintain surveillance for several years to prevent future relapse and outbreaks, even though the importation of camels from Africa is currently prohibited.

This study can be used as a basis for the future revision of the World Organization for Animal Health (WOAH) protocol to implement recommendations of control strategies for animal Trypanosomosis in camels amongst other studies (Diall et al., 2022), providing evidence that can facilitate the surveillance and control of the African Animal Trypanosomosis (ATT) in endemic areas of the world.

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CRediT authorship contribution statement

María Teresa Tejedor-Junco: Conceptualization, Investigation, Methodology. **Adrián Melián Henríquez:** Writing – original draft, Project administration. **Pedro Peláez Puerto:** Investigation, Formal analysis. **María Dolores Ramos:** Investigation, Resources. **Margarita González-Martín:** Investigation, Resources. **Manuel Morales Doreste:** Investigation, Formal analysis. **Geoffrey Gimonneau:** Investigation, Formal analysis. **Marc Desquesnes:** Methodology, Writing – review & editing. **Sergio Martín Martel:** Investigation, Formal analysis. **Juan Alberto Corbera:** Conceptualization, Investigation, Methodology, Writing – original draft, Project administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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