



Epidemiological investigations on *Trypanosoma evansi* infection in dromedary camels in the South of Algeria

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ABSTRACT

An epidemiological study of *Trypanosoma evansi* (*T. evansi*) infection in dromedaries was conducted in four wilayate (localities) of Southern Algeria: Béchar, El Bayadh, Ouargla, Tamanrasset. Between February 2014 and April 2016, 1056 camels of different ages and both sexes from 84 herds were sampled. The prevalence was determined through parasitological examination (Giemsa stained thin smear, GST), serological tests (CATT/*T. evansi*, ELISA/VSG RoTat 1.2, immune trypanolysis), and molecular tests (*T. evansi* type A specific RoTat 1.2 PCR and *T. evansi* type B specific EVAB PCR).

The overall prevalence was 2.4 % with GST, 32.4% with CATT/*T. evansi*, 23.1% with ELISA/VSG RoTat 1.2, 21.0% with immune trypanolysis (TL), 11.2 % with RoTat 1.2 PCR and 0% with EVAB PCR.

El Bayadh was the most affected wilaya with 11.8% positives in GST, 74.9% in CATT/*T. evansi*, 70.1% in ELISA/VSG RoTat 1.2 and 62.2% in immune trypanolysis. Only in Béchar, a non-significantly higher prevalence (13.6%) was observed with RoTat1.2 PCR than in El Bayadh (13.0%). We didn't find any evidence of the presence of *T. evansi* type B in the study area.

1. Introduction

Trypanosoma evansi (*T. evansi*) causes trypanosomosis called "surra" in many countries (OIE, 2018). It affects a large number of wild and domesticated animal species in Asia, Africa and Central and South America (Luckins and Dwinger, 2004; OIE, 2018). It is especially pathogenic in camelids and equids (Desquesnes et al., 2013b). *Trypanosoma evansi* is a protozoan parasite transmitted mechanically by hematophagous biting flies, mainly tabanids (Atarhouch et al., 2003).

In camels, surra usually occurs in its chronic form but it may be acute with high mortality when the animal is under stress. The chronic form is characterised by reduced fertility, generalised loss of body condition, oedema, particularly of the lower parts, neuropathy and immune suppression, anaemia and eventually death (Al-Qarawi et al., 2004; Luckins,

1998; Parsani et al., 2008). In affected countries, surra is an economically important disease, which causes high mortality, low milk and meat production, poor carcass quality, reduced reproductive performance, decreased draught power and manure production (Desquesnes et al., 2013a).

Algeria covers an area of 2,381,741 km², of which 87% is occupied by the Sahara where 344,015 camels live (Ministère de l'Agriculture, du Développement Rural et de la Pêche (MADRP), 2014).

The first case of trypanosomosis in Algeria was reported by the Sergeant brothers in 1903 (Sergent and Sergent, 1905). A year later, 10% of 282 camels sampled from the South-Eastern regions of the country, showed *Trypanosoma* in their blood (Sergent and Sergent, 1905; Sergent and Donatien, 1921). The disease was well known by herders who associated it with the presence of biting flies. Indeed, its local name is "El

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Dhebab" which means "fly" in Arabic. However, no extended studies were conducted until 1999 when a parasitological survey was carried out on 1125 dromedary camels with a reported prevalence of 0.5 % in Ouargla (Marfoua, 1999). More recently, between 2005 and 2006, we sampled 1074 dromedary camels of the South of Algeria in the wilayate of El Bayadh, Ouargla, Béchar, Tindouf, Adrar and Tamanrasset. We observed 2.3% positives in Giemsa stained thin blood smears (GST) and 20.3% in CATT/*T. evansi* with the highest parasitological (12.3%) and serological (68.7%) prevalences in Béchar and no single positive in El Bayadh (Boushaki, 2007).

Absence of pathognomonic signs of surra necessitates laboratory analysis to confirm the infection, either by microscopy or by molecular tools to demonstrate the parasite and thus active infection or by serological tools to reveal antibodies induced by a present or past infection (Büscher, 2001, 2014; Tehseen et al., 2015). Given the low sensitivity of parasitological examinations in chronic infections, DNA amplification techniques, such as the polymerase chain reaction (PCR), are often applied as surrogate (Büscher, 2014). For surra, *Trypanozoon*-specific primers targeting satellite DNA or ribosomal DNA are the most sensitive (Gari et al., 2010; Masiga et al., 1992; Njiru et al., 2005) while the distinction between *T. evansi* type A and type B can be made with PCRs specific for the type A RoTat 1.2 gene and specific for type B minicircles (Claes et al., 2004; Njiru et al., 2006; Urakawa et al., 2001). In addition to parasitological or molecular diagnostics, serological tests are useful to provide indirect evidence of the presence of *T. evansi* in a susceptible population or individual. The most specific tests to detect antibodies against *T. evansi* type A make use of the variant surface glycoprotein (VSG) RoTat 1.2 as antigen (Bajyana Songa and Hamers, 1988; Urakawa et al., 2001). These tests comprise the direct agglutination test CATT/*T. evansi* (Bajyana Songa and Hamers, 1988), the ELISA/VSG RoTat 1.2 (Lejon et al., 2005; Rogé et al., 2013; Verloo et al., 2000) and the immune trypanolysis (Van Meirvenne et al., 1995; Verloo et al., 2001). *Trypanosoma evansi* type B typically lacks the RoTat 1.2 gene and

therefore does not induce anti-RoTat 1.2 antibodies (Ngaira et al., 2005; Njiru et al., 2006). So far, *T. evansi* type B seems only to occur in camels in Kenya, Chad, Ethiopia and Sudan (Birhanu et al., 2015; Ngaira et al., 2005; Njiru et al., 2006; Salim et al., 2011).

In order to create awareness about surra in Algeria and to overcome the economic losses it can cause, it is necessary to acquire recent and accurate information on the epidemiology of this disease using sensitive and effective diagnostic tools. Hence, the aim of the study was to estimate the prevalence of *T. evansi* infection in dromedary camels in Southern Algeria, and to assess the associated risk factors.

2. Materials and methods

2.1. Ethical statement

Authorisation to conduct the survey was obtained from the Direction des Services Vétérinaires (DSV, Ministry of Agriculture, Rural Development and Fisheries). At each wilaya, the study was authorised and supervised by the respective Inspection Vétérinaire de Wilaya (IVW Béchar, El Bayadh, Ouargla, and Tamanrasset), operating under the umbrella of the Direction des Services Vétérinaires.

2.2. Study area

The study was conducted in four wilayate of Southern Algeria: El Bayadh, Béchar, Ouargla, Tamanrasset (Fig. 1).

El Bayadh is divided into three areas: the steppe high plains, the Saharan atlas zone, and the Pre-Saharan zone. The average temperatures range from 0 °C in January to 35.1 °C in August. The vegetation is composed mainly by degraded forests of *Pinus halepensis* and *Ziziphus lotus* in the mountains. Unsalted steppe formations are characterised by grasses like *Artemisia herba alba*, *Stipa tenacissima* and *Aristida pungens*. The vegetation of salty soils is characterised by *Atriplex* sp (Direction des

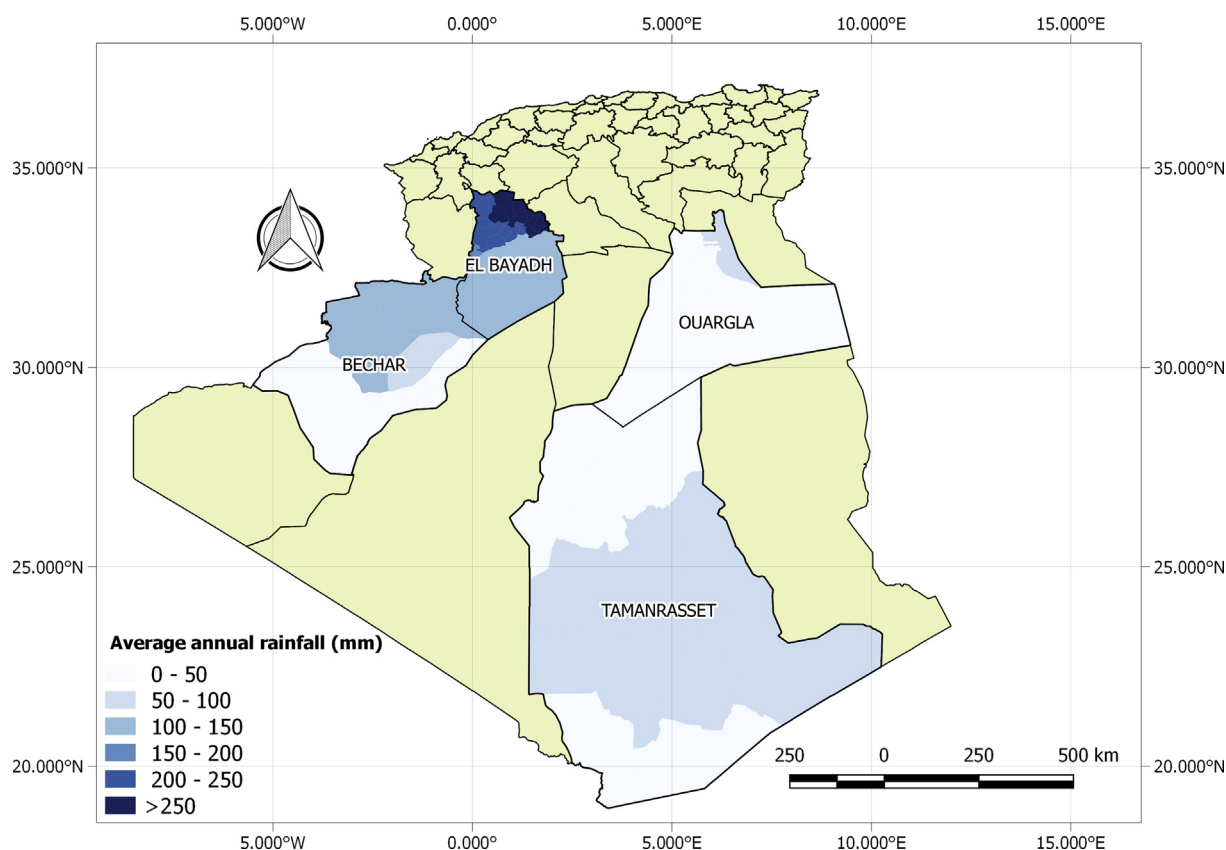


Fig. 1. Map of the study area showing the four wilayate and the average annual rainfall measured during 2014, 2015 and 2016.

Services Agricoles d'El Bayadh, 2016; Office National de la Météorologie (ONM), 2018).

Béchar is characterised by a continental desert climate. The temperature varies between 3.7 °C in January and 45 °C during July and August. The valleys are very rich in woody and perennial grasses such as *Aristida pungens*, and *Cenchrus biflorus* (Direction des Services Agricoles de Béchar, 2018; Office National de la Météorologie (ONM), 2018).

Ouargla is characterised by a Saharan climate with rare and irregular rainfall. Temperatures range from 4 °C in January to 44.6 °C in August. The plant canopy is composed of herbaceous species like *Cenchrus biflorus*, and *Aristida pungens*, shrubby vegetation with *Retama raetam* and *Calligonum azel* and woody species, mainly *Acacia sp.* (Direction des Services Agricoles de Ouargla, 2016; Office National de la Météorologie (ONM), 2018).

Tamanrasset is divided into three regions: the north composed of plateaus and plains, the centre a region of relief, and the south with plains bordering the Sahel countries. Temperature varies between 4.7 °C in January and 47.6 °C in August. The vegetation is composed of steppe formations such as *Artemisia herba alba* in the north and desert formations in the south with *Tamarix aphylla*, *Acacia sp.* and *Trichodesma africanum* (Direction des Services Agricoles de Tamanrasset, 2016; Office National de la Météorologie (ONM), 2018).

2.3. Study characteristics

This cross-sectional study was conducted between February 2014 and April 2016 on the dromedary camel population of the selected localities. In these localities, herds were encountered in the camps, and especially in rangelands (pastures and watering wells). Ten percent of the animals in each herd were sampled randomly without stratification according to breed, sex or age. With an expected prevalence of 25% (Boushaki, 2007) and a desired precision of 10%, the calculated sample size was 1153 animals (Toma et al., 2010). The following parameters were recorded for each animal: origin, age, sex, fur colour, breeding system and presence of clinical symptoms (cachexia, conjunctival mucosae, lachrymation, oedema and lymph nodes). The breeder's knowledge of the disease, previous trypanocidal treatment, history of abortions and transhumance routes were also recorded. The age of the animals recorded was based on information from the owners. Animals were grouped into five age classes: less than one year, between one and two years, between two and four years, between 4 and 10 years, older than 10 years.

2.4. Blood sampling

For each animal, two blood samples were taken from the jugular vein: 4.5 ml on ethylene diamine tetra acetate (EDTA) and 10 ml in a dry tube. The sample on EDTA was used to measure the packed cell volume (PCV) and to extract DNA using the QIAamp DNA Blood Mini Kit 250 from Qiagen, Hilden, Germany according to the manufacturer's instructions. Extracted DNA was preserved at -20 °C till analysis. Blood taken in the dry tube was allowed to clot where after it was centrifuged for 15 min at 480 g. The obtained serum was tested with CATT/*T. evansi* and subsequently aliquoted into cryotubes and frozen at -20 °C for further serological tests.

2.5. Parasitological examination

Parasitological examination was performed on Giemsa stained thin smears (OIE, 2018). A smear was considered positive when at least one trypanosome was observed.

2.6. Packed cell volume

Packed cell volume was assessed after centrifugation of capillary tubes (75 mm, Hirschmann, Germany) filled with EDTA blood in a microhaematocrit centrifuge (OIE, 2018). An animal was considered

anaemic when it had a PCV of less than 24% (Dioli and Stimmelmayer, 1992). For technical reasons, the PCV was carried out on only 904 samples.

2.7. CATT/*T. evansi*

The sera were tested for the presence of anti-*T. evansi* antibodies using the card agglutination test for trypanosomosis (CATT/*T. evansi*) (Institute of Tropical Medicine, Antwerp, Belgium) (Bajjana Songa and Hamers, 1988).

2.8. ELISA/VSG RoTat 1.2

Indirect ELISA/VSG RoTat 1.2 was carried out according to Lejon et al. (2005) and Verloo et al. (2000). Minor modifications were the coating of the native VSG RoTat 1.2 (Institute of Tropical Medicine, Antwerp, Belgium) diluted at 2 µg/ml in phosphate buffer (PB 0.01 M, pH 6.5, NaH₂PO₄·H₂O 0.95 g/l, Na₂HPO₄·2H₂O 0.55 g/l), the incubation of 150 µl of test sera diluted 1/100 in PBS-Blotto without shaking and reading of the absorption at 405 nm. Corrected ODs were expressed as percentage positivity (PP) of the OD_{corr} obtained with positive control serum included in each plate. The cut-off value was set at 30% PP, based on the bimodal distribution observed in the histogram constructed with data from the whole study cohort (Supplementary material S1).

2.9. Immune trypanolysis

Immune trypanolysis (TL) was performed at the OIE Reference Laboratory for Surra at the Institute of Tropical Medicine, Antwerp, Belgium, according to Van Meirvenne et al. (1995) and Verloo et al. (2000).

2.10. Molecular diagnosis

Two molecular tests were carried out to detect *T. evansi* DNA in blood. The RoTat 1.2 PCR targets the *T. evansi*-specific gene coding for RoTat 1.2 VSG; the EVAB PCR targets *T. evansi* type B minicircle kinetoplast DNA (Birhanu et al., 2015; Urakawa et al., 2001) (Table 1). Compared to the cited references, minor changes were the activation of HotStar Taq DNA polymerase at 95 °C for 15 min and, in the EVAB PCR, the final extension for 10 min at 72 °C.

As positive control, DNA (10 ng/ml) of *T. evansi* type A (MCAM/ET/2013/MU/02) and type B (MCAM/ET/2013/MU/10) were included in each PCR run, together with pure water as negative control (Birhanu et al., 2016). The amplified products were visualised under UV after electrophoresis in a 2% agarose gel (Sigma, USA) at 135 V for 30 min and staining with ethidium bromide (Sigma, USA).

2.11. Data analysis

All data were recorded in Microsoft Excel. R version 3.4.1 (R Core Team, 2017) was used for statistical analysis. Percentages with 95% confidence interval (CI) were used to express prevalence. Concordance between test results was expressed in terms of indices of positive and negative agreement (Cicchetti and Feinstein, 1990) and their 95% confidence intervals were calculated (Adel et al., 2015; Graham and Bull, 1998). It was noticed that despite a high concordance between two tests, the kappa coefficient may paradoxically be low (Cicchetti and Feinstein, 1990; Feinstein and Cicchetti, 1990). Therefore, levels of agreement between diagnostic tests were indicated by indices of positive and negative agreement since these indices are not influenced by prevalence, unlike the kappa coefficient.

The t-test was used to compare between the mean PCV in the group of positive animals and negative animals according to their different diagnostic test results. Logistic regression was applied for assessing differences in prevalence of *T. evansi* infection according to the wilaya, the age, the sex, the fur colour, and the breeding system. P values < 0.05 were

Table 1

Details on target gene, primer sequences, amplicon lengths of the RoTat 1.2 PCR and the EVAB PCR.

Taxon	Target gene	Primers	Primer sequences	Amplicon length	Reference
<i>T. evansi</i> type A	RoTat 1.2 VSG	ILO7957 ILO8091	5'-GCC ACC ACG GCG AAA GAC -3' 5'-TAA TCA GTG TGG TGT GC-3'	488 bp	Urakawa et al. (2001)
<i>T. evansi</i> type B	minicircle	EVAB-1 EVAB-2	5'-ACAGTCCGAGAGATAGAG-3' 5'-CTGTACTCTACATCTACCTC-3'	436 bp	Birhanu et al. (2015)

considered as significant. The map was constructed on QGIS 2.18.15.

3. Results

3.1. Study cohort

A total of 1056 dromedaries from 84 herds at 68 sites were sampled. A coverage rate of 10% was observed within all prospected herds. The number of animals sampled according to wilaya, sex and age class is represented in Table 2. About two thirds of the animals (69.9%) were adults (>4 years). Most animals were females (80.5%).

Out of the 1056 animals sampled, some did not undergo all the available diagnostic tests because of technical reasons (i.e. losses, haemolysis), leading to variations in the number of samples per diagnostic test.

3.2. Mortality and morbidity

During the study period the camel owners reported 293 abortions, 60 stillbirths and 96 deaths (Table 3). The highest number of abortions, stillbirths and deaths were recorded in El Bayadh.

3.3. Clinical findings

Clinical examination revealed signs of both the acute and chronic form of disease, with lacrimation (30.2%), lymph node hypertrophy (28.4 %), rough coat (20%), cachexia (17%) and pale conjunctival mucosa (11%). Other signs were observed with lower frequency: diarrhoea, oedema, petechial haemorrhages of the oral mucous membranes, ulcerative keratitis, respiratory complications, and sterno-abdominal decubitus due to paralysis of the hind quarters. In El Bayadh, 78.9% of the parasitologically positive animals and 81.8% of the PCR positive animals showed clinical signs of surra.

3.4. Laboratory diagnostic test results

The observed overall parasitological prevalence in GST was found to be 2.4% (95% CI:1.5–3.3). Overall serological prevalence ranged from 32.4% (CI: 29.6–35.2) in CATT/*T. evansi* over 21.0% (CI: 18.5–23.5) with TL to 23.1% (CI: 20.5–25.7) in ELISA/VSG RoTat 1.2. Overall molecular prevalence was 11.2% (CI: 8.5–13.9) in RoTat 1.2 PCR (Table 4). No single animal was positive in EVAB PCR. Overall prevalence recorded in the other tests were statistically different from each other ($p < 0.0001$) except between TL and ELISA/VSG RoTat 1.2 ($p = 0.28$).

Irrespective of the test used, the observed trypanosomosis prevalence was significantly higher in El Bayadh than in the other wilaya ($p <$

Table 2

Number of dromedary camels sampled according to wilaya, sex and age class.

Wilaya	Sex		Age class (years)				
	Female	Male	<1	≥1 ≤ 2	>2 to ≤4	>4 and ≤10	>10
El Bayadh	152	19	9	12	12	65	73
Ouargla	280	24	12	27	33	141	91
Béchar	201	20	0	34	12	86	89
Tamanrasset	217	143	96	49	32	126	57
Total	850	206	117	122	89	418	310

Table 3

Mortality and morbidity recorded in the wilaya.

Wilaya	Period	Abortions	Stillbirths	Deaths
El Bayadh	February 2014–November 2014	132	26	59
Ouargla	February 2014–December 2015	30	10	10
Béchar	January 2015–January 2016	110	18	15
Tamanrasset	February 2014–April 2016	21	6	12

0.0001) except for RoTat 1.2 PCR where the prevalences in El Bayadh and Béchar were similar (respectively 13.0 and 13.6%). The epidemic situation in El Bayadh is reflected by the high observed prevalence in GST (11.8%, CI: 6.8–16.8) and very high serological prevalences in CATT/*T. evansi* (74.9%, CI: 68.4–81.4), ELISA/VSG RoTat 1.2 (70.1%, CI: 62.4–77.9) and TL (62.2%, CI: 54.0–70.4). The logistic regression shows that the animals of El Bayadh were 80 times more at risk than in Tamanrasset (OR = 80.5, CI: 37.6–200.4). Camels in Béchar were 21 times (OR = 21.5, CI: 10.5–52.0) more likely to be infected than in Tamanrasset. Camels in Ouargla were almost 7 times (OR = 6.9%, CI: 3.1–17.5) more likely to be infected than in Tamanrasset.

Regarding observed parasitological and molecular prevalences, no statistical difference was found between male and female animals. This is in contrast with observed serological prevalences. For example, according to TL, females are almost 5 times more at risk to be infected than males (OR = 4.9, CI: 2.8–9.4).

For most of the diagnostic tests, prevalence did not significantly differ between the age classes although there is a tendency to find a lower prevalence in calves (<1 year) and a higher prevalence in older animals (>10 years) (Table 4). For example, none of the calves under one year was found positive in GST but parasitological prevalence was not significantly different between the other age classes. On the other hand, TL seroprevalences in the animals between 1 and 2 years old and above 4 years old were significantly different from the calves under one year. They were respectively, four (OR = 4.2, CI: 1.7–11.8, $p < 0.01$), five (OR = 4.7, CI: 2.1–12.3, $p < 0.001$) and eight (OR = 8.3 CI: 3.8–21.7, $p < 0.0001$) times more at risk to be positive than the <1 year old calves.

The cohort consisted of 1019 transhumant camels, 20 semi-intensive and 17 sedentary animals. The twenty five parasitologically positives were all transhumant. A significant difference was observed between prevalence in sedentary camels and semi-intensive ones ($p < 0.01$) with CATT/*T. evansi* (OR = 10.1, CI: 2.1–77.3). Also in the transhumant camels, the seroprevalence with CATT/*T. evansi* was higher than in the other breeding systems (borderline significant, $p = 0.05$). In the other diagnostic tests, the observed prevalence was not significantly different between breeding systems; With reference to local language, five fur colours were observed: azeghrif (bicolored), baida (white), hamra (red), sefra (yellow), and zerga (blue). Observed prevalence in the different diagnostic tests was not significantly different except in CATT/*T. evansi* where zerga camels were 3 times more at risk of being infected than the others (OR = 3.2, CI: 1.2–9.8).

3.5. Degree of agreement between the different diagnostic tests

A contingency table and degree of agreement between the different diagnostic tests using indices of positive and negative agreement and

Table 4

Number (n) of animals tested, number (p) and percentage (%) of positives in different tests according to wilaya, breeding system, sex, age and fur colour of the animals. CATT = CATT/T. *evansi*, ELISA = ELISA/VGS RoTat 1.2, TL = immune trypanolysis, PCR = RoTat 1.2 PCR

		GST		CATT		ELISA		TL		RoTat 1.2 PCR	
		n	p (%)	n	p (%)	n	p (%)	n	p (%)	n	p (%)
Wilaya	El Bayadh	161	19 (11.8)*	171	128 (74.9)*	134	94 (70.1)*	135	84 (62.2)*	169	22 (13.0)
	Ouargla	221	2 (0.9)	221	27 (12.2)*	219	25 (11.4)*	218	27 (12.4)*	50	5 (10.0)
	Béchar	304	4 (1.3)	304	112 (36.8)*	304	99 (32.6)*	304	93 (30.6)*	162	22 (13.6)
	Tamanrasset	360	0 (0.0)	360	75 (20.8)	349	14 (4.0)	349	7 (2.0)	155	11 (7.1)
	Overall	1046	25 (2.4)	1056	342 (32.4)	1006	232 (23.1)	1006	211 (21.0)	536	60 (11.2)
Breeding system	sedentary	17	0 (0.0)	17	9 (52.9)*	16	2 (12.5)	16	1 (6.3)	15	1 (6.7)
	semi-intensive	20	0 (0.0)	20	2 (10.0)	19	0 (0.0)	18	1 (5.6)	6	1 (16.7)
	transhumant	1009	25 (2.5)	1019	331 (32.5)*	971	230 (23.7)	972	209 (21.5)	515	58 (11.3)
Sex	female	841	21 (2.5)	850	288 (33.9)*	813	212 (26.1)*	813	199 (24.5)*	439	49 (11.2)
	male	205	4 (2.0)	206	54 (22.2)	193	20 (10.4)	193	12 (6.2)	97	11 (11.3)
Age class (year)	<1	117	0 (0.0)	117	28 (23.9)	115	9 (7.8)	115	6 (5.2)	52	3 (5.8)
	≥1 ≤ 2	121	4 (3.3)	122	38 (31.1)	117	27 (23.1)*	117	22 (18.8)*	67	7 (10.4)
	>2 to ≤4	87	2 (2.3)	89	27 (30.3)	85	16 (18.8)*	85	11 (12.9)	51	2 (3.9)
	>4 and ≤10	414	7 (1.7)	418	121 (28.9)	401	92 (22.9)*	401	82 (20.4)*	193	29 (15.0)
	>10	307	12 (3.9)	310	128 (41.3)*	288	88 (30.6)*	288	90 (31.3)*	173	19 (11.0)
Fur colour	azeghruf	26	1 (3.8)	26	5 (19.2)	26	4 (15.4)	26	5 (19.2)	9	2 (22.2)
	baida	211	3 (1.4)	211	44 (20.9)	205	31 (15.1)	205	22 (10.7)	105	13 (12.4)
	hamra	334	9 (2.7)	337	108 (32.0)	315	67 (21.3)	316	63 (19.9)	157	16 (10.2)
	sefra	252	3 (1.2)	252	86 (34.1)	248	58 (23.4)	248	55 (22.2)	132	12 (9.1)
	zerga	223	9 (4.0)	230	99 (43.0)*	212	72 (34.0)	211	66 (31.3)	133	17 (12.8)

* = Significant difference.

their 95% confidence interval are shown in Table 5.

The indices of negative agreement are invariably higher than those of positive agreement. TL and ELISA/VSG RoTat 1.2 showed the highest concordance with the highest index of negative agreement (0.96, 95% CI: 0.95–0.97) followed by GST and RoTat 1.2 PCR (0.95, CI: 0.93–0.96). The indices of negative agreement between GST and TL, GST and ELISA/VSG RoTat 1.2, and CATT/T. *evansi* and TL were 0.89, 0.88, and 0.87 respectively. The lowest negative index of agreement was observed between CATT/T. *evansi* and RoTat 1.2 PCR (0.57). TL and ELISA/VSG

Table 5

Contingency table and degree of agreement, with 95% confidence interval, between the different diagnostic tests using indices of positive and negative agreement (Ppos, Pneg). CATT = CATT/T. *evansi*, ELISA = ELISA/VGS RoTat 1.2, TL = immune trypanolysis, PCR = RoTat 1.2 PCR.

		CATT		ELISA		TL		PCR	
		Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg
GST	Pos	20	5	19	2	18	3	16	9
	Neg	314	707	213	772	193	792	44	457
	Total	334	712	232	774	211	795	60	466
	Ppos	0.11		0.15		0.16		0.38	
	Pneg	(0.07–0.16)		(0.09–0.21)		(0.10–0.22)		(0.23–0.51)	
CATT	Pos			183	132	173	142	41	275
	Neg			49	642	38	653	19	201
	Total			232	774	211	795	60	476
	Ppos			0.67		0.66		0.22	
	Pneg			(0.62–0.71)		(0.61–0.70)		(0.16–0.28)	
ELISA	Pos					191	41	42	170
	Neg					20	753	15	263
	Total					211	794	57	433
	Ppos					0.86		0.31	
	Pneg					(0.82–0.89)		(0.24–0.38)	
TL	Pos							42	159
	Neg							15	275
	Total							57	434
	Ppos							0.33	
	Pneg							(0.25–0.40)	

RoTat 1.2 showed the highest index of positive agreement (0.86), while the lowest was seen between GST and CATT/T. *evansi* (0.11).

3.6. Packed cell volume

Table 6 shows the average PCV values and standard deviations (SD) according to the status of the camels in the GST, CATT/T. *evansi*, TL, ELISA/VGS RoTat 1.2, and RoTat 1.2 PCR. For all tests except the RoTat 1.2 PCR, positive animals had a significantly lower average PCV when compared to negative camels. It is to be noted that 13% of all camels were anaemic (PCV <24%).

4. Discussion

This study was undertaken to investigate the epidemiological situation of *T. evansi* infection in dromedary camels in the South of Algeria by means of parasitological, serological and molecular diagnostic tests, including those recommended by the OIE.

The overall prevalence was found to be 2.4% with GST. This is equivalent to the parasitological prevalence estimated by Boushaki (2007) in the same region with the same technique, but is much lower than molecular prevalence (11.2%). This difference is not unexpected given the low sensitivity of the blood smear with a lower detection limit of 10⁵ trypanosomes/ml (Desquesnes et al., 2013a). More parasitologically positive animals could have been detected if more sensitive techniques like the hematocrit centrifugation technique (Woo, 1969) or the dark ground buffy coat examination (Murray et al., 1977) or mouse inoculation would have been used, which was not possible under the field conditions in all the wilaya studied. Therefore, the zero parasitological prevalence observed in Tamanrasset does not necessarily indicate the absence of surra in that wilaya since 7% of the 360 examined

Table 6

Average packed cell volume values (in %) ± the standard deviation (SD) according to the animal status in the different diagnostic tests.

Test	negative	positive	P
GST	27.3 ± 4.3	22.2 ± 2.7	<0.001
CATT/T. <i>evansi</i>	28.1 ± 3.5	25.0 ± 5.3	<0.001
ELISA/VSG RoTat 1.2	27.7 ± 3.7	25.0 ± 4.9	<0.001
TL	27.7 ± 4.0	24.6 ± 3.7	<0.001
RoTat 1.2 PCR	26.2 ± 5.1	25.2 ± 6.9	0.202

camels were positive in RoTat 1.2 PCR and in a previous survey parasitologically positive animals were identified with GST (Boushaki, 2007).

On the other hand, overall seroprevalence estimates obtained with CATT/*T. evansi* (32.4%), ELISA/VSG RoTat 1.2 (23.1%), and immune trypanolysis test (21%) were much higher than parasitological and molecular prevalence estimates. This can be explained by the fact that animals with chronic infections may remain false negative in both parasitological and molecular tests when parasite loads are very low. Also, it is known that after successful cure, antibodies can remain in the circulation for several months, thus leading to serologically false positive animals in herds that are treated with trypanocides (OIE, 2018; Verloo et al., 2001). Indeed, one third of the examined camels have a history of treatment with drugs containing diminazene diaceturate (Fa.Try.Banil®, Fa.Try.Banil R.T.U.® FATRO, Italy), the only molecule with trypanocidal activity that has been authorised for over 19 years on the national Algerian market to treat domestic animals but not dromedary camel. So, it is probable that of the 151 TL positive animals that were negative in RoTat 1.2 PCR, some were cured after recent trypanocidal treatment. Yet, three camels with positive GST and RoTat 1.2 PCR were negative in TL. It may be that those animals carried recent infections and that RoTat 1.2 specific antibodies were not yet detectable (Verloo et al., 2001).

On 133 animals, of which three were positive in GST, we were able to run *T. evansi* type B specific EVAB PCR. None of them were positive. Thus, so far we have no evidence that *T. evansi* type B, that has been isolated only in Eastern Africa, has already spread towards the West (Birhanu et al., 2015; Ngaira et al., 2005; Njiru et al., 2006; Salim et al., 2011).

The overall seroprevalence was higher than in Morocco, where, among 1460 dromedaries examined with CATT/*T. evansi* and ELISA/VSG RoTat 1.2, respectively 14.1% and 18.2% were positive (Atarhouch et al., 2003). Similarly, in Mauritania, out of 254 dromedary camels examined, 14.2% were positive with CATT/*T. evansi* (Dia et al., 2011).

Our results demonstrate that all wilayate in the study area are affected by *T. evansi*. Tamanrasset seems the least affected. Noteworthy is that in Tamanrasset, only 8 of the 25 interviewed farmers knew the disease. On the other hand, El Bayadh, which was previously free of disease (Boushaki, 2007; Marfoua, 1999), has experienced an outbreak during this investigation with high frequencies of acute cases. The outbreak is reflected by the high number of deaths (59 cases), high observed parasitological prevalence (12%) and very high serological prevalence in CATT/*T. evansi* (75%), ELISA/VSG RoTat 1.2 (70%) and TL (62%). This epidemic episode originated probably from the introduction of *T. evansi* with transhumant dromedaries from Béchar, a surra endemic wilaya bordering El Bayadh (Boushaki, 2007). In Béchar, repeated abortions over the years associated with considerable seroprevalence (30.6–36.8%) and a molecular prevalence of 13.6% are probably related to all forms and stages of the disease, from recent infection to subclinical and chronic complications. Indeed, mortality is frequent during the first two to three years of disease evolution. Some live up to four years with a subclinical infection and some resist and eventually heal themselves (Dia et al., 1997a; Röttcher et al., 1987). It should be mentioned that in 94% of the investigated farms in El Bayadh and in 63% of the farms in Béchar, treatment with Fa.Try.Banil R.T.U.® is applied although diminazene diaceturate is toxic to camels and can be fatal if the dose is higher than 3.5 mg/kg (Cuisance et al., 2001; Homeida et al., 1981 in Röttcher et al., 1987). In none of the farms in Ouargla and Tamanrasset, treatment of camels with trypanocides is recorded.

Sex was found to be a risk factor associated with trypanosomosis in our study population when using serological tests only. With TL, females are five times more likely to be infected than males. This finding is in agreement with Dia et al. (1997b) but in contradiction with Boushaki (2007) and Njiru et al. (2004) where no difference was found in seroprevalence between males and females. Rather than a real biological predisposition, differences in husbandry practices applied to male and female animals should be considered as an explanatory factor (Antoine-Moussiaux and Desmecht, 2008). Seroprevalences estimated with TL and ELISA/RoTat 1.2 were also significantly higher in adult camels as

compared to calves under one year old. This is in agreement with previous studies (Atarhouch et al., 2003; Dia et al., 1997c; Gutierrez et al., 2000; Ndoutamia et al., 1999) and is probably related to the chronic nature of the infection, the increasing exposure to re-infection over time, and the persistence of antibodies after curative treatment. The finding of a 20 days old calf that was positive for TL and CATT/*T. evansi* can be explained by the *in utero* passage of maternal antibodies. Vertical transmission of the parasite may be an alternative cause although usually this leads to stillbirth (Röttcher et al., 1987). Within the group of young animals aged one year, 4 of the 121 were found with trypanosomes in the blood, as was also reported by Dia et al. (1997c) but is in contrast to observations of Diall et al. (1993) and Jacquet et al. (1994).

The breeding system in the studied wilayate is dominated by transhumance, which is a system for the rational exploitation of vegetation and water for the camel. Transhumance may favour transmission by dry season migrations to areas with bioclimatic conditions favorable to the survival of vectors.

In camels, the PCV threshold or the minimum physiologically acceptable PCV value is 24% (Dioli and Stimmelmayer, 1992). The high proportion (13%) of animals in our study cohort that had anaemia is probably related to *T. evansi* infection, as was observed in other studies (Birhanu et al., 2015; Boushaki, 2007; Fikru et al., 2015; Gutierrez et al., 2005). Indeed, trypanosomosis is an anaemic infection associated with intravascular hemolysis (Chaudhary and Iqbal, 2000; Gutierrez et al., 2005). In our study, we found a significantly lower PCV in test positive than in test negative animals for all diagnostic tests, except for RoTat 1.2 PCR, which is in contrast to what was observed by Birhanu et al. (2015) and Fikru et al. (2015) who reported also lower PCV values in PCR positive camels. Taking into account that anaemia may be caused by other conditions and infections, PCV has only a limited additional diagnostic value when it is applied together with serological tests.

Regarding these serological tests, we observed high positive (0.86) and negative (0.96) indices for TL and ELISA/VSG RoTat 1.2. For TL and CATT/*T. evansi* the positive (0.67) and negative (0.88) indices were somewhat lower. Yet, TL is a reference test and is only performed in the OIE Reference Laboratory for Surra in Antwerp, Belgium (Birhanu et al., 2015; Fikru et al., 2015; Holland et al., 2002; Tehseen et al., 2015; Verloo et al., 2000). For testing in the field or in endemic country laboratories, less demanding serological tests like CATT/*T. evansi* and ELISA/VSG RoTat 1.2 are available. With TL as gold standard for detection of RoTat 1.2 specific antibodies (100% sensitivity and specificity), the relative sensitivity and specificity of ELISA/VSG RoTat 1.2 with 30% PP cut-off were respectively 91% and 95%, while the relative sensitivity and specificity of CATT/*T. evansi* were 82%.

In the light of the results obtained, the need to implement a control strategy is obvious. Veterinary laboratories should be provided with accurate diagnostic tools in order to detect infected animals that represent potential reservoirs at an early stage. Especially during the seasons with abundant vectors, a reduction of the impact of trypanosomiasis in an endemic area may be obtained by implementing an integrated prophylactic plan based on insect trapping and treatment of all serologically positive dromedaries with an effective trypanocide such as melarsomine (Cymelarsan), irrespective of whether they are actually infected or not. Such a control plan was implemented in Morocco as part of a study programme that proved the utility of Cymelarsan to reduce surra prevalence in the pilot region (Rami et al., 2003). Care should be taken however to monitor the appearance of drug resistance when only one type of drug is used over a longer period (Luckins, 2000). Indeed, resistance of *T. evansi* strains to drugs like suramin and antrycide (pyrimethamine) has been reported in different countries in Africa and Asia (El Rayah et al., 1999; Zhou et al., 2004).

5. Conclusion

Our study confirms the high prevalence of surra, caused by *T. evansi* type A, in the dromedary population in South Algeria and its potential to

spread into previously non-endemic areas like El Bayadh. There is an obvious need to implement control measures, including diagnosis, treatment and vector control, in order to reduce the incidence of the disease, not only in the study area but all over the country.

Declarations

Author contribution statement

Djamila Boushaki: Conceived and designed the experiments; Performed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Amel Adel: Analyzed and interpreted the data; Wrote the paper.

Mamadou Lamine Dia, Nadia Kechemir Issad: Conceived and designed the experiments.

Philippe Büscher: Performed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Hafsa Madani: Performed the experiments; Contributed reagents, materials, analysis tools or data.

Brahim Aymard Brihoum, Hassiba Sadaoui, Nadera Bouayed: Performed the experiments.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

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