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Research paper

Comparison of serological and molecular tests for detection of *Trypanosoma evansi* in domestic animals from Ghardaïa district, South Algeria

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ABSTRACT

Trypanosoma evansi (*T. evansi*) is a hemoflagellate parasite that affects a broad range of mammalian hosts and that causes a disease called surra. Diagnosis of surra based on clinical symptoms alone is inaccurate. Therefore, a variety of serological and molecular diagnostic tests are used to assist in the detection of *T. evansi* infections.

The aim of this study was to compare the diagnostic performance of four serological tests (CATT/*T.evansi*, immune trypanolysis, ELISA with purified variant surface glycoprotein RoTat 1.2 and with whole cell lysate) and two molecular PCR tests targeting sequences within the ribosomal genes locus (ITS1 TD PCR and 18S qPCR). Tests were carried out on blood samples from 161 dromedary camels, 93 horses, 129 goats, 168 sheep, 127 bovines and 76 dogs. Latent class analysis was carried out to calculate the sensitivity and specificity of each diagnostic test. Cohen's Kappa test was used to assess the concordance between the different diagnostic tests.

Overall positivity rates observed with the serological tests were as follows: 3.1 % with CATT/*T.evansi*, 4.9 % with ELISA/RoTat 1.2, 3.4 % with ELISA/whole lysate and 2.0 % with immune trypanolysis (TL). Among the 754 samples tested with the molecular tests, 1.7 % were positive with 18S qPCR and 1.3 % with ITS1 TD PCR. Cohen's Kappa test showed agreement ranging from fair to substantial (k = 0.2-0.8) between serological diagnostic tests. However, it showed a perfect agreement (k = 0.868) between molecular diagnostic tests. Latent class analysis showed that all serological tests were 100 % sensitive, in contrast to the molecular tests with 47 % sensitivity. All tests, though, were highly specific (≥ 97 %).

Given the persistence of circulating antibodies after cure, detectable by serological tests, it is recommend combining a serological and a molecular diagnostic test for accurate diagnosis of infection with *T. evansi* in domestic animals.

1. Introduction

Surra is a trypanosomosis due to *Trypanosoma (T.) evansi*, the first ever pathogenic trypanosome described in horses and dromedaries from India (Hoare, 1972). *Trypanosoma evansi* is a flagellated protozoan parasite transmitted mechanically by different fly species like *Tabanus* sp. and *Stomoxys* sp. (Luckins, 1988; Brun et al., 1998) and in South America also by vampire bats (Hoare, 1972). *Trypanosoma evansi* affects a huge range of domestic and wild mammals around the world and especially camels and horses in Africa (Dia et al., 1997; Njiru et al., 2004; Gari et al., 2010; Birhanu et al., 2015; Fikru et al., 2015); camels, horses, water buffalo and cattle in Asia (Abo-Shehada et al., 1999; Verloo et al., 2000; Hasan et al., 2006; Elshafie et al., 2013; Tehseen et al., 2015; Alanazi et al., 2018; Yadav et al., 2019) and horses, cattle and dogs in South America (Herrera et al., 2004; Jaimes-Dueñez et al., 2017; Ramírez-Iglesias et al., 2017). Sporadically, the parasite has caused outbreaks in Europe by importation of infected animals from endemic countries as was recently the case in Spain and France (Gutierrez et al., 2006; Desquesnes et al., 2009; Tamarit et al., 2010).

Clinical signs differ from one host species to another and vary from unapparent to lethal. In camels, surra causes intermittent fever, weakness, abortion and oedema. It is sometimes fatal within a few months

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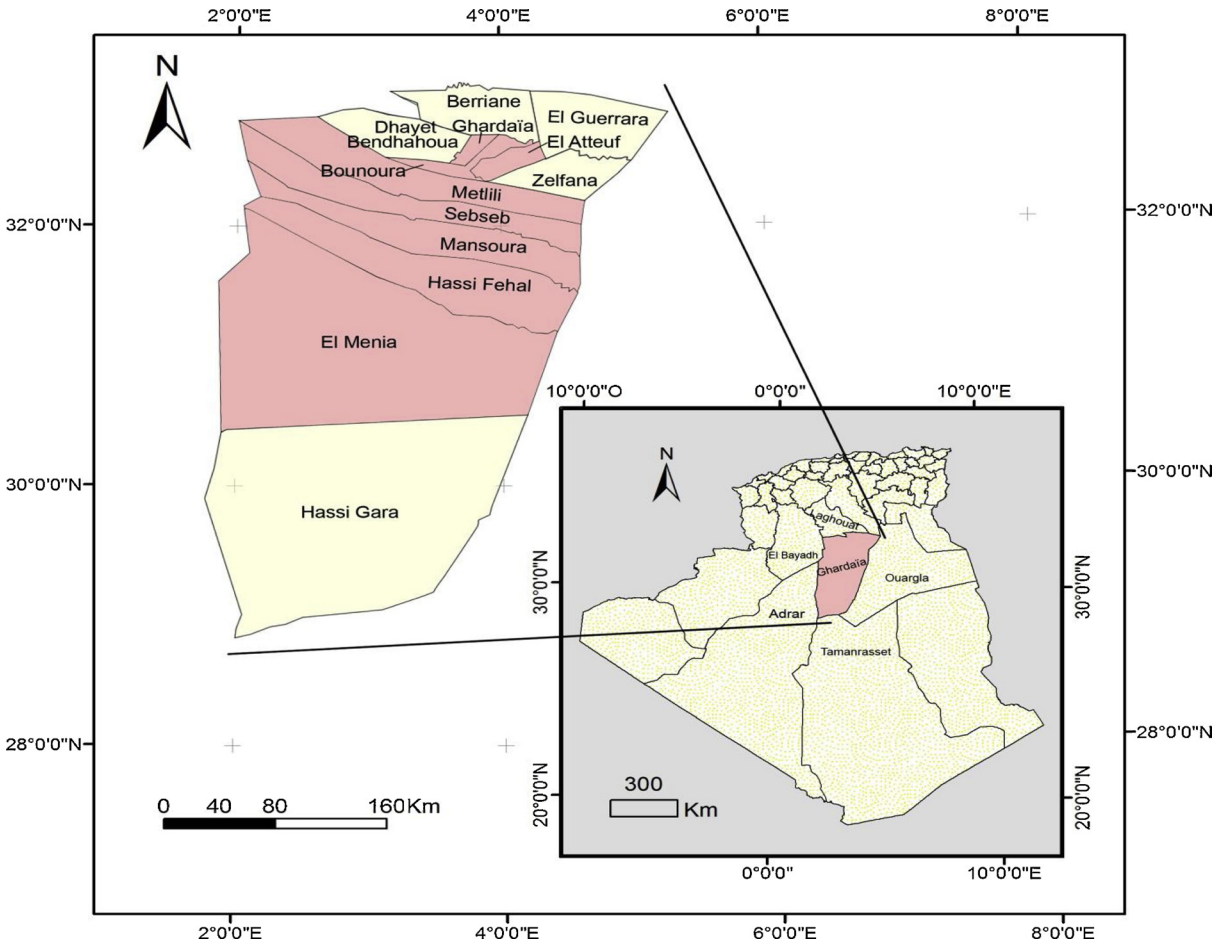

Fig. 1. Map of Algeria showing the wilaya of Ghardaïa and localisation of sampling sites.

but more chronic evolution lasting 2 to 3 years is demonstrated (Singh and Momin, 2008). Infection in horses is characterised by anaemia, icterus, weight loss and neurological signs with a mortality rate of about 50 % (Rodrigues et al., 2009). In cattle and water buffaloes, surra is usually a chronic disease but clinical signs occur like abortion, weight loss and neurological disorders (Luckins, 1988). Sheep and goats are mainly asymptomatic (Desquesnes et al., 2013). In dogs, *T. evansi* causes a serious disease, often accompanied with blindness, and frequent fatal outcome (Echeverria et al., 2019).

Clinical signs are not pathognomonic and diagnosis can only be confirmed by laboratory tests. In routine practice, parasitological examination is usually limited to microscopic observation of a Giemsa stained thin blood smear or thick drop which are poorly sensitive in chronic infections with low numbers of parasites circulating in peripheral blood. Serological antibody detection tests on the other hand, have been proven to be very useful, especially for epidemiological surveys on surra (Atarhouch et al., 2003). Recommended serological techniques listed in the Terrestrial Manual of the World Organisation for Animal Health (OIE) are: ELISA with native *T. evansi* variant surface glycoprotein (VSG) RoTat 1.2 as antigen (ELISA/RoTat1.2), ELISA with whole *T. evansi* cell lysate as antigen (ELISA/WCL), the Card Agglutination Test for *T. evansi* (CATT/*T.evansi*), and an antibody-mediated complement lysis test (immune trypanolysis or TL) with *T. evansi* variable antigen type (VAT) RoTat 1.2 (OIE, 2018). Major disadvantages of serological tests are cross-reactivity with non-specific antibodies caused by other infections and the persistence of specific antibodies for weeks or months after successful treatment. As surrogate for parasitological diagnosis, molecular tests detect parasite-specific DNA or RNA and therefore are very specific. Moreover, DNA and RNA disappear within days after successful treatment and RNA presence indicates active infection. Molecular tests for the diagnosis of surra are either specific for the subgenus Trypanozoon, to which *T. evansi* belongs together with *T. brucei* and *T. equiperdum* or specific for a certain *T. evansi* subtype (Claes et al., 2004; Njiru et al., 2006; Carnes et al., 2015). The OIE Terrestrial Manual recommends TBR PCR which targets a 177 bp repetitive microsatellite sequence present in Trypanozoon (Masiga et al., 1992; OIE, 2008). The high copy number of the 177 bp repeats renders the TBR PCR very sensitive but makes is also very prone to contamination during sampling, especially in large scale surveys (unpublished observations). Alternative tests for Trypanozoon detection, such as 18S PCR targeting a ribosomal gene and ITS1 PCR targeting the internal transcribed spacer 1 within the ribosomal locus have been described (Desquesnes et al., 2001). The former is Trypanozoon specific while the latter has the advantage that it detects the ITS1 in the genome of *T. congolense, T. vivax*, and Trypanozoon subgenus (including *T. brucei, T. evansi, T. equiperdum*). To replace the conventional 18S PCR, Deborggraeve and co-workers (2011) developed a quantitative real time PCR targeting the 18S gene (18S qPCR). Recently, a touchdown variant of the ITS1 PCR (ITS1 TD PCR) was developed to reduce non-specific reactions often observed with specimens from cattle (Tran et al., 2014). It detects the ITS1 in the genome of *T. congolense, T. vivax*, and Trypanozoon subgenus (including *T. brucei, T. evansi, T. equiperdum*).

The aims of this study were: i) to determine the prevalence of *T. evansi* in dromedary camels, ruminants and dogs, with particular interest in detection of subclinical infections and putative reservoir hosts, ii) to compare the accuracy and to assess the concordance between six diagnostic methods used for diagnosis of *T. evansi*: TL, ELISA/RoTat 1.2,

ELISA/WCL, CATT/*T.evansi*, 18S quantitative PCR and ITS1 touchdown PCR.

2. Materials and methods

2.1. Study area

The study was carried out from May 2017 to February 2018 in the wilaya of Ghardaïa (Fig. 1). Ghardaïa is located in the north of the Algerian Sahara, about 600 km from the capital Algiers, between 33° and 31°15′ N, 2°30′ and 5 °E. The climate is arid with very low rainfall (160 mm/year), very high summer temperatures (20 °C–45 °C) and low winter temperatures. The wilaya of Ghardaïa covers a total area of 86,560 km^2 (4 % of the Algerian territory). The pastoral area of Ghardaia covers an area of 1,344,303 ha (15 % of the total area of the wilaya). It consists of: oued, daya, hamada, reg and erg and contains floristic diversity with *Anabasis articulata*, *Helianthemum lippii*, *Calligonum comosum* and *Stipagrostis pungens*.

2.2. Sampling procedure

A cross sectional survey was conducted on 161 dromedary camels, 93 horses, 127 cattle, 168 sheep, 129 goats, and 76 dogs. All animals were clinically healthy at the time of blood collection, but some had a history of clinical signs. Blood samples were collected from the jugular vein into plain vacuum tubes and EDTA tubes. Sera were prepared from blood in plain tubes after centrifugation. Serum and blood samples were stored at -20 °C until transported to the Institute of Tropical Medicine, Antwerp, Belgium.

2.3. Diagnostics tests

The CATT/*T.evansi* (Institute of Tropical Medicine, Antwerp, Belgium) is a direct agglutination test for detection of specific antibodies in blood, plasma and sera. It was used according to the manufacturer's instructions. Briefly, 25 µl of serum, diluted 1:4 in phosphate buffered saline, and 45 µl of CATT antigen are mixed in a reaction zone of a plastic card. The card is placed on an electric rotator and rocked at 70 rpm for 5 min. The reaction is scored positive if blue agglutinates are visible by naked eye.

Immune trypanolysis (TL) is an antibody-mediated complement lysis test with a cloned *T. evansi* population expressing the RoTat 1.2 VSG. It is highly specific and therefore used as serological reference test for *T. evansi* type A (OIE, 2018). It was performed according to Van Meirvenne et al. (1995). Briefly, 25 µl of serum is diluted 1:2 in guinea pig serum (GPS, complement source) in a well of a microtiter plate. Thereafter 50 µl of a 10^{-7}/ml suspension of live *T. evansi* RoTat 1.2 trypanosomes in GPS are added. The microtiter plate is shaken for a few seconds and kept at ambient temperature. After 90 min, 5 µl of the reaction mixture is dispensed on a microscope slide, covered with a cover slip and examined under a phase contrast microscope at 10 × 25 magnification. The test result is considered positive for anti-RoTat 1.2 antibodies when at least 50 % of the trypanosomes are lysed.

Indirect ELISA/RoTat 1.2 was carried out according to Verloo et al. (2000) and Lejon et al. (2005) and with minor modifications. VSG RoTat 1.2 was coated at 2 µg/ml in PBS (0.01 M; pH 7.4; 0.14 M NaCl). Half of the plate was left empty and served as antigen-free control. Dog, camel and horse sera were diluted 1:150 in PBS-Blotto (0.01 M; pH 7.4; 0.2 M NaCl; 8 mM NaN_3; 10 g/l skimmed milk powder) while cattle, goat and sheep sera were diluted 1:200 in the same buffer. Hundred fifty µl of each serum were applied in duplicate in an antigen-containing and an antigen-free well and incubated at ambient temperature for 30 min. After washing, horse radish peroxidase HPO conjugates were added in each well. For camel: protein A-HPO Sigma, P8651, 1:10,000, horse: goat anti horse IgG H + L-HPO Jackson Immunoresearch 108-035-003, 1:40,000, for dog: rabbit anti dog IgG H + L-HPO Jakson Immunoresearch 304-035-003, 1:40,000, for sheep and goat: donkey anti goat IgG H + L-HPO Jackson Immunoresearch 705-035-003, 1:40,000, for cattle: goat anti bovine IgG H + L-HPO Jackson Immunoresearch 101-035-003, 1:40,000. Reactions were revealed with 1-Step Ultra TMB ELISA Substrate, Thermo Scientific 34029 and stopped by adding 150 µl of 2 M H_2SO_4. The optical density (OD) was read at 450 nm with a Multiskan RC ELISA reader. The corrected optical density (OD_{corr}) of each serum was calculated by subtracting the mean OD in the two antigen-free wells from the mean OD in the two corresponding antigen containing wells. These corrected OD are expressed as percentage of the OD obtained with the strong positive control (percent positivity, PP). Indirect ELISA/WCL was carried out as described above but with a whole cell lysate coated at 2 µg/ml in PBS (0.01 M; pH 7.4; 0.14 M NaCl) (OIE, 2017). The WCL was prepared as follows: *T. evansi* RoTat 1.2 is grown in rats and separated from the rat blood by anion exchange chromatography on a DEAE cellulose column according to (Lanham and Godfrey, 1970). A 1 ml sediment of pure trypanosomes is lysed in ice cold hypotonic phosphate buffer (10 mM) and sonicated on ice. After cold centrifugation at 14,000 x g for 10 min and 17,000 x g for 10 min, the supernatant (WCL) is collected and protein content measured by UV absorption. This WCL is divided in small aliquots and stored at −80 °C prior to use.

As template in the molecular tests, DNA was extracted from 400 µl whole blood of each animal sample with the Maxwell® 16 DNA purification kit, according to the instructions of the manufacturer (Maxwell 16 DNA purification kit, Promega). DNA was stored at −80 °C until amplification.

ITS1 TD PCR was used to detect to allow simultaneous detection of all non-tsetse transmitted trypanosomes (*T. evansi, T. equiperdum* and *T. vivax*). Specifications of the PCR are represented in Table 1. Amplifications were carried out in 50 µl final volume containing 5 µl of purified DNA and 45 µl of the master mix (800 µM of dNTPs, 0.2 µM of each primer (ITS-1-Forward, ITS-1-Reverse), 1 unit of HotStar Taq Plus DNA polymerase (Qiagen), 1× Coral Load PCR buffer). Each PCR run included a non-template negative control (5 µl nuclease free water), a positive control (0,5 ng of each parasite DNA) and controls of DNA extractions. The reaction conditions were as follows: denaturation at 95° for 5 min, three cycles at 94 °C for 30 s, 70 °C for 30 s, 72 °C for 30 s; three cycles at 94 °C for 30 s, 69 °C for 30 s, 72 °C for 30 s; three cycles at 94 °C for 30 s, 68 °C for 30 s, 72 °C for 30 s; three cycles at 94 °C for 30 s,

Table 1
Taxon specificity, target sequence, primers and amplicon lengths for the 18S qPCR, the ITS1 TD PCR and the EVAB PCR.

Target group	Sequence	Primers/Probe	Primers/probe sequences	Amplicon length	Reference
Trypanozoon	18S	M18SF	5′- CGTAGTTGAACTGTGGGCCACGT -3′	150 bp	(Deborggraeve et al., 2011)
		M18SR	5′- ATGCATGACATGCGTGAAAGTGAG -3′		
		M18S probe	5′- TCGGACGTGTTTTGACCCACGC-MGB-VIC-3′		(Wagemans, 2011)
Trypanosoma sp	ITS1	ITS1 F	5′-TGTAGGTGAACCTGCAGCTGGATC-3′	*T. vivax*:150 bp,	(Tran et al., 2014)
		ITS1 R	5′-CCAAGTCATCCATCGCGACACGTT- 3′	Trypanozoon: 450 bp, *T. congolense*: 650 bp	
Trypanosoma evansi type B	minicircle class B	EVAB1	5′-CACAGTCCGAGAGATAGAG-3′	436 bp	(Njiru et al., 2006)
		EVAB2	5′- CTGTACTCTACATCTACCTC-3′		

67 °C for 30 s, 72 °C for 30 s; three cycles at 94 °C for 30 s, 66 °C for 30 s, 72 °C for 30 s; three cycles at 94 °C for 30 s, 65 °C for 30 s, 72 °C for 30 s; 22 cycles at 94 °C for 30 s, 64 °C for 30 s, 72 °C for 30 s and a final extension step at 72 °C for 5 min. PCR assays were performed in a Biometra T3000 cycler (Germany). PCR products were analysed in UV illumination after electrophoresis in 2 % agarose gel and staining with ethidium bromide.

For specific detection of Trypanozoon taxa, the 18S qPCR assay used the M18S primers described by Deborggraeve et al. (2011) in combination with a novel probe predicted by Primer3 (Table 1). The reactions were conducted in a final volume of 20 µL, consisting of 5 µL of DNA template, 0.3 µM of each primer, 0.1 µM of a MGB-VIC labelled probe (Thermo Fischer Scientific) in Perfecta® qPCR UNG Low-Rox ToughMix® Quantabio qPCR was performed on a Quantstudio 5 (Applied Biosystems) as follows: 50 °C for 2 min, followed by 35 cycles of denaturation at 95 °C for 15 s and annealing and detection at 60 °C for 60 s. The cycle quantification values were calculated automatically and compared between runs using a *T. brucei* positive control.

To assess whether *T. evansi* type B also circulates in Algeria, the minicircle type B PCR, which is specific for *T. evansi* type B (Njiru et al., 2006), was performed exactly as described in Birhanu et al. (2016).

Statistical analyses were performed using R (version 3.5.1, R Foundation for Statistical computing, Vienna, Austria) via RStudio (version 1.1.383, RStudio Inc., Boston, MA). Results for the six diagnostic tests were analysed to establish associations between all possible combinations of test pairs. Cohen's kappa coefficients (Cohen, 1960) were used as a measure of concordance between each pair of tests using irr package in R (Gamer et al., 2012). Interpretation of the kappa coefficient was according to Landis and Koch (Landis and Koch, 1977) poor agreement: < 0.00; slight: 0.00–0.20; fair: 0.21–0.40; moderate: 0.41–0.60; substantial: 0.61–0.80; almost perfect: 0.81–1.00. By absence of a gold standard test for diagnosis of *T. evansi* infection, sensitivity and specificity of the different tests was assessed using the latent class analysis (LCA) via random LCA package in R (Beath, 2017). Latent class analysis assumes that the results obtained by imperfect tests are influenced by a common latent variable, which is the real infection status not directly measurable by each test separately.

3. Results

3.1. Prevalence

The number of positive samples, according to the various diagnostic methods and host species, are shown in Table 2. Regarding the serological tests, the highest proportion of positive animals was recorded with ELISA/RoTat 1.2, ranging from 0.8 % in goat to 9.9 % in camels and overall positivity rate of 4.9 %. With CATT/*T.evansi*, the overall positivity rate was 3.1 % ranging from 0.6 % in sheep to 9.3 % in camels. With ELISA/WCL, the overall positivity rate was 3.4 % ranging from 0% in horses to 9.3 % in camels. The lowest overall positivity rate (2.0 %) was observed with TL and positive animals (9.3 %) were only observed within the camel group.

Among the 754 tested samples, the 18S qPCR detected 12 positive camels and 1 positive goat while the ITS1 TD PCR detected only 10 positive camels which were all positive for Trypanozoon and not for *T. vivax* or *T. congolense*. *Trypanosoma evansi* type B specific PCR remained negative for all tested animals, i.e. all dromedary camels and the 18S qPCR positive goat.

Table 3
Specificity and sensitivity (in percentage) with 95 % confidence intervals (CI) of each test based on Latent Class Analysis.

	Sensitivity (95 % CI)	Specificity (95 % CI)
Immune trypanolysis	100 (100-100)	100 (100-100)
ELISA/RoTat 1.2	100 (100-100)	97 (95–98)
ELISA/WCL	100 (100-100)	99 (97–99)
CATT/*T.evansi*	100 (100-100)	99 (95–100)
18S qPCR	47 (21–73)	98 (96–99)
ITS1 TD PCR	47 (22–75)	100 (87–100)

3.2. Sensitivity and specificity of the diagnostic tests, assessed by latent class analysis

As shown in Table 3, all serological tests were found to be 100 % sensitive while both molecular tests were 47 % sensitive. Specificities ranged from 97 % to 100 %.

3.3. Concordance between the different diagnostic tests

The data on agreement between the six different tests are represented in Table 4.

Among the serological tests, substantial agreement was observed between TL on the one hand and CATT/*T.evansi* and ELISA/WCL on the other hand while all other combinations showed moderate agreements with k between 0.515 and 0.599. In contrast, almost perfect agreement (k = 0.868) was observed between the two molecular tests. The agreements between the serological tests and molecular test were fair to moderate.

4. Discussion

Our study confirms that *T. evansi* is present in Algeria particularly in dromedary camels, as observed in other studies carried out elsewhere in this country (Bennoune et al., 2013; Boushaki et al., 2019). Depending on the diagnostic test, prevalences ranged from 6.2 %–13 % which is much lower than what is reported from some other countries. For example, observed prevalences of *T. evansi* in dromedaries in Egypt were 31 % and 71 % in respectively RoTat 1.2 PCR and TBR PCR (Elhaig and Sallam, 2018), 26.6 % using Trypanozoon-specific and *T. evansi* minicircle-specific primer sets in Kenya (Njiru et al., 2004), 30 % and 32 % with respectively RoTat 1.2 PCR and TBR1/2 PCR in Pakistan (Tehseen et al., 2015). Also with serological tests, extremely high prevalences were recorded in Egyptian camels: 82 % with CATT/*T.evansi* and 64 % with ELISA/*T.evansi* (Zayed et al., 2010). In Mauritania and Ethiopia, seroprevalences obtained in CATT/*T.evansi* were between 14 % and 24 % (Dia et al., 1997; Birhanu et al., 2015; Fikru et al., 2015). The relatively low prevalences in our study, together with the apparent health

Table 2
Number and percentage of positive results obtained in each test for each host species.

Host	Number	TL	ELISA/RoTat 1.2	ELISA/WCL	CATT/*T.evansi*	18S qPCR	ITS1 TD PCR
Camel	161	15 (9.3 %)	16 (9.9 %)	15 (9.3 %)	15 (9.3 %)	12 (13 %)	10 (6.2 %)
Horse	93	0	1 (1%)	0	3 (3.2 %)	0	0
Goat	129	0	1 (0.8 %)	4 (3.1 %)	2 (1.5 %)	1 (0.8 %)	0
Sheep	168	0	11 (6.5 %)	4 (2.4 %)	1 (0.6 %)	0	0
Cattle	127	0	4 (3.1 %)	2 (1.6 %)	1 (0.8 %)	0	0
Dog	76	0	4 (5.3 %)	1 (1.3 %)	1 (1.3 %)	0	0
Total	754	15 (2.0 %)	37 (4.9 %)	26 (3.4 %)	23 (3.1 %)	13 (1.7 %)	10 (1.3 %)

Table 4
Agreement between the different diagnostic tests to detect *T. evansi*. k = kappa (< 0 = poor, 0–0.2 = slight, 0.21–0.4 = fair, 0.41–0.6 = moderate, 0.61–0.8 = substantial, 0.81–1 = almost perfect); P = positive; N = negative.

		ELISA/RoTat 1.2		ELISA/WCL		CATT/*T.evansi*		18S qPCR		ITS1 TD PCR	
		P	N	P	N	P	N	P	N	P	N
Immune trypanolysis	P	15	0	15	0	15	0	7	8	7	8
	N	22	717	11	728	8	731	6	733	3	736
	K	0.565		0.725		0.784		0.491		0.553	
ELISA/RoTat 1.2	P			18	19	16	21	7	30	7	30
	N			8	709	7	710	6	711	3	714
	K			0.553		0.515		0.261		0.283	
ELISA/WCL	P					15	11	7	19	7	19
	N					8	720	6	722	3	714
	K					0.599		0.344		0.377	
CATT/*T.evansi*	P							7	16	7	16
	N							6	725	3	728
	K							0.375		0.413	
18S qPCR	P									10	3
	N									0	741
	K									0.868	

of the sampled dromedaries, suggest that *T. evansi* is endemic in Ghardaïa district.

All horses were negative in the molecular tests, TL and ELISA/WCL. The few animals that were positive in ELISA/RoTat 1.2 and CATT/*T.evansi* were not confirmed in TL and therefore may be considered as false positives. This result is in sharp contrast with the prevalence of 45 % observed in a previous study conducted in El-Bayadh district in south-western Algeria using CATT/*T.evansi* (Benfodil et al., 2019). In the same El-Bayadh district, a high mortality rate was recorded in dromedaries (Boushaki et al., 2019) suggesting an ongoing epidemy of surra that also may have affected horses. In a survey conducted in northern Ethiopia, 7 of 25 horses were positive in PCR RoTat 1.2 but also none were positive in TL thus casting doubt on the specificity of the molecular test (Birhanu et al., 2015). *Trypanosoma evansi* not only affects horses in Africa but also in other continents, for example in Asia where 13 % of horses tested in Malaysia were found positive in CATT/*T.evansi* (Elshafie et al., 2013) and 27 % of horses in India were positive in ELISA/*T.evansi* (Laha and Sasmal, 2008). In Brazil, Herrera and coworkers recorded even 73 % (234/321) IFAT positive horses while 10 % were positive in the microhaematocrit centrifugation technique (Herrera et al., 2004).

Since we were interested in putative reservoirs of *T. evansi* in the study area, we also tested ruminants and dogs. None of the goats, sheep, cattle and dogs were positive in TL and both molecular tests, with the exception of 1 goat that was positive in 18S qPCR. Seroprevalences in the other serological tests ranged from 0.6 % of the sheep in CATT up to 6.5 % of the sheep in ELISA/RoTat 1.2. Again, the negative TL and PCR results suggest false positivity in CATT and the ELISAs, for example due to infections with the non-pathogenic *T. melophagium* in sheep or *T. theileri* in cattle.

The possibility that in the study area, *T. evansi* strains circulate that do not express the RoTat 1.2 VSG like in Kenya and Ethiopia (Njiru et al., 2006; Birhanu et al., 2015) might have been the cause that 6 and 3 camels are positive in respectively 18S qPCR and ITS TD PCR but negative in TL and the other serological tests. However, when tested with the *T. evansi* type B specific PCR, all dromedary camels and the 18S qPCR positive goat were negative, making it improbable that seropositive animals were infected with a *T. evansi* type B strain. Thus, with the current data, we cannot confirm that ruminants and dogs act as reservoir of *T. evansi* in the study area, notwithstanding that small ruminants and dogs are susceptible to the infection (Aregawi et al., 2019).

To assess the diagnostic accuracy of diagnostic tests in the absence of a gold standard, we used an LCA approach that revealed 100 % sensitivity of all serological tests and < 50 % sensitivity of both molecular tests. The high sensitivity of the antibody detection tests results from the fact that infected animals produce high amounts of persistent anti-*T. evansi* antibodies (Verloo et al., 2000). The lower sensitivity of the molecular tests, on the other hand, can be explained by the usually low parasite load in chronic surra cases resulting in parasite DNA concentrations below the lower detection limit of the PCRs. In addition, successful treatment will clear an animal from *T. evansi* parasites or their DNA within two days (Desquesnes et al., 1995; Clausen et al., 1999; Desquesnes and Dávila, 2002).

The result will be animals that are negative in DNA detection tests but still positive in antibody detection tests. As mentioned above, the concordance analysis shows quite a number of camels that were positive in the molecular tests but negative in the serological tests which may suggest recent infections sampled before the apparition of specific antibodies as observed in experimental studies (Ramírez-Iglesias et al., 2011).

Regarding specificity, LCA shows that all tests are highly specific with overlapping 95 % intervals except for the TL with absolute specificity. This result is in line with previous studies that showed TL as gold standard for detection of antibodies against *T. evansi* in buffaloes (Holland et al., 2002). However, the equally high specificities of the other tests are in contrast with the above discussed assumption of false positive reactions occurring in ELISA/WCL, ELISA/RoTat 1.2 and CATT/*T.evansi* with the horses, ruminants and dogs.

The concordance analysis showed a moderate to substantial agreement between TL, ELISA/WCL, ELISA/RoTat 1.2 and CATT/*T.evansi*, which is expected since all serological tests are detecting antibodies against the same *T. evansi* strain used for preparing the antigen, as observed in other studies in Ethiopia and Pakistan (Birhanu et al., 2015; Tehseen et al., 2015). Agreement is highest between TL and CATT/*T.evansi* (k = 0.784), thus confirming the interest of using CATT/*T.evansi* for serodiagnosis of surra in the field as alternative to the use of TL or ELISA which are tests that are restricted to well-equipped laboratories. Agreement between the molecular tests was almost perfect and both tests have similar diagnostic sensitivity and specificity of both tests are similar. Therefore, one can choose either test to confirm a *T. evansi* infection in a serologically or clinically suspect animal.

The results obtained in this study confirm that surra is prevalent in the study area and that it is present mainly in dromedary camels. We have no firm evidence of other domestic animal species that may be infected with *T. evansi*. Diagnosis of surra in Algeria could be based on a combination of screening for *T. evansi*-specific antibodies followed by confirmation of infection by a molecular tests and treatment of confirmed animals. Diagnosis preferably is confirmed as close as possible to the point-of-care (POC). Serological screening at POC is possible with CATT/*T.evansi* but not yet with the molecular tests that we used in this

study, thus necessitating further research on the development of field-applicable molecular diagnostics (Chiweshe et al., 2019).

CRediT authorship contribution statement

Karima Benfodil: Conceptualization, Methodology, Resources, Writing - original draft, Writing - review & editing, Visualization, Supervision. **Philippe Büscher:** Conceptualization, Methodology, Resources, Writing - original draft, Writing - review & editing, Visualization, Funding acquisition. **Amine Abdelli:** Writing - review & editing, Formal analysis. **Nick Van Reet:** Writing - review & editing, Validation. **Abdellah Mohamed-herif:** Methodology, Validation. **Samir Ansel:** Methodology, Validation. **Said Fettata:** Methodology, Validation. **Sara Dehou:** Methodology, Validation. **Nicolas Bebronne:** Methodology, Validation. **Manon Geerts:** Methodology, Validation. **Fatima Balharbi:** Methodology, Validation. **Khatima Ait-Oudhia:** Conceptualization, Methodology, Resources, Writing - original draft, Writing - review & editing, Visualization, Supervision.

Declaration of Competing Interest

None.

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