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

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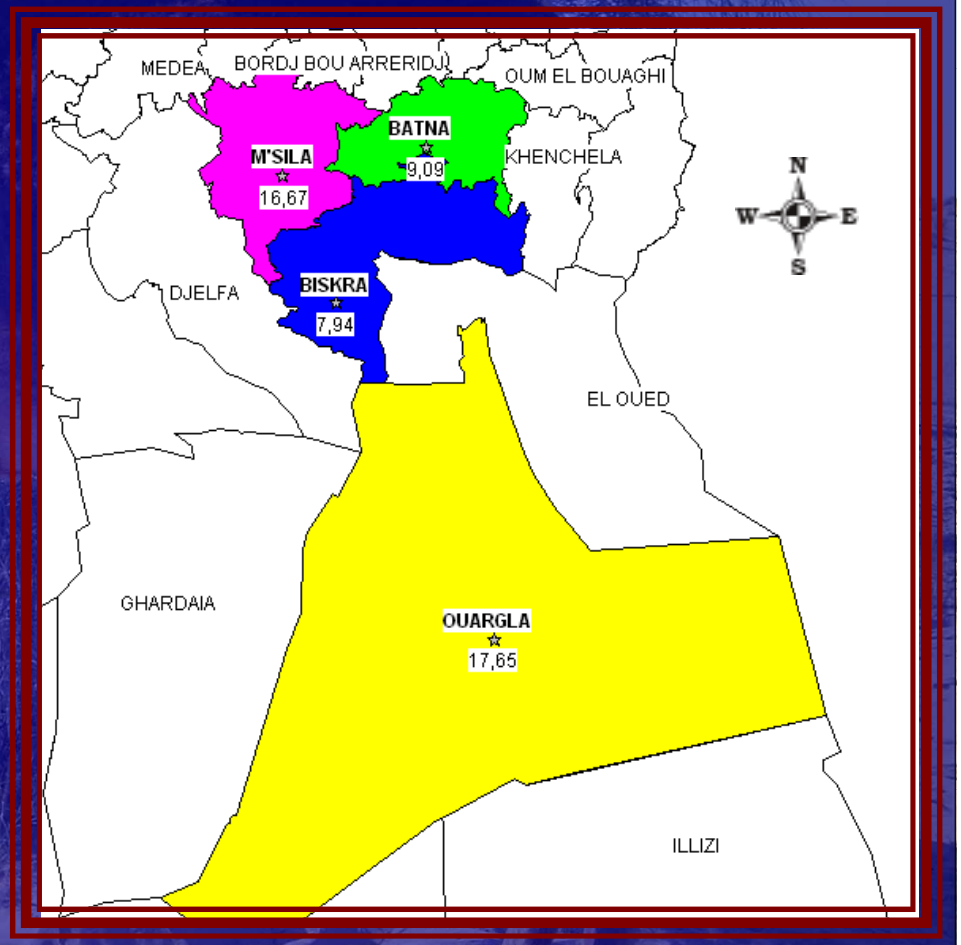
Background

- Trypanosomosis caused by *Trypanosoma evansi* is one of the major diseases in camels.
- This parasitic infection is transmitted mechanically by haematophagous biting flies, especially tabanids.
- The disease causes a great impairment in productivity in camel herds and even mortality in the acute form. It is widespread in different parts of the world

Background: in Algeria

In Algeria, the first case of trypanosomosis was reported by the Sergent brothers in 1903.
In 1904, they conducted a research on camels from the southeast of the country: 10% of the 282 investigated animals were positive to the parasitology.
The disease was well known by herders who associated it with the presence of biting fly.
Its local vernacular name is «El Debab »

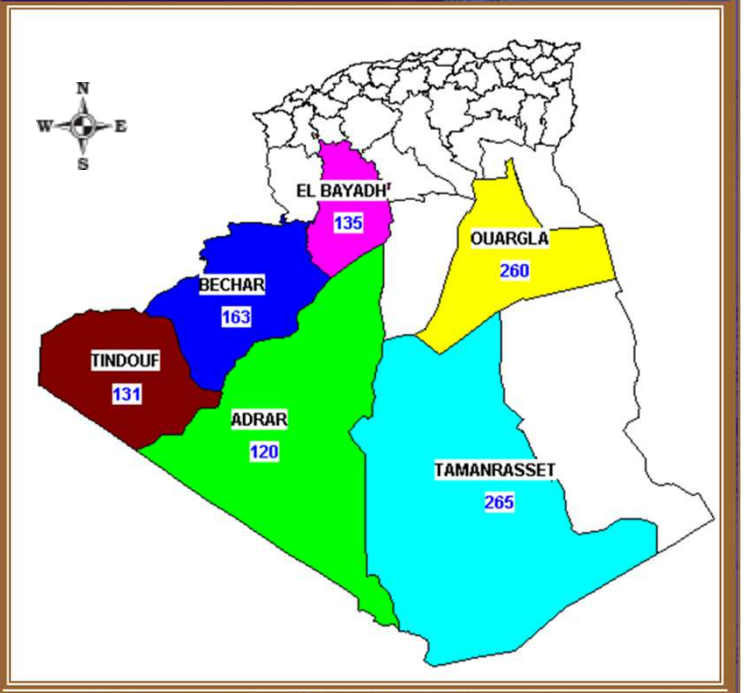


In 1999

In 1999, a parasitological survey was carried out in an important herd of dromedary camels with a reported prevalence of 0.5 % in Ouargla (6 positives out of 1125 animals tested) (Marfoua, 1999).

2005-2006

- Out of 1074 dromedaries camel of the South of Algeria were sampled in the localities of El Bayadh, Ouargla, Bechar, Tindouf, Adrar, and Tamanrasset (as shown on the map). As a result:
  - 2.33% of the animals were positive to parasitological examination,
  - And 20.30% of the animals were positive to serology using CATT/*T. evansi*.
- Infection rate varied according to region:
  - Bechar was the most infected (12.27% STBS *vs* 68.71% CATT/*T. evansi*)
  - Whereas El Bayadh was negative irrespective of the applied test.



Objectives

Overall objective: to make a contribution towards a better understanding of the epidemiology of dromedary camel trypanosomosis in Algeria.

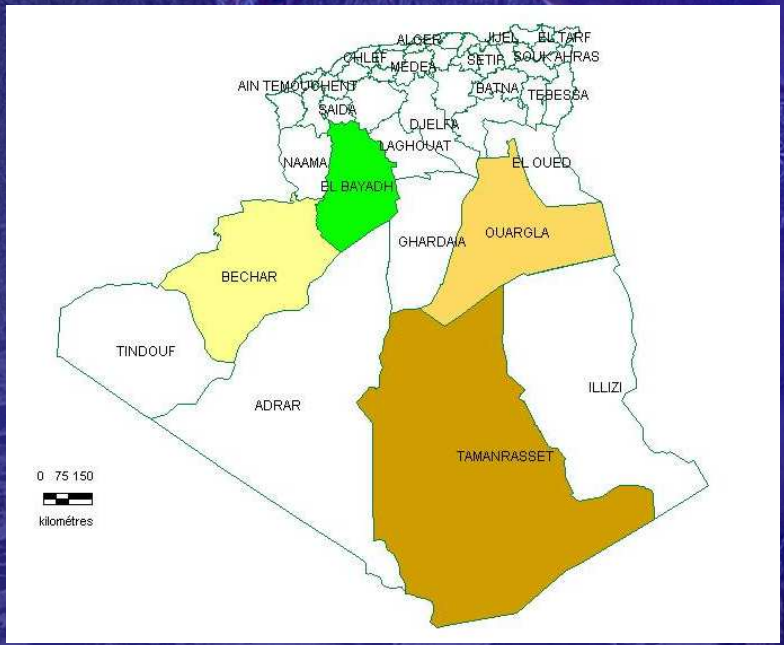
Specific objectives:

- Estimation of the prevalence of *Trypanosoma evansi* infection in South of Algeria, using parasitological, serological and molecular tests,
- Assessment of the associated risk factors,
- Screening other animal species to decide on their likely role as a reservoir of *T. evansi*
- Inventory of biting insects responsible for the transmission of camel trypanosomosis in Algeria.

Material and methods

Study area

- A cross-sectional study was conducted in four Wilayate (localities) of the camel region of Algeria: three of the great South (Bechar, Ouargla, Tamanrasset) and one of the sub-Saharian region (El-Bayadh).
- The surveys were carried out between November 2014 and March 2017.



Wilayas	Effectif Camelin
Adrar	46 998
Laghouat	1950
Djelfa	6440
Batna	43
Biskra	3025
Bechar*	**24 320**
Tamanrasset*	**85 745**
Tebessa	410
Tiaret	230
Tindouf	51 342
El Oued	36 700
M'Sila	1620
Ouargla*	**31787**
El Naama	1013
El-Bayadh*	**10 060**
Illizi	31 182
Ghardaia	11 150
Total Algérie	**344 015**

Material and methods

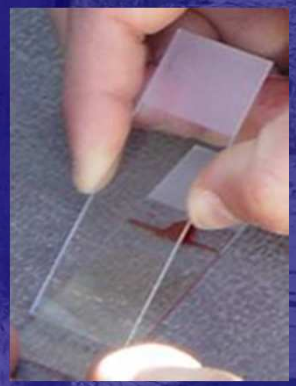
Animals

- A total of 1046 dromedary camels of different ages and both sexes, originating from 83 herds, were sampled *at random.*
- Our sample size was estimated with a maximum prevalence of 25% and an absolute precision of 10 %.
- The samples were accompanied by an epidemiological questionnaire detailing ethnicity, herd management, health status, knowledge of the disease, vector presence and locations, treatment, etc.
- Age, sexe, race, and colour were recorded.
- Five animal age groups were defined as follows: less than one year, from one to two years, from two to four years, from four to ten years and more than ten years.

Material and methods

Blood sampling and diagnostic tests

- Five ml of blood were collected from the jugular vein into a tube containing EDTA for **packed cell volume** (PCV) measurement and for DNA extraction. PCV, expressed in percentage, was estimated after 5 minutes centrifugation at 12000 rpm of blood into capillary tubes.
- For serological tests, ten ml of blood were collected in vacutainer® tubes.
- The antibody detection was performed with the **Card Agglutination Test for Trypanosomosis** (CATT/*T. evansi*) and **Enzyme-Linked Immunosorbent Assay ELISA/RoTat 1.2 VSG** (Lejon *et al.*, 2005, ITM protocols).
- For the **parasitological examination** , thin blood smears were prepared by spreading a drop of blood on slides, air dried, fixed with methanol and stained with Giemsa (**STBS**).

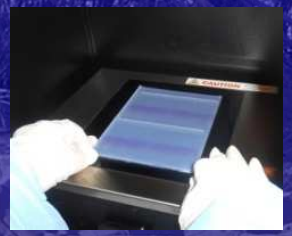


Material and methods

Molecular analysis

Polymerase Chain Reaction (PCR) : tow PCRs were performed:

- **RoTat 1.2 PCR for detection of T. evansi:** specific for *T. evansi* type A (adapted from Claes *et al.*, 2004).
- **EVAB PCR** was used for the detection of *T. evansi* type B (Birhanu *et al.*, 2015).



Results

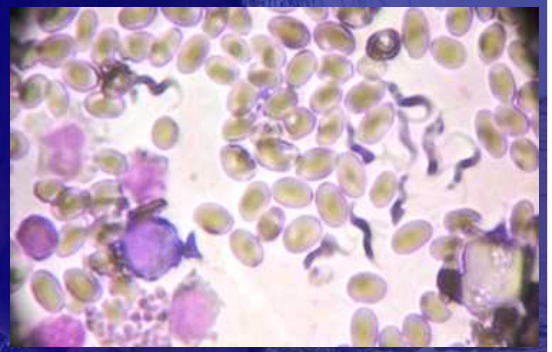
Clinical signs

The observed symptoms revealed that the infection varied from an acute form with a high number of mortality and stillbirths in El Bayadh, to a chronic form in Bechar and Ouargla.

Results

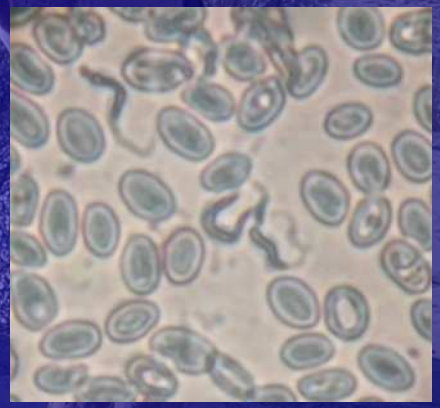
Parasitology

- STBS examinations showed 23 positives out of 1036 animals.
- With an apparent prevalence of **2.22% (95% CI (1.3 - 3.1))**
- Parasitemia ranged from few trypanosomes per blood smear slide to over 09 per microscopic field.



Serology

- CATT/*T. evansi* : **31.74% 95% CI (28.9 - 34.6)**
- ELISA/ RoTat1.2 VSG : **18.43% 95% CI (16 -20.8)**

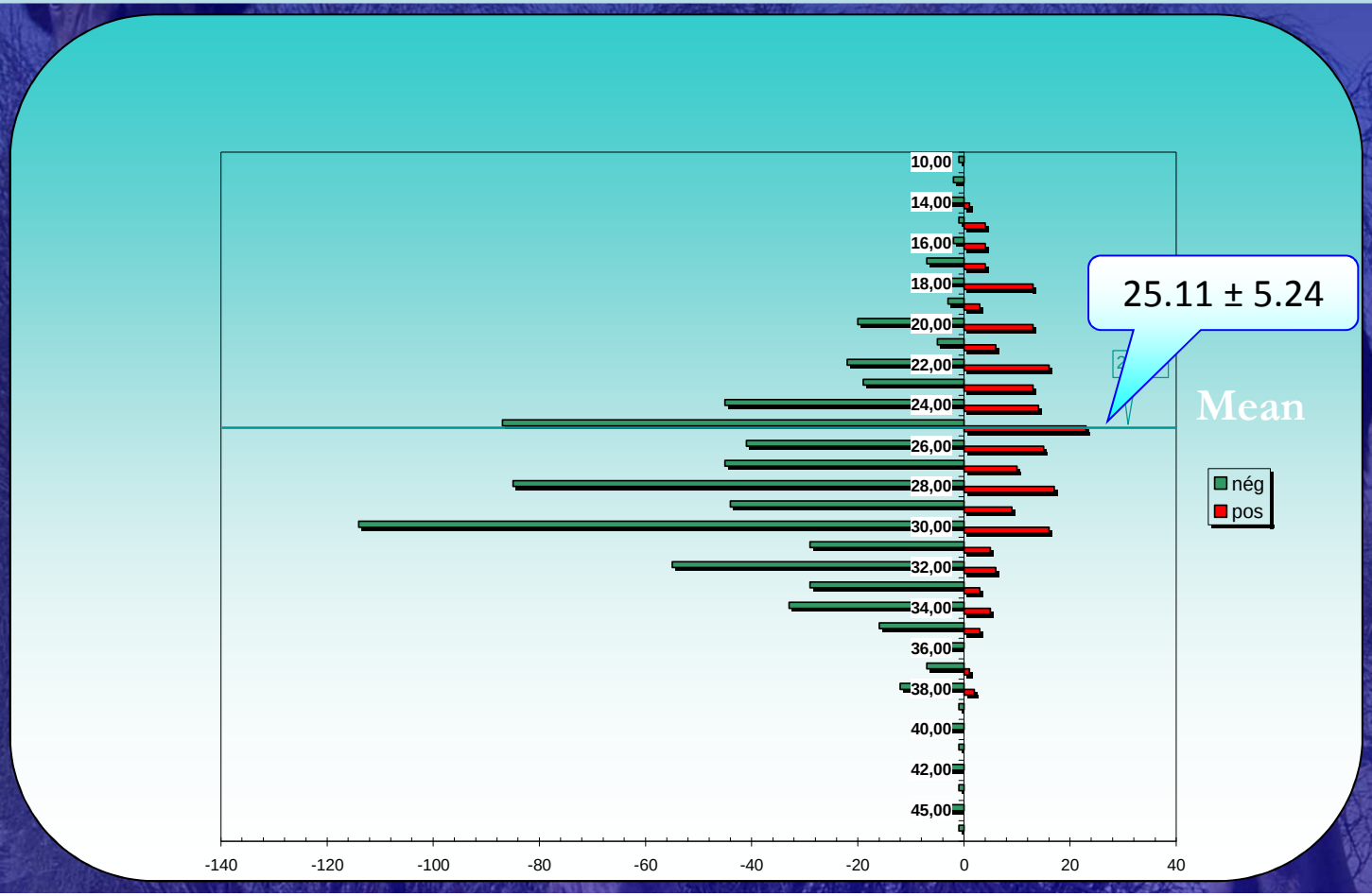


Molecular test

- RoTat 1.2 PCR : **5.79% 95% CI(3.3- 8.3)**

Using different tests, the overall prevalence estimates were statistically different from each other ($p < 0.05$). X-squared = 361.85, df = 3, p-value < 2.2e-16

Distribution of the PCV according to the result of the CATT/*T.evansi*



The dromadaries positive for the CATT/*T.evansi* had a very significantly lower mean of PCV than the negatives (**p-value = 3.939e-15)**

Animals positive for ELISA /RoTat 1.2 VSG and the parasitological test also had a significantly lower mean PCV than the negative animals (**p < 0.05)**

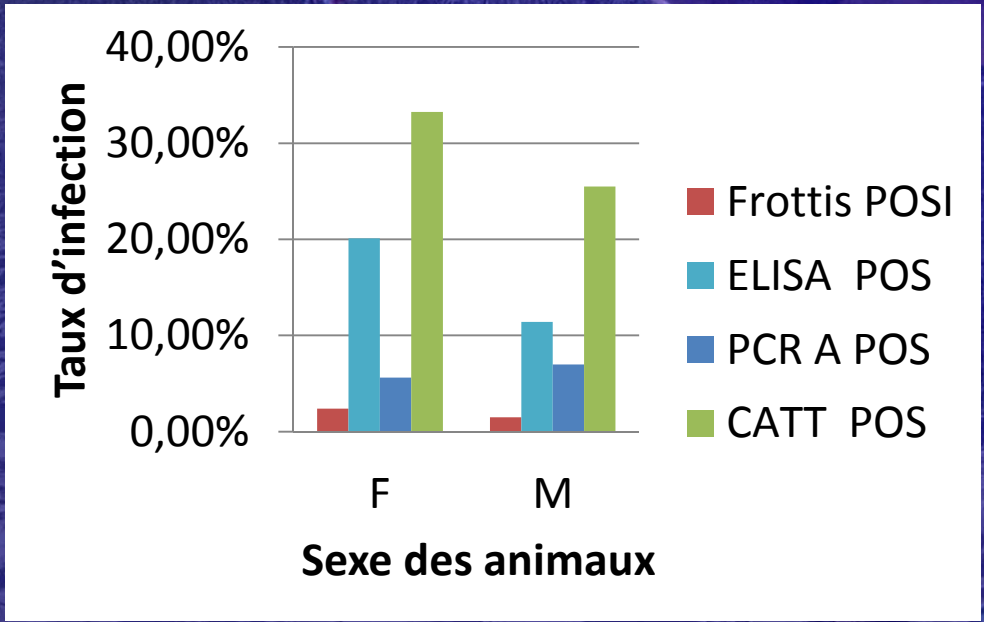
Prevalence according to Wilaya

	STBS		CATT/*T. evansi*		ELISA/RoTat 1.2 VSG		RoTat 1.2 PCR	
wilaya	Testés	Pos (%)	testés	Pos (%)	testés	Pos (%)	testés	Pos (%)
El Bayadh	151	17 (11.26)	161	118 (73.29)	128	53 (41.41)	145	6 (4.14)
Ouargla	221	2 (0.90)	221	27 (12.22)	220	32 (14.55)	20	0 (0.0)
Béchar	304	4 (1.32)	304	112 (36.84)	303	68 (22.44)	110	13 (11.82)
Tamanrasset	360	0 (0.0)	360	75 (20.83)	353	32 (9.07)	53	0 (0.0)
Total	**1036**	**23 (2.22)**	**1046**	**332 (31.74)**	**1004**	**185 (18.43)**	**328**	**19 (5.79)**

- Tamanrasset was negative to both parasitology and PCR.
- The highest prevalences were observed in El Bayadh :
  - with 11.26% using STBS (Ficher test, $p < 0.05$)
  - 73.29% and 41.41 % respectively with CATT/*T.evansi* and ELISA Rotat 1.2 VSG (X-squared, $p < 0.05$)
- Bechar showed the higest prevalence with RoTat 1.2 PCR type A (11.82%) but without a significant statistical difference.

Prevalence according to gender

	STBS		CATT/*T. evansi*		ELISA/ RoTat 1.2 VSG		RoTat 1.2 PCR	
	Testés	Pos (%)	testés	Pos (%)	testés	Pos (%)	testés	Pos (%)
Sexe								
Femelle	833	20 (2.40)	842	280 (33.25)	811	163 (20.10)	285	16 (5.61)
Mâle	203	3 (1.47)	204	52 (25.49)	193	22 (11.40)	43	3 (6.98)
Total	1036	23 (2.22)	1046	332 (31.74)	1004	185 (18.43)	328	19 (5.79)



- When using CATT / T. evansi ($p = 0.0332 < 0.05$) we observed that males were 0.68 times less at risk of infection than females (odds ratio = 0.6866)
- With ELISA / RoTat 1.2 VSG ($p = 0.0057 < 0.05$), males were also 0.51 times less at risk of infection than females (odds ratio = 0.5114).

Prevalence according to age

	STBS	CATT/*T. evansi*	ELISA/ RoTat 1.2 VSG	RoTat 1.2 PCR
< 1 year	0.00	23.93	09.57	4.35
≥ 1year ≤ 2 year	1.68	30.00	26.72	5.26
> 2 year ≤ 4 year	2.30	30.34	16.28	0.00
> 2 year ≤ 4 year	1.71	28.26	19.30	8.33
> 10 year	3.96	40.52	18.06	5.34

Both STBS and CATT / *T. evansi* showed that risk of infection increases significantly with age, the highest rate observed in older camel over 10 years ($p < 0.05$)

Conclusion and recommendations

- This is the first study *on T. evansi* infection in dromedary camel in the South of Algeria, using parasitological, serological and molecular diagnostic tests, including those recommended by the OIE.
- This study indicates that dromedary trypanosomosis in Algeria is caused by *T. evansi* type A, and that it is endemic and widespread.
- Given the high prevalence observed and spread to new non-infected areas (El Bayadh), we recommend urgent action to reduce the incidence of the disease by treating infected animals with an effective drug and controlling vectors.
- it is desirable to isolate strains of *T. evansi* circulating in Algeria for studies on their pathogenicity and susceptibility to existing drugs.

Acknowledgements

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Thank you!