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Short Communication

First serological investigation of peste-des-petits-ruminants and Rift Valley fever in Tunisia

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ABSTRACT

This study, carried out between September 2006 and January 2007, is the first cross-sectional serological investigation of peste-des-petits-ruminants (PPR) and Rift Valley fever (RVF) in Tunisia. The objective was to assess the potential need to develop a dual, recombinant PPR–RVF vaccine and how such a vaccine might be utilised in Tunisia. An overall PPR seroprevalence of 7.45% was determined, a finding supported by the high specificity (99.4%) and sensitivity (94.5%) of the ELISA used. On assessment of the diversity and density of mosquitoes in the sampling area, four species of RVF-vectors of the genus *Aedes* and *Culex* were identified. However, no serological evidence of RVF was found despite the use of a highly sensitive ELISA (99–100%). Larger scale investigations are underway to confirm these findings and the continuation of the emergency vaccination program against these two diseases remains valid.

The ongoing, un-regulated movement of livestock between Algeria and Libya poses a risk to the health of animals in intervening Tunisia, in particular to diseases such as peste-des-petits-ruminants (PPR) and Rift Valley fever (RVF). In consequence, the development and use of a dual, recombinant capripox vector vaccine against these diseases has been proposed (Faye et al., 2007). To determine the current prevalence of these diseases in Tunisia a cross-sectional serological study was conducted in six regions considered high-risk areas for PPR and RVF because of their ecology and livestock density. A total of 610 serum samples were randomly collected from animals near water sources between September 2006 and January 2007 (Table 1). Serological analysis was carried out at the Pasteur Institutes at Tunis and Paris and at CIRAD Montpellier, France.

To assess the seroprevalence of PPR, blood samples from 263 sheep and 119 goats were analysed by competitive ELISA as previously described (Libeau et al., 1995). In addition, 28 lung samples harvested from slaughtered animals (25 sheep; 3 goats) from different regions were tested using a PPR virus-specific RT-PCR (Couacy-Hymann et al., 2002). All sera were also analysed using a RVF competitive ELISA as previously described (Paweska et al., 2005). In order to assess the extent and prevalence of potential mosquito vectors, larvae from potential breeding sites in each region (the main wetland areas), were collected and identified. Statistical analysis was carried out using Winepiscope software (De Blas et al., 2000).

The overall apparent seroprevalence of PPR was 7.6% (95%, confidence interval, CI, 4.9–10.1), a finding supported by the high specificity (99.4%) and sensitivity (94.5%) of the ELISA (Libeau et al., 1995). The Kairouan (95%, CI 8.7–22.3) and Kebili (95%, CI 3.1–20.9) regions had the highest seroprevalence, a finding that was not unexpected given the abundant animal movement within these areas (Table 2). The difference in seroprevalence between goats (11.8%) and sheep (5.7%) was statistically significant ($\chi^2 = 4.3$, 1df, $P = 0.04$), which was consistent with the pathogenesis of PPR in these species. The lung samples were all negative on RT-PCR (Couacy-Hymann et al., 2002). Although this is the first serological evidence of PPR in Tunisia, no clinical signs were reported in the sampled animals. The similarity in clinical presentation between PPR with other diseases such as bluetongue and sheep-pox, endemic in Tunisia, may account for this finding (Ozmen et al., 2009).

In our serological assessment of RVF, given an assumed prevalence of at least 1% (CI 99.5%), a sample size of 527 was required to detect at least one positive animal. This number was considered as an indicator given our sampling was not random but focused on high-risk areas. All of the 610 samples tested were found negative (De Blas et al., 2000). Since the ELISA we used to screen for RVF is highly sensitive (99–100%) (Paweska et al., 2005) we concluded

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Table 1
The geographical distribution of serum and mosquito samples. The numbers in brackets refer to the total number of animals species in the sampled regions. *Ae, Aedes; An, Anopheles; Cx, Culex.*

	Sheep	Goats	Cattle	Camels	Total	Mosquito spp.	%
Bizerte	44 (6000)	0 (1500)	49 (6050)	0 (0)	93 (13,550)	*Ae. caspius* *Ae. detritus* *Cx. pipiens* *An. labranchiae*	29 29 38 4
Kairouan	76 (102,300)	33 (9000)	82 (7000)	0 (0)	191 (118,300)	*Ae. caspius* *Ae. detritus* *Cx. pipiens* *Cx. theileri* *Cx. perexiguus* *Cx .laticinctus*	33.5 11 33.5 11 5.5 5.5
Kébili	21 (14,500)	27 (13,800)	0 (110)	28 (1930)	76 (30,340)	*Cx. pipiens* *Cx. theileri* *An. sergenti*	33.5 33.5 33
Médenine	0 (130,000)	1 (50,000)	0 (230)	7 (1700)	8 (181,930)	*Cx. pipiens* *Cx. theileri*	50 50
Nabeul	67 (39,980)	49 (7430)	59 (17,480)	0 (0)	175 (64,890)	*Ae. caspius* *Ae. detritus* *Cx. pipiens* *Cx. theileri*	26 36 26 9
Sousse	55 (103,500)	9 (7300)	3 (1600)	0 (0)	67 (112,400)	*Ae. caspius* *Ae. detritus* *Cx. pipiens* *Cx. theileri*	41.5 11.5 41.5 5.5
Total	263 (396,280)	119 (89,030)	193 (32,470)	35 (3630)	610 (521,410)		

Table 2
Seroprevalence of peste-des-petits-ruminants in sheep and goats in six regions of Tunisia. SP, seroprevalence.

	Total sheep/positive sheep (% SP)	Total goats/positive goats (% SP)	Total animals/positive animals (% SP)
Bizerte	44/3 (6.8%)	0/0 (0%)	44/3 (6.8%)
Kairouan	76/8 (10.5%)	33/9 (27.3%)	109/17 (15.6%)
Kébili	21/2 (9.5%)	27/4 (14.8%)	48/6 (12.5%)
Médenine	0/0 ([a])	1/1 ([a])	1/1 ([a])
Nabeul	67/1 (1.5%)	49/0 (0%)	116/1 (0.9%)
Sousse	55/1 (1.8%)	9/0 (0%)	64/1 (1.6%)
Total	263 (15%)	119 (14%)	382/29 (7.6%)
Percentage	5.7%	11.8%	7.6%

[a] Seroprevalence not representative.

that there was no serological evidence of infection in these regions despite the presence of several potential mosquito vectors (Table 1; Fig. 1) (Moutailler et al., 2008).

Culex theileri was present in all regions except Bizerte. Two species of *Anopheles* (*An. sergenti* and *An. labranchiae*) were found at very low density in Kebili and Bizerte, respectively. Few larvae of *Culex perexiguus* or *laticinctus* were identified in Kairouan. Species of the *Aedes* (*Ae. caspius* and *Ae. detritus*) and *Culex* (*Cx. pipiens* and *Cx. theileri*) genus frequently found are considered important in the transmission and persistence of RVF (Gad et al., 1987). RVF virus has been isolated from both *Cx. pipiens* and *Cx. theileri* during outbreaks in Egypt and South Africa (Gear et al., 1955; Hoogstraal et al., 1979). However, although these vectors are present in Tunisia, our serological data did not indicate animal infection.

The abundant trading in ruminants between Tunisia and neighbouring Algeria, Libya, Mauritania and Mali, emphasise the potential high risk of RVF transmission. A larger national survey will be required to validate the findings of this pilot serological study and sentinels herds should be established to monitor the possible introduction of RVF in Tunisia. The justification for the continuation of the emergency vaccination program against these two diseases remains valid.

Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

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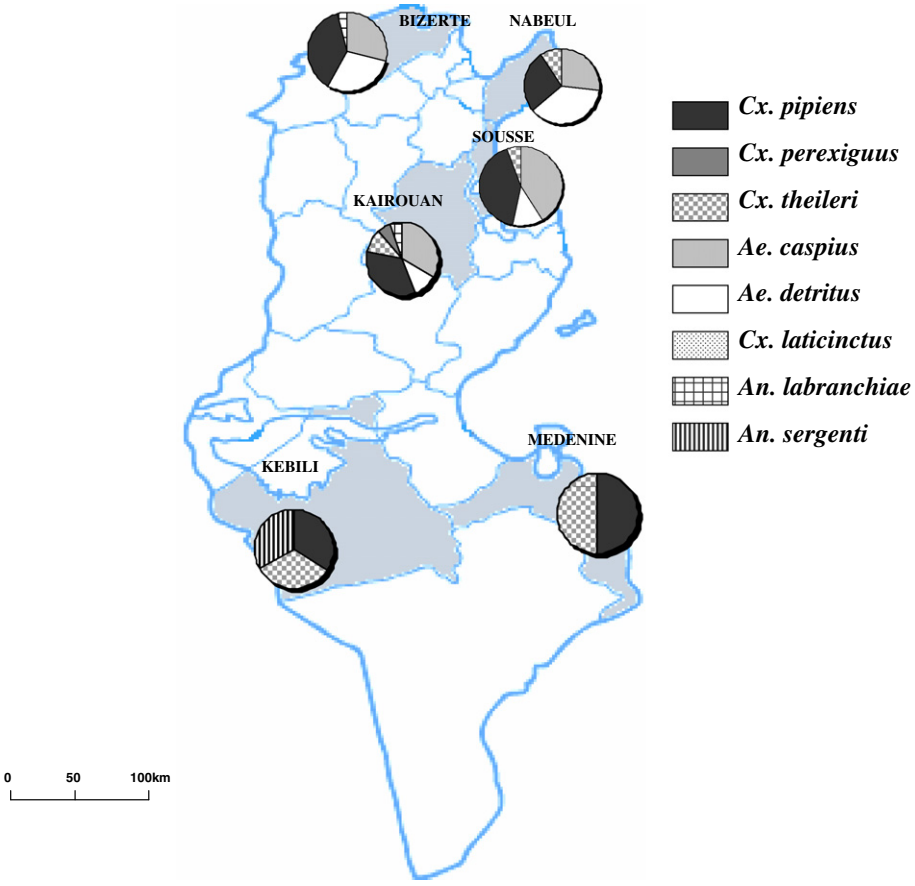

Fig. 1. Map illustrating the six regions of Tunisia sampled (grey shading). Pie charts represent the distribution of mosquito species identified. *Ae, Aedes; An, Anopheles; Cx, Culex*.

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