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Rift Valley fever virus: a serological survey in Libyan ruminants

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Abstract
A serological survey was carried out in Libya to investigate the circulation of Rift Valley fever virus (RVFV) among domestic ruminants. A total of 857 serum samples were collected from year 2015 to 2016 in eleven provinces of Libya belonging to five branches of the country. Samples were tested for RVFV antibodies using a competitive Enzyme-Linked Immunosorbent Assay (c-ELISA). Antibodies specific for RVFV were not detected in any of the 857 samples. However, a statistical analysis was carried out to assess the maximum expected number of infected animals and the maximum expected prevalence of RVFV among Libyan ruminants' populations according to the sampled population. The overall maximum expected prevalence was estimated to be 1.8% for cattle and 0.4% for small ruminants. Results seem to exclude the circulation of RVFV, however, a surveillance plan should be implemented in areas at risk of RVFV introduction.
Keywords: c-ELISA, Libya, RVF, Sero-prevalence.

Introduction

Rift Valley fever (RVF) is an acute arthropod-borne zoonotic disease caused by a *Phlebovirus* belonging to the family *Bunyaviridae*. Although direct transmission (contact with infectious animals or animal tissues) might occur and plays a significant role in the human infection, mosquitoes usually represent the most common way for viral transmission (Abdo-Salem *et al*., 2011; EFSA, 2013) among a wide range of domestic and wild ruminant hosts.

The number of competent RVF vectors is far to be defined, at least 30 species of mosquitoes have been found infected in course of outbreaks (Meegan and Bailey, 1988; Fontenille *et al*., 1998) or have demonstrated their capability to transmit the virus in laboratory conditions (Pages *et al*., 2009). However, a crucial role in the occurrence of RVF is played by mosquitoes belonging to *Aedes*, *Culex* and *Anopheles* genera (Smithburn *et al*., 1948; Meegan *et al*., 1980; Davies *et al*., 1985).

RVF is endemic in most of the sub-Saharan territories (Meegan, 1979; Aradaib *et al*., 2013) with different cyclic reoccurrence usually associated to exceptionally heavy rains and floods (Mondet *et al*., 2005; Abdallah *et al*., 2016) leading to severe epidemics with human and animal fatalities (Caminade *et al*., 2014). The same devastating consequences are known to occur every time the virus spread outside the traditional endemic territories as reported in Egypt (Kamal, 2011), in Saudi Arabia and Yemen (Madani *et al*., 2003).

The capability of the virus to infect a huge variety of vertebrate hosts coupled with its capability to be transmitted by a wide range of potential vectors (e.g. *Aedes vexans*, *Aedes albopictus*, *Ochlerotatus caspius*, *Ochlerotatus detritus*, *Culex pipiens*, *Culex theileri*, *Culex perexiguus*, *Culex antenantus*, *Culex tritaeniorhynchus*) poses RVF virus (RVFV) as one of the major threat for Mediterranean territories (EFSA, 2013). Eventhough RVF has been described in Egypt and is considered endemic in Mauritania (Faye *et al*., 2014), nevertheless, the viral circulation has never been reported in the ruminant population of the Maghreb region (Ayari-Fakhfakh *et al*., 2011).

The aims of this study were, firstly, to investigate the sero-prevalence of RVF in cattle, sheep and goats of eleven Libyan provinces belonging to five Branches (Green Mountain, Benghazi, Tripoli, West Mountain, Sabha) of the country, secondly to assess the maximum number of positive animals and RVF sero-prevalence that could be expected according to the sampled population.

Material and Methods

During the 2015-2016 period, 171 cattle and 686 small ruminants sera were collected from domestic ruminants in 96 farms located in eleven provinces distributed in five regions of Libya (Fig. 1).

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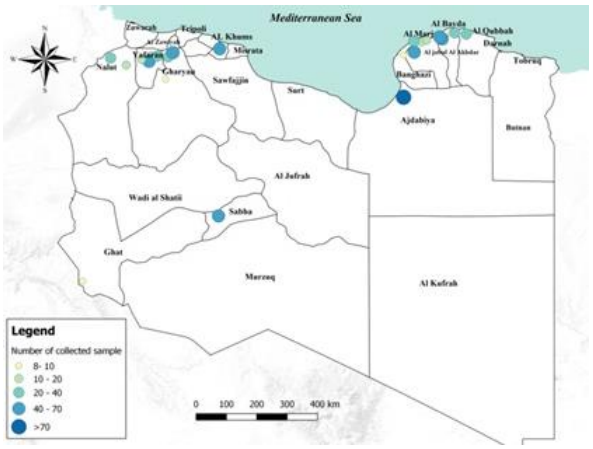

Fig. 1. Number of collected samples (small and Large ruminants) represented by sampled areas and sample size classes in Libya.

In each farm a maximum of 10 serum samples were gathered regardless the herd size. Unfortunately, the socio-political particular situation in the country limited the possibilities of samples collection to suitable and easily accessible provinces within each region, therefore, the criteria used for sampling strategy may have biased the representativeness of data in a number of the provinces tested.

To investigate the sero-prevalence of RVF, all the samples were tested with a competitive Enzyme-Linked Immunosorbent Assay (c-ELISA) commercially available according to the manufacturer's protocol (ID screen® Rift Valley Fever Competition Multi-species, IDvet, Grabels, France).

The maximum expected prevalence of antibodies against RVFV (a) and the maximum number of positive animals among cattle and small ruminants (b) for each province were estimated with the statistical approach described by Cannon and Roe (1982), based on the following formulae:

a) $P = 1 - (1-\alpha)1/n$

b) $D = [1 - (1-\alpha)^{1/n}][N- n/2)]+1$

Where N= number of individuals in a population, n= number of samples, D= the maximum number of positive individuals, P= the maximum expected prevalence of RVF in the target population and α = the desired confidence level (95% used).

Results and Discussion

Antibodies against RVFV were not detected in any of the 857 sera tested. Therefore, the overall maximum expected prevalence of RVF was estimated at 1.8% for cattle and 0.4% for small ruminants, which lead to the estimation of not more than 1,875 positive cattle and not more than 13,869 positive small ruminants in the Libyan population (FAO, 2018) (Table 1 and Fig. 2). The interpretation of the statistical analysis results should take into account the efforts in collecting samples under the peculiar country's situation resulting in a non-random sampling, which may lead to a certain level of bias in the observed results.

The circulation of RVFV has never been reported in Libya neither in humans nor in livestock populations. Thus, the lack of any serologically reactive ruminant among the 857 samples from the 11 provinces included in the study was to some extent expected. The negative result obtained from the serological survey may also be ascribed to the performances of the test used to screen the sera even though the sensitivity of the assay has been considered satisfactory with domestic ruminant sera (Monaco *et al*., 2015).

Even if the RVFV circulation in Libya could be excluded in the period considered, nevertheless, the results should be carefully evaluated since it is well known that the virus is endemic in many of the bordering countries as Egypt (Mroz *et al*., 2017), Sudan (Hassan *et al*., 2011) and Niger (WHO, 2016), where the viral activity has been documented.

However, no outbreaks of RVF were notified during the study period in these countries. It is well known that RVFV environmental cycle is characterized by inter-epidemic periods (IEPs) with no apparent viral circulation, lasting even for decades (LaBeaud *et al*., 2008; Lichoti *et al*., 2014). During IEPs the occurrence of clinical manifestation of the disease is often absent and the low-level enzootic circulation in livestock could be detected by active surveillance activities, mainly relying on the use of sentinel ruminants (LaBeaud *et al*., 2008; Rissman *et al*., 2016; Mroz *et al*., 2017).

Thus, to prevent the potential spread of the disease from bordering regions it would be advisable to focus the surveillance activities in the areas at risk for RVFV introduction. In particular, the Southern part of Libya is the theatre of an intense animals movement, often uncontrolled or with fragile quarantine measures.

The lack of any report referring RVFV circulation in Libya supports the negative results obtained during the study period, as well as the lack of an appropriate sampling strategy could represent a weak point in the serological survey. Even though we are aware of these limits linked to the political instability of the country, we consider the obtained results may be of value for understanding the RVF epidemiological situation in Libya.

Conclusion

This study was conducted to investigate the epidemiological situation of RVFV in Libya. The viral circulation has not been detected in 11 provinces of the country as determined by absence of RVFV antibodies in animals tested during the sampling period. However, the evidence of RVFV activity in countries bordering the Southern part of Libya supports the hypothesis of a continuous risk of introduction of RVFV through animals imported from endemic neighbor countries.

Table 1. Detail of the maximum number of infected animals and the maximum expected sero-prevalence of RVFV by province and species in Libyan ruminant population.

Province ID	Province name	Cattle				Sheep and Goats			
		Population	Tested	MI	MSP(%)	Population	Tested	MI	MSP(%)
1	Ajdabiya	75	10	19	25.9%	400,000	88	13,387	3.3%
8	Al Marj	61,861	29	6,071	9.8%	580,000	61	27,795	4.8%
9	Al Marqab	8,611	70	360	4.2%	495,135	0	nc	nc
10	Al Qubbah	8,225	7	2,863	34.8%	220,000	25	24,844	11.3%
3	Benghazi	13,315	24	1,562	11.7%	880,000	134	19,455	2.2%
19	Gharyan	1,050	0	nc	nc	57,829	100	1,706	3.0%
22	Mizdah	0	0	nc	nc	153,000	10	39,606	25.9%
24	Nalut	120	0	nc	nc	192,554	60	9,377	4.9%
25	Sabha	2,000	0	nc	nc	185,000	48	11,193	6.1%
32	Yafran - Jadu	117	10	30	25.9%	94,563	131	2,137	2.3%
4	Al-Jabal Al-Akhdar	15,793	21	2,099	13.3%	420,000	29	41,220	9.8%
	Total	111,167	171	1,875	1.7%	3,678,081	686	13,869	0.4%

(MI): Maximum number of infected animals; (MSP): Maximum expected sero-prevalence; (nc): Not calculable.

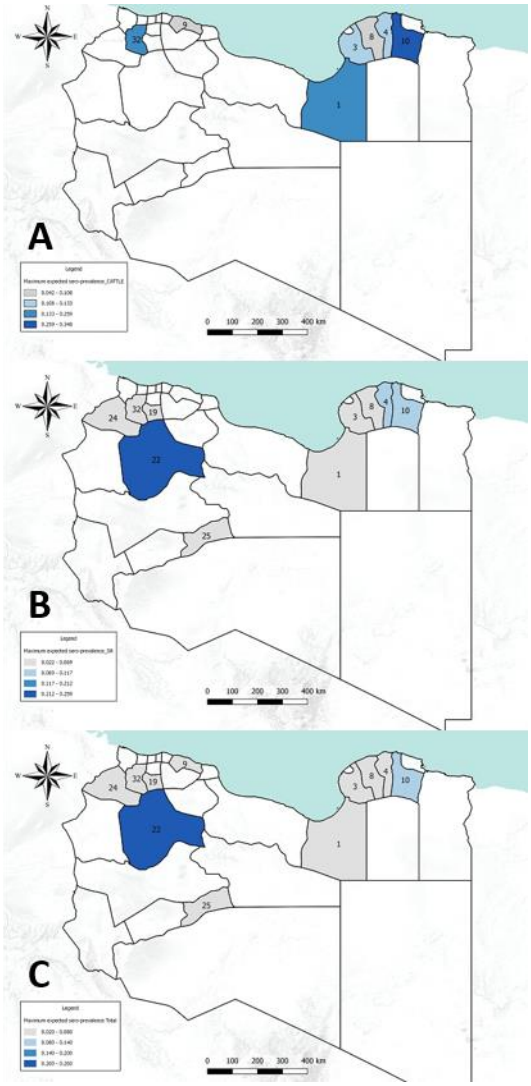

Fig. 2. Maps of maximum expected sero-prevalence by Provinces (the numbers indicated ID provinces in Table 1). **(A):** Cattle. **(B):** Small ruminants. **(C):** Total maximum expected sero-prevalence for small and large ruminants.

Future surveillance activities and animals movement control should be foreseen in the areas at risk, *i.e.* where imported animals and vectors population may create the environmental suitable condition for RVFV circulation.

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Conflict of interest

The Authors declare that there is no conflict of interest.

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