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Sero-prevalence and epidemiology of peste des petits ruminants in Libya

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Summary

We conducted a cross-sectional study during 2013 to quantify the serological prevalence of peste des petits ruminants (PPR) infection and to investigate host factors associated with PPR infection in small ruminants in Libya. A two-stage sampling design was carried out. A total number of 148 flocks owning at least 100 heads each were randomly selected. Sixteen to forty-eight samples were collected from each selected flock. A total number of 3,508 serum samples from unvaccinated animals were collected and analysed at IZSLER Brescia, Italy, by using competitive ELISA, IDvet innovative diagnostics (IDvet 310, France). The overall serological prevalence among SR was 33% (95% CI: 31.4–34.5). Significant differences between the prevalence in the geographical branches were observed. The lowest prevalence level was observed in Zawiyah branch (16.1%), whereas the highest value was obtained for the Sabha branch (56.8%). Considering the age, a serological prevalence of 24.7%, 31.5% and 42.1% was observed in SR <1 year, between 1 and 2 years and more than 2 years, respectively. Statistically significant differences ($p < .001$) in the sero-prevalence levels were also observed between the age groups. Our findings suggest that the southern part of Libya could be more exposed to the infections coming from the neighbouring countries and this should be better investigated to correctly identify wherever specific entry points can be considered at higher risk than others. The results also confirmed the endemic status of PPR in Libya, with a constant exposure to the infection of the animals during their life. In the framework of the global strategy for control and eradication of PPR, our results, even if obtained by a preliminary study, can contribute to the assessment of the epidemiological situation of PPR in Libya as required by the Stage 1 of the plan.

KEYWORDS

Libya, peste des petits ruminants, risk factors, sero-prevalence, small ruminants

1 | INTRODUCTION

Peste des petits ruminants (PPR) is a highly contagious viral disease of goats and sheep caused by a *Morbillivirus* in the family Paramyxoviridae (Radostits, Gay, Hinchcliff, & Constable, 2007). The clinical signs are fever, sores in the mouth with discharges, diarrhoea, pneumonia and sometimes death. The disease causes high morbidity

and mortality in susceptible small ruminants, with goats being more susceptible than sheep (Singh, Saravanan, Sreenivasa, Singh, & Bandyopadhyay, 2004). Cattle and several wild ruminants are experimentally susceptible to infection, but naturally occurring infections are rare (Mornet, Orue, Gilbert, Thiery, & Mamadou, 1956).

Peste des petits ruminants is transmitted by close contact between susceptible and infected animals during the febrile stage of disease (Braide, 1981). This is when the discharges from eyes, nose, mouth and faeces contain large amount of the virus and droplets are aerosolized from these secretions and excretions, particularly when infected animals cough or sneeze (Bundza et al., 1988; Taylor, 1984).

The first detection of PPR was in 1942 in western Africa (Côte d'Ivoire) (Gargadennec & Lalanne, 1942). Subsequently, the disease was detected in many countries including: India (Shaila, Purushothaman, Bhavasar, Venugopal, & Venkatesan, 1989); Saudi Arabia (Abu Elzein, Hassanien, Al-Afaleq, Abd Elhadi, & Housawi, 1990); sub-Saharan Africa (Lefèvre & Diallo, 1990); and Jordan (Lefèvre, Diallo, Schenkel, Hussein, & Staak, 1991).

The PPR virus is separated into four lineages (I–IV), based on the genetic comparison of a fragment of the nucleoprotein or the fusion protein (Banyard et al., 2010). Historically, the four lineages follow a geographical distribution: lineages I and II found in western and central Africa; lineage III is present in eastern Africa and in the southern part of the Middle East; and lineage IV is distributed in the Middle East and southern Asia. Historically, lineage IV was primarily restricted to Asian continent, but since the 1990s, this lineage has been found in African countries, including: Cameroun; Central Africa Republic; Sudan; Morocco; Egypt; Tunisia; Algeria; and Uganda (Banyard et al., 2010; Kwiatek et al., 2011).

The virus is transmitted by close contact between susceptible and infected animals in the febrile stage (Braide, 1981). The discharges from eyes, nose, mouth and loose faeces contain large amount of the virus. Fine infected droplets are released into the air from these secretions and excretions, particularly when infected animals cough or sneeze (Bundza et al., 1988; Taylor, 1984).

Despite the detection of PPR in all neighbouring countries such as Tunisia (Ayari-Fakhfakh et al., 2011; Sghaier et al., 2014), Algeria (De Nardi et al., 2012), Tchad (Bidjeh, Bornarel, Imadine, & Lancelot, 1995), Sudan (Osman, Ali, A/Rahman, & Fadol, 2009) and Egypt (Abd El-Rahim, Sharawi, Barakat, & El-Nahas, 2010), little information concerning PPR in Libya is available. A first notification to OIE of PPR occurrence in Libya was made in 2012 and then in 2013. Since 2014, no official report on animal diseases status was submitted to OIE by Libya; however, PPR is a notifiable disease included into the OIE-listed diseases, infections and infestations in force in 2017 and reporting any occurrence of the disease mandatory (OIE, 2016).

The aim of this study was to quantify the serological prevalence of PPR infection in the whole Libya and to investigate possible risk factors associated with PPR infection in small ruminants (sheep and goats).

2 | MATERIALS AND METHODS

2.1 | Study area, sample size and sampling strategy

The small ruminant population in Libya is around 6.5 million heads (Communication Plan in Animal Health in the frame of REMESA/RECOMSA). Among susceptible population to PPR reared in Libya, small ruminants are the most represented species in the country. The Libyan National Center of Animal Health (NCAH) has subdivided the country in seven regional animal health branches (administrative units) named Green Mountain, Benghazi, Middle Area, Zawiyah, Tripoli, West Mountain and Sabha. Each branch contains several districts.

The study was conducted in all NCAH's seven branches (Figure 1) during 2013. Serum samples were collected from sheep and goats by the National Veterinary Services in 39 Libyan cities area (Figure 1). A two-stage sampling design was carried out. In the first stage, we calculated the number of flocks we needed to sample to detect a 2% prevalence with a 95% level of confidence (Dohoo, Martin, & Stryhn, 2003). We estimated the total number of flocks in Libya using an average herd size of 250 heads and 75% of animals bred in flock with more than 100 small ruminants. Finally, we randomly selected 148 farmers from all over the country that owned at least 100 heads. In the second stage, we collected 16 samples from each flock. This allowed us to detect a 15% prevalence with 95% of confidence; from each selected flock, a total number of 16 samples were collected (Martin, Shoukri, & Thorburn, 1992). If the selected flock had different age cohorts, we collected 16 samples from each age category as follows: group (I) including animals from 6 months to less than 12 months of age; group (II) including animals from 12 to 24 months of age; and group (III) including animals of more than 24 months of age. We collected a total of 3,508 samples, from 148 flocks, from 85, 55 and 8 farms of one age group, two age groups and three age groups, respectively. Some very interesting results can be seen when different age groups are compared and when the prevalence was observed within-herd variability at the different ages.

2.2 | Serological testing

We collected ~5 ml of blood from each animal. We collected blood from the jugular vein using identified Vacutainer tubes without an anti-coagulant. We stored the blood tubes in a cooler on wet ice and transferred to the Libyan Animal Health Laboratory within 24 hr of collection. We then centrifuged the clotted blood samples, and we aliquoted the sera into 2-mL cryovials and preserved at −20°C until use.

We detected antibodies to PPR using the ID Screen® PPR Competition ELISA kits supplied by IDvet innovative diagnostics (IDvet 310, France). The competitive ELISA is based on the reaction between a monoclonal antibody and a recombinant nucleoprotein expressed in baculovirus (Libeau et al., 1995). Optical density values observed in the presence of sera were converted to percentage of a negative control serum, and according to the manufacturer's ELISA cut-off, percentage values of ≤60% were considered positive.

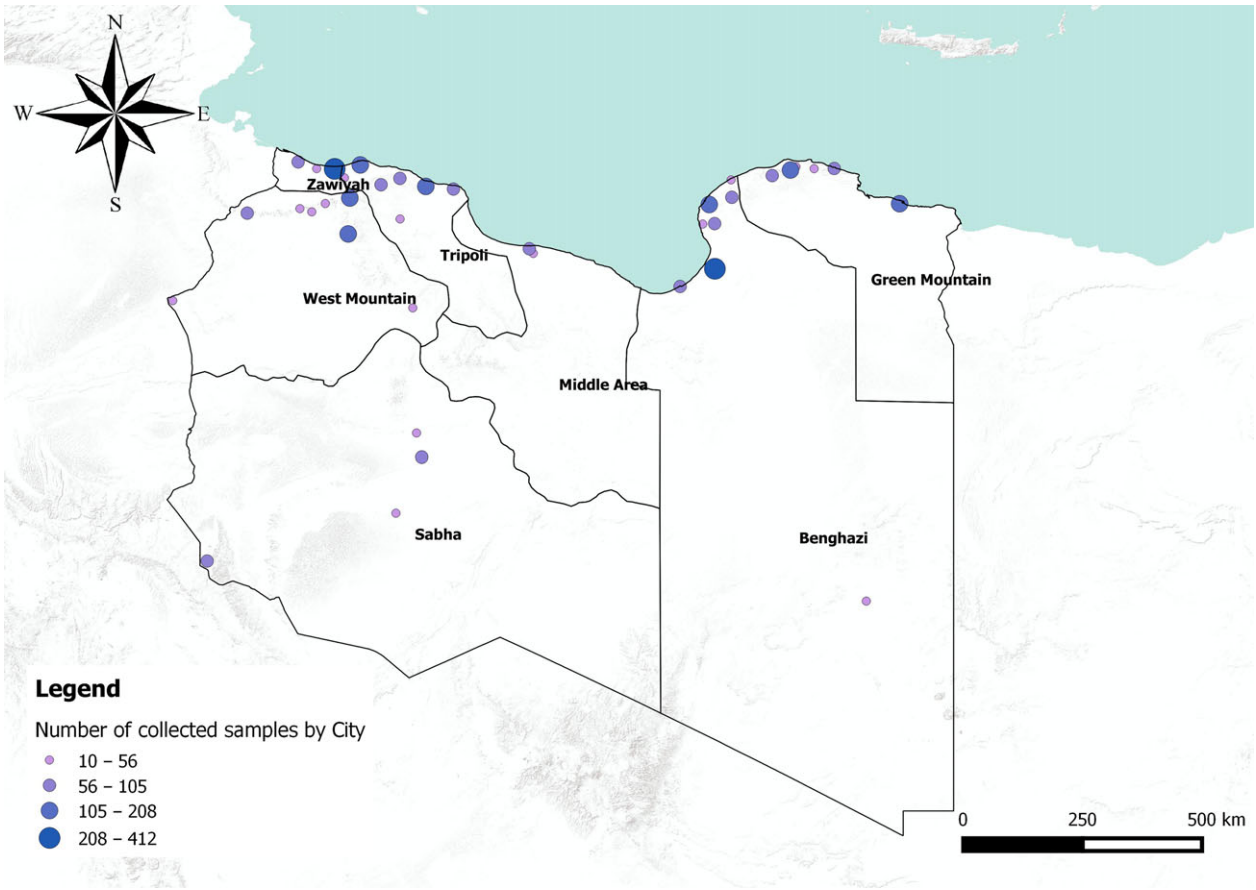

FIGURE 1 Map of Libya according to the administrative division into Branches with distribution of collected samples

2.3 | Data analysis

We entered the data into Microsoft Excel® spreadsheet and coded for analysis. Statistical analysis was performed using XLSTAT© (Addinsoft, New York, USA). We calculated the prevalence and 95% confidence intervals (CI) using a Bayesian approach based on beta distribution. If we detected least one positive animal in a one flock or a city, then this flock or city was considered as PPR positive. We used a Chi-squared test to verify significant differences in serological prevalence among branches, animal gender and age groups. In all statistical analysis, the confidence level was set at 95% and $p < .05$ set for significance.

None of the animals sampled were vaccinated against PPR. The risk factors assessed included administrative division (the Branch) and host factors such as age group and gender.

3 | RESULTS

The location and prevalence values base on our results are shown in Table 1 and Figure 1. We detected at least one infected flock in every city we sampled (38/38) with sero-prevalence ranged from 4.2% to 76.6% (Table 1 and Figure 2). The overall infected flock prevalence was 87.5% (126/148). Mean within-flock PPR prevalence was found to be 48.5% (95% CI: 36.8%–47.2%) with PPR sero-prevalence in an infected flock ranged from 3% to 100% (Table 1 and Figure 3).

The overall serological prevalence among small ruminants in the seven Branches was 33% (95% CI: 31.4–34.5). Significant differences between the prevalence in the geographical branches were observed (Table 1). When compared with the rest of branches, the lowest prevalence level (χ^2: 76.45, $p < .01$) was observed in Zawiyah branch (16.1%; 95% CI: 13.2%–19.6%), whereas the highest value (χ^2: 62.89, $p < .01$) was obtained for the Sabha branch (56.8%; 95% CI: 50.3%–63.0%).

Considering the age, a serological prevalence of 24.7% (95% CI: 22.1%–27.4%), 31.5% (95% CI: 29.1%–34.0%) and 42.1% (95% CI: 39.2%–45.0%) was observed in small ruminants from 6 months to less than 1 year, between 1 and 2 years and more than 2 years, respectively (Table 1). We detected statistically significant differences (χ^2: 75.07, $p < .001$) in the sero-prevalence levels among the age groups (Table 1), but we did not detect a significant difference by gender (χ^2: 1.27, $p = .260$) and we detected prevalences of 34.7% (95% CI: 31.3%–38.2%) in males and 32.5% (95% CI: 30.8%–34.3%).

TABLE 1 Number of tested and positive samples

	Tested samples	Prevalence (%) [95% CL][b]	df[a]	Chi-square	p-value
City area	**38**	**100**			
Flock	**148**	**86.5**			
Within flock		**48.5** [36.8–47.2]			
Branch area	**3,508**	**33.0** [31.4–34.5]	**6**	**169.51**	**<.001**
Green Mountain	571	34.0 [30.2–38.0]			
Benghazi	828	28.6 [25.6–31.8]			
Middle Area	96	27.1 [19.2–36.8]			
Zawiyah	509	16.1 [13.2–19.6]			
Tripoli	668	32.5 [29.0–36.1]			
West Mountain	607	44.5 [40.6–48.5]			
Sabha	229	56.8 [50.3–63.0]			
Age	**3,508**	**33.0** [31.4–34.5]	**2**	**75.07**	**<.001**
Less than 1 year	1,001	24.7 [22.1–27.4]			
Between 1 to 2 years	1,375	31.5 [29.1–34.0]			
More than 2 years	1,132	42.0 [39.2–44.9]			
Gender	**3,508**	**33.0** [31.4–34.5]	**1**	**1.27**	**.260**
Male	747	34.7 [31.3–38.2]			
Female	2,761	32.5 [30.8–34.3]			

[a]*df*: "degrees of freedom".
[b]CL: "confidence limits".
The bold font representing the overall variable measurement.

4 | DISCUSSION

PPR is one of the most relevant transboundary animal diseases that have a negative socio-economic impact at national and international levels for both countries where the disease is endemic and for countries with a high number of small ruminants.

At the global level, the OIE and the FAO have set the goal of eradicating the disease by 2030. PPR is also one of the priority diseases of the FAO–OIE Global Framework for the Progressive Control of Transboundary Animal Diseases (GF-TADs). Between 2012 and 2013, a FAO regional TCP project aiming at assisting the Maghreb countries (Algeria, Libya, Mauritania, Morocco and Tunisia) in the prevention and control of PPR was implemented. In Maghreb countries (except Libya where field activities could not be implemented), serological prevalence varied from 37% to 62% (http://www.fao.org/3/a-i4484e.pdf).

Due to the paucity of data and information on PPR virus circulation and PPR distribution in Libya, we focused our efforts to understand the possible extent of infection within the country and to preliminary investigate some possible risk factors linked to PPR infection in sheep and goats.

We detected the highest prevalence in the Sabha branch, in the south-west part of Libya. The high prevalence rate recorded in Sabha branch could be explained because this region is more exposed to cross-border animal movements with neighbouring countries. This could indicate increased transmission due to the illegal importation of animals into this southern region of Libya. In addition, many of these herds are nomadic which would also increase risk of contact with more herds.

Nomadic lifestyles are relatively common in some parts of Libya, especially in the south. The Tuareg and Toubou people inhabit southern Libya. The Tuareg peoples are a large group of nomadic peoples that inhabit a vast area in the Sahara, stretching from far south-western Libya (from Ghat to Ghadamis) to southern Algeria, Niger, Mali and Burkina Faso whereas the Toubou peoples inhabit northern Chad, southern Libya, north-eastern Niger and north-western Sudan. In Libya, the Toubou are found to the east of Fezzan (south of Libya), as well as in and around Tibesti Mountains. Their main towns include Tazerbu, Kufra, Bezzima, Qatroun and Tajerhi. Generally, the western side of the Libyan Sahara is inhabited by the Tuareg (alongside with the Algerian border), while the eastern side is inhabited by the Toubou (Shoup, 2011).

The introduction of the disease to the country could be explained by the illegal importation of animals from the south and west borders due to the movement of tribes with their animals from the neighbour countries, whereas the spread of PPR within Libya is more likely due to the internal animal's movements. The data on the sero-prevalence by city (Figure 2) showed that the highest sero-prevalence was found in Ghadamis 76.6% (boarder with Algeria and Tunis) and Ghat 69.4% (the nearest city to the boarder with Chad, Niger and Algeria) which are inhabited with Tuareg. The biggest city in the south is Sabha (75.3%) with big animal market. Gharyan (68.9%) is the biggest city in the West Mountain branch with large market for animals brought from the south and Ghadamis. Unfortunately, we only sampled one city inhabited by the Toubou peoples (Kufra 45.2%, south-east Libya with border with Sudan). The sero-prevalence of Sabha can be used as indirect estimation of the effect of illegal importation of animals as Sabha sero-prevalence is an aggregated of the PPR sero-prevalence of the surrounded cities with its own sero-prevalence.

The highest prevalence of infection was recorded in adult animals (more than 24 months), indicating that older animals have more chance to be exposed with infectious agent than younger animals. This finding confirms the endemic status of PPR in Libya, with a constant exposure to the infection of the animals during their life.

Maternal antibodies in young animals are detectable up to 6 months of age, but fell below the protection threshold level at 3.5 and 4.5 months in lambs and kids, respectively (Awa, Ngagnou, Tefiang, Yaya, & Njoya, 2002). In our study, the young class comprised animals aged between 6 months and 1 year (averaged 8.7 months). Therefore, it was unlikely that the serological positive response from animals of this age class was due to the presence of maternal antibodies. This aspect should be considered in further investigations for vaccination purposes that should be focused initially on high-risk group animals, for example young animals (6 months–1 year), goat or/and sheep population and migratory flocks (Singh, 2011).

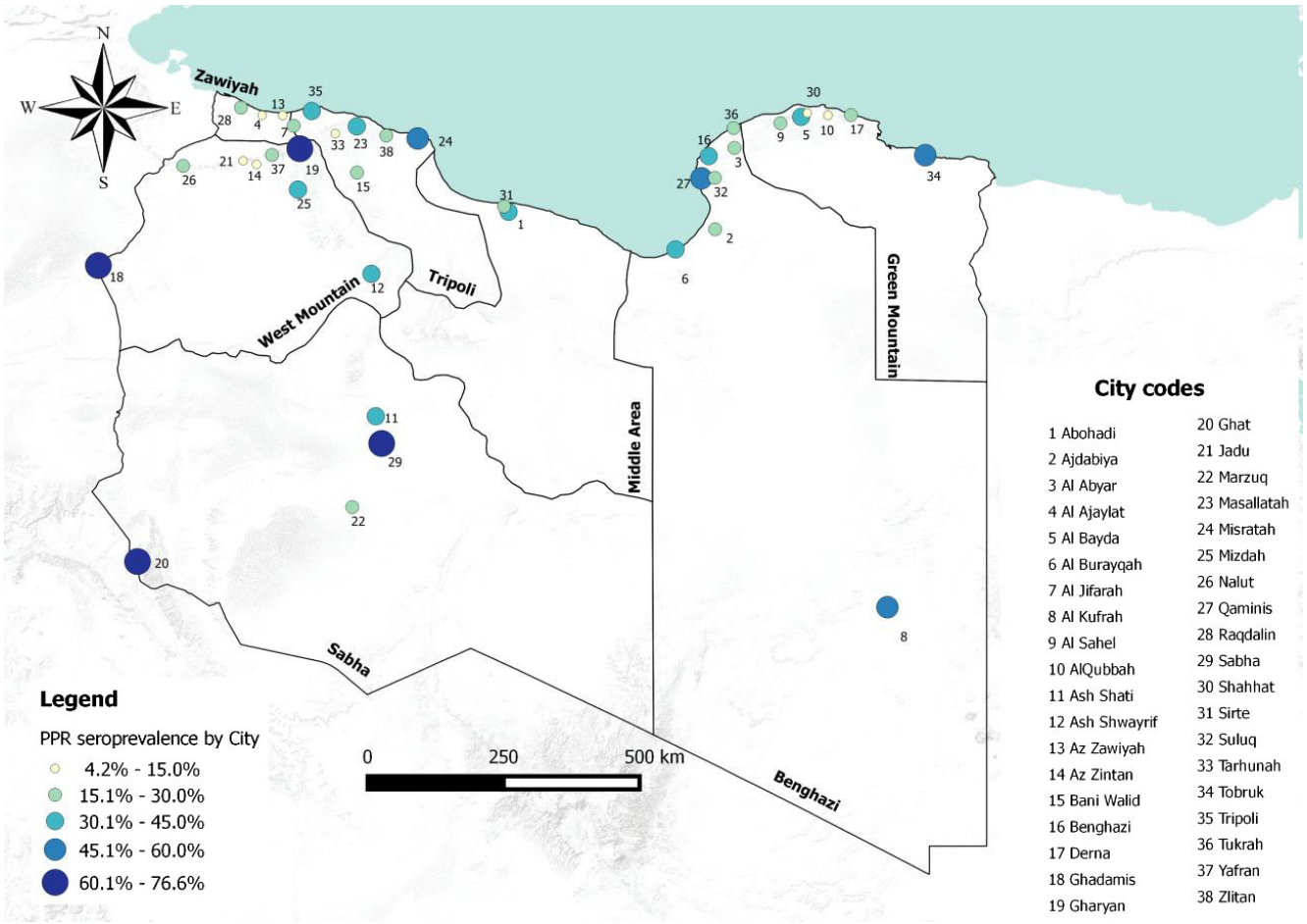

FIGURE 2 Serological prevalence of peste des petits ruminants by City in Libya

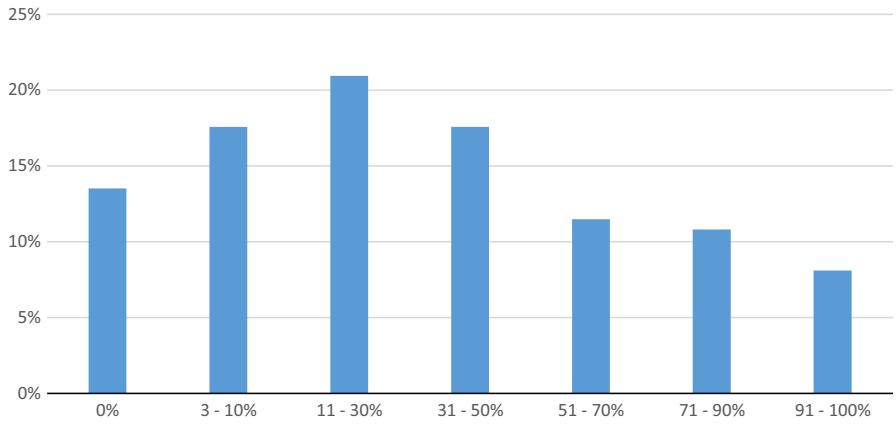

FIGURE 3 The relative frequency distribution of sero-prevalence of peste des petits ruminants within herd

The detection of antibodies in young animals (>6 months) in the absence of vaccination indicates that PPR was circulating in the population during the study period. Moreover, we can assume that Libya has experienced PPR virus circulation since 2011 at least or earlier, as high levels of serological prevalence were detected in animal older than 2 years.

No statistical differences were observed between males and females. This result is consistent with the epidemiology of PPR and could confirm that male and female small ruminants are equally exposed to the virus. Although goats and sheep are the primary hosts for the virus, goats seem to be more susceptible to the disease than sheep (Nanda et al., 1996), with some breeds of goat are considered to be more susceptible than others (Couacy-Hymann et al., 2007). Therefore, the species and the breed may play an important role in the epidemiology of PPR. In this study, a comparison between prevalence values in sheep and goats or in different breeds could

not be assessed, due to the lack of registration of this information during the blood sampling activities.

Furthermore, different prevalence rates of antibodies in cattle and buffaloes have been reported in various regions and countries (Balamurugan et al., 2012; Raghavendra et al., 2008; Zahur et al., 2011), suggesting the evidence of PPR infection in cattle and buffaloes (Abraham et al., 2005). Further studies should be conducted in Libya to understand also the virus circulation among cattle populations.

Higher levels of serological prevalence were reported in sub-Saharan countries such as Nigeria (55%; Lefèvre & Diallo, 1990), Cameroon (46.5%; Ekue, Tanya, Ndi, & Saliki, 1992), Sudan and Ethiopia (Abraham et al., 2005; Osman et al., 2009) than we detected in Libya.

PPR is currently present in some countries in the North Africa region, where the situation has evolved in recent years. The disease occurred for the first time in Morocco in 2008, with a virus belonging to lineage IV (a virus present notably in South Asia and the Middle East), and the same lineage IV is also present in Tunisia and Algeria and is widespread in Egypt. On the other side, in Mauritania, PPR infection has been linked to the circulation of virus of lineage I. As disease outbreaks have also been reported across North Africa during the past years, another relevant aspect that further researchers should consider in future epidemiological investigations is the identification of current circulating lineages in Libya. The molecular identification of PPRV lineages circulating in Libya is of paramount importance to elucidate the possible pathways of introduction from neighbouring countries. Furthermore, animal movements across the national borders should be mapped to design an effective national risk-based strategic control plan for PPR as well as for other transboundary diseases affecting livestock. Our findings seem to suggest that the southern part of Libya, namely Sabha branch, could be more exposed to the infections coming from the neighbouring countries and this should be better investigated to correctly identify wherever specific entry points can be considered at higher risk than others.

As different epidemiological situations may prevail in different countries, differential approaches are needed to be considered in accordance with a regional common strategy for PPR control and eradication.

In the framework of the global strategy for control and eradication of PPR, our results, even if obtained by a preliminary study, can contribute to the assessment of the epidemiological situation of PPR in Libya as required by the Stage 1 of the plan.

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