An epidemological study on Peste des petits ruminants in Tripoli Region, Lybia

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Veterinaria Italiana 2017, **53** (3), 235-242. doi: 10.12834/VetIt.964.5025.3
Accepted: 08.09.2016 | Available on line: 29.09.2017

Keywords
Libya,
Peste des petits ruminants,
Risk factors,
Seroprevalence,
Small ruminants,
Tripoli.

Summary
A cross-sectional study was conducted in Libya in 7 areas of Tripoli to determine the seroprevalence of Peste des Petits Ruminants (PPR) Virus (PPRV) in small ruminants (sheep and goats) between June and August 2013, and to identify the potential risk factors associated with the infection. The study involved 10% of small ruminant herds with ≥ 50 animals in the Tripoli region. They were selected randomly (15 herds), and 35 to 58 samples, depending on its size, were collected from each selected herd. Seven-hundred and twenty-one serum samples from unvaccinated animals (601 of sheep and 120 of goats) were collected and then tested using cELISA commercial kit in the National Center of Animal Health Laboratory in Tripoli, Libya. The overall seroprevalence was 46.7% [(sheep 44.3% (266/601) and goats 59.2% (71/120)]. Mean within-herd prevalence was 48.5% (95% CI: 32.1% - 64.8%), and the herd prevalence was 93.3% (14/15). Various risk factors at the animal and herd levels were analysed by multivariable logistic regression model (forward stepwise). The results identified breed, source of animal, and region as significant risk factors ($p < 0.05$). As for the source of new animal to the farm, PPRV seroprevalence was highest in illegally imported animals (90.9%), followed by the seroprevalence in animal legitimately acquired (55.8%), and by the seroprevalence in animals belonging to the same herd (4.7%). The seroprevalence among breeds was 69.5% (228/328) in illegally imported animals, whereas 27.7% (109/393) was found to be in local breed. Seroprevalence in the areas considered in this study was higher (66.2%) in Al-Mashroa area followed by Ein-zara (57.8%), Arada (50%), Ben-Own (47%), AL-Naem (37.5), Ber-Alalem (24.5) and in Tajora (0%). The results indicated that PPRV virus was actively circulating in Tripoli regions and that the illegal importing of animals was the main source of spreading PPR in Tripoli regions, showing that better efforts should be made to raise public awareness with respect to biosecurity.

Studio epidemiologico sulla Peste dei piccoli ruminanti nella regione di Tripoli, Libia

Parole chiave
Libia,
Peste dei piccoli ruminanti,
Fattori di rischio,
Sieroprevalenza,
Piccoli ruminanti,
Tripoli.

Riassunto
Tra giugno e agosto 2013 è stato condotto in Libia uno studio trasversale in 7 aree nella regione di Tripoli per determinare la sieroprevalenza del virus della Peste dei piccoli ruminanti (PPR) nelle pecore e capre della regione e per individuare i potenziali fattori di rischio associati all'infezione. Lo studio è stato condotto sul 10% (15 mandrie) degli allevamenti di piccoli ruminanti presenti nella regione di Tripoli che contavano ≥ 50 animali, gli allevamenti sono stati selezionati casualmente. Da ciascun allevamento sono stati prelevati da 35 a 58 campioni a seconda della dimensione della mandria. Sono stati raccolti complessivamente 721 campioni di siero da animali non vaccinati (601 pecore e 120 capre) e poi testati nel Centro Nazionale del Laboratorio di Salute Animale a Tripoli (Libia) utilizzando un'ELISA competitiva commerciale. La sieroprevalenza complessiva è stata del 46,7% (ovini 44,3% (266/601) e caprini 59,2% (71/120)], negli allevamenti del 93.3% (14/15) mentre all'interno degli allevamenti del 48.5% (95% CI: 32.1% - 64.8%). L'influenza dei vari fattori di rischio sulla prevalenza rilevata negli animali e in allevamento è stata analizzata con il modello di regressione logistica multipla (*forward stepwise*). I risultati hanno mostrato che

la razza, la provenienza dell'animale e la regione sono fattori di rischio significativi ($p < 0,05$). Se si considera la provenienza degli animali, la sieroprevalenza è stata più alta negli animali importati illegalmente (90,9%), seguita dalla sieroprevalenza rilevata in animali acquisiti legittimamente (55,8%) e da quella in animali nati in allevamento (4,7%). La sieroprevalenza tra le razze è risultata essere del 69,5% (228/328) negli animali illegalmente importati, mentre il 27,7% (109/393) si riscontra in razze locali. Secondo le aree geografiche prese in considerazione in questo studio, la sieroprevalenza è risultata superiore (66,2%) nell'area di Al-Mashroa, seguita da Ein-zara (57,8%), Arada (50%), Ben-Own (47%), AL-Naem (37,5) Ber-Alalem (24,5) e in Tajora (0%). I risultati hanno indicato che nelle regioni studiate il virus PPR circolava attivamente e che per quelle aree l'importazione illegale di animali costituisce la principale fonte di diffusione del virus PPR, dimostrando che occorrono maggiori sforzi per sensibilizzare l'opinione pubblica ad adottare tutte le misure igienico sanitarie necessarie per ridurre la diffusione di queste infezioni emergenti.

Introduction

Peste des petits ruminants (PPR), also known as 'goat plague', is a viral disease of goats and sheep characterized by fever, sores in the mouth, diarrheal, pneumonia, and sometimes death. It is caused by a morbillivirus of the family of paramyxoviruses, which is related to rinderpest, measles and canine distemper (Radostits *et al.* 2007). Cattle and several wild ruminants have been infected most often experimentally (Mornet *et al.* 1956), but goats and sheep are the common hosts. Peste des petits ruminants is a disease listed in the OIE Terrestrial Animal Health Code, and countries are obligated to report the disease to the OIE.

The PPR virus strains can be classified into 4 groups: 3 from Africa and 1 from Asia (Kwiatek *et al.* 2011). The Asian strain was recently introduced to some African countries including Cameroun, Central Africa Republic, Sudan, Morocco, Egypt, Tunisia, Algeria, and Uganda (Kwiatek *et al.* 2011).

The virus requires 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 (Taylor 1984, Bundza *et al.* 1988). Animals inhaling the droplets are likely to become infected.

To the best of our knowledge, despite the detection of PPR in all neighbouring countries – Tunisia (Ayari-Fakhfakh *et al.* 2011), Algeria (De Nardi *et al.* 2010), Tchad (Bidjeh *et al.* 1995), Sudan (Osman *et al.* 2009) and Egypt (Abd Elrahim *et al.* 2010) – no data were available concerning PPR in Libya. Therefore, the aim of this study was to investigate the seroprevalence of the disease in Tripoli region and to highlight the risk factors associated with PPR infection in sheep and goats on animal and flock levels.

Materials and methods

Study area and source of data

This cross-sectional study was conducted in the region of Tripoli (Libya). The region has a hot subtropical semi-arid climate with long, hot, and dry Summer with relatively wet and mild Winter. Temperatures vary between 18°C and 45°C throughout the year, the average annual rainfall is less than 400 millimeters and varies from year to year and from season to season. Tripoli region has been chosen for the study for several reasons, such as presence of large numbers of sheep and goats with a mass movement of small ruminants, the presence of many live animal markets, and wide pastures. The data regarding animal population were taken from the National Center of Animal Health (NCAH) of Libya.

Sampling protocol and the number of samples per farm

Sampling method was carried out according to the distribution of small ruminants in Tripoli areas; 10% percent of small ruminant herds, which have more than 50 animals were selected randomly from each area. For each farm, the number of samples necessary to detect 5% prevalence with 95% confidence was calculated with the following formula (Martin *et al.* 1992):

$$n \geq [1 - (1 - C^{\frac{1}{SexPrxN}}] x [N^{\frac{PrxN-1}{2}}]$$

Where n is the number of samples needed per farm, C is the confidence level, Se is the sensitivity of the test, Pr is the prevalence that should be detected and N is the total number of PPR susceptible animals on the farm. Thirty five to fifty eight samples were collected from each herd depending on herd size, as shown in Figure 1.

From each animal, about 5 mL of blood sample was collected from the jugular vein using identified vacutainer tubes without an anticoagulant. The blood tubes were kept in a cold box with ice and transferred to the Libyan Animal Health Laboratory within 24 hours from collection. The clotted blood samples were centrifuged and the sera were aliquoted in 2 mL cryovials and preserved at -20°C until use.

Risk factors and information were collected by filling the relevant questionnaires during the collection of blood samples to record data on farm location, history of recent movement of purchased animals, contact with other animals through shared grazing and water, feeding and breeding systems, source of animals, distance between the farm and nearest veterinary clinic, distance between farm and animal markets, and dealing with manure.

Seven areas within Tripoli region; 10% of farms with more than 50 animals were selected randomly.

Animals

Sheep and goats were identified, and the number of target samples to collect from each farm was calculated. Thus, a total of 721 animals (601 sheep and 120 goats) were tested for PPR. The sampled animals were local Libyan breeds (Barbary sheep and local goats breed) or illegally imported animals from Tunisia (Arbi goats breed). Animals used in this study were not vaccinated against PPR as both countries, Libya and Tunisia, do not include PPR vaccination in their vaccination programme (FAO 2012, OIE 2015). Therefore, all PPR positive sera will represent PPR infected animals.

Serological technique and statistical analyses

Serum samples were tested for the presence of PPRV antibodies by using ID Screen® PPR Competition ELISA kit [IDvet innovative diagnostics (IDvet) 310, Grabels, France]. The kit is based on PPRV nucleoprotein (NP) antigen and specific monoclonal antibody (Libeau *et al.* 1995). The relative sensitivity of the test is 94.5%; whereas the specificity is 99.4 % (Saliki *et al.* 1993).

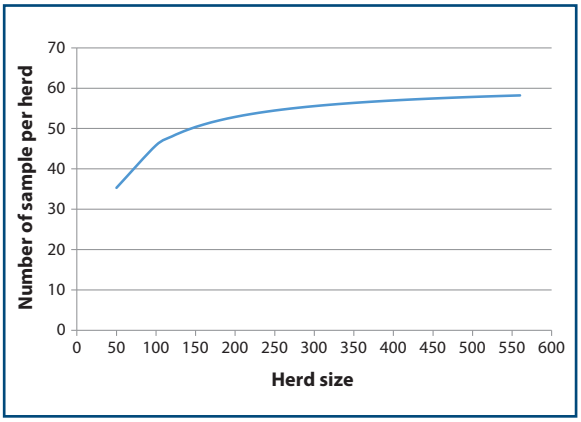

Figure 1. *The number of samples per herd according to its size.*

Optical density values were converted to inhibition percentage, according to the ELISA cut-off value, inhibition percentages of ≤ 60% were considered positive.

Data analysis was performed using the statistical software program SPSS for windows (Version 17.0 and SPSS Inc.).

Initially, descriptive analysis was used for the collected samples. Univariate analysis using Chi square (2 tailed) test was used to access the association between the occurrence of PPR and the suggested risk factors. For each variable, the p value and odds ratio (OR) with a 95% confidence interval were calculated.

Then logistic regression model was used to evaluate the association between all risk factors and PPR. All risk factors and other important variables [such as region, date of sampling, distance between farm and animal market, distance between farm and veterinary clinic, type and number of animals purchased during the last 12 months, the source of new animals, method of transport, species of live animals (sheep or goats) left at the farm during the past 12 months, dealing with manure of animal during the last 12 months, species of animals that were slaughtered on the farm during the past 12 months, visit to the farm during past year, and number of visits to the farm by veterinarians] were all included in the subsequent multivariable logistic regression model for small ruminant.

A forward Stepwise (Likelihood Ratio) method, with sequential manual removal of variables based on a lack of statistical significance and biological plausibility, was used to produce the best-fit model. For the factors to remain in the final model, a significance level was set at the likelihood ratio of p-value of 0.05 for entry and 0.10 for removal.

Results

Sample description

Sheep accounted for 83% (601/721) of the collected samples, 54% (390/721) of the samples were males; 36% (256/721), 38% (272/721), and 27% (193/721) of the samples were less than 12 month, 12-24 month, and more than 24 month old, respectively.

Seroprevalence

The herd prevalence was 93.3% (14/15). The mean of within-herd prevalence in infected herds for small ruminants was found to be 48.5% [32.1-64.8%] whereas the overall seroprevalence in this study was 46.7% (337/721).

By species: seropositive samples were 44.3% (266/601) and 59.2% (71/120) in sheep and goats, respectively.

By gender: seropositive samples were 50.3% (196/390) and 42.6% (141/331) for males and females, respectively.

By age: seropositive samples were 44.9% (115/256), 49.6% (135/272), and 45.1% (87/193) for age groups: less than 12 months, between 12 and 24 months, and more than 24 months of age, respectively.

The highest seroprevalence was 91% in imported animals; PPR antibodies were not detected in small ruminant populations in Tajora region as shown in Table I.

Table I. *Results of univariate associations with cELISA PPR seropositivity in small ruminants in Tripoli region.*

Variable	Total	Positive	Prevalence [CI]	*p-value*	Odds Ratio [CI]
Breed					
Local	393	109	27.7	0.0000	*0.16[0.12-0.23]*
Imported	328	228	69.5		
Species					
Sheep	601	266	44.3	0.0028	*0.5 [0.37-0.82]*
Goats	120	71	59.2		
Sex					
Male	390	196	50.3	0.04	1.36 [1.01-1.83]
Female	331	141	42.6		
Age					
< 12 months	256	115	44.9		
12-24 months	272	135	49.6	*0.84*	
> 24 months	193	87	45.1		
Breeding system					
Closed	422	218	51.7		
Open	52	16	30.8	0.0025	
Semi-closed	247	103	41.7		
Animal source					
Animal market	538	300	55.8		
Imported	33	30	90.9	< 0.001	
Same herd	150	7	4.7		
Region					
Enzara	173	100	57.8		
Arada	46	23	50.0		
Tajora	53	0	0		
Almshro	148	98	66.2	< 0.0001	
Benon	100	47	47		
Beralalem	49	12	24.5		
Anem	152	57	37.5		
Total	**721**	**337**	**46.7**		

[CI]: 95% Confidence Interval

Univariate association analysis for the risk factors

The result showed that all the important risk factors have a strong statistical association with the seropositivity, with the only exception of the age of the animals (Table I).

Multivariable regression

Only breed, source of animal, and region were identified to be significant risk factors (Table II).

Discussion

Small ruminants have an important role in the economy of the region, both as a source of income for farmers and as an important source of meat and wool in Libya. Located in North-Western part of Libya, Tripoli is characterised by a large population of small ruminants. The region was selected for conducing this study due to the presence of intensive illegal animal movement, high number of live animal markets, and a harbour favouring the import of live animals, as well as for the uncertainty about PPR situation in Libya.

In this study, the overall cELISA PPR seropositivity in small ruminant serum samples, collected from 7 investigated sub-regions of Tripoli, was 46.7%. This finding suggests that the disease may have been circulating in some areas in Libya without having been reported to the veterinarian authority and escaping the control of national disease surveillance systems, which, thus, proved to be inadequate. Epidemiological studies conducted in Middle East and Africa revealed that prevalence of PPR varies

Table II. *Final logistic regression model showing variables associated with seropositivity to PPR in small ruminants.*

Steps	Variables	Model Log Likelihood	Change in 2 Log Likelihood	df	Sig. of the change
Step 1	Breed	-498.226	128.968	1	.000
Step 2	Source	-433.742	68.950	1	.000
	Breed	-439.902	81.270	1	.000
Step 3	Source	-433.739	85.192	1	.000
	Breed	-411.227	40.168	1	.000
	Region	-399.267	16.247	1	.000

Veterinaria Italiana 2017, **53** (3), 235-242. doi: 10.12834/VetIt.964.5025.3

between 50 and 58% (Taylor 1979, Lefevre *et al.* 1991) – this is within our result range.

The PPR prevalence in our study was higher than those reported in some Libyan neighbouring countries. Seroprevalence reported in Tunisia was 8% (Ayari-Fakhfakh *et al.* 2011), 33% in Algeria (De Nardi *et al.* 2010), and 34 % in Tchad (Bidjeh *et al.*, 1995). Conversely, PPR prevalence in this study was lower than those reported in Sudan (51%) (Osman *et al.* 2009) and in Egypt (63%) (Abd Elrahim *et al.* 2010). The variation of seroprevalence reports could be attributed to several factors, such as differences in management system of small ruminants; different levels of immunity, types of diagnostic tests and sampling used, level of technical knowledge of the investigators (Roeder and Obi 1999, Singh *et al.* 2004a, Waret Szukuta *et al.* 2008), vaccination against PPR that leads to presence of antibodies or subclinical infection as earlier reported by Diop and colleagues (Diop *et al.* 2005).

Furthermore, cELISA PPR sero-positivities are influenced by many factors including: number of animals, age, time of sampling, animal husbandry and feeding.

There was no statistically significant difference among the age group PPR prevalences. The higher proportion of positive animals was recorded among animals between 1 and 2 year old. The high prevalence in young animals could stand for a recent circulation of the virus in the Country. Moreover, these findings also suggest that PPR has been circulating for a long time in the region. However, this result is in contrast with most studies carried out on PPR showing difference in age-susceptibility (El Rashid 1992, Saliki *et al.* 1993, Abubakar *et al.* 2011).

It was suggested that the PPR virus is highly immunogenic, as infected animals remain serologically positive for a long period. Similar infection rates in the age groups indicate an early exposure of the animals to the PPR infection. We conclude that the virus circulated for a long period in Tripoli region without being noted by the local veterinarians. Sarker and Hemayeatul (Sarker and Hemayeatul 2011) reported that PPR was significantly higher in young animals (31%) in comparison to suckles (13%) and adult (10%). Taylor (Taylor 1984) and Obi and colleagues (Obi *et al.* 1983) reported PPR infections most frequently in animals between 1 and 2 years of age. In enzootic areas, the prevalence is higher in older animals (Tounkara *et al.* 1996).

This study revealed that the prevalence of PPR in males (50.3%) was higher than females (42.6%). These results were almost similar to those reported by Abubakar and colleagues (Abubakar *et al.* 2008) in Nigeria, Sarker and Hemayeatul (Sarker and Hemayeatul 2011) in Bangladesh, Rahmanand and colleagues (Rahman *et al.* 2004) in Pakistan, Ozkul and colleagues (Ozkul *et al.* 2002) in Turkey who also found that males are apparently more prone to the infection than females. It has been hypothesised that males are more prone to disease because of genetic variation (Razmi *et al.* 2006). Nonetheless, Swai and colleagues (Swai *et al.* 2009) in Tanzania and Osman (Osman 2005) in Sudan found higher seroprevalence in females than in males. These results were explained considering that males are sold for meat earlier at 1 to 2 years of age, while females are used for flock reproduction and, therefore, kept for a longer period of time.

Imported breeds were found to be most susceptible to PPR infection than local breeds. The open grazing system in rural areas around water sources during the dry season may also play an important role in spreading the disease.

The prevalence of PPR was found to be significantly different among breeding system categories, animal kept in a close system (52%) were mostly infected, followed by semi-closed system (43%), and lower prevalence was found in the open system (31%). Taylor and Barrett found that close contact between infected and susceptible animals is the main mode of virus transmission (Taylor and Barrett 2007).

The interactive breeding between goats and sheep especially in high density grazing could affect the PPR prevalence.

The role of wild ruminants in transmitting PPR virus has been described in Pakistan (Zahur *et al.* 2008), in Ethiopia (Gopilo 2005), and in Saudia Arabia (Housawi *et al.* 2004). Infected small ruminant herds in open grazing systems can infect other herds in pastures and water points. This finding is in agreement with the observations conducted in Uganda (Kihu *et al.* 2010).

Seroprevalence of PPR was significantly associated with breeds. The number of imported animals is considerably increasing (in term of sheep and goat population) in the last 4 years due to uncontrolled animal movement through borders with neighbouring countries. This study revealed that prevalence in imported animals was 70% and in local animals 28%. Guinean breeds (West African dwarf, logoon, kindi, and Djallonke) are known to be highly susceptible to PPR virus (Abubakar *et al.* 2011). In Pakistan, the prevalence of PPR is higher in indigenous Black Bengal goats (27%) than in Jamunapari (12%) and exotic breeds (10%) (Mondal *et al.* 1995). This may be due to immunosuppression or cross breed vaccination (Mondal *et al.* 1995).

In the present study, local prevalence is ranging from 0% to 66%. The highest prevalence of PPR was found in Al-Mashroa (66%) followed by Ein-Zara (58%), Arada (50%), Ben-Own (47%),

Al-Naem (38%), Ber-Alalem (25%), and Tajora (0%). These dissimilarities among different areas may be attributed to variations in the husbandry system and biosecurity, and animal production practiced in each locality, to farm density and to number of illegally imported small ruminants in each region. They could also be attributed to the continuous animal movement from one locality to another. This finding is in agreement with that previously reported in Tanzania (Muse *et al.* 2012, Shuaib 2011). In Ethiopia, Szkuta and colleagues (Szkuta *et al.* 2008) explored the spatial distribution and risk factors of PPR and found that seroprevalence was very heterogeneous across regions, with prevalence rates ranging from 0% to 53%.

Uncontrolled animal movement throughout the country is one of the main causes of PPR infection and spread among small ruminant population. These results are in agreement with the findings of Zahur and colleagues (Zahur *et al.* 2008) in Pakistan. Also in Uganda, PPR seroprevalence ranged from 43% to 88% as observed in Mbulu, Siha, Longido, and Ngorongoro districts, while seroprevalence with less than 40% to none was found in Monduli, Karatu, and Simanjiro, respectively (Singh *et al.* 2004a).

The difference among seropositivity to PPR from one locality to another may be attributed to some factors including the management systems, biosecurity measures, and levels of immunity (Singh *et al.* 2004b, Waret-Szkuta *et al.* 2008). According to OIE report in 2011, variation in seroprevalence is probably related to the intensity of movement of livestock and trade of small ruminants. In addition, the higher seroprevalence in some localities could be due to the sample size or may be attributed to the number of PPR- affected animals from which blood samples were collected, as well as geographical differences.

Our results confirmed that the source of new animals to the farm is a risk factor: PPR seroprevalence was highest in illegally imported animals (91%) playing a major role as source of infection in sheep and goats in Tripoli, followed by the seroprevalence of animal legitimately acquired (55.8%), and by the seroprevalence of animals belonging to the same herd (4.7%). Difference in prevalence may be related to the intensity of trade of illegally imported small ruminants (Naeem *et al.* 2000). This study revealed that the introduction of new animals purchased from live animal market was one of the main sources for PPR infection and spread. This finding is in agreement with the results reported by Singh and colleagues (Singh *et al.* 2004a) and Muhammad and colleagues (Muhammad *et al.* 2009).

Visiting live animal markets was identified as a major risk factor (Shankar *et al.*, 1998). Peste des petits ruminants may be associated with introduction of recently purchased sick animals from markets or contact in a close/village flock with sheep and/or goats that had been sent to market, but returned unsold (Radostits *et al.* 2000). Introduction of new animals from animal market is also considered as main cause of PPR infection of small ruminants in Saudi Arabia (Anderson 1995, Taylor and Barrett 2007). According to anecdotal reports from the animal owners, illegally imported goats and sheep were widely used for breeding purposes. This might be the main cause for the higher percentages of PPRV seropositive animals found in Tripoli.

In Jordan, Abraham and colleagues (Abraham *et al.* 2005) reported that large size was a risk factor for PPR seropositivity in both sheep and goat flocks.

There was evidence that exposure of goats and sheep to PPRV was high and widespread, despite considerable variation among species. Since no vaccination has been carried out against PPR in Libya, our results confirm for the first time natural transmission of PPRV under field conditions in Tripoli, especially in the local breed. The seroprevalence of PPRV in Tripoli reflects the image of the disease in the Western part of Libya and breed, source of animal and region were identified to be significant risk factors.

In conclusion, the present study revealed illegally imported animals as the main source of infection in Tripoli. As most of the farms have no biosecurity measures and because of the lack of sufficient veterinary services in the local live animal markets, PPR and other diseases can spread easily. Further efforts should be carried out to raise public awareness with respect to this new ri-emerging disease. Research targeting virus isolation and molecular epidemiology, involving large areas of the country, and exploring seasonal occurrence is obviously needed to define the epidemiology of PPR in Libya.

Acknowledgements

The study was supported by the FAO project TCP/RAB/3302.

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