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Seroprevalence, distribution and risk factor for peste des petits ruminants (PPR) in Algeria

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ABSTRACT

Peste des petit ruminants (PPR) is a highly contagious and infectious viral disease of small ruminants with severe socio-economic implications. The disease was first reported in the Southern part of Algeria in 2011 and by February 2012 it has reached the central part of the country. Estimating national prevalence, distribution and identification of risk factors remains a key component in understanding the epidemiology and control of the disease. The present study was carried out between January and June 2014, to include a detailed description of flock and within-flock seroprevalence and risk association between PPR seropositivity and various flock management factors in Algeria. A total of 150 flocks randomly sampled across the country were investigated and 4552 serum samples were collected from 3336 sheep and 1216 goats, respectively. C-ELISA was used to detect the presence of antibodies in small ruminant animals as an indicator of PPRV exposure. The results showed an overall true flock seroprevalence of 30.45% [95% CI 23.76–37.14] with a mean of the true within-flock prevalence as 29.87% ± 2.11. The mean of the true within-flock prevalence in mixed flocks (12.93% ± 1.85) was however found to be significantly higher than sheep flocks (5.74% ± 1.06). Also the mean of the true within-flock prevalence was found to be significantly higher in adult (35.36% ± 3.13) compared to young animals (21.83% ± 2.47) and in females (33.11% ± 2.87) compared to males (22.14% ± 2.31). The univariate analysis revealed that PPR overall flock seroprevalence was significantly higher ($P < 0.20$) in large flock (50.61%) than in small flock (33.33%), in mixed flock (56.7%) than in sheep flock (35.35%) and in the flocks that had contact with other flocks (46.5%) compared to those who had not (30.6%). However the differences among studied regions and grazing system were not statistically significant. For the risk factor analysis, univariate analysis of variables followed by a multiple logistic regression identified mixed flocks [OR = 2.64, 95% CI 1.30–5.38; $P = 0.007$] and contact with other flocks [OR = 2.27, 95% CI 0.99–5.21; $P = 0.053$] as risk factors in the spread of the disease. In conclusion, this study revealed a high seroprevalence of PPR in Algerian small ruminants, therefore the establishment of early warning systems and comprehensive implementation of control measures are advocated to improve animal welfare and reduce economic losses.

1. Introduction

Peste des petits ruminants (PPR) is a disease of sheep and goats caused by a *Morbillivirus* that belongs to the family *Paramyxoviridae*. PPR is an important viral disease that is described as an acute, highly contagious and fatal disease of sheep and goats (Leferve and Diallo, 1990). Overtime, the disease has spread across Africa, the Middle East and Asia (Banyard et al., 2010; Kwiatek et al., 2011; Albina et al., 2013). The disease is characterized by high fever, stomatitis, purulent ocular and nasal discharges, pneumonia, and diarrhea with severe dehydration often leading to death (Diallo et al., 2007). The morbidity and mortality rates can be as high as 90% depending on some intrinsic and extrinsic factors (Ezeokoli et al., 1986). Considering the importance of sheep and goats in the socio-economic livelihood of the poor communities, PPR has been associated with the achievement of food security and poverty alleviation (Chauhan et al., 2009). Therefore, PPR has been classified as a notifiable disease by the World Organization for Animal Health (OIE).

However in 2011, De Nardi et al. (2011) demonstrated the presence of PPR virus (PPRV) circulating in Sahrawi refugee camps

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Table 1
Flock selection according to the distribution of small ruminants in the five delimited Algerian regions.

Region	Latitudes	Longitudes	Number of small ruminants	% of small ruminants	Number of selected flocks	% of selected flocks	Number of selected animals	% of selected animals
North-Central	35.3°–36.8°N	1°E–4.7°E	3.72 millions	13%	20	13.34%	617	13.55%
North-Western	35°–36.3°N	2°W–1°E	3.55 millions	11%	17	11.33%	541	11.90%
North-Eastern	35.3°–37°N	4.7°E–8.5°E	4.87 millions	15%	23	15.33%	695	15.26%
Steppe	33°–35.3°N	2°W–8.5°E	17 millions	55%	80	53.34%	2373	52.13%
Sahara	19°–33°N	8.8°W–12°E	1.86 millions	6%	10	6.66%	326	7.16%
Overall			31 millions	100%	150	100%	4552	100%

in Tindouf district southwestern border of Algeria with Western Sahara, Mauritania and Morocco. A year later, Kardjadj et al. (2015) described the first serological and molecular typing of the PPRV strain implicated in an outbreak in Ghardaïa district, in the center of Algeria. The strain was clustered with lineage IV of PPRV and shared 97–99% similarity with the strain implicated in neighboring Morocco and Tunisia. These findings stress the importance of an epidemiological survey at a national level to establish the status of the disease in Algeria. As a result, a national survey was carried out to establish the status of the disease in Algeria and to recommend an adequate control strategy.

In Algeria, small ruminants are one of the main sources of meat production (≈31 millions heads; 26 millions sheep and 4 millions goats) and consequently, an important source of financial income for a large number of Algerian families. The fact that both sheep and goats are not vaccinated against PPRV makes this disease a serious threat to the livelihood of Algerian farmers. Therefore, the aim of this study was to conduct a nationwide study (between January and June 2014) in order to estimate the flock and within flock seroprevalence of PPR infection in Algerian small ruminants' population at the national level, to describe its regional distribution and to identify risk factors associated with its seropositivity in the country.

2. Materials and methods

2.1. Study area

Algeria is the largest country in Africa and the Mediterranean Basin. It is located between latitudes 19° and 37°N and longitudes 9°W and 12°E. It has more than 1600 km coastline at the Mediterranean Sea; where most of the coastal area (northern region) is hilly and sometimes even mountainous. South of the northern region is a steppe landscape and farther south is the Sahara desert. Administratively, Algeria is divided into 48 districts (wilayas) but for study purposes and based on the geographical and agro-ecological peculiarities, five regions were delineated (Table 1) where each region contained 7–12 districts; north-central (11 districts), north-western (7 districts), north-eastern (10 districts), steppe (12 districts) and Sahara region (8 districts).

2.2. Study design and sample collection

A cross-sectional study with a two-stage selection designed as described by Toma et al. (2009) was carried out across the country between January and June 2014 to investigate PPR flock seroprevalence in Algerian small ruminants' flocks. The simple size ($n = 150$ flocks) and the number of animals to be sampled within each flock ($m = 30$ animals) was determined at a 95% confidence level using an expected prevalence of 27% (Kardjadj et al., 2015), an absolute precision of 6.6 % and an estimated within-class coefficient $P = 0.8$ with an inflation coefficient of 25. The number of flocks and animals to be sampled from each region was proportional to the percentage of small ruminants in that region (Table 1). Flocks were selected from each region using random numbers generated by an electronic calculator and within each flock; animals were selected randomly by lottery.

A total of 4552 blood samples were collected (3336 sheep and 1216 goats) via jugular venous puncture and 5 ml blood was collected using sterile vacutainer tubes and needles (Venoject, UK). Blood samples were allowed to coagulate and transported on ice to Institut National de Médecine Vétérinaire (INMV), Algiers, Algeria for analysis. The samples were centrifuged and the serum separated into a sterile tube and stored at −20 °C until tested.

2.3. Data collection

A tested questionnaire was administered in an interactive approach to all selected flocks with the principal objective of revealing the multi-factorial background of the disease. Flock owners participating in the study were informed about the purpose of the study and their consent was obtained. The questionnaire contained an individual animal's information such as species (sheep, goats), age (young < 18 months and adult > 18 months), sex (male, female) and flock management risk factor attributes including size (small flocks < 100 heads, large flocks > 100 heads), grazing system (sedentary and transhumant flocks), history of contact with other flocks (yes or no), and type of flocks; sheep flock (sheep only) and mixed flock (both sheep and goats). There was no goat alone flocks in the study area.

2.4. Laboratory analysis

Detection of antibodies to PPRV in sheep and goats sera was analyzed using a competitive ELISA (c-ELISA) according to the manufacturer's instructions (ID Screen® PPR Competition, ID vet, Montpellier, France). This diagnostic kit detects antibodies against the nucleoprotein of PPRV with an individual level sensitivity (Se) and specificity (Sp) of 94.5% and 99.4% respectively (Libeau et al., 1995).

Briefly, PPR antigen was diluted in a coating buffer (PBS-0.01 M, pH 7.4) and each well of the microtitre plate charged with 50 μl of diluted antigen followed by 1 hour of incubation at 37 °C. After the incubation, the plates were washed to eliminate non-specific antibody binding. Anti-NP-HRP conjugate was added and then incubated followed by a repeated washing. Substrate was added into each well and the samples were considered negative when color change was observed. Sulfuric acid was added to stop the colorimetric reaction. Optical Density (OD) was measured at 450 nm and calculations were done using the following formula to determine the Inhibition Percentage (PI) PI = 100×(OD sample/OD negative control). The cut off for seropositivity used was ≤50 per cent as recommended by the manufacturer (ID Screen® PPR Competition, ID vet, Montpellier, France). Test samples having PI values between 50 and 60 per cent were considered doubtful (as recommended by the manufacturer). All samples were analysed in duplicates for confirmative purposes.

Table 2
Distribution of the 64 positive flocks in the five studied region according to the gravity of PPR apparent within-flock prevalence.

Apparent within flock prevalence	North-Central	North-Western	North-Eastern	Steppe	Sahara	Overall
<20%	2	3	2	8	1	16
20–40%	4	4	4	18	3	33
40–60%	3	4	1	7	0	15

2.5. Statistical analysis

Any flock with at least one animal found seropositive by c-ELISA was considered positive. The apparent within-flock prevalence was obtained for each "positive flock" by dividing the number of positive animals by the number of animals tested. The flock true prevalence was calculated using the following formula: Flock true prevelance = FAP + fsp − 1/fSe + fSp − 1 where FAP is the flock Apparent Prevalence and fSp and fSe are test flock specificity and sensitivity; $fSp = Sp^m$ and $fSn = 1 - (AP)^m$. The true within flock prevalence was calculated using the following formula: WAP + Sp − 1/Se + Sp − 1 where WAP is the mean of the within-flock apparent prevalence and Sp and Se were the test individual specificity and sensitivity (Rogan and Gladen, 1978). The comparison between the means of the within flock prevalence was carried out by a Student *t*-test using SPSS software version 20.0.

Confidence Interval (CI) at 95% = *P* +/− Pa, where *P* is obtained prevalence and Pa is the absolute precision, was determined for a two-stage random sampling, taking into account the variability that is likely to exist between and within flocks, using the following formula: $Pa = 2 \times \sqrt{(1 + pm) \times p(1 - p)/nm}$ where *p* is a within-class coefficient, *n* is the flock size and *m* is the mean of sampled animals within each flock (Toma et al., 2009).

For the risk factor analysis, an initial exploratory analysis of the data (univariable) was conducted for selection of variables with $P \leq 0.2$ by the chi-square test or Fisher's exact test; subsequently, the variables that passed this cut-off were subjected to logistic regression (Hosmer and Lemeshow, 2000). The fit of the final model was verified with the Hosmer and Lemeshow test, and collinearity between independent variables was verified by a correlation analysis. For those variables with a strong collinearity (correlation coefficient > 0.9), one of the two was excluded from the multiple analysis according to biological plausibility (Dohoo et al., 1996). The calculations were performed using SPSS software version 20.0. The variables were considered as risk factors when the odds ratio is greater than 1 and the *P* value is less or equal to 0.05.

3. Results and discussion

Although infection with PPRV in Algeria was previously described (De Nardi et al., 2011; Kardjadj et al., 2015) but the epidemiology of the disease was not well understood. The current study was to our knowledge the first to address the flock and the within flock seroprevalence of PPR in Algeria, its distribution across the country and risk factors associated with PPRV seropositivity in the Algerian small ruminant's flocks.

3.1. Seroprevalence estimation

3.1.1. Flock level

The overall apparent flock seroprevalence of PPR was 42.66% [95% CI 34.67–50.65]. After adjusting to the PPR c-ELISA flock sensitivity and flock specificity, the true flock seroprevalence was 30.45% [95% CI 23.76–37.14]. Our results showed that PPR is widely prevalent in the Algerian small ruminant's flocks. Such a high prevalence implies the contagious nature of the disease, covering wide geographic areas and infecting perhaps most of the susceptible animals in affected flocks as the disease spreads from the index case at the southern borders in 2011 due to trade and free movement of animals. Agreeably, several authors had previously reported high prevalence in some African countries such as Cameroon and Nigeria (Majiyagbe et al.,1992), Ethiopia (Abraham et al., 2005; Megersa et al., 2011), Burkina Faso (Sow et al., 2008), Tanzania (Swai et al., 2009), Morocco (FAO, 2009), Sudan (Saeed et al., 2010), Tunisia (Ayari-Fakhfakh et al., 2010, Mauritania (El-Arbi et al., 2014) and in Asian countries such as Turkey (Ozkul et al., 2002), Saudi Arabia (Al-Afaleq et al., 2004), India (Singh et al., 2004; Khan et al., 2008; Raghavendra et al., 2008), Jordan (Al-Majali et al., 2008), China (Wang et al., 2009) and Pakistan (Zahur et al., 2011).

3.1.2. Within-flock level

The mean of the apparent within-flock prevalence was 29.87% with a standard deviation of 2.11 and a median of 28.81%. The range of the apparent within-flock prevalence was between 16.66% and 53.33%. Seventy five percent (75%) of the tested flocks have apparent within-flock prevalence greater or equal to 20% (Table 2). The mean of the true within-flock prevalence was 31.8% with a standard deviation of 2.12%. The mean and the median of the true within-flock prevalence were very close and estimated at 31.8% and 28.5%, respectively. Our findings showed that PPR is extensively present in small ruminants' flocks in Algeria. Such high within-flock prevalence underlines the notable contagious nature of the disease.

The mean of the true within-flock prevalence in mixed flocks (12.93% ± 1.85) was found to be significantly higher (*P* = 0.012) than in sheep flocks (5.74% ± 1.06). A number of researchers in Jordan, Ethiopia, Tanzania and Tunisia (Al-Majali et al., 2008; Waret-Szkuta et al., 2008; Swai et al., 2009; Ayari-Fakhfakh et al., 2010) reported higher sero-prevalence in goats than sheep. However, inside the positive mixed flocks, the mean of the true within-flock prevalence in sheep (5.55% ± 1.23) was found to be statistically comparable (*P* = 0.63) with goats (7.37% ± 1.15). The seropositivity difference between sheep and goats remains debatable in literature; conversely, some authors have reported a higher seroprevalence in sheep than in goats in Turkey, India and Burkina Faso (Ozkul et al., 2002; Khan et al., 2008; Sow et al., 2008).

The mean of the true within-flock prevalence in adult animals (35.36% ± 3.13) was found to be significantly higher (*P* = 0.027) than in young animals (21.83% ± 2.47). This observation is common for many infectious diseases as older animals exposed to infectious agents from the environment for a longer period tends to seroconvert and to be better protected than the young (Singh et al., 2004; Sow et al., 2008; Al-Majali et al., 2008 Zahur et al., 2011).

The mean of the true within-flock prevalence in females (33.11% ± 2.87) was found to be significantly higher (*P* = 0.019) compared to males (22.14% ± 2.31). Abubakar et al. (2009) attributed the difference in seroprevalence between sexes to physiological differences where females reveal some degree of infection preponderance as a result of production and reproduction related stresses. Furthermore, Waret-Szkuta et al. (2008) and Megersa et al. (2011) reported in a PPR national survey in Ethiopia that for reproductive purposes, females get older than males and invariably exposed to several diseases due to production and reproduction stresses compared to males which only a few are favored within a flocks.

Table 3
Individual animal PPR positivity rates in the five studied regions according to species, age, and sex.

Variables		North-Central	North-Western	North-Eastern	Steppe	Sahara	Positivity overall
Species	Sheep	(66/475) 13.99%	(83/401) 20.69%	(28/503) 5.56%	(168/1756) 9.56%	(17/201) 8.45%	(362/3336) 10.85%
	Goats	(31/142) 21.83%	(45/140) 32.14%	(23/192) 11.97%	(95/617) 15.4%	(20/125) 16%	(214/1216) 17.59%
Age	Young	(27/238) 11.34%	(40/213) 18.77%	(14/282) 4.96%	(80/866) 9.23%	(8/128) 6.25%	(169/1727) 9.78%
	Adult	(70/379) 18.46%	(88/328) 26.28%	(37/413) 8.95%	(183/1507) 12.14%	(29/198) 14.64	(407/2825) 14.4%
Sex	Male	(16/153) 10.54%	(23/137) 16.78%	(17/236) 7.2%	(63/699) 9.01%	(5/95) 5.26%	(124/1320) 9.39%
	Female	(81/464) 17.45%	(101/404) 26%	(34/459) 7.4%	(200/1674) 11.94%	(32/231) 13.85%	(452/3232) 13.98%
Individual animal overall		(97/617) 15.72%	(128/541) 23.66%	(51/695) 7.33%	(263/2373) 11.08%	(37/326) 11.34%	(576/4552) 12.65%

3.1.3. Individual animal level

As a result of the fact that small ruminants kept in flock's makes individual animal's seroprevalence invalid for comparison. Therefore we could not compare the PPR seroprevalence at the individual animal level. However Table 3 presents a detailed description of the positivity rates among sheep and goats, males and females, adults and young animals.

3.1.4. Distribution

The flock seroprevalence of PPR in the north-western region of Algeria was the highest (64.7%), following by the north-central (45%), the steppe (41.2%), the Sahara (40%) and the north-eastern region (30.4%). This difference could be attributed to the fact that the north-western region borders two endemic countries Morocco and Mauritania, besides African borders are considered to be very porous and free animal movement is practiced frequently. However, this differences in PPR seroprevalence among the studied regions was found not significant ($P = 0.295$) (Table 4) suggesting a relatively high and uniform distribution of PPR seroprevalence among Algeria's five regions. Arguably, this could be due to sample size deficiencies to estimate the distribution of PPRV infection in Algeria or to animal's movement that can aid the dissemination of the virus.

3.2. Risk factors for infection

Cross sectional studies helps to evaluate both the presence of disease and the risk factors associated with the disease at the same time. Therefore, cross sectional studies take a snapshot of the situation at a specific moment (Toma et al., 2009). The use of cross sectional study as a tool for the evaluation of risk factors is adequate mainly for constant factors. Actually, in this study all the investigated factors were constant.

3.2.1. Univariate analysis

An initial exploratory analysis of the data with the univariate analysis was conducted for the selection of variables with $P \leq 0.2$ by chi-square test or Fisher's exact test; subsequently, the variables that passed this cut-off were utilized in the risk factor study by logistic regression.

PPR seropositivity (Table 4) was observed to be significantly higher ($P = 0.049$) in large flocks (50.6%) compared to smaller flocks (33.3%). Similar observation was reported in Turkey and Jordan by Ozkul et al. (2002) and Al-Majali et al. (2008). Moreover, Albina et al. (2013) reported that high population density and intensive farming system might increase the chances of contracting contagious diseases.

Our study showed that the seropositivity was significantly higher ($P = 0.019$) in mixed flocks (56.9%) than in sheep flocks (35.4%). Similar findings have been reported by other investigators such as Anderson et al. (1991) and Al-Majali et al. (2008) in Northern Jordan. In addition, Lefevre and Diallo (1990) reported that goats are more sensitive to PPRV therefore; the presence of goats in a sheep farm may increase the risk of PPR transmission.

The findings of this study also reported a higher seroprevalence in transhumant flocks (53.1%) compared to sedentary flocks (39.8%) but without been statistically significant ($P = 0.251$). Other researchers' have observed a higher PPR seropositivity in transhumant flocks compared to sedentary flocks (Zahur et al., 2011; Megersa et al., 2011). Arguably, this may be due to the fact that the number of transhumant flocks tested in our study may not be enough to demonstrate significant differences between the two grazing system. Further studies are therefore advocated to establish the role of the transhumance on the seroprevalence of PPR in Algeria. Our findings also reported that PPR seropositivity (Table 4) is significantly higher ($P = 0.13$) in flocks that had contact with other flocks (46.5%) compared to those who have not (30.6%). Our results are in agreement with that of Al-Majali et al. (2008) and Zahur et al. (2011) in Jordan and Pakistan. They demonstrated that contact between flocks increases the chances of transmission among different flocks.

At the end of the univariate analysis (Table 4); large flocks, mixed flocks and flocks that had contact with other flocks were subjected to logistic regression for the risk factor study as potential risk factors.

Table 4
Univariate analysis for risk factors associated with PPR flock seropositivity.

Variables	Categories	No. of flocks sampled	No. of positive flocks (%)	*P*
Region	North-Cenral	20	9 (45%)	0.295
	North-Western	17	11 (64.7%)	
	North-Eastern	23	7 (30.4%)	
	Steppe	80	33 (41.2%)	
	Sahara	10	4 (40%)	
Flock size	Small flocks	69	23 (33.3%)	0.049*
	Large flocks	81	41 (50.6%)	
Type of flocks	Sheep flocks	99	35 (35.4%)	0.019*
	Mixed flocks	51	29 (56.9%)	
Grazing system	Sedentary flocks	118	47 (39.8%)	0.251
	Transhument flocks	32	17 (53.1%)	
Contact with other flocks	No	36	11 (30.6%)	0.136*
	Yes	114	53 (46.5%)	

* Variables selected and used in the multiple analysis ($P \leq 0.2$).

Table 5
Risk factor (logistic regression final model) associated with PPR flock seropositivity.

Risk factor	*B*	SE	Odds ratio	95% CI	*P*
Mixed flocks	0.972	0.363	2.64	1.30–5.38	0.007
Contact with other flocks	0.821	0.424	2.27	0.99–5.21	0.053
Constant	−1.269	0.414	–	–	–

Hosmer and Lemeshow test: chi-square = 4.145; df = 2; $P = 0.126$, $R^2 = 0.108$.

3.2.2. *Multivariable analysis (logistic regression)*

Surprisingly, the risk factor analysis using multivariable logistic regression did not recognize the flock size as a risk factor for PPR seropositivity. Dissimilar observation was reported previously by Ozkul et al. (2002) in Turkey where larger flocks health management is more difficult to maintain compared to small flocks which eventually increases the chances of contracting infectious diseases.

However our study showed that mixed farming was identified as a risk factor (Table 5) and the odds of being seropositive to PPR was 2.64 [95% CI 1.30–5.38; $P = 0.007$] times higher in mixed flocks compared to sheep flocks. Our findings also identified contact with other flocks as a risk factor for PPR seropositivity (Table 5). The odds of being seropositive to PPR was 2.27 [95% CI 0.99–5.21; $P = 0.053$] times higher in the flocks who had contact with other flocks compared to those who had not. Raising sheep with goats and contact with other flocks were found to be risk factors for PPR seropositivity by several investigators (Anderson et al., 1991; Al-Majali et al., 2008; Zahur et al., 2011; Megersa et al., 2011).

In conclusion, our findings documented serological evidence of a widespread distribution and endemic establishment of PPR in Algerian small ruminant population. The risk factors implicated in the spread of the disease in the Algerian scenario were mixed flocks and contact with other flocks. Therefore, the establishment of early warning systems and proper implementation of control measures are needed, including regular surveillance and vaccination, to improve animal welfare and reduce economic losses associated with outbreak episodes.

Conflict of interest

The authors declare that they have no conflict of interest.

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