Evidence and predisposing factors of Peste des Petits Ruminants in Nineveh Governorate, Iraq

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ABSTRACT

The objective of this epidemiological investigation was to explore the evidence of Peste des Petits Ruminants (PPR) and to model the factors associated with the disease in sheep and goats in Nineveh Governorate, Iraq. A total of 400 animals (335 sheep and 65 goats) Nineveh Governorate, Iraq, were randomly selected. The PPR was found in them, when their serum samples were tested positive using the sheep PPR ELISA kit. Results indicated that 49% of sheep exhibited anti-PPR seropositivity, while only 12% of goats were positive. Anti-PPR seropositivity was 38% and 81% in the east and west sides of the Governorate, respectively. The odds of anti-PPR seropositivity were 2.00 and 6.11 times greater in ages 6-12 and >12 months, respectively, compared to those <6 months. The odds of anti-PPR seropositivity were 4.46 times greater in females compared to males, and about two times higher in flocks with <50 or 50-100 heads compared to those with >100 heads. In conclusion, the presence of PPR was considerable in sheep and goats in Nineveh Governorate, Iraq, with age, sex, and flock size being potential predisposing factors for the infection.

Keywords: PPR, Nineveh, Iraq

INTRODUCTION

Many viral diseases affect sheep in Mosul city (Al-Baroodi *et al*., 2022), and one of the most important is PPR is a contagious disease affecting small ruminants caused by the PPR virus; a Morbillivirus belonging to Paramyxo viridae (Parida *et al*., 2015). The main method of transmission among susceptible animals is inhaling aerosols exhaled, sneezed, or coughed from the affected animals (Jones *et al*., 2020). The disease is characterized by different gastrointestinal and respiratory symptoms, as the virus has the affinity to both lymphoid and epithelial tissues where the initial multiplication occurs at the site of entry within the local lymph nodes, and subsequently, the virus enters the circulation resulting in viremia and targeting the epithelial cells of the alimentary and respiratory systems (Pope *et al*., 2013 and Constable *et al*., 2017). While the disease shows a moderate course of illness in sheep, it can lead to death in goats due to severe diarrhea and dehydration, and the animals of four months to one year age are more severely affected compared to other ages, highlighting the economic importance of the disease, particularly in the countries where sheep and goats are considered an essential source of animal protein (Constable *et al*., 2017).

PPR became a transboundary and widespread devastating disease affecting small ruminants in Africa, the Middle East, the Arabian Peninsula, and Asia (Dhar *et al*., 2002 and Munir, 2015). The disease is present in Iraq and its surrounding countries. In Kuwait, an outbreak was reported in 1989 (Benfield et al., 2023). Moreover, the first instance of the disease in Turkey was observed in 1993 in a flock of 6 to 7-month-old lambs from the eastern region (Alcigir et al., 1996), although the first official documentation of the disease was in 1999 (Özkul *et al*., 2002). In Iran, the first outbreak was reported in 1995 in Ilhan area, a border province in Iraq (Bazarghani *et al*., 2006). In Iraq, the first sero-evidence of the PPR was reported in 1997 in sheep in central and northern parts of the country, and it was

officially documented first time in 1998. Finally, although there is a lack of official report of PPR in Syria, the disease has become endemic in bordering and other surrounding countries (Benfield *et al*., 2023).

Evidence of PPR is usually investigated using serological methods, although definitive diagnosis is currently based on molecular techniques. Different types of ELISA demonstrated sensitivity and specificity greater than 90% compared to the virus neutralization test (Singh *et al*. (2004a), Singh *et al*. (2004b) and Balamurugan *et al*. (2007). These tests are basically based on detecting antibodies to the PPR virus protein, which is the N-protein, a major and most conserved protein or the H-protein, a surface glycoprotein responsible for recognizing the receptors of the host cells (Renukaradhya *et al*. (2002) and Dechamma *et al*. (2006). In Mosul, a competitive ELISA was used to study the prevalence and risk factors of the disease in sheep and goats in the city (Hussain, 2021), where serum samples were collected in the year of 2013 and about 47.5% of sheep and 49% of goats exhibited seropositivity against PPR, with evidence of risk of the disease in older animals than 1-year-old ones. Further studies are required to estimate the evidence of the disease in the entire Nineveh Governorate, and to thoroughly examine the epidemiological factors associated with the disease in the governorate, particularly since the disease is present in the neighbouring Iraqi Governorates and countries (Benfield *et al*., 2023). Therefore, the objective of this investigation was to explore the evidence of PPR and examine the epidemiological factors contributing to the spread of the disease in sheep and goats in Nineveh Governorate, Iraq.

MATERIALS AND METHODS

Ethical Approval

The present study was approved by the scientific committee at the Department of Microbiology, College of Veterinary Medicine, University of Mosul. The study animals were handled, and the blood samples were collected according to standard procedures.

Study Animals

A total of 400 animals, including 335 sheep and 65 goats from different regions in Nineveh Governorate, Iraq, were used in the study. Study animals were raised by private owners. The study flocks included different age groups, sexes, and breeds. Some flocks consisted of a mix of both sheep and goats. The animals were usually kept in the open and grazed mainly on the natural grassland growth throughout the year. Some flocks were vaccinated against PPR and Enterotoxemia.

Data collection

The data collected include: type (sheep, goat), sex (male, female), age (months), geographical location, (east, west; relative to Tigris River), source (local, imported), animals in the flock (sheep, goats, mixed), flock size (< 50, 50-100, > 100), PPR vaccination history (yes, no), in addition to the clinical findings related to the disease like mouth lesions (yes, no), respiratory symptoms (yes, no), and diarrhea (yes, no). Finally, based on the date of data and sample collection, the season was assigned into autumn, winter, spring, and summer as previously determined (Dahl *et al*., 2021).

Evidence of anti-PPR virus seropositivity

In the investigation, PPR was found in the study animals when their serum samples tested positive using the sheep PPR ELISA kit (Sunlong Biotech Co., Ltd, China), which applies Sandwich-ELISA as a method of detection. According to the manufacturer's protocol, serum samples were added to a PPR-specific antibody pre-coated micro-ELISA strip plate. After incubation for 30 minutes, the wells were washed 5 times, the PPR-specific Horseradish Peroxidase (HRP)-conjugate reagent was added, and the plate was incubated for 30 minutes. Later, the

wells were washed 5 times, and the tetramethyl-benzidine (TMB) chromogen solution was added to produce a blue colour within the wells. Finally, the reaction was terminated using the provided stop solution, resulting in a colour change to yellow. The Optical Density (OD) was measured spectrophotometrically at a wavelength of 450 nm, and then the concentration of the anti-PPR antibodies was calculated according to the manufacturer's instructions.

Statistical Analysis

Descriptive Analysis: Evidence of anti-PPR seropositivity was calculated as the proportion of animals that tested positive in ELISA. The proportion of animal type among positive cases and the proportion of positive cases in each type of animal (i.e., sheep, goats) were calculated. Additionally, the proportions of positive cases in each side of the Governorate, as well as during each season (seasonality), were calculated. The correlation (rs) between anti-PPR seropositivity and clinical findings observed on study animals (i.e., mouth lesions, diarrhea, and respiratory symptoms) was assessed via Spearman's rank correlation coefficient.

Epidemiological Analysis: The conditional logistic regression analysis was applied to identify the predisposing factors of the anti-PPR virus seropositivity, where the type of animal (sheep, goats) served as a variable that stratified the data. Initially, variables with a P-value ≤ 0.20 in the univariable analysis were considered for the multivariable analysis. The exposure variable among pairs of associated variables identified by a two-tailed chi-square test and considered the most biologically plausible was subsequently examined in the multivariable analysis. Variables selection method was used in the multivariable analysis as a strategy for model-building by applying a manual forward selection procedure (Hosmer and Lemeshow, 2000). In this procedure, one variable at a time was added, the model goodness-of-fit was evaluated using the likelihood ratio test statistic, and the confounding effect was examined considering the ≥ 20% changes in odds ratio (Szklo and Nieto, 2007). In the final model, the adjusted odds ratio (OR), the 95% confidence interval (CI), and the two-tailed P-value were reported. All analyses were conducted using STATA 13.0 (StataCorp., College Station, TX, USA).

RESULTS

Descriptive Analysis

Anti-PPR seropositivity was identified in a total of 171 animals (43%), where 95% of them were sheep and 5% were goats (Fig. 1A). In addition, 49% of sheep were positive (Fig. 1B), whereas only 12% of goats were positive (Fig. 1B). The proportion of anti-PPR seropositivity was 38% and 81% in the east and west sides of the governorate, respectively (Fig. 2). We observed a pattern in the seasonality of the anti-PPR seropositivity, where it gradually increased as the autumn approach to stay high in the winter then decreased in the spring and summer (Fig. 3). Finally, the correlation was high (rs = 0.92; P< 0.01) between the anti-PPR seropositivity and mouth lesions, whereas it was moderate (rs = 0.42 and 0.40; P< 0.01) with both diarrhea and respiratory symptoms, respectively.

Epidemiological Analysis

In the univariable analysis, the variables for age, sex, geographical location, season, flock size, and mixed animals were statistically significant (P ≤ 0.05, Table 1). The variable for source (local, imported) was not significant (Table 1). The modelling process revealed that the variables for geographical location and mixed animals did not improve the model goodness-of-fit; therefore, they were removed from the multivariable analysis. In addition, the variable for season was associated with the variable of age (chi-square P-value < 0.01, and rs=-0.60 with P-value < 0.01). The variable of age was

considered the most biologically plausible; therefore, it stayed in the multivariable analysis, whereas the variable for season was removed. The final model indicated that the odds of anti-PPR seropositivity were increased with the age of the animals; adjusted OR were 2.00 and 6.11 for ages 6-12 and >12 months, respectively, compared with those <6 months (Table 2). Furthermore, the odds of anti-PPR seropositivity were 4.46 times greater in females compared to males (P< 0.01), adjusted for age and flock size (Table 2). Moreover, the odds of anti-PPR seropositivity were about two times higher in flocks with <50 or 50-100 heads compared to those with >100 heads (P< 0.01), adjusted for age and sex (Table 2). Finally, no confounding effects were revealed in the variables retained in the final model, and the interaction terms between variables of the final model were not significant; thus, removed from the model.

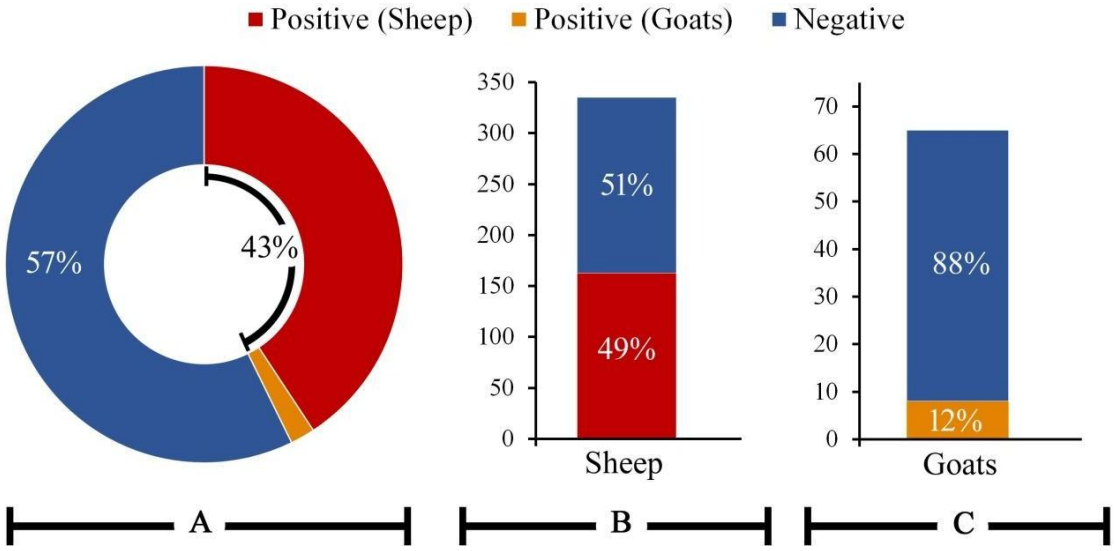

Figure 1. Descriptive analysis of anti-PPR seropositivity in sheep and goats in Nineveh governorate (A) Proportions of positive and negative animals, (B) Proportion of positive sheep, and (C) Proportion of positive goats.

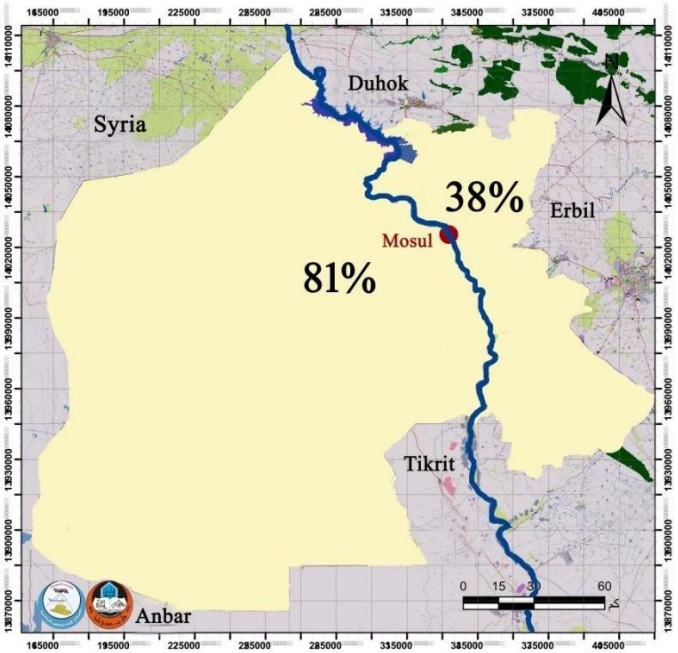

Figure 2. Proportion of anti-PPR seropositivity cases in each side of Nineveh governorate

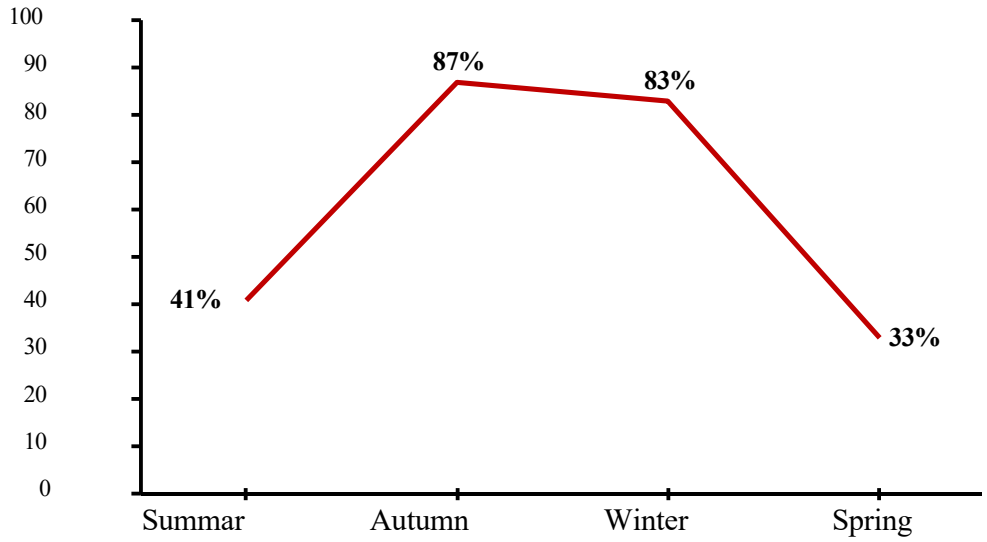

Figure 3. Seasonality of anti-PPR seropositivity in sheep and goats in Nineveh governorate

Table 1. Conditional univariable logistic regression analysis for the odds of exposure factors associated with anti-PPR seropositivity in sheep and goats in Nineveh governorate, Iraq

Variables	Anti-PPR		Odds Ratio	95% CI	P-value
	Positive (n=171)	Negative (n=229)			
Age (months)					
< 6	56	109	1.00	Reference	NA
6 – 12	72	99	1.55	0.98, 2.45	0.06
> 12	43	21	7.60	3.59, 16.08	<0.01
Sex					
Male	117	203	1.00	Reference	NA
Female	54	26	5.93	3.16, 11.14	<0.01
Source					
Local	44	86	1.00	Reference	NA
Imported	127	143	0.72	0.42, 1.23	0.23
Geographical Location					
East	136	221	1.00	Reference	NA
West	35	8	5.58	2.50, 12.43	<0.01
Season					
Summer	102	146	1.00	Reference	NA
Autumn	20	3	7.40	2.14, 25.65	<0.01
Winter	10	2	5.55	1.19, 25.93	0.03
Spring	39	78	0.69	0.43, 1.11	0.13
Flock size (head)					
< 50	50	76	2.06	1.18, 3.59	0.01
50 – 100	64	58	1.67	1.02, 2.72	0.04
> 100	57	95	1.00	Reference	NA
Mixed animals					
No	155	213	1.00	Reference	NA
Yes	16	16	2.49	1.07, 5.80	0.03

Table 2. Multivariable conditional logistic regression analysis for the odds of exposure factors associated anti-PPR seropositivity in sheep and goats in Nineveh governorate, Iraq

Variables	Adjusted Odds Ratio	95% CI	P-value
Age (months)			
< 6	-	-	-
6 – 12	2.00	1.21, 3.30	<0.01
> 12	6.11	2.66, 14.04	<0.01
Sex			
Male	-	-	-
Female	4.46	2.19, 9.06	<0.01
Flock size (head)			
< 50	1.91	1.00, 3.65	0.05
50 – 100	1.96	1.14, 3.35	0.02
> 100	-	-	-

DISCUSSION

PPR was highly present in Nineveh Governorate, Iraq, with relatively high prevalent in sheep. Age, sex, and flock size were the risk factors influencing the odds of infection in sheep and goats. The study conducted here is the first local study that modeled the odds of anti-PPR seropositivity as a function of different potential predisposing factors. The conditional logistic regression indicated to control the disease in sheep and goats.

The anti-PPR seropositivity was identified in sheep more than in goats, in line with Özkul *et al.* (2002) and AL-Afaleq et al. (2004). However, a few studies observed contrary results (Hussain, 2021 and Al-Majali *et al*., 2008). The reasonable explanation to this study is that goats are usually affected with peracute and acute forms of the disease with a case-fatality rate between 55%-85%, whereas sheep are more commonly affected with subacute form with a case-fatality rate less than 10% (Constable *et al.*, 2017).

This study has some limitations. One is that this study considered a convenience sample. Another limitation is that local flocks usually did not keep organized records; therefore, the age of the animals, number of heads inside the flock, and other information were only approximates in the current study.

CONCLUSION

This study supports previous studies that PPR is considerably present in both sheep and goats in Nineveh Governorate, Iraq, and the disease cannot be neglected when oral lesions are observed. Moreover, >1-year-old sheep and goats are at high risk of PPR infection. Finally, the owners with small flocks (<100 heads) should follow the flocks' biosafety to minimize the opportunity of PPR spread.

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