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Determination of Peste Des Petits Ruminants Seroprevalence in Large Ruminants in Egypt with Analysis of Associated Risk Factors in Buffaloes

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Abstract

PESTE des petits ruminants (PPR) is a highly contagious, notifiable viral disease that can cross the species barrier. This study assessed the seroprevalence of PPR in buffalo and cattle across seven governorates in Egypt. Blood samples were randomly collected from 345 clinically healthy animals (229 buffaloes and 116 cattle) coexisting with small ruminants. Sera were tested for PPR virus (PPRV) specific antibodies using a commercial competitive ELISA kit. The overall seroprevalence was 8.116% (28/345) (95% confidence interval [95% CI] 5.7- 11.5%), with 6.897% (8/116) (95% CI 3.5- 13%) of cattle and 8.734% (20/229) (95% CI 5.7-13.1%) of buffaloes tested positive. These results suggest exposure and seroconversion to PPRV in both cattle and buffalo mixed with small ruminants. There was no significant difference in seroprevalence between cattle and buffalo (P=0.555). In buffaloes, age and sex were significantly associated with PPR seropositivity (P< 0.005), while location had no effect (P = 0.437). Female buffaloes and older animals (>4 – 6 and >6 years) showed significantly higher seropositivity than males (odds ratio [OR]= 4.347, 95% CI 1.603, 11.79) and younger buffaloes (2–4) (OR= 8.063, 95% CI 1.835, 35.42 and OR= 8.716, 95% CI 2.584, 29.4, respectively). The significantly higher seropositivity in older and female buffaloes is possibly due to frequent and cumulative virus exposure and stressors that female buffaloes are exposed to. This study highlights the importance of large ruminants serosurveillance to assess subclinical virus circulation in small ruminants, particularly in endemic area where routine vaccination program is applied.

Keywords: PPR, PPRV, Buffalo , Competitive ELISA , Seropositive.

Introduction

Peste des petit ruminants (PPR) is a highly contagious viral disease that predominantly affects small ruminants, such as sheep and goats. It is caused by the peste des petit ruminants virus (PPRV), which belongs to the genus *Morbillivirus* of the family *Paramyxovirdea* [1]. Based on the fusion (F) gene sequence, PPRV is genetically divided into four lineages: Lineages I to III, found in Africa, and lineage IV, found in Asia.[2].

Initially, PPR was identified in the Ivory Coast, West Africa. The disease has expanded its geographical distribution and become endemic across extensive areas in Africa, Asia, and the Middle East, making PPR a transboundary emerging disease of small ruminants [3].

PPR causes significant economic losses due to high morbidity and mortality of sheep and goats [1], [4], [5].Consequently, the World Organization for Animal Health (WOAH) has categorized it as a notifiable disease [6]. Recently, the disease has spread to Thrace (the European part of Turkey), representing a major threat to other European countries [4].

One of the remarkable features of PPRV is its ability to cross species barriers and infect different hosts [7]. Small ruminants (sheep and goats) are the most common clinical hosts for PPR. The clinical symptoms include fever, ocular and nasal discharges, mucosal erosions, oral ulcers, respiratory distress, diarrhea, and high mortality. Goats usually show more severe clinical illness than sheep [8].

PPRV can induce seroconversion in camels, cattle and buffalo [7]. There are several reports of PPRV infection in camelids in different countries, with seroprevalence ranging from 7 to 15% [9]. In

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2004, an outbreak of PPR in camels occurred in Sudan. This was the first time that PPR was confirmed in this species, raising concerns about the potential spread of the disease in camel populations under field conditions [10]. The virus can also infect cattle and pigs, but they do not show any clinical signs. Previous experimental study in cattle indicated that PPRV can induce seroconversion without showing obvious clinical signs [11].

Seroprevalences of PPR in domestic water buffalo (*Bubalus bubalis*) were previously reported in different countries [12]–[15].There was only one reported PPR clinical outbreak in water buffalo in India [13]. PPR was reported in various wild ruminant species, although the role of these hosts in the epidemiology of the disease remains inadequately understood [9].

These previous findings support the hypothesis of the subclinical circulation of PPRV among large ruminants. However, the role of these species in the epidemiology of PPR and their effect on the evolutionary dynamics of the virus is still unknown. The host range diversity of PPRV suggests that it can adapt to novel hosts and overcome their immune defenses. This poses a challenge for the control and eradication of PPRV, as well as a potential risk for human health [7].

After the global eradication of rinderpest, the Food and Agricultural Organization (FAO) and WOAH targeted PPR to be eradicated globally by 2030 [16].Sero-surveillances in large ruminants are crucial for detecting virus circulation in regions where PPR vaccination campaigns in small ruminants are implemented [17]. As a result, there is a need for serological, molecular and epidemiological investigations of the disease among large ruminants in endemic regions to achieve the goals of disease control and eradication programs.

In Egypt, PPR is prevalent and has a high mortality rate in sheep flocks, with the circulating virus belonging to lineage IV, which is also shared with the Ethiopian variant [18].The seroprevalence of PPR in Egypt was determined in cattle imported from Sudan [19]. To the best of our knowledge, no previous research has been conducted in Egypt to investigate the seroprevalence of PPRV antibodies in buffalo.

This study aims to establish initial data on the seroprevalence of PPR in cattle and buffalo in Egypt from 2023 to 2024 by conducting a cross sectional sero-epidemiological study. The study designed to determine the seroprevalence of PPRV antibodies in Egyptian water buffaloes (*Bubalus bubalis*) and cattle across different Egyptian governorates and to detect the association between PPR seroprevalence in buffalo and certain risk factors, such as animal species, age, sex, and location. Data obtained from this study will help in i) understanding the role of buffalo and cattle in the epidemiology of PPR, ii) determining the current sub-clinical virus circulation in regions where mixed farming practices are conducted, and iii) consequently, improving PPR control program in Egypt.

Material and Methods

Ethical approval

Our study was conducted according to the guidelines of the Institutional Animal Care and Use Committee of the Cairo University CU-IACUC (Ethics approval number: Vet CU08072023733).

Animals and samples

The number of required serum samples was calculated using the OpenEpi online software, Version 3.01 [20]. based on 95% confidence level, 10% precision, and 50% expected proportion in population of unknown previously estimated seroprevalence. A total of 345 apparently healthy animals (229 buffaloes and 116 cattle) were randomly selected for blood sampling. Samples were collected from slaughterhouses and one dairy farm during the period between July 2023 and July 2024. Two milliliters of blood was collected individually form each animal for serum separation. Serum samples was preserved at -20°C until testing.

Data collection and grouping

Samples were classified according to animals' species (cattle and buffalo). Serum samples from buffaloes were grouped according to the collected data on, age (< 2, 2– 4, >4–6,and >6 years old), sex (female and male), and location (Ismailia, Giza, Kafr El-Sheikh, Sharkia, Fayoum, Qualyobia and Menoufia). Data were collected from farm records and from animal's owners.

Competitive enzyme-linked immunosorbent assay (c-ELISA) for detection of PPRV antibody

Each serum sample was tested using PPR competitive ELISA kit (ID Screen® PPR Competition. IDvet, Montpellier, France) for the presence of PPRV-specific antibodies [21]. The assay was performed and analysed following the manufacturer's instructions. The optical density (OD) of each sample was recorded at a wavelength of 450 nm and the competition percentage (S/N) was calculated as follow: S/N = (OD of the sample / OD Negative Control) × 100. Samples with (S/N) ratio of ≤50% was regarded positive, and those with S/N ratios greater than 60% was declared negative.

Statistical analyses

The overall PPR seroprevalence among large ruminants (cattle and buffalo), as well as the PPR seroprevalence among cattle and buffalo populations separately, were calculated according to [22].

The Chi-square test of independence was used to determine the effect of species on PPR seroprevalence, and to assess the association between different risk factors (age, sex, and location) and PPR seroprevalence in buffalo. *P* values <0.05 were regarded as significant results. The odds ratio (OD) estimates were calculated for risk factors that found to be significantly associated with PPR seropositivity in buffalo. Male buffaloes and buffaloes aged 2-4 years were used as the baseline groups for sex and age variables, respectively. The calculations and statistical analysis of the obtained data were performed using JASP, Version 0.19, Version 23.0 [23].

Results

Out of 345 tested serum samples, 28 samples were positive for PPR antibodies using competitive ELISA with a total seroprevalence of 8.116% (95% CI 5.7- 11.5%) in both cattle and buffalo. The seroprevalence in cattle was 6.897% (8/116) (95% CI 3.5- 13%), while the seroprevalence in buffalo was 8.734% (20/229) (95% CI 5.7- 13.1%). All sampled animals were in contact with small ruminants (sheep and goat). The frequencies of PPR seropositive samples in different sex, age, and location groups in buffalo are illustrates in table 1. According to animal location, samples from buffaloes in Qualyobia (n= 6) and Menoufia (n=12) were all seronegative (Table1).

There was no significant difference (χ^2= 0.348, *P* = 0.555) in PPR seroprevalence between cattle and buffalo. Among buffalo, a statistically significant relationship was found between PPR seroprevalence and the risk factors of age and sex (*P* <0.05). On the other hand, there was no effect of location (*P*= 0.437) on PPR seroprevalence (Table 1).

Odds ratio estimates showed that the seroprevalence among female buffaloes was significantly higher than that of male buffaloes (Table 2). According to the odds ratio estimates among different age groups, older buffaloes (>4 – 6 and >6 years) were found to have significantly higher seroprevalence than younger ones (2– 4) (Table 2).

Discussion

The first aim of this study was to assess the serological status of PPR in buffalo and cattle across different Egyptian governorates. In Egypt, both small and large ruminants are important sources of meat production [24] Moreover, mixed species farming practices are conducted on some farms and among livestock holders in different governorates. Screening large ruminants for PPRV antibodies is a valuable method for detecting the subclinical (asymptomatic) circulation of the virus in small ruminants, especially in areas where vaccination is applied to small ruminants, as there are no available marker vaccines that allows the differentiation of infected animals from vaccinated ones [17].

All sampled animals in this study were in contact with small ruminants. Our results demonstrate the presence of specific antibodies against PPRV in both cattle and buffalo, indicating the circulation of PPRV in regions where mixed animal species are farmed together. This finding is crucial as it suggests that, in addition to small ruminants, large ruminants such as buffalo and cattle could play a role in the epidemiology of PPR. Since these animals were not vaccinated and the PPRV vaccinal strain cannot be transmitted from vaccinated small ruminants to exposed susceptible animals [25], the detected antibodies implies that cattle and buffaloes are exposed to PPRV naturally either directly or indirectly [12].

The tested animals were apparently healthy with no observed clinical signs of PPR. This finding is consistent with previous studies, which have documented the presence of PPRV specific antibodies in large animals that do not exhibit PPR clinical symptoms[14], [26], [27].

A key observation from the study was the significant association between PPR seroprevalence and the sex of buffaloes. Female buffaloes exhibited a notably higher seroprevalence compared to males. This finding indicates that females might be more susceptible to PPRV infection, potentially due to stressors related to reproduction and lactation that may compromise their immune responses. The higher seroprevalence in females could also be influenced by management practices that result in longer life spans for females, thereby increasing their cumulative exposure to the virus [15].

Arecently published study on PPR seroprevalence in Indonesian buffaloes have found opposite results, reporting higher seroprevalence in male buffaloes. However, the authors mentioned that this result could be biased due to small sample size and the long maintenance of small proportions of males by the farmers [6]. This assumption is consistent with the cumulative exposure hypothesis, despite the results difference.

Age is also detected to be a significant factor influencing PPR seroprevalence among buffalo. The odds ratio analysis reveals that older buffaloes (>4 years) had a significantly higher seroprevalence than younger animals (2-4 years), with those older than 6 years showing the highest seroprevalence. This result supports the cumulative exposure hypothesis, where older animals are more likely to have been exposed to the virus over time. The influence of maternal antibodies is not discussed because the youngest age of the sampled buffaloes was 18 months. PPRV maternal antibodies are declined significantly within the first 6 months after birth [28].

The seroprevalence of PPR in buffalo was slightly higher than in cattle, although this difference was not statistically significant. The lack of

significance suggests that both species have a similar susceptibility to PPRV and may play similar roles in the epidemiology of PPR. This result agrees with a previously published one [17]. In contrast, other studies reported a significantly higher seroprevalence of PPR in buffalo compared to cattle[14], [26].

Certain areas, such as Giza, showed higher seroprevalence in buffalo compared to others, like Qalyubia and Menoufia, which reported no seropositive cases. Nevertheless, statistical analysis revealed no significant effect of location on PPR seroprevalence in buffalo. The absence of the significant effect of location refers to uniform exposure of buffalo to PPRV across different regions in Egypt. This uniform exposure could be due to relatively similar farming practices, and vaccination status of small ruminants across different governorates in Egypt.

Conclusion

In conclusion, farming different animal species together is considered an ideal condition for virus transmission from small to large ruminants. Buffalo and cattle coexisting with small ruminants are exposed and seroconvert to PPRV. This suggests potential virus adaptation in these species. Such adaptation may increase the risk of changes in virus virulence, particularly in female and older buffaloes, which have a higher chance of cumulative exposure to the virus. Ongoing surveillance is essential to monitor the clinical state of PPR among cattle and buffalo, and to detect the likelihood of virus circulation among large ruminants that are not in contact with small ruminants

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Declaration of Conflict of Interest

The authors declare that there is no conflict of interest.

Tables

TABLE 1. Frequencies of PPR seropositive buffaloes among various sex, age, and location groups with the values of Chi-square test of independence in relation to each variable. Frequencies of PPR seropositivity were used for the Chi-square test of independence (x^2).

Variables (buffalo)	Groups	No. of collected samples	Positive n (%)	Chi-square test of independence results
I) Sex	Male	142	6 (4.225)	X^2= 9.531, P= 0.002
	Female	87	14 (16.092)	
II) Age	<2	29	2 (6.897)	X^2= 18.055, P< .001
	2– 4	133	4 (3.008)	
	>4 – 6	20	4 (20)	
	>6	47	10 (21.277)	
III) Location	Ismailia	50	3 (6)	X^2= 5.881, P= 0.437
	Giza	78	10 (12.821)	
	Kafr El-Sheikh	6	1 (16.667)	
	Sharkia	45	5 (11.111)	
	Fayoum	32	1 (3.125)	
	Qualyobia	6	0	
	Menoufia	12	0	

TABLE 2. Odds ratio (OR) estimates of PPR seroprevalence in buffalo among sex and age groups. The significantly high groups are bold.

Variables		Odds ratio	95% CI
I) Sex			
	Male	**1 (baseline group)**	
	Female	**4.347**	**1.603, 11.79**
II) Age			
	<2	**2.389**	**0.4163, 13.71**
	2– 4	**baseline group**	
	>4 – 6	**8.063**	**1.835, 35.42**
	>6	**8.716**	**2.584, 29.4**

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تحديد الانتشار المصلي لمرض طاعون المجترات الصغيرة في المجترات الكبيرة في مصر مع تحليل عوامل الخطر المرتبطة به في الجاموس

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الملخص

طاعون المجترات الصغيرة (PPR) هو مرض فيروسي شديد العدوى وواجب الإبلاغ عنه، ويمكن أن يتخطى حاجز الفصائل. تم تقييم الانتشار المصلي لمرض طاعون المجترات الصغيرة في الجاموس والأبقار عبر سبع محافظات في مصر. تم تجمع عينات دم عشوائيا من 345حيوانًا سليمًا اكلينيكيا (229جاموس و 116بقرة) ومخالطة للمجترات الصغيرة. تم اختبار الامصال للكشف عن وجود الأجسام المضادة الخاصة بفيروس طاعون المجترات الصغيرة (PPRV) باستخدام اختبار ال ELISA التنافسية. وجد أن إجمالي الانتشار المصلي هو 8.116% (28/ 345) (ب 95% فاصل ثقة 5.7، 11.5) حيث أظهرت الأبقار نسبة إيجابية قدرها 6.897% (8/ 116) (ب 95% فاصل ثقة 3.5، 13)، والجاموس نسبة إيجابية قدرها 8.734% (20/ 229) (ب 95% فاصل ثقة 5.7، 13.1). تشير هذه النتائج إلى أن كل من الأبقار والجاموس المخالطين للمجترات الصغيرة يتعرضون لفيروس طاعون المجترات الصغيرة ويكونون اجساما مناعية مضادة له. لم يكن هناك فرق ذات دلالة في نسب الانتشار المصلي بين الأبقار والجاموس (P=0.555). في الجاموس، وجد أن العمر والجنس مرتبطان ارتباطا ذات دلالة بالإيجابية المصلية لمرض طاعون المجترات الصغيرة ($P < 0.005$). بينما لم يوجد للموقع تأثير على الانتشار المصلي لمرض طاعون المجترات الصغيرة (P=0.437). أظهرت اناث الجاموس والحيوانات الأكبر سنًا (<4 – 6 سنوات و >6 سنوات) نسبًا من الإيجابية المصلية أعلى بشكل ذات دلالة مقارنة بالذكور (نسبة الأرجحية [OR]=4.347، ب 95% فاصل ثقة 1.603، 11.79) والجاموس الأصغر سنًا (2-4 سنوات) (نسبة الأرجحية 8.063، ب 95% فاصل ثقة 1.835، 35.42 ونسبة الأرجحية 8.716، ب 95% فاصل ثقة 2.584، 29.4، على التوالي). قد تكون نسبة الانتشار المصلي المرتفعة بشكل ذات دلالة في الجاموس الأكبر سنًا والإناث ناتج عن التعرض المتكرر والمتراكم للفيروس، والضغوط التي تتعرض لها إناث الجاموس. تسلط هذه الدراسة الضوء على أهمية تتبع نسب الانتشار المصلي في المجترات الكبيرة لتقييم انتشار الفيروس التحت الاكلينيكي بين المجترات الصغيرة، خاصة في المناطق التي يستوطن فيها المرض والتي يُطبق فيها برنامج التطعيم دوريا.

الكلمات الدالة: طاعون المجترات الصغيرة ، الجاموس، ايليزا تنافسية ، ايجابية الانتشار المصلي.