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Serological Investigation of Peste Des Petits Ruminants in Lambs in Iraq-Kirkuk Region

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ABSTRACT This study was carried out to determine the prevalence of Peste des Petits Ruminant (PPR) in sheep herds in different areas in the Iraq - Kirkuk region by using the ELISA test technique. According to clinical findings, the presence of seropositive samples by PPR test kit and ELISA method was revealed in the sera obtained from blood samples of lambs suspected of PPR disease. According to the obtained the test results, 47% of the antibodies against the PPR virus of all lambs used in the study were positive. Presence of PPR antibody was detected as 41.5% in 2-6 month old lambs. The disease was detected at the highest level in April with a rate of 56.50%. As a result, it was revealed that PPR is endemic in the Kirkuk-Iraq region. In conclusion; In the fight against Peste des Petits Ruminant (PPR) disease; It was concluded that in addition to preventive vaccine studies, disinfection systems, quarantine, training of sheep breeders and development of a plan for global disease prevention are required.

Keywords: *Diseas, Kirkuk-Iraq, Lamb, PPR.*

ÖZ

Irak-Kerkük Bölgesindeki Kuzularda Küçük Ruminant Vebası Virüsünün Serolojik Araştırılması

Bu çalışma, Irak - Kerkük bölgesindeki farklı alanlardaki koyun sürülerinde Peste des Petits Ruminant (PPR) hastalığının yaygınlığını ELISA test tekniği kullanılarak hastalığın varlığın ortaya koymak amacıyla yapılmıştır. Klinik bulgulara göre PPR hastalığında şüphelinen kuzularda kan numunelerinde elde edilen serumlarda PPR test kiti ile ELISA yöntemi seropozitif olan numunelerin varlığı ortaya konuldu. Elde edilen test sonuçlarınra göre çalışmada kullanılan tüm kuzuların PPR virüsüne karşı antikor varlığının oranın %47'si pozitif tespit edildi. PPR antikor varlığı 2-6 aylık kuzularda %41.5 olarak saptandı. Hastalık Nisan ayında en yüksek düzeyde ve %56.50 oranında tespit edildi. Sonuç olarak, PPR'nin Kerkük- Irak bölgesinde endemik olarak görüldüğü ortaya konuldu. Sonuç olarak; Peste des Petits Ruminant (PPR) hastalığıyla mücadelede; koruyucu aşı çalışmalarının yanı sıra dezenfeksiyon sistemleri, karantina, koyun yetiştiricilerin eğitimi ve küresel boyutta hastalıkta korunmada plan geliştirilmesi gerekli olduğu sonucuna varılmıştır.

Anahtar Kelimeler: *Hastalık, Kerkuk-Irak, Kuzu, PPR.*

INTRODUCTION

Peste des petits ruminants (PPR) is infectious sickness of domestic and small ruminants, which is a highly contagious, infectious, and fatal viral disease. Fever, necrotic mouth, gastroenteritis, and pneumonia are all symptoms (Abdalla et al. 2012). This family of viruses, the Paramyxoviridae (RPV and Canine Distemper) is closely related to the PPRV (Ozkul et al. 2002). According to reports, the disease has been most prevalent in sub-Saharan in Africa, the Middle East, and South Asia (Omani et al. 2019). The Food and Agriculture Organization and the World Organization for Animal Health have suggested a control program for the illness, with 2030 set as the goal year for elimination (FAO and OIE 2016). PPRV is rapidly spreading throughout continents, especially in North and East Africa and Asia, which creates a difficult environment for efforts to eradicate PPRV before 2030. Epidemics that reoccur in North Africa's Maghreb region (Libya, Tunisia, Algeria, Morocco, Mauritania, and the Western Sahara) provide as a good example of these difficulties. PPRV appears to have been eradicated in Morocco as a result of widespread vaccination when it was first discovered there in 2008. Following the relaxation of these vaccination tactics, the World Organisation for Animal Health (OIE) was informed in 2015 of the re-emergence of PPRV in Morocco (Baazizi et al. 2017).

The genetics of the inflaming viral strain, the infectious dosages of the virus, the infection routes, the type and breed of infected animals, and the immunological and

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nutritional status of an infected animal may all influence the severity of the illness (Parida et al. 2015). The research reported that young animals provided higher positive percentages of PPR than older animals (Rahman et al. 2004).

There is evidence that females have greater antibody titers than males in Bangladesh. In terms of PPR, male goats are somewhat more susceptible than females (Abdalla et al. 2012). Despite this, data from Pakistan show no significant difference in susceptibility between males and females (Kozat and Sepehrizadeh 2017). Environmental factors that promote PPR virus persistence and propagation can also have an impact on the seasonal distribution of PPR outbreaks. PPRV outbreaks were most frequent in the first and fourth quarters of the year, with March being the most common, followed by April (Abubakar et al. 2009).

The current study aimed to investigate and confirm the prevalence of PPR illness using a competitive ELISA test, as well as to identify the primary risk factors in Kirkuk governorate, Iraq.

MATERIAL AND METHODS

This research was carried out with the permission numbered 73 on 22.12.2021 from the Republic of Iraq Ministre of Agriculture Veterinary Directorate/Kirkuk Ethics Committee.

Animals

A cross-sectional study was done in Iraq's Kirkuk governorate, which is located at 35.46° north latitude and 44.38° east longitude. It borders Salahiddin province to the north and west, AS-Sulimania province to the east, and Erbil and Nineveh province to the north. Two hunderd male and female local breed lambs between the ages of 1 and 6 months who had a fever, diarrhea, lacrimation, and mouth lesions were given blood samples. Between February and April 2022, samples were collected from several locations within the city of Kirkuk and its surrounding regions (Shwan, Laylan, Daquq, and Rashad).

Clinical Examination

Blood samples were taken from 200 lambs in various parts of Kirkuk province that had fever, oral lesions, and diarrhea from February to June in 2022. These samples came from lambs that were 1 to 6 months old. Following a clinical examination, clinical results were acquired and documented, including the sampling date, address, animal breed, clinical examination (age, body temperature), and clinical signs (mouth lesions, diarrhea, and lacrimation).

Collecting Samples

For this purpose, 5 ml of blood was taken from the jugular vein and kept in the non-anticoagulant tube. Then, blood samples were transported to the Kirkuk veterinary laboratory in cold chain within the hours of collection and the blood was centrifuged at 3000 RPM for 15 min. Each sample's serum was separated into two Eppendorf tubes and kept at -20 °C in a deep freezer. Samples were transferred to (Biological laboratory in the Faculty of Education in the Salahiddin University in Erbil) in order to perform analyses.

Laboratory Analysis

All serum samples were examined using the company's specified methodology. The identification of antibodies against the Peste des Petits Ruminants (PPR) virus in serum from lambs was carried out using a commercial test kit (PPR ELISA Kit Sunlong® Biotech Co. Ltd, China).

Statistical Analysis

The SPSS package program was used in this study to evaluate the statistical analysis. In terms of sex, age, area, season, and clinical findings, ELISA results and percentages of acquired results were computed. The statistical significance level was taken as 5% and SPSS (ver: 26) statistical package program was used for calculations.

RESULTS

Two hundred distinct local breeds of sheep with PPR symptoms had blood samples taken. These animals were of various sexes, ages, and geographical origins. Laboratory testing for PPR disease was done on blood samples using the ELISA technique. The research found that of 200 samples, the percentages of positive, suspected, and negative findings were 94 (47%), 8 (4%) and 98 (49%), respectively (Table 1).

Table 1: Serological results according to ELISA.

Results	Number of samples	Percentage %
Positive	94	47
Suspected	8	4
Negative	98	49

Findings According to Sex

A total of 200 blood samples were collected at random from 114 female and 86 male animals. While 39 of 86 female animal samples (45.3%) were seropositive, 55 of 114 male animal samples (48.2%) were seropositive. Table 2 show the sample variances by gender.

Table 2: ELISA findings based on the sex.

	Sex	
	Male	**Female**
Number of samples	114	86
Positive	55	39
Suspected	4	4
Negative	55	43
Percentages of positive (%)	48.2	45.3

Findings According to Age

In this investigation, 200 serum samples were collected from animals aged 1 to 6 months. According to the findings, the seropositivity ratios of age groups are shown in the table and figure. When the table and figure were analyzed, the disease's seropositivity rate increased with age (Table 3).

Table 3: ELISA findings based on the ages.

	Age (Day)		
	1-60	**61-90**	**91-180**
Number of samples	57	69	74
Positive	18	37	39
Suspected	4	4	0
Negative	35	28	35
Percentages of positive (%)	19.1	39.4	41.5

Findings According to Regions

In this study, 200 blood samples were collected from animals in the Kirkuk city areas of Daquq, Lalan, Rashad, and Shown. Table4 show the number of samples and seropositivity rates by area. When Table 4 were analyzed, Rashad had the greatest seropositivity rate (66%). Other regions' seropositivity rates were 45.8%, 47.2%, and 28.6%, respectively (Table 4).

Findings According to Clinical Symptoms

Clinical symptoms in animals were documented, and blood was drawn following a general examination. Serological data in terms of clinical symptoms such as fever, oral lesion, diarrhea and lacrimation were presented in tabular and figure form (Figure 1 and Figure 2). The percentages of fever, oral lesions, diarrhea and lacrimation were 90.4%, 76.5%, 82.9%, and 44.7%, respectively, according to these data (Table 5).

Figure 1: Diarrhea symptoms caused by PPR in a lamb.

Table 4: ELISA findings based on the location.

Location	Number of samples	Positive	Suspected	Negative	Percentages of positive (%)
Shown	48	22	0	26	45.8%
Laylan	53	25	0	28	47.2%
Daquq	49	24	3	32	28.6%
Rashad	50	33	5	12	66%

Table 5: ELISA findings based on the clinical sings.

Clinical sings	Positive showing clinical sings	Suspected showing clinical sings	Negative showing clinical sings	Percentages of positive (%)
Fever	85/94	2/8	45/98	90.4%
Mouth lesion	72/94	2/8	35/98	76.5%
Diarrhea	78/94	5/8	47/98	82.9%
Red eye membrane and lacrimation	59/94	4/8	69/98	44.7%

Figure 2: Severe lacrimation in a lamb.

Results According to Season

Between February and April 2022, samples were collected from several locations in Kirkuk and surrounding regions. We have seen that the infection rate rises in March and then again in April (Table 6).

Table 6: ELISA findings based on the season.

	Month		
	February	**March**	**April**
Positive	7	35	52
Suspected	3	0	5
Negative	20	43	35
Percentages of positive (%)	23.3%	44.9%	56.5%

DISCUSSION AND CONCLUSION

In Iraq as in many parts of the Middle East, PPRV remains unknown, with outbreaks in Iraq the most often recorded. A virus with significant morbidity but low mortality was identified in Iraq in 2000, and that's when PPRV was originally characterized (Banyard et al. 2010).

According to the OIE and FAO, Iraq reported an epidemic of peste des petits ruminants (PPR) in its northern governorates in September 1998. Despite the fact that this disease had been suspected in the central and northern governorates for some years and was known to exist in neighboring countries, this was the country's first official report of PPR, which prompted tremendous alarm. Iraqi veterinary officials have insufficient resources to deal with this highly infectious illness of small ruminants due to international sanctions imposed on the country. A FAO TCP project was launched in 1999 to help eliminate the disease through targeted vaccination, strengthen laboratory-assisted surveillance, improve field veterinary staff diagnostic capacity, and establish a national network for surveillance and early warning systems against transboundary animal diseases (Alwan and Alsaad 2022).

PPR has been detected clinically in sheep and goats in Irbil and Dahuk Governorates in recent years, and has been suspected clinically in Mosul, As-Sulaimaniyah, and Ta'amim (Kirkuk), all of which are in the country's north (FAO 2000). Between August 2010 and February 2011, the Erbil Governorate in northern Iraq lost approximately 750 wild goats. The participation of the peste des petits ruminants' virus (PPRV) was hypothesized based on the clinical symptoms and post-mortem results. Laboratory testing supported this and revealed a virus with a similar resemblance to a Turkish variant discovered in 2000. There were no cases of illness in domestic animals during the wild goat epidemic (Hoffmann et al. 2011). In then As-Sulaimaniyah Governorate in the north of Iraq, an outbreak of peste despetitst ruminants (PPR) in sheep was investigated between 2012 and 2013. The findings gave the PPRV lineage linked to lethal PPR infections in small ruminants its first molecular characterization. The diagnosis was made using RT-PCR (Babashek et al. 2014).

In 1995, a peste des petits ruminants (PPR) pandemic was found serologically and virologically in Ilam province, Iran, near the Iraqi border. PPR has been identified throughout the country despite all control attempts, costing Iranian sheep and goat owners at least $1.5 million US (Bazarghani et al. 2007).

PPRV infection was first officially documented in neighboring Turkey in southern and eastern Anatolia in 1999. From 1999 to 2018, the OIE documented roughly 1,000 PPR outbreaks in Turkey. These outbreaks peaked in 2007 and 2011, particularly in Turkey's Marmara and Aegean regions (Altan et al. 2019).

All of these reported results reflect a considerable occurrence of PPR. It is necessary to establish a surveillance system, to monitor and manage this illness. Because of the huge economic ramifications of PPR in Iraq, analyzing PPR prevalence and risk factor data is crucial. Concerns concerning PPR epidemiology are crucial in attempts to control, manage, and eliminate the illness effectively.

The current study was conducted to assess and construct an epidemiological history of PPR prevalence in the Kirkuk region of Iraq. Despite the spread of the disease in such a wide way in most of the governorates of Iraq and the neighboring countries of Iraq, there are no adequate studies on the disease in Iraq in general and in Kirkuk governorate in particular, so to understand the epidemiology of this disease in Iraq, it is important to perform this study as an attempt to complete this deficiency.

The utilization of quick, targeted, and responsive diagnostic techniques is required for the effective implementation of PPR control strategies. Small ruminant PPR infection is frequently identified by clinical assessment, gross anatomy, histological findings, and laboratory confirmation. A range of serological and molecular diagnostic assays are used to find PPR virus (Munir et al. 2014). For quick diagnostic and control measures, pen-side trials are particularly tempting. These include chromatographic strip tests, dot ELISA, and others. They may be performed without the need for specific equipment or technical expertise (Balamurugan et al. 2012).

In this study, according to the findings obtained from ELISA (regardless of sex, age and animal's regions), blood samples reflect that positive, suspected and negative

results were determined as 47%, 4% and 49%, respectively of PPR (Table 1). It appears to be very similar as a result of a study conducted in the neighboring governorate (Nineveh) in 2021 which was 47.46% (Hussain 2021).

It appears to be greater than comparable to the prevalence documented in bordering countries. These findings are consistent with previous epidemiological research. PPR was found in 3.1% of sheep in the Kingdom of Saudi Arabia (KSA) using a microtiter neutralization test (AL-Afaleq el at. 2004). The frequency of PPR in sheep was found to be 96% in Syria, much higher than in Jordan (60%) (Al-Majali et al. 2008). PPR incidence rates in Turkey varied from 0.87% to 82.6%, with sheep (29.2%) outmatched goats (20%) (Ozkul et al. 2002).

The risk factors including sex, age and season reflected reasonable effects; that males (48.2%) were more impacted and seropositive for PPR than females (45.3%). (Table 2). There were several possible explanations for this result; According to Bangladeshi results, females are more likely than males to have higher antibody titers. Male goats are somewhat more vulnerable to PPR than females (Abdalla et al. 2012); but others showed that; while female goats and sheep are kept alive for breeding and milking, male animals are slaughtered at an early age (Khan et al. 2008).

Abubakar et al. (2015) reported that there was a positive correlation between PPR seropositivity and age and the animal's coming to pasture in their research on PPR, and they found that PPR seropositivity was higher in sheep aged 2 years and older. According to research from Turkey, India, Kenya, Pakistan, and Ethiopia, young animals are more susceptible to PPRV because they have lower antibody titers that serve as a protective barrier (Kozat and Sepehrizadeh 2017). The research reported that young animals provided higher positive percentages of PPR than older animals (Rahman et al., 2004). Animals aged 3 months to 2 years are the most seriously impacted in endemic regions. The severity of the illness is determined by the age, species, and immunity of the host (Kozat and Sepehrizadeh 2017). In addition, 200 serum samples were collected from animals aged 1 to 6 months for this investigation. According to the findings. The seropositivity ratios of age groups are shown in the table and figure. The disease's seropositivity rate rises between the ages of 2 and 6 months (Table 3). It can be interpreted that the increase in the presence of the disease is due to the lack of attention to adequate prophylactic applications.

Morbidity region that recorded the highest seropositivity rate of PPRV was Rashad region (66%) compared with other studied regions as Shown, Laylan, and Daquq as 45.8%, 47.2% and 28.6% respectively; PPR is most contagious when it manifests in a vulnerable population for the first time. In endemic regions, outbreaks can often happen, especially when additional animals are mixed in with the herd or introduced there (Table 4). We also explain the high rate of infection in the Rashad region compared to the rest of the areas due to the security strikes there in previous years, which led to the failure of veterinary teams to reach it to conduct vaccination campaigns organized by the government. The same is the case in the Shwan region, which is ranked second in terms of the high incidence of infection, and the reason for this is that it is a border area and it is difficult for the authorities in the province to control the movement of animals in it.

In this study, the incidence of clinical findings such as fever, mouth lesions, diarrhea and lacrimation was 90.4%, 76.5%, 82.9% and 44.7%, respectively (Table 5). Many researchers report that PPR will be evaluated as acute, subacute or subclinical according to clinical findings such as fever, mouth lesions, diarrhea and lacrimation (Munir 2014; Kozat and Sepehrizadeh 2017). Depression, mucous membrane congestion, oculo-nasal discharge, dyspnea, and a lot of aquatic diarrhoea are symptoms of infected animals dying within 4-5 days (Munir et al. 2013). Moreover; infants and lambs frequently experience acute sickness soon after their passive immunity becomes depleted (Munir 2014). Subacute or subclinical kinds appear to be particularly common in specific regions. In such circumstances, the sickness has intermittent effects and lasts 10 to 15 days. Later on, papules or pustules resembling infected ecthyma may develop. Because they encourage respiratory conditions that cannot be identified as PPR, invisible kinds are more hazardous. Typically, the only means to detect them is by serological surveys (Lefèvre et al. 1991).

Between February and April 2022, samples were collected from several locations in Kirkuk and its regions for this investigation. The Percentages of positivity were February 23.3%, March 44.9%, and April 56.5%. Iraq in general, and Kirkuk governorate in particular, suffered during the past two years from a great drought and a lack of rain, which led to a lack of green pastures, a lack of moisture and a large number of dust storms, starting from the March and April months, and this is the explanation for the high incidence of the disease in these months (Table 6).

A result, we conclude from this study that PPR is endemic in Kirkuk Governorate in Iraq and that the animals do not enjoy great protection against the disease because the strain used in vaccination is a non-local strain and the risk factors of age, gender, and geographical area played a role in the spread of the disease. It was necessary to establish a global plan to combat and eradicate small ruminant plagues by using several monitoring and preventative methods whether by following disinfection systems, immunization, quarantine, and applying health awareness among young farmers in all world regions.

CONFLICTS OF INTEREST

The authors report no conflicts of interest.

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AUTHOR CONTRIBUTIONS

Idea / Concept: SKR, SK
Supervision / Consultancy: SK
Data Collection and / or Processing: SKR
Analysis and / or Interpretation: SKR, SK
Writing the Article: SKR, SK
Critical Review: SKR, SK

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