Iranian Journal of Ruminants Health Research (2018), 3(1): 27-33
Doi: 10.22055/ijrhr.2020.32873.1032

Serological survey of *Small ruminant morbillivirus* (SRM) infection of slaughtered Water Buffaloes (Bubalus bubalis) in Ahvaz abattoir in south of Iran

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Abstract

Pest des petits ruminants (PPR) is one of the important viral diseases of goats and sheep. The viral agent of PPR, *Small ruminant morbillivirus* (SRM), has been shown to infect several species among domestic and wild ruminants. In order to investigate the occurrence of SRM infection in Water Buffalo (Bubalus bubalis), blood samples were collected from 150 buffaloes (98 males, 52 females) referred to Ahvaz abattoir and then their sera were tested for the presence of anti-SRM specific antibody. Sera were tested by Virus Neutralization Test (VNT) in Vero cells, using the live attenuated virus of a commercial PPR vaccine. Based on the results of VNT, 11.33% of the studied buffaloes had anti- SRM antibodies. Statistical analysis of the results revealed no correlation between infection and age and sex. Given the seropositivity of some of the slaughtered buffaloes for SRM, it can be concluded that *Small ruminant morbillivirus* has the ability for transition from small ruminants to Water Buffaloes. Therefore, in planning to control and prevent the disease in domestic small ruminants, the possibility of SRM infection in other ruminant species, including Water buffaloes, should be considered.

Keywords: PPR, Water buffalo, Seroprevalence, Ahvaz, Iran

Introduction

Pest des petits ruminants (PPR) is an acute and highly contagious disease of small ruminants characterized by high fever, eye and nasal discharge, pneumonia, necrosis and ulceration of mucous membranes and severe diarrhea (Khan *et al*. 2008). The viral agent of PPR, *Small ruminant morbillivirus* (SRM), previously named as *Peste-des-petits ruminants virus* (PPRV), is a negative sense single-stranded RNA virus of the family *Paramyxoviridae*, genus *Morbillivirus* (Rima *et al*. 2016). Like other paramyxovirses, SRM is enveloped and pleomorphic.

PPR was first reported in West Africa in 1942 (Gargadennec *et al*. 1942) and then spread to other parts of the world. In the recent years, PPR has been clinically observed in the Middle East, the Arabian Peninsula, Iran, Iraq, Israel, Jordan, Kuwait, Lebanon, Syria, Oman,

the United Arab Emirates, Yemen and Turkey (Koshemetov, 2009). In addition, the cases of infection with SRM have been reported in European countries like Georgia and Bulgaria (World Organisation for Animal Health, 2020). PPR, in addition to domestic and wild small ruminants (Furley *et al*. 1987; Couacy-Hymann *et al*. 2007; Bao *et al*. 2011), has the ability to cause infections in large ruminants (cattle, buffalo and camels) and pigs (EMPRES, 1999). Definitive diagnosis of SRM infections is based on laboratory methods such as virus isolation, viral antigen and nucleic acid detection and serology. The neutralization assay and competitive ELISA are common serum tests for diagnosis of the disease (World Organisation for Animal Health, 2013). Both of these tests can distinguish anti- SRM antibodies from anti-*Rinderpest morbillivirus* (RM) antibodies.

Apparently, SRM has been spreading in Iran for many years, leading to an extensive spread of the virus throughout the country and huge economic burden (Bazarghani *et al*. 2006). PPR, for the first time, was officially reported in Iran, from Ilam province in 1994, then it became one of the most important diseases of small ruminants until 2005 (Barani *et al*. 2008).

The purpose of the present study is to provide an overview of the prevalence of SRM infection among Water buffaloes referred to Ahvaz abattoir and thus a better understanding of the epidemiology of PPR in south of Iran.

Materials and Methods

Sampling

In order to carry out the present study, blood samples were collected from 150 buffaloes (98 male, 52 female) referred to Ahvaz abattoir in the period from January to March of 2015. Age of the sampled buffaloes was determined based on dental formula. According to the mandibular incisive dental formula (FAO, 1977) the buffaloes were divided into four age groups of under 2.5 years old (all deciduous teeth), 2.5-3.5 years old (a pair of permanent teeth), 3.5-4.5 years (two pairs of permanent teeth) and over 4.5 years (all permanent teeth). In this study, the blood samples were also collected from 10 sheep vaccinated against PPR, as the positive controls. After clotting the blood samples, the sera were separated and stored at -20°C to be tested for anti-SRM antibody.

Culture and titration of the vaccine strain of SRM

A lyophilized live attenuated PPR vaccine (Nigeria 75/1 strain) was obtained from Razi Vaccine and Serum Research Institute of Iran and was dissolved in 5 ml of RPMI medium (Bio-Idea, Iran) containing 2% fetal bovine serum (FBS) (Bio-Idea, Iran). After dilution (1:10) in RPMI medium containing 2% FBS, 0.5 ml of the prepared vaccine was inoculated into a 25 cm^2 flask of Vero cell monolayer, subcultured on the previous day. After observing the wide spread cytopathic effects in the cell monolayer and one cycle of freezing and thawing, the cell culture medium (considered as the virus) was centrifuged and

frozen at -70°C in 0.5 ml aliquots. For titration, one of the virus aliquots was removed from the freezer and thawed at ambient temperature to prepare serial dilutions of 10^{-1} to 10^{-8} in RPMI medium containing 2% FBS. Then, 100 µl of each dilution was transferred into a 96-well cell culture microplate, in three replicates. In the next step, about 10^4 Vero cells in 0.1-ml of RPMI medium containing 2% FBS were added into each well. The plate was incubated in a CO2 incubator (Memmert, Germany) for about 5 days, and then was observed under microscopy to record the presence or absence of the the virus cytopathic effects in the wells. Finally, according to the obtained results, the titer of the virus was calculated using the Spearman and Kairber formula (Muthannan Andayar, 2016).

Virus Neutralization Test (VNT)

For testing the sera by VNT, all 150 buffalo serum samples and sheep sera (positive controls) were first inactivated by 30 minutes incubation at 56°C. The VNT was performed using a serum dilution of 1:20 in duplicate, based on the method presented in the OIE guidelines (World Organisation for Animal Health, 2013), using 100 $TCID_{50}$ of SRM. To this end, 5 µl of each serum was first added into two adjacent wells of a 96-well microplate. Then, 95 µl of RPMI medium without FBS was added into each well and thus a 1:20 dilution of serum samples was prepared. Next, 100 $TCID_{50}$ of SRM in 100 µl of RPMI medium containing 2% FBS was added to each well. Following 1 hour incubation at 37°C, 10^4 Vero cells in 50 µl of RPMI medium containing 2% FBS, was added to the wells and incubation at 37°C was continued up to 5 days. The microplate was daily observed microscopically. The serum samples were determined to be either positive or negative based on the absence or presence of cytopathic effects. Chi-square test was used to compare the seroprevalence of SRM infection in both sexes in different age groups.

Results

Based on the results of the virus neutralization test, 17 out of 150 buffalo serum samples at 1:20 dilution were able to neutralize the SRM infectivity (preventing the cytopathic effects of the virus in cell cultures); in other words, the seroprevalence of anti-SRM antibody in the studied population was 11.33%. All the 10 sheep sera, collected from vaccinated animals, were able to neutralize the virus and thus had anti-virus antibody. The seroprevalence of SRM infection in buffaloes according to different age and gender groups is presented in Tables 1 and 2, respectively.

Relative frequency of positive cases in buffaloes with all deciduous teeth, a pair of permanent teeth, two pairs of permanent teeth and all permanent teeth was 7.46, 15.62, 9.52 and 16.66% respectively (Table 1). Statistical analysis showed no significant difference between different ages ($p<0.05$, df =3, X^2 =2.503). The frequency of positive cases in males and females was 10.2% and 13.46% respectively (Table 2). Again, statistical analysis revealed no significant difference between male and female buffaloes in terms of infection with SRM ($p<0.05$, df =1, X^2 =0.359).

Table 1: Seroprevalence of *Small ruminant morbillivirus* infection in Water Buffaloes in terms of age

Infected / Sex	VN+	VN-	Total
Male	10 (10/2%)	88 (89/8%)	98
Female	7 (13/46%)	45 (86/54%)	52
Total	17 (11/33%)	133 (88/67%)	150

Table 2: Seroprevalence of *Small ruminant morbillivirus* infection in Water Buffaloes in terms of sex

Infection / Age	VN+	VN-	Total
all deciduous teeth	5 (7/46%)	62 (92/54%)	67
a pair of permanent teeth	5 (15/63%)	27 (84/37%)	32
two pairs of permanent teeth	2 (9.5%)	19 (90/5%)	21
all permanent teeth	5 (16/67%)	25 (83/33%)	30
Total	17 (11/33%)	133 (88/67%)	150

Discussion

Serious determination has been adopted in many countries to eradicate PPR due to its huge losses to the population of small ruminants and wildlife threats (Albina *et al*. 2013). To this end, it is essential to measure anti-SRM antibody in ruminant populations in different geographical regions to determine the prevalence of infection with the virus in the natural state, and thus to consider a suitable strategy for vaccination programs. In this regard, Khalafi *et al*. (2018) for the first time reported that the seroprevalence of SRM infection in sheep and cattle populations in Ahvaz in south of Iran was 58% and 23%, respectively. Following that study, in the current investigation, the presence and prevalence of anti-SRM antibody in water buffaloes referred to Ahvaz abattoir was studied.

The results of the present study indicated that 11.33% of water buffaloes referred to Ahvaz abattoir had neutralizing antibody against SRM. This is the first report of the seropositivity of native Water buffaloes in south of Iran against SRM, although the susceptibility of buffaloes to SRM infection has been reported previously, elsewhere (Khan *et al*. 2008; Balamurugan *et al*. 2012; Balamurugan *et al*. 2014).

Since the PPR vaccine virus cannot be transmitted from vaccinated small ruminants to exposed animals, the seropositivity of buffaloes in the current study and the presence of anti-SRM antibodies in the sera of cows in the study of Khalafi *et al*. (2018) is certainly due to the contact of these animals with the wild SRM strains and the occurrence of a natural infection in these animals (Diallo *et al*. 2007). On the other hand, the cross-reaction of SRM with anti-RM antibodies mentioned in the study of Anderson and McKay (1994) could not play a role in this observation, because Rinderpest was eradicated from the region several years ago. Certainly, the animals that were sampled in the study of Khalafi *et al*. (2018) and the present study were born several years after stopping

vaccination against Rinderpest and none of them have been vaccinated against SRM or RM. Therefore, the seroprevalence observed in these animals can only be due to a natural infection with SRM.

Despite the serologic findings, there has been no report on the incidence of SRM-related disease in cows and buffaloes in the region. In fact, SRM infections appear to be subclinical in these animals. However, there is a report of isolation of SRM from an epidemic of a Rinderpest-like disease in Indian buffaloes in the Tamil Nadu region, where 50 out of 385 buffaloes have been clinically affected. The affected buffaloes showed conjunctival hyperaemia, severe salivation and depression, but none had a fever (Govindarajan *et al*. 1997). In an experimental study, a 15-month-old calf inoculated with the SRM showed a fever of 42°C for 48 hours. These observations indicate that SRM, like other *Morbillivirus* members with immunosuppressive behavior, may overcome the innate resistance of large ruminants, depending on the age and physical condition of the host animal, and cause clinical symptoms.[36] In general, the ability of SRM to infect large ruminant species in endemic regions can be a serious threat[4], because following the successful global program for eradicating Rinderpest and stopping anti-RM vaccination, the persistence and repetition of SRM infections in large ruminants may increase the compatibility of this virus with large ruminants and lead to the appearance of a RM-like virus circulating in large ruminant populations independent of small ruminants. In this regard, increased prevalence of antibodies in the buffalo population during two studies conducted by Balamurugan *et al*. in India in 2012 (4.22%) and 2014 (16.2%) and a much higher incidence of antibodies in the Pakistani buffalo population (67.42%) compared to the small ruminants (43.33%) of that region (Khan *et al*. 2008) may be due to the viral circulation within the buffalo population independent of the small ruminants.

Khalafi *et al*. (2018) showed that the SRM seroprevalence among sheep in the age group of over 4 years was significantly higher than that of younger sheep. Kardjadj *et al*.(2015) also reported significantly higher SRM seroprevalence in adult small ruminants compared to young animals. This observation is common in many infectious diseases, as older animals are more likely to be exposed to infectious agents for a longer period. However, there was no discrepancy in the SRM seroprevalence in different age groups of the buffalo and cattle populations in the present study and in the study of Khalafi *et al*.(2018), respectively. The reason for this observation may be due to the fact that the SRM is not currently a primary pathogen for cows and buffaloes and the prevalence rate of its infection in the population of these animals is dependent on the direct or indirect contact of these animals with small ruminants. This may also be an explanation for the different rates of SRM seroprevalence in cattle and buffalo populations in different regions.

In conclusion, based on the results of this study and our previous work (Khalafi *et al.* 2018), in planning of programs for controlling and preventing the disease in small ruminants

of the region, the possibility of SRM infection or circulation in other ruminant species, including Water buffaloes should be considered.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

This project was supported by research grant 96.3.02.16670 of Shahid Chamran University of Ahvaz (Ahvaz, Iran), awarded to Masoud reza Seyfi Abad Shapouri.

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