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SERO-PREVALENCE OF FOOT-AND-MOUTH DISEASE IN SMALL RUMINANTS OF PAKISTAN

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ABSTRACT

Foot-and-mouth disease (FMD) is a contagious viral disease of cloven-footed animals. It is endemic in Pakistan with huge economic losses. The aim of the study was to estimate the sero-prevalence of FMD in small ruminants (sheep and goats) kept in proximity to large ruminants with a recent history of FMD outbreak. The study was conducted in seven purposively selected districts of Pakistan. A total of 1478 sera were collected from small ruminants during 2010-2012. Serum samples were tested for the presence of antibodies against non-structural protein (3ABC) of FMD virus using CHEKIT FMD-3ABC bo-ov kit (IDEXX laboratories, USA). The data were analyzed using χ^2 test and multiple logistic regression methods. The sero-prevalence of FMD in small ruminants was 22.8% (337/1478). The FMD sero-prevalence in goats (282/1094; 25.8%) was significantly higher (χ^2=21.18; p<0.001) than that in sheep (55/384; 14.3%). After adjusting for other factors, the risk of FMD in sheep was 0.56 times of that in goats (CI; 0.4-0.78, p=0.001). The sero-positivity in male animals was higher 17.8% (76/428) than female animals 24.9% (261/1050). Female animals were more likely to be positive for FMD infection compared to male animals (χ^2=8.71; p<0.003). The sero-prevalence in district Islamabad (11.8%) was significantly lower than other districts (p<0.001). The findings indicate that FMD is endemic in small ruminants kept in close contact with large ruminants and these animals may serve as reservoirs of FMD virus and possible sources of infection in susceptible livestock.

Key words: Foot-and-mouth disease; sero-prevalence; Sheep; Goats; Pakistan.

INTRODUCTION

Foot-and-mouth disease (FMD) is a highly infectious viral disease of cloven-footed animals including cattle, swine, goat and sheep (Bachrach, 1968). FMD virus (FMDV) belongs to *Aphthovirus* of family *picornaviridae* with seven serotypes namely A, O, C, Asia-1, SAT 1-3. It is a small and un-enveloped virus with a positive sense RNA genome and a large open reading frame encoding for four structural and ten non-structural proteins (Mason *et al*., 2003; Ryan *et al*., 1991).

In addition to large ruminants the disease has also been reported in sheep and goats (Kitching and Hughes, 2002). However, the clinical signs of the disease in these animals are so mild (Pay, 1998) that it mostly goes undiagnosed in these animals (Hughes *et al*., 2002). The role of sheep and goats in epidemiology of FMD is not well studied. It may be due to in- apparent and self-limiting nature of the disease in small ruminants (Pay, 1998; Anderson *et al*., 1976). Sheep and goats may act as carriers of FMD. For example, a study indicated that sheep and goats may become FMDV carriers for 9 months and 4 months, respectively (Kitching, 2002). Therefore, studies are required to understand the role of sheep and goats in epidemiology of FMD.

FMD is endemic in Pakistan and three different serotypes (A, O and Asia-1) mostly cause outbreaks in cattle and buffaloes (Zahur *et al*., 2006). Although in Pakistan, mostly small and large ruminants are reared together but few FMD outbreaks have been reported in sheep and goats (Anjum *et al*., 2006). In order to gain complete information about the complex epidemiology of FMD in Pakistan, it is necessary to understand the role of small ruminants in overall epidemiology of FMD in Pakistan. This information would help in devising an appropriate FMD control strategy in the country.

The objective of this study was to determine the sero-prevalence of antibodies against non-structural proteins (NSP) of FMDV in sheep and goats kept in close contact with cattle and buffaloes. The information obtained from this study might help in understanding the extent of FMDV circulation in sheep and goat flocks under field conditions and also their role in FMD outbreaks.

MATERIALS AND METHODS

Samples and sampling area: This study was conducted to estimate sero-prevalence of FMD in small ruminants of Pakistan.

A cross sectional survey was conducted during 2010-12 across 7 districts. A multi stage sampling design with three hierarchal stages was used.

The sample size was calculated for simple random sampling using the following formula (Dohoo *et al*., 2003):

$$n = z^2_{\alpha 0.05} .[p \, . \, (1\text{-}p)/L^2]$$

where $z = 1.96$, $p = 0.21$ and $L = 0.05$ (the desired level of precision or accuracy). The required sample size was accordingly calculated as $n = 255$. This sample size was adjusted to account for clustering by the following formula (Dohoo *et al*., 2003):

$$n_{new} = n \times [1+p \times (m - 1)]$$

where $n = 255$, $\rho = 0.156$ and $m = 30$, representing the average number of animals to be sampled from each village Therefore, the new sample size was equal to 1408.62 animals and 49 villages were to be included in this study.

Seven districts were purposively selected as primary sampling unit (PSU). Within each PSU, 7 villages were randomly selected as secondary sampling units and 30 animals (either sheep or goats) were randomly selected from 49 villages. Following this sampling plan, a total of 1486 samples (sheep = 384 and goats = 1102) were collected from 2010 to 2012 (Table 1).

The blood samples were collected by jugular vein puncture using vacutainer tubes (BD Vacutainer® Serum, REF 367812, BD Franklin Lakes, NJ, USA). Blood samples were allowed to clot overnight and sera were harvested in labeled 2ml cryo-vials after centrifugation at 600g for 10 minutes. All the sera (n=1486) were stored at -20°C till further use.

The information regarding the age, sex, breed of animal, vaccination against FMDV and husbandry practices was recorded on a pre-designed proforma. Our Institutional Animal Ethics Committee approved this study.

Detection of FMDV specific antibodies in sera: Collected serum samples (n=1486) were tested by Indirect Enzyme Linked Immuno-Sorbent Assay (I-ELISA; CHEKIT FMD-3ABC bo-ov kit, IDEXX Laboratories, USA) for the presence of NSP antibodies against FMDV (Brocchi *et al*., 2006). The micro-titration plate was read in ELISA plate reader (Immunoskan MS, BDSL) using 450 nm filter.

Data Analysis: The data obtained were analyzed using χ^2 test and multiple logistic regression methods.

RESULTS

The overall sero-prevalence of FMD in small ruminants (sheep and goats) was 22.8% (337/1478). District wise FMDV NSP sero-conversions in small ruminant (sheep and goats) are presented in Table 1.

The sero-prevalence was significantly (χ^2=21.18; p<0.001) higher in goats (25.8%) compared to sheep (14.3%). Our study found a lower risk of FMD infection in sheep: After adjusting for other factors, the risk of FMD in sheep was 0.56 times of that in goats (CI; 0.4-0.78, p=0.001).

The study found significant inter-breed differences in susceptibility to FMDV infection among goat breeds (χ^2=58.19; p<0.001). The sero-prevalence of 80% was recorded in Potohari, 78.57% in Kamori, 23.9% in Beetal and 23.53% in Teddy goats. However, there was no significant difference (p=0.95) in FMD sero-prevalence in various sheep breeds. The sero-prevalence of 17% was observed in Balkhi followed by Thalli (15.79%), Gojal (14.18%), Poonchi (12.82%), Kooka (12.5%) and Kajli (11.36%).

Table 1. Sero-prevalence of Foot-and-mouth disease (FMD) in seven districts of Pakistan. The samples were analyzed using ELISA.

	FMD NSP Positive animals		
District	**Sheep**	**Goat**	**Total**
Muzaffarabad	5/39 (12.8)	43/170 (25.3)	48/209 (22.9)
Attock	8/63 (12.7)	61/147 (41.5)	69/210 (32.9)
Gilgit	13/89 (14.6)	17/116 (14.7)	30/205 (14.6)
Islamabad	12/85 (14.1)	15/143 (10.5)	27/228 (11.8)
Karachi	8/56 (14.3)	46/155 (29.7)	54/211 (25.6)
Lahore	5/35 (14.3)	48/170 (28.2)	53/205 (25.9)
Toba Tek Singh	4/17 (23.5)	52/193 (26.9)	56/210 (26.7)
Total	55/384 (14.3)	282/1094 (25.8)	337/1478 (22.8)

The values in parenthesis are percentages

The sero-positivity in female animals was higher (24.9%; 261/1050) than male animals (17.8%; 76/ 428). In preliminary analysis, sex of the animal was found as an important risk factor for FMD in small ruminants; female animals were more likely to be positive for FMD infection compared to male animals (χ^2=8.71; p<0.003).

However, in the logistic regression analysis this effect was not observed after accounting for other confounding factors (Odds ratio: 0.78; C.I 95%; 0.58-1.07).

The risk of FMDV infection appears to increase with age. A significant difference in sero-conversion was observed in kids, young and adult animals (χ^2=14.98; p<0.001). The sero-conversion was highest in adult animals (28.6%) followed by young animals (21.4%) and kids (16.3%).

The sero-prevalence in district Islamabad was significantly lower (11.8%) than other districts (p<0.01) and there were no differences in the sero-prevalence of FMD in small ruminants in other districts.

DISCUSSION

FMD is one the most prevalent transboundary viral disease of animals in Pakistan knowingly affecting mainly large ruminants (cattle and buffaloes). Although communal grazing and mixed production system is practiced in the country, only few FMD outbreaks are diagnosed and documented in sheep and goats (Ur-Rehman *et al*., 2014). The small ruminant population (93.7 million) in Pakistan is higher than large ruminant population (72 million) however; a national project on the control of FMD in Pakistan, being executed by Food and Agriculture Organization (FAO) of the United Nations, Islamabad, Pakistan, only includes vaccination and sero-surveillance in bovines; totally ignoring the role of small ruminants in transmission of FMD. In this study, sero-prevalence against FMDV was recorded in sheep and goats to understand the potential role of small ruminants in the epidemiology of FMD.

Serum samples were tested for NSP antibodies using NSP ELISA as easy to perform and suitable for large scale serological surveillance. It also can differentiate between vaccinated and infected animals (De Diego *et al*., 1997; Rodríguez *et al*., 1994). Moreover, it can detect 3ABC antibodies, which generally have a higher concentration in serum than other NSPs (Sørensen *et al*., 1998). Additionally, 3ABC ELISA has already been used in ruminants as a DIVA (Differentiation between Infected and Vaccinated Animals test (Bergmann *et al*., 1993; Mackay *et al*., 1998).

The findings of this study indicate FMD viral activity in small ruminants as evident by reported sero-conversion detected by ELISA. The overall sero-conversion against FMDV NSP in small ruminants was recorded as 22.8%, ranging from 11.8-32.9% in various districts of the country. Similar to our findings, 21% sero-conversion in small ruminants was reported in a recent study conducted in three districts (Chakwal, Faisalabad and Khanewal) of Punjab province (Ur-Rehman *et al*., 2014). The sero-conversion reported in our study is lower than in a study conducted in Nigeria and it may be due to the difference in the sample size, locality, husbandry practices and genetic differences among the population.

In the present study, the FMDV NSP sero-positivity in female animals was significantly higher (24.9%) than in male animals (17.8%). A previous study conducted in three districts of Punjab also reported higher prevalence of FMD in female animals (26.26%) compared to male animals (11.46%) (Ur-Rehman *et al*., 2014). However, these results are in contrast to an Ethiopian study where 15.7% and 8.27% sero-conversions were reported in male and female animals respectively (Gelaye *et al*., 2009). Furthermore, another study reported no difference in risk associated with FMD transmission between male and female animals (Jenbere *et al*., 2011). The male and female animals have the equal chance of getting the disease. However, the difference in disease status may be due to the longer captivity of female animals as compared to male animals, which are sold at an earlier age. Another possible explanation for this difference may be physiological stress experienced by female animals due to pregnancy, lactation and nutrition.

In this study higher sero-prevalence was observed in goats (25.8%) as compared to sheep (14.3%; 55/384). These results are in accordance with the Nigerian study, which also reported a higher sero-prevalence of FMD in goats (15%) compared to sheep (9.3%) (Ehizibolo *et al*., 2010). The Indian study also reported similar findings (Rout *et al*., 2014). However, our results are different from another Nigerian study, which reported higher sero-prevalence of FMD in sheep (41.66%) compared to goats (21.8%) (Lazarus *et al*., 2012). A Jordanian study also reported, higher sero-prevalence of FMD in sheep (10.4%) compared to goats (6.3%) (Al-Majali *et al*., 2008). In another Pakistani study, higher sero-prevalence was observed in sheep (19.44%) compared to goats (21.27%) (Ur-Rehman *et al*., 2014).

The difference in sero-prevalence of sheep and goats in our study is probably due to inter species differences to FMDV susceptibility in sheep and goats. There is also a possibility that the FMDV behaves differently in sheep and goats. It has been reported that the susceptibility of sheep and goats to the FMD can vary with the breed of the animals and strain of the virus (Kitching and Hughes, 2002) and also it has been documented that the sheep to sheep spread of FMDV is restricted in a flock (Mackay *et al*., 1995). Furthermore, the duration of experimental persistence in sheep is 9 months (McVicar and Sutmoller, 1969) and persistence has been demonstrated to occur in goats with relatively lower prevalence compared to sheep (Sutmoller and McVicar, 1972).

The sero-prevalence in Islamabad district was significantly lower (11.8%) than other districts in this study, while there were no differences in the sero-

prevalence of FMD in small ruminants in other districts. There are no grazing areas in Islamabad, therefore nomads do not bring their animals for grazing to this area. In addition, the population of small ruminants in this area is less and farmers mostly feed their animals at home. The highest sero-prevalence was recorded in Attock (32.9%) followed by Toba Tek Singh (26.7%), Lahore (25.8%), Karachi (25.6), Muzaffarabad (22.9%), Gilgit (14.6%) and Islamabad (11.8%). The prevalence of the disease in Attock is higher due to the mixing of nomadic flocks with the local animal population during grazing.

Our findings indicated that small ruminants, kept in close contact with large ruminants may serve as reservoirs of FMD virus and possible sources of infection for susceptible livestock. The study also indicated that goats were more likely to be positive for FMD compared to sheep. However, these are the results of a small scale study and further large scale studies are required to get better understanding of role of small ruminants in transmission of FMD.

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