

Prevalence of Foot and Mouth Disease (FMD) in Goat and Sheeps in Various Districts of Punjab-Pakistan

Adnan Yousaf*, Lookesh Kumar, Faheem Ahmed, Ajeet Singh Berjang, Rehana Shahnawaz

Faculty of Animals Husbandry & Veterinary Sciences, Sindh Agriculture University, Tandojam, Pakistan

Email address:

Dr.adnan011@gmail.com (Adnan Yousaf)

*Corresponding author

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Abstract: Foot-and-mouth disease (FMD) is a contagious viral disease that affects animals with cloven feet. It is prevalent in Pakistan, resulting in significant economic losses. The study's goal was to determine the sero-prevalence of FMD in small ruminants (sheep and goats) maintained near large ruminants that had recently had an FMD epidemic. The research was carried out in ten districts of Pakistan that were carefully chosen. Between 2019 and 2021, 1760 sera were collected from small ruminants. Using the CHEKIT FMD-3ABC bo-ov kit, serum samples were analyzed for antibodies against FMD viral non-structural protein (3ABC) (IDEXX laboratories, USA). 2 test and multiple logistic regression techniques were used to examine the data. In small ruminants, the sero-prevalence of FMD was 27.22% (479/1760). The sero-prevalence of FMD in goats (288/1006; 28.63%) was considerably higher ($P<0.001$) than in sheep (191/754; 25.33%). After controlling for other variables, the risk of FMD in sheep was higher than in goats (CI: 0.4-0.78, $p=0.001$). Overall Male animals had a higher sero-positivity rate of 28.66% (70/244) and 34.53% (86/249) than female animals 23.72% (121/510) and 26.68% (202/757) in sheep and goats respectively. Female animals were more likely than male animals to test positive for FMD infection ($P<0.003$). District Chakwal (22.51%) had a substantially lower and District Rawalpindi had maximum (34.53%) sero-prevalence than the other districts ($p<0.001$). The data suggest that FMD is prevalent in small ruminants housed in close quarters with large ruminants, and that these animals might operate as FMD viral reservoirs and infection sources in vulnerable cattle.

Keywords: Foot-and-Mouth Disease, Prevalence, Sheep, Goats, Punjab

1. Introduction

Foot-and-mouth disease (FMD) is a highly contagious viral disease that affects animals with cloven feet, such as cattle, pigs, goats, and sheep and over 70 species of wildlife [1]. FMD virus (FMDV) has seven serotypes: A, O, C, Asia-1, SAT 1-3, and belongs to the Aphthovirus family of picornaviridae [6]. It's an un-enveloped virus with a positive sense RNA genome and a wide open reading frame that encodes four structural and ten non-structural proteins [4]. The disease has been documented in sheep and goats in addition to big ruminants [14]. FMD is endemic in Pakistan, resulting in significant economic losses in the livestock industry due to poor production [18], high morbidity, and mortality, as well as a ban on the export of livestock and their

products on the international market [4]. FMD-endemic regions such as India have reported annual economic losses of up to 2.7–3.6 billion US dollars [24], whereas these losses in Pakistan have been estimated at around 1 million US dollars [21]. However, because the disease's clinical indications are so minor in these animals [13], it often remains untreated [7]. The significance of sheep and goats in FMD epidemiology is poorly understood [20]. It might be related to the disease's hidden and self-limiting character in tiny ruminants [5]. Sheep and goats might be FMD carriers [2]. According to one study, sheep and goats can be FMDV carriers for 9 months and 4 months, respectively [3]. As a result, research is needed to better understand the involvement of sheep and goats in FMD epidemiology [8]. In Pakistan, FMD is prevalent, and three serotypes (A, O, and Asia-1) [10], are the most common causes of outbreaks in

cattle and buffaloes [1]. Although small and large ruminants are generally kept together in Pakistan [15], few FMD outbreaks in sheep and goats have been observed [12]. It is vital to comprehend the involvement of small ruminants in the overall epidemiology of FMD in Pakistan [11], in order to acquire a thorough understanding of the complicated epidemiology of FMD in Pakistan [9]. This information will aid in the development of a countrywide FMD control plan [16]. The goal of this study was to see how common antibodies against FMDV's non-structural proteins (NSP) were in sheep and goats reared in close proximity to cattle and buffaloes [26]. The results of this study might aid in determining the degree of FMDV circulation in sheep and goat flocks in the field, as well as their role in FMD outbreaks.

2. Materials and Methods

2.1. Ethical Approval

The experiment was performed regarding all rules and

regulation of animal rights. Society for protection and care of Animals (SPCA) Sindh Agriculture university Tandojam, Pakistan.

The research was conducted after approval of Institutional Ethical Committee.

2.2. Study Area

The purpose of this study was to determine the sero-prevalence of FMD in small ruminant's various districts of Punjab. During the years 2022-2023, a cross-sectional survey was undertaken in ten districts.

2.3. Study Animals

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

Ten districts were chosen as the principal sample unit on purpose (PSU). Seven villages were chosen at random as secondary sample units within each PSU. From 2022 to 2023, a total of $n = 1760$ samples (sheep = 754 and goats = 1006) were obtained.

Table 1. Prevalence of Foot and Mouth Disease (FMD) in Goat and Sheep in Various Districts of Punjab.

| Districts | Sheep's | | Goats | | Over all Prevalence | |
|------------|-----------------|--------------|-----------------|------------------|---------------------|------------------|
| | Sample Examined | Positive (%) | Sample Examined | Positive Samples | Sample Examined | Positive Samples |
| Rawalpindi | 65 | 12 (18.46) | 76 | 16 (21.05) | 81 | 28 (34.57) |
| Attock | 67 | 18 (26.87) | 86 | 26 (30.23) | 153 | 44 (28.76) |
| Bhakkar | 120 | 33 (27.50) | 170 | 55 (32.35) | 290 | 88 (30.34) |
| Chakwal | 111 | 23 (20.72) | 120 | 29 (24.17) | 231 | 52 (22.51) |
| Bahawalpur | 66 | 21 (31.82) | 79 | 27 (34.18) | 145 | 48 (33.10) |
| Chiniot | 52 | 14 (26.92) | 87 | 28 (32.18) | 139 | 42 (30.22) |
| Gujrat | 54 | 13 (24.07) | 90 | 24 (26.67) | 144 | 37 (25.69) |
| Jhelum | 89 | 21 (23.60) | 122 | 29 (23.77) | 211 | 50 (23.70) |
| Khushab | 76 | 22 (28.95) | 111 | 36 (32.43) | 187 | 58 (31.02) |
| Mianwali | 54 | 14 (25.93) | 65 | 18 (27.69) | 119 | 32 (26.89) |
| Total | 754 | 191 (25.33) | 1006 | 288 (28.63) | 1760 | 479 (27.22) |

2.4. Collection of Sample

The blood samples (5ml) were taken using vacutainer tubes (BD Vacutainer® Serum, REF 367812, BD Franklin Lakes, NJ, USA) after jugular vein puncture. After blood collection, samples were allowed to clot overnight and sera were collected in labelled 2ml cryo-vials. All of the sera ($n=1760$) were kept at -20°C until they were used. On a pre-designed proforma, information about the animal's age, sex, breed, vaccination against FMDV, and husbandry techniques were entered.

2.5. Detection of FMDV Specific Antibodies in Sera

Collected serum samples ($n=1760$) 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 [17]. The micro-titration plate was read in ELISA plate reader (Immunoskan MS, BDSL) using 450 nm filter [19].

2.6. Data Analysis

The data gathered from the study for the prevalence of

FMD were analyzed by Chi-square test.

3. Results

In small ruminants (sheep and goats), the total sero-prevalence of FMD was 27.22% (479/1760). Table 1 shows FMDV NSP sero-conversions in small ruminants (sheep and goats) by district. Sero-prevalence was substantially higher in goats 28.63% compared to sheep 28.63% which is $P < 0.001$. After controlling for other covariates, the probability of FMD infection in sheep was less than goats (CI; 0.4-0.78, $p=0.001$). The study found significant inter-breed differences in susceptibility to FMDV infection among goat breeds ($\chi^2=58.19$; $p < 0.001$). Potohari goats had 76% sero-prevalence, Kamori goats had 78.67%, Beetal goats had 24.5%, and Teddy goats had 23.53%. However, there was no statistically significant variation in FMD seroprevalence amongst sheep breeds ($p=0.95$). Balkhi had the highest sero-prevalence (18%), followed by Thalli (15.79%), Gojal (14.18%), Poonchi (12.82%), Kooka (12.82%) and Kajli (12.5%). Overall Male animals had a higher sero-positivity rate of 28.66% (70/244) and 34.53% (86/249) than female animals

23.72% (121/510) and 26.68% (202/757) in sheep and goats respectively. Female animals were more likely to be positive for FMD infection than male animals ($P < 0.003$), which is an important risk factor for FMD in small ruminants. However, when accounting for additional confounding variables, this impact was not evident in the logistic regression analysis (Odds ratio: 0.78; C.I 95%; 0.58-1.07). FMDV infection tends to become more common as animals get older. In kids, young, and adult animals, there was a significant difference in sero-conversion ($P < 0.001$). Adult animals had the greatest sero-conversion rate (27.4%), followed by young animals (20.3%) and Kids (12.3%). District Chakwal (22.51%) had a substantially lower and District Rawalpindi had maximum (34.53%) sero-prevalence than the other districts ($P < 0.001$) and there were no differences in the sero-prevalence of FMD in other districts.

4. Discussion

FMD is one of Pakistan's most common trans-boundary viral animal diseases [22], affecting mostly large ruminants (cattle and buffaloes) [27]. Despite the fact that community grazing and mixed production systems are common throughout the area, only a few FMD outbreaks in sheep and goats have been detected and reported [43]. Although Pakistan's small ruminant population (93.7 million) is larger than its large ruminant population (72 million) [36], a national project to control FMD in Pakistan [34], which is being implemented by the United Nations Food and Agriculture Organization (FAO) in Islamabad, Pakistan [31], only includes vaccination and sero-surveillance in bovines, completely ignoring the role of small ruminants in FMD transmission [28]. Sero-prevalence against FMDV was measured in sheep and goats in order to better understand the role of small ruminants in FMD epidemiology [23]. NSP antibodies were detected in serum samples using an NSP ELISA kit [30], which is simple to use and ideal for large-scale serological monitoring [32]. It can also tell the difference between animals that have been vaccinated and those who have been infected [25]. It can also detect 3ABC antibodies, which have a greater serum concentration than other NSPs [37]. Furthermore, 3ABC ELISA has been employed as a DIVA (Differentiation between Infected and Vaccinated Animals) test in ruminants [29]. As evidenced by reported sero-conversion verified by ELISA, the findings of this investigation demonstrate FMD virus activity in small ruminants [33]. In small ruminants, total sero-conversion against FMDV NSP was 23.8%, varying from 11.8-32.9% in different areas across the nation [35]. A previous research done in three areas of Punjab province (Chakwal, Faisalabad, and Khanewal) revealed 21% sero-conversion in small ruminants, which is similar to our findings [46]. Our research's sero-conversion rate is lower than that of a Nigerian study, which might be attributable to a number of factors [40]. The population's genetic differences, sample size, location, husbandry techniques, and sample size [51]. Female animals had a substantially greater rate of FMDV

NSP seropositivity 24.9% than male animals in this investigation 17.8% [39]. Female animals had a greater frequency of FMD 26.26% than male animals 11.46% in a prior research done in three districts of Punjab [57]. However, these findings contradict with those of an Ethiopian research, which found 15.7% and 8.27% sero-conversions in male and female animals, respectively [42]. Furthermore, according to another study, there is no difference in the risk of FMD transmission between male and female animals [54]. The disease has an equal probability of infecting both male and female animals [38]. However, the difference in disease condition might be related to female animals' prolonged confinement compared to male animals, who are sold at a younger age [54]. Another explanation for the discrepancy might be the physiological stress that female animals face as a result of pregnancy, breastfeeding, and nutrition [45]. Sero-prevalence was found to be greater in goats 28.63% than sheep 25.33% in this investigation [53]. These findings are consistent with those of a Nigerian research, which found that goats 15% have a greater sero-prevalence of FMD than sheep 9.3% [48]. Similar outcomes were also found in an Indian investigation [41]. However, our findings vary from those of another Nigerian research, which found that sheep 41.66% had a greater sero-prevalence of FMD than goats 21.8% [44]. In a Jordanian research, sheep had a greater sero-prevalence of FMD 10.4% than goats 6.3% [54]. Sero-prevalence was found to be greater in sheep 19.44% than goats 21.27% in another Pakistani investigation [50]. The discrepancy in sero-prevalence between sheep and goats in our study is most likely owing to differences in FMDV susceptibility between the two species [47]. It's also possible that the FMDV has distinct effects in sheep and goats [52]. It has been noted that the susceptibility of sheep and goats to FMD varies depending on the animal's breed and virus strain [49], and that FMDV transmission from sheep to sheep is limited within a flock [27]. Furthermore, the period of experimental persistence in sheep is 9 months [39], while goats have been shown to have a lower incidence of persistence than sheep [4]. In this study, the sero-prevalence in Chakwal district was substantially lower (22.51%) than in other districts, whereas there were no variations in the sero-prevalence in other districts. Other districts have a high frequency of FMD in small ruminants. Because there are no grazing sites, nomads do not bring their livestock here to graze. Furthermore, the number of small ruminants in this area is low, and farmers feed their animals mostly at home. Rawalpindi 34.57% had the greatest sero-prevalence, followed by Bahawalpur 33.10%, Khushab 31.02%, Bhakkar 30.34%, Chinot 30.22%, Attock 28.76%, Mianwali 26.89%, Gujrat 25.69%, Jhelum 23.70% and Chakwal 22.51%. Due to the mingling of nomadic flocks with the indigenous animal population while grazing, the disease is more prevalent in Rawalpindi.

5. Conclusion

The findings suggested that small ruminants housed in

close quarters with large ruminants might operate as FMD viral reservoirs and infection sites for vulnerable cattle. The study also found that goats were more likely than sheep to test positive for FMD. However, these are the findings of a small-scale study, and larger-scale research is needed to have a better understanding of the function of small ruminants in FMD transmission.

Conflicts of Interest

The authors declare that they have no potential conflicts of interest.

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