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Serological evidence of foot-and-mouth disease virus infection in goat breeds in the Samsun province of Turkey

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ABSTRACT. Foot-and-mouth disease (FMD) is one of the major endemic trans-boundary livestock diseases of socio-economic importance in Turkey and worldwide. Goats constitute the third largest susceptible population of domestic livestock in Turkey. FMD surveillance and control strategies in the country largely ignore small ruminants, known to be critical in the epidemiology of the disease. In this study, blood samples were randomly collected from different domestic goat breeds (Anatolian black goat, Maltese and Saanen). The material consisted of 368 domestic goats, including 121 Anatolian black, 125 Maltese and 122 Saanen goats from Samsun province. The serum samples were examined for the presence of antibodies to foot and mouth disease virus using non structural proteins (NSPs) competitive enzyme-linked immunosorbent assay (ELISA). Out of 368 serum samples examined, 12 (3.26%) were positive for FMD. Seropositivity rates in Anatolian black, Saanen and Maltese breeds were 0.83%, 0.82% and 8.00% for FMD, respectively. Although, seropositivity rate in Maltese goat breed was higher than others, this result was not attributed to breed susceptibility. The results of the investigation indicate that FMD is less widespread in goats than sheep and cattle in Samsun province. The results, supported for the first time in Turkey the hypothesis that goats act as a potential reservoir of FMD virus and thus have a role in the epidemiology of FMD.

Key words: FMDV, ELISA, goat, seroprevalence, Turkey

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INTRODUCTION

Foot-and-mouth disease (FMD) is endemic in many parts of the world, particularly in Asia and Africa. Foot-and-mouth disease virus (FMDV) is a causative agent of highly infectious and economically important disease in cloven-hoofed animals characterized by the formation of vesicles in, and around, the mouth, on udder and on the feet. The disease set significant constraints to international trade of live animals and animal products (Grubman et al., 2004; OIE, 2012). FMDV is a single-stranded RNA virus belonging to the genus *Aphthovirus* in the family *Picornaviridae*. There are seven serotypes (each including several strains), with each serotype showing clinical disease, indistinguishable from that caused by the others (Knowles et al., 2012). FMD is endemic in Turkey with outbreaks occurring frequently; cattle can show overt clinical signs than small ruminants. The control strategies for FMD outbreaks include quarantine and ring vaccination of cattle using trivalent vaccines (O, A and ASIA 1). However, the success of these efforts is hindered by uncontrolled and excessive animal movements (Klein et al., 2006; Parlak et al., 2007). Regular vaccination is one of the well-known methods for the control of FMD. Use of inactivated whole-virus vaccines has been successful in the control and eradication of FMD in several parts of the world. Inactivated vaccines mainly consist of capsid proteins, thus less likely induce antibody responses to non-structural proteins (NSP) after vaccination. The detection of antibodies to NSP is, therefore, the preferred diagnostic method to distinguish virus infected carrier animals from vaccinated animals. Non-structural protein serology assays are routinely used to monitor virus activity in endemic countries where vaccination is performed (Clavijo et al., 2004; Rweyemamu et al., 2008; Hosamani et al., 2015).

Currently, there is no recorded data regarding prevalence and distribution of FMDV infection among goat breeds in Turkey. Therefore, the objective of this study was to investigate FMDV infection serologically in rural areas of the Samsun province of northern Turkey, and to detect some factors influencing the epidemiology of FMDV infections (age, breed, sex).

MATERIALS AND METHODS

The study protocols and experimental procedures were approved by the Samsun Veterinary Control In-

stitute Scientific Ethics Committee (No: 36).

Blood samples were collected from goats housed in 9 herds, 7 in Tekkekoy area and 2 in Havza area of Samsun province in the northern part of Turkey between January and September 2012 (Figure 1). As regards to the breed, 121, 125 and 122 of the samples were originating from Anatolian black, Maltese and Saanen goats, respectively. The age of the animals varied from 6 months to 7 years. Blood samples were collected from the jugular vein of the animals. Blood tubes were centrifuged at $3,000\times g$ for 10 min, and the samples were transferred to sterile tubes and stored in -20°C until used. The commercial FMD NSP competitive ELISA kits (ID.Vet Innovative Diagnostics, Montpellier, France, FMDNSPC ver 0914 GB) were used. Plates were read with an ELISA reader at 450 nm and results were calculated. According to manufacturer's instructions, the mean value of the negative control (NC) optical density (OD) is greater than 0.7 and the mean value of the positive control (PC) OD is less than 30% of the OD_{NC} ($\text{OD}_{\text{NC}} > 0.7$ and $\text{OD}_{\text{PC}} / \text{OD}_{\text{NC}} < 0.3$). Furthermore, the competition percentage: " $\text{S/N}\% = \text{OD}_{\text{sample}} / \text{OD}_{\text{NC}} \times 100$ " had been calculated for each sample. Samples presenting S/N% less than or equal to 50% were considered as positive, while greater than 50% as negative ($\text{S/N}\% \leq 50\%$: Positive; $\text{S/N}\% > 50\%$: Negative).

Chi-square test to determine significance differences in percentages had been used.

RESULTS

As a result of ELISA test, out of 368 goats, twelve (3.26%) were found to be seropositive for FMD. Seropositivity rates in Anatolian black, Saanen and Maltese breeds were detected as 0.83%, 0.82% and 8.00% for FMD, respectively (Table 1). The use of the chi-square test to determine significance of FMD percentage in Maltese goats was higher than indigenous Anatolian Black and composite Saanen goats ($p < 0.01$). In addition, FMD percentage in adult goats was higher than kids ($p < 0.01$). The prevalence of antibodies did not differ between male and female goats for FMD ($p < 0.01$).

The seroprevalence rate for FMD were on the different level in Havza (8.0 %) and Tekkekoy towns (0.82 %). In Havza town, we detected a higher seroprevalence for FMDV than Tekkekoy town.

Table 1. Seroprevalence of FMD in Samsun area.

		Number of Seropositive animals/Total number of animals (%)				
Goat breeds	Towns	Female	Male	Kids < 1 year old	Adults > 1 year old	Total (%)
Saanen	Tekkekoy	1/108(0.93)	0/14(-)	0/15(-)	1/107(0.93)	1/122(0.82)
Maltese	Havza	8/105(7.62)	2/20(10.00)	(-)	10/125(8.00)*	10/125(8.00)*
Anatolian Black	Tekkekoy	1/96(1.04)	0/25(-)	0/35(-)	1/86(1.16)	1/121(0.83)
Total		10/309(3.24)	2/59(3.39)	0/50(-)	12/318(3.77)	12/368(3.26)

*, Indicates difference among breeds in the same column, (p<0.001)

DISCUSSION

Turkey is of special interest for the European countries, because it is the natural connection to Asia, where FMD is endemic and thus serves as buffer zone for the disease. Small ruminants should be given due importance, as FMD epidemics in various countries have previously highlighted their epidemiological significance (Ranabijuli et al., 2010; Rout et al., 2014). When infected with FMDV, unvaccinated cattle and pigs usually develop clear signs of FMD and a presumptive diagnosis can be made on the basis of clinical signs without a history of contact between the herd and an infected animal. However, clinical diagnosis in sheep and goats is more difficult because FMD is often mild or subclinical and lesions may be similar to those caused by other common diseases (Knowles et al., 2012; OIE, 2012), so that the infection can very often go unnoticed. As these animals have an unrestricted access to local cattle population, the interspecies transmission of virus cannot be ignored (Ranabijuli et al., 2010). This is because, the interspecies interaction in shared grazing areas or along the livestock migratory routes poses threats for undetected virus circulation in small ruminants. As most of the goats were not vaccinated against FMD, further routine prophylactic

vaccination to maintain an optimum level of herd immunity against FMD is suggested in the region.

The prevalence of antibodies in goat breeds varied among our study sites from intense to sparse, suggesting that FMDV seroprevalence was either spatially focal or temporally sporadic. Seroprevalence of FMDV in goat breeds were most intense in the Havza town wherein the highest number of FMD outbreaks was reported during last period. The lowest prevalence (0.82%) of antibodies in our sample of goat breeds from Tekkekoy town where the lowest number of FMD cases was reported during last period. Although seropositivity rate in Maltese goats was presently higher than in goats of the other breeds, it cannot be attributed to breed susceptibility, but is rather a result of higher FMDV exposure numbers (Table 1). Indeed since 2008, a total of 39 FMD outbreaks have been documented in Havza town, in contrast to only 4 FMD outbreaks documented in Tekkekoy town. The apparent overall seroprevalence in Havza town was 8.00%, which signifies a very high level of FMD virus circulation in the Maltese goat breed population despite the lack of clinical signs in this species. The apparent prevalence of test result was positively correlated in the sampling areas.

Figure 1. Sampling areas (each red dot represent one herd)



Sevik (2013) has reported that female animals had higher antibody responses to inactivated FMD vaccine than male animals, but we did not detect any differences between male and female goats in terms of sex susceptibility for FMD.

CONCLUSIONS

Although here only 368 goat serum samples were tested in NSP ELISA, this preliminary work has gathered serological evidence of viral activity in goat

population of Turkey, concurrent FMD virus infection and asymptomatic goats in outbreak areas, all of which underscores the fact that unrecognized FMDV-infected goats could pose a potential risk of virus dissemination. The results could be helpful to veterinary authorities in assessing the risk factors of FMDV, but to understand the role of goats in the FMDV outbreaks in Turkey better, additional studies using a larger sample size supplemented with virological examination data are needed.

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CONFLICT OF INTEREST STATEMENT

None of the authors of this article has any conflict of interest.

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