Research Article

Investigation of the Epidemiology of Foot and Mouth Disease in Sheep and Cattle with NSP ELISA and LPBE

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Abstract | Control of Foot and Mouth Disease depends on the disease control policies and epidemiological status of the country. In countries where the disease is endemic, protective vaccinations are performed with inactive vaccines of suitable serotype and measures are taken in order to reduce the prevalence of the disease. In this study, blood samples were collected from clinically healthy sheep and bovine in the surrounding region of the southeast of Konya province that were classified as non-vaccinated, single-vaccinated, multiple vaccinated, aged 0-1 and 1-3 and male and female starting from the third month following the vaccination. In the study aimed to show the disease-related status of the selected region, to investigate the active virus circulation and determine the antibodies formed against the nonstructural proteins (NSP) of Foot and Mouth Disease and evaluation the carrier rate in the region and the risk of infection and given information on the immune ratio of the animals. As a result, prevalence was observed to be 6.6% for breeding enterprises, 13.3% in bovine animals and 1% in ovine animals individually. Prevalence was found 13.3% throughout Konya. Generic immune ratio in bovine animals was found 58.8% for serotype O and 61.1% for serotype A; and in sheep, 51% for serotype O and 55% for serotype A. Difference was detected to be significant (p<0.001) in bovine by groups' vaccine, age and gender. It was significant in sheep varying by groups vaccine-age (p<0.001).

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Introduction

Foot and mouth disease is an acute and highly infectious viral disease of cloven-footed animals that negatively affects the transnational livestock and animal product trade and causes economic losses (Dill et al., 2017). The foot and mouth disease virus antigenically varies and has 7 different serotypes (AOC, Asia 1, SAT1, SAT2, SAT3). The foot and mouth disease virus also have a high mutation rate under natural conditions, as in other single-stranded RNA viruses (Sobrino et al., 1986; Steinhauer et al., 1987; Tekleghiorghis et al., 2014). The disease causes significant economic losses in milk production and also limits the international trade of livestock and animal products (Ko et al., 2009).

At the OIE General Assembly held in Paris, France in May 2010, the Thrace region of Turkey gained the status of "Free zone where vaccinated from Foot and Mouth Disease" (Tarım, 2010). In the examinations performed in the outbreaks of foot and mouth disease in Turkey, it has been reported that the most common route of transmission of the disease is direct

transmission. In this form of transmission, animal movements and livestock markets are generally thought to play an important role (Aktas, 1988). Animals, such as dogs and horses, that are resistant to foot and mouth disease may play a role in the mechanical transport of the disease (Alexandersen et al., 2005).

It has been reported that the pathogenicity of infectious virus particles scattered by persistently infected sheep with their secretions and extracts for cattle and buffalos does not change and plays an important role in the epidemiology of foot and mouth outbreaks (Moonen et al., 2004). NSP ELISA kits were prepared to identify the detection of antibodies formed against non-structural virus proteins of the foot and mouth disease virus. Since NSP ELISA kits are not type-specific, as a result of test positivity, the fact that the animal has foot and mouth disease is only determined, but the serotypes of the virus cannot be determined (Dekker et al., 2003).

NSP ELISA kits are used in distinguishing the animals that have had disease from vaccinated animals and in eradication and control studies to determine the carriage status in the field, especially in epidemiological studies (Bergmann et al., 2000; Clavijo et al., 2004; OIE, 2004).

With this study, it was aimed to reveal the disease-related status of the selected region and to investigate the active virus circulation in blood seras of the cattle and sheep in the region surrounding the southeast of Konya province in the Central Anatolia Region that were classified as unvaccinated, single-vaccinated, multiple vaccinated, aged 0-1 and 1-3 and male and female gender groups starting from the third month following the vaccination, to reveal seroprevalence and to get information about the immune ratio of the animals by determining the antibodies formed against non-structural proteins of foot and mouth disease and assessing the carriage ratio in the region, in other words, the risk of disease. Thus, it is thought that contribution can be made within the framework of the policies related to the action plans to be formed and the eradication programs to be developed in the Epidemiology of Foot and Mouth Disease and fighting against it.

Material and Methods

Blood Sera Samples

The cattle and sheep were grouped by age (0-1 years, 1-3 years), number of vaccinations (unvaccinated, single-vaccinated, multiple (two and more than) vaccinated) and gender (female, male) to investigate the active virus circulation in the southeastern part of Konya province and to determine the immune ratio in animals after vaccination against foot and mouth disease (Table 1). By sampling, blood samples were taken from the animals' *V. jugularis* into vacuum blood collection tubes separately for each animal. This study was approved by the ethics board of the Faculty of Veterinary Medicine, Selçuk University, Konya, Turkey (Approval No: 2008/10-081).

Table 1: *Data of samples collection.*

District	Animal description							
	Cattle				Sheep			
	Number of farm	Age		Total	Number of farm	Age		Total
		0-1 years	1-3 years			0-1 years	1-3 years	
Emirgazi	18	16	14	30	20	19	21	40
Ereğli	12	16	14	30	10	10	10	20
Karapınar	15	18	12	30	20	21	19	40
Total	45			90	50			100

NSP ELISA kit

In the study, the ready ELISA kit (PrioCHECK ® FMDV NS, Prionics Lelystad BV, Netherlands) was used for the determination of non-structural proteins to distinguish the animals that had diseases from vaccinated animals.

Trapping antibody

This sera is the antisera obtained by the injection of the 146S antigen of the foot and mouth disease virus into rabbits. It was used in the coating of ELISA plates.

Control antigens

Serotype O, A serotypes of the foot and mouth disease virus (O_1 Manisa, A_{22} Iraq strains) were of cell culture origin and used as the control antigen.

Control Sera

These seras were used as antibody strong positive, antibody weak positive and antibody negative according to the amount of antibody they contain to the extent of appropriate inhibition values against foot and mouth disease virus O and A serotypes.

Detecting antibody

This sera is the antisera obtained by the injection of the 146S antigen of the foot and mouth disease virus into guinea pigs. It was used as a detecting antibody.

Conjugate

It was obtained by binding rabbit anti-guinea pig antibodies to horseradish peroxidase enzyme. It is not type-specific and was used as an indicator.

Liquid phase blocking ELISA test

It was performed according to the method adopted by the OIE and reported by Hamblin et al. (1986). ELISA plates were coated with serotype-specific (serotype O and A) rabbit antisera and kept at 4 °C overnight. The plates were incubated overnight so that the antibodies present in rabbit antisera would be absorbed into the plate. In the first stage, 1/16 dilution of the test sera and control sera was performed on U-based carrier plates. 1/32 initial dilution was achieved by adding an equal amount of the viral antigen. It was incubated at 4ºC for overnight neutralization. Then, the ELISA plates with rabbit antisera were washed 3 times with 0.05% Tween 20 PBS. Sera-antigen mixtures in the carrier plates were transferred to the ELISA plates. In the blocking solution prepared, guinea pig antisera were type-specifically added to plates for O and A serotypes. They were incubated for 1 hour. At the end of the incubation period and after washing, the conjugate was added and washed after incubation. Orthophenylene diamine (OPD) and 0.05% H_2O_2 were added to each plate. After 15 minutes, 1.25 M H_2SO_4 stop solution was added to the plates to stop the reaction. It was read in accordance with the absorbance criteria at 492 nm wavelength with a spectrophotometer using Softmax Pro-312ex program, and the % Inhibition Value was calculated from the Optical density (OD) determined as a result of the reading process. The inhibition values of 50% and above were considered positive.

Results and Discussion

NSP ELISA test

Among 90 cattle blood sera taken from 45 businesses in the southeastern region of Konya province, it was determined that 12 blood sera belonging to Emirgazi and Karapınar districts and villages were positive. Of the 12 positive results, 6 (6.6%) were multiple vaccinated, 1 (1.1%) was unvaccinated, 5 (5.5%) were single-vaccinated, 11 (12.2%) were female, and 1 (1.1%) was male, 6 (6.6%) were between 0-1 years old and 6 (6.6%) were between 1-3 years old. 1 of 100 sheep blood sera obtained from sheep businesses in Emirgazi district was determined to be positive. It was determined that 1 (1%) positive result was an unvaccinated female aged between 0-1 years. According to these results, the percentage of NSP positive animals in total sera was determined to be 1% in cattle and 13.3% in sheep. After the NSP ELISA test performed, the businesses with positive NSP were visited, and the field research was carried out to investigate the cause of positive results and the presence of disease risk. Interviews were conducted with veterinarians and animal owners in the region and positive businesses were examined.

Liquid phase blocking ELISA

As a result of the test, it was evaluated that positivity was 5.49 at the base of $\log_2$ and protection was 6.58 at the base of $\log_2$. In the data analysis, the percentage frequency tables were formed, and the significance level of the test results (p-value) was interpreted by using the Pearson's chi-square statistic and Fisher's exact test in statistical comparisons. In the study, the level of significance was considered to be $p<0.001$. The general immunity ratio for cattle in the region was found to be 58.8% for O serotype and 61.1% for A serotype. For sheep, it was found to be 51% for O serotype and 55% for A serotype.

In cattle, the protective immunity ratio according to age groups was found to be 46% in the 0-1 age range and 80% in the 1-3 age range for O serotype and 46% in the 0-1 age range and 85% in the 1-3 age range for A serotype. The protective immunity ratio according to gender was found to be 77.2% in female animals and 45.6% in male animals for O serotype and 79.5% in female animals and 47.8% in male animals for A serotype. The protective immunity ratio according to the vaccination status was found to be 10% in unvaccinated cattle, 55% in single-vaccinated cattle and 80% in multiple vaccinated cattle for O serotype and 10% in unvaccinated cattle, 55% in single-vaccinated cattle and 85% in multiple vaccinated cattle for A serotype.

In sheep, the protective immunity ratio according to age groups was found to be 26% in the 0-1 age range and 76% in the 1-3 age range for O serotype and 28% in the 0-1 age range and 82% in the 1-3 age range for A serotype. The protective immunity ratio according to gender was found to be 60% in female sheep and 42% in male sheep for O serotype and 62% in female sheep and 48% in male sheep for A serotype. The protective immunity ratio according to the vaccination status was found to be 5% in unvaccinated

sheep, 45% in single-vaccinated sheep and 80% in multiple vaccinated sheep for O serotype and 5% in unvaccinated sheep, 50% in single-vaccinated sheep and 85% in multiple vaccinated sheep for A serotype.

The diagnosis of foot and mouth disease makes a significant contribution to the determination of outbreak in a short time and the monitoring and prevention of epidemic spread in the countries where the disease is epidemic like Turkey. Vaccination is an important tool to be considered first in the fight against foot and mouth disease. Protection from the disease is ensured in vaccinated herds, and also, active virus circulation and persistence will be suppressed and minimized. Here, the most important thing is that the virus source is not taken into the vaccinated areas to prevent the risk of the disease from becoming permanent. Otherwise, the carriage will appear as a risk for the region. The carriage is one of the most important problems in fighting against the disease.

In the study carried out by Gürhan et al. (1993), it was determined that the carriage ratio for sheep was 16.8% in the Central Anatolia Region of Turkey, and it was reported that the carriage ratio decreased from the Eastern Anatolia, Southeastern Anatolia, and Central Anatolia regions towards north and west, which was related to the prevalence of the disease. In an experimental study carried out by Fondevila et al. (1996), the carriage ratio was found to be 100% in sheep and goat with a low antibody level while it was found to be 42% in animals with high antibody levels. In the study on the seroprevalence of foot and mouth disease in sheep and goat carried out by Balinda et al. (2009), in 2007, 14% prevalence and 22% prevalence were determined in goats and sheep, respectively. They reported that this difference was probably due to different levels of exposure to foot and mouth disease and different animal husbandry practices. The ratios determined in this study are compatible with the study carried out by Leon et al. (2003), to investigate the circulation of foot and mouth disease in sheep and cattle. The results of the study are similar to the findings of other studies reported in the world (Gil, 2003; Megersa et al., 2008; Mannan et al., 2009; Molla et al., 2010; Mwiine et al., 2010).

With respect to the level of immunity, in a study carried out by Psikal et al. (1995), the researchers investigated the antibodies of 50 blood cattle sera, collected from 20 animals vaccinated with the inactive trivalent vaccine and 30 animals unvaccinated for more than 1 year, formed against the foot and mouth disease virus O, A and C serotypes by the LPBE method, and they determined that they had 58%, 66% and 58% antibodies, respectively.

The findings related to the level of immunity in this study are compatible with the study in Argentina in which Smitsaart et al. (1998) determined the amounts of antibodies in sera obtained from vaccinated cattle aged 0-1 years, 1-2 years and 2 years and above by LPBE. In the cattle aged between 1-3 years, it was determined that the immunity formed against A serotype of the foot and mouth disease virus was higher than the immunity formed against O serotype. In sheep, similarly to that in cattle, it was determined that the immunity formed against A serotype of the foot and mouth disease virus was higher than the immunity formed against O serotype among different age groups. Furthermore, it was observed that the ratio of the foot and mouth disease virus with the protective antibody against O and A serotypes increased proportionally with age. These results obtained in the present study are consistent with previous studies (Sil et al.1999; Şevik, 2013).

In terms of age groups, it was found to be 46%, 80% - 46% and 85% in cattle and 26%, 76% - 28% and 82% in sheep against O and A types. Moreover, in the 0-1 age group that was vaccinated for the first time, the ratios were found to be below the general average as expected. The fact that the level of immunity was found to be low since young animals in this group were vaccinated for the first time and booster vaccination was not performed was considered to be normal. These results obtained in the present study are consistent with the determination that female animals (68.56%) were more resistant against foot and mouth disease compared to male animals (64.45%) in the study carried out by Sil et al. (1999). The results related to the level of immunity are similar to the findings of other studies carried out around the world (Woodbury et al., 1995; Reid et al., 1998; Smitsaart et al., 1998; Sil et al., 1999; Reid et al., 2000; Blanco et al., 2002; Grindharan et al., 2005).

In conclusion, it was determined that the vaccination administered in the region ensured a sufficient level of immunity and that a herd immunity of over 60% in regularly vaccinated animals and in general a sufficient level of immunity were found. Since foot

and mouth disease has a highly infectious, wide host spectrum and has numerous types and subtypes and has a complex antigenic structure, vaccination campaigns can be successful if they are applied with other control measures. Furthermore, it is necessary to follow the vaccination rules and program required to ensure appropriate immunity since the vaccine administered provides short-term immunity.

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