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An Investigation of the Seroprevalence of Pestivirus Infection in Goats in Denizli Province of Turkey

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Abstract: Pestiviruses cause important infections in ruminants worldwide. Pestivirus infections are economically important due to their direct effects on reproduction in the sheep and goat breeding sector. The pestivirus genus of the Flaviviridae family is single-stranded, icosahedral symmetrical, membranous RNA viruses, among which there are also Bovine Viral Diarrhea Virus (BVDV type 1 and 2), Border Disease Virus (BDV) and Classical Swine Fever (CSFV) virus. BDV is the main cause of congenital infections in sheep and it can cause acute, fetal and persistent infections. BDV infections are common in countries where BVDV infections are endemic in cattle worldwide. Clinical disease is rarely reported in goats. Persistently Infected (PI) offspring in goats are less common compared to sheep or cattle. Vertical transmission plays an important role in the epidemiology of the infection. Serological studies have demonstrated that natural pestivirus infections in goats are common in many countries. In this study, 274 blood samples were collected via. random sampling from goats in 34 livestock enterprises in agroecological sub-regions of Denizli Province, since, there is no sufficient information on the seroprevalence of pestiviruses in goats in Denizli Province of Turkey. The samples were centrifuged at 3000 rpm and serum was obtained. Blood serum was inactivated at 56°C for 30 min and then stored at -20°C in a deep freezer until being tested. Serum samples were investigated in terms of the presence of antibodies by the Serum Neutralization Test (SNT) using the BVDV reference strain NADL as a virus strain. The Madin-Darby Bovine Kidney (MDBK) cell culture was used for testing. The infectious power of the BVDV-NADL strain was determined as the Tissue Culture Infective Dose ($TCID_{50}$/0.1 mL) value of 10^5. According to the test results, the individual seroprevalence was 10.58% (29/274) and the flock-based

seroprevalence was 14.71% (5/34). When the seropositivity rates of the animals were evaluated according to their sex, 12.85% of the female goats and 3.12% of the male goats were found to be antibody-positive. According to the sex of the goats, the seropositivity rates of the female and male animals were found to be statistically significant (χ^2:4.90; p:0.02). A significant difference was found in terms of age groups (6-24, 24-48 and 48 months<) (χ^2:11.77; p:0.002). As a result, pestivirus infection was detected in goats in livestock enterprises in Denizli Province, although, it was not common. Therefore, large-scale studies are important for the economy of the region and the country to prevent the spread of the infection in question and to determine its prevalence rates.

INTRODUCTION

Pestiviruses are pathogens with a worldwide distribution in which signs of infection in ruminants can range from subclinical to severe clinical manifestations. Pestiviruses causing infections in small ruminant (sheep and goat) species characterized by the findings of the reproductive system have been classified in the pestivirus genus of the Flaviviridae family. There are generally four viruses in the pestivirus genus including Border Disease Virus (BDV), Bovine Viral Diarrhoea Virus 1 (BVDV-1), Bovine Viral Diarrhoea Virus 2 (BVDV-2) and Classical Swine Fever Virus (CSFV). These viruses are antigenically and genetically closely related to each other[1, 2].

Viruses in the pestivirus genus contain a single-stranded, positive-polar RNA molecule with a length varying between 12.3-12.5 kb. The virion is enclosed in an envelope of nucleocapsid lipid structure with an Open Reading Frame (ORF), approximately 40-60 nm in diameter and surrounded by untranslated regions (5' and 3', untranslated region) from both ends[3-5].

Acute pestivirus infections in adult sheep and goats usually do not show any clinical findings other than mild fever. The infection generally shows a subclinical course in non-pregnant goats. Abortion cases occurring in sheep are shaped more than persistent offspring birth[6]. However, persistent goats are not common in natural infection. However, the studies conducted show that goats are susceptible to BVDV infection[7, 8]. Especially in sheep, PI offspring births may occur following fetal infection. PI animals are of great importance in the epidemiology of the infection. PI sheep and especially cattle play the role of the suppliers of pestivirus infections in goats[9]. Pestiviruses have been obtained from only a few naturally infected goats[10, 11].

Serological studies in many parts of the world demonstrate that natural pestivirus infection is common in sheep and goats[7,9]. Since, PI is not very common in goats and acute infection is temporary, seroepidemiological studies are the most beneficial approach to evaluate pestiviruses spreading among ruminants. In the studies, conducted on the presence of pestiviruses in Turkey, it was determined that pestiviruses cause economic losses in small ruminants and the presence of factors at rates varying between 0.7-3% was identified in flocks where abortion also took part[12-15]. Within this framework, the diagnosis of viral diseases and the investigation of their epidemiology are an important criterion for eradication and control strategies. In this study, the seroepidemiology of pestivirus infection in livestock enterprises dealing with goat breeding in Denizli Province was investigated.

MATERIALS AND METHODS

Sample collection and serum processing: Within the scope of this study performed in Denizli Province located in the Inner Aegean Region of Turkey, between 28°30'-29°30' Eastern meridians and 37°12'-38°12' Northern parallels, none of the goats investigated was vaccinated against pestivirus infection. Blood samples were taken into tubes to obtain serum from the vena jugularis of 274 clinically healthy goats by random sampling from goats in 34 livestock enterprises in the agroecological sub-regions of Denizli Province. The samples were centrifuged at 3000 rpm and serum was obtained. Blood serum was inactivated at 56°C for 30 min and then stored at -20°C in a deep freezer until being tested.

Virus and cell culture: In the study, NADL, the reference strain of the BVD virus was used to detect antibodies formed against pestivirus. The Madin-Darby Bovine Kidney (MDBK) cell culture was used for the production of BVD virus, calculation of infectious power, calculation of the SN_{50} value and virus neutralization test. In the production of cell cultures, Eagle's Minimal Essential Medium (EMEM) was used by adding 10% fetal calf serum and Dulbecco's Modified Minimal Essential Medium (DMEM) was used for the production of the virus, the calculation of the infectious power of the virus and the calculation of the VNT and SN_{50} values.

Virus neutralization and Serum Neutralization$_{50}$ (SN_{50}) test: To this end, the method reported by Frey and Liess[16] was used. The infectious power of the BVDV-NADL strains produced in the cell culture was determined as the Tissue Culture Infective Dose ($TCID_{50}$/0.1 mL) by the microtitration method according to the method of Kaerber. The serum obtained from 274

blood samples collected in the study was tested according to the microneutralization method, following the previously reported procedure for the presence of specific antibodies responsible for neutralization for BVDV[16]. To determine the antibody titer, serum dilutions of 1:5, 1:10, 1:20, ..., 1:320 were prepared for BVDV, starting from a titer of 1:5[17]. After the samples were diluted in medium at a ratio of 1:5, 50 μL was placed in two wells of the 96-well microplate. It was diluted with an infectious power of 100$TCID_{50}$ and placed in equal proportions (50 μL serum+50 μL virus). After incubation for 1 h in the oven (5% CO_2, 37°C), MDBK cell suspension (300.000 h mL^{-1}) was added to all wells and removed to the oven. One day later, positive samples evaluated under the microscope were subjected to the SN_{50} test. The neutralization test was accepted as positive and was evaluated as seropositive since antibodies in serum samples in the wells of the microplate in which CPE could not be detected were homologous for virus strains and blocked virus growth. Since, antibodies and virus strains were not specific for each other in the wells with CPE, virus growth was not blocked and the result was evaluated as seronegative.

Statistical analysis: Data processing was performed using the SPSS (Statistical Package for Social Sciences) package program. Chi-square tests were used to analyze age, sex and sub-regions. The value of $p<0.05$ was accepted to be statistically significant.

RESULTS AND DISCUSSION

According to the test result in this study, the $TCID_{50}$/0.01 mL value of the BVDV virus was found to be 10^5. It was determined that the antibody titers of the blood serum with BVDV specific antibodies had antibodies at the titers varying between 1:5 and 1:160. For BVDV, the intensity was found to be between 1:5-1:20.

As a result of the serum neutralization test applied to 274 goat blood serum samples in the study area, specific antibodies against BVDV were detected in 29 samples (10.58%). The lowest and highest values for BVDV infection were found to be 8.54% (7/82-sub-region I) and 15.91% (7/44-sub-region IV), respectively. Flock-based BVDV infection rates varied between 10.00-25.00%, respectively. No statistically significant difference was found between the sub-regions (sub-region I-IV) ($p>0.05$). Table 1 shows in detail the number and prevalence rates of antibody-positive animals against pestivirus in goats. According to the test results, the individual seroprevalence was 10.58% (29/274) and the flock-based seroprevalence was 14.71% (5/34). Of a total of 274 seropositive serum samples, SN_{50} values were determined as 1:5 in 34.48% as 1:10 in 27.59% as 1:20 in 17.24% as 1:40 in 10.34% as 1:80 in 6.9% and as 1:160 in 3.45% (Fig. 1). When the seropositivity rates of the animals were evaluated according to their sex, 12.85% (27/210) of the female goats and 3.12% (2/64) of the male goats were detected to be antibody-positive. According to the sex of the goats, the difference between the seropositivity rates of the female and male animals was found to be statistically significant (χ^2: 4.90; p:0.02) (Table 2). A significant difference was found in terms of age groups (6-24 months, 24-48 months, 48 months<) (χ^2:11.77; p:0.002) (Table 3).

Pestivirus infections are quite common in Turkey as in many countries in the world and cause economic losses. Due to the antigenic affinity of BVDV and BDV isolates, their physical and biological similarities, both viruses are identified as the same species within the Pestivirus genus[18]. In this study, the detection of BVDV antibodies in goat flocks shows the presence of pestivirus infections in the sampled flocks. Pestiviruses were added to the World Organization for Animal Health (OIE) list of priority diseases for international trade. Furthermore, the identification of pestiviruses in goats and sheep necessitates regular serological studies for pestivirus in small ruminants[19-21]. Pestivirus infections in ruminants involve a complex scenario, since, subclinical, genetically related and cross-species transmission is possible[22].

Studies conducted around the world show that the prevalence of pestivirus infections varies between and

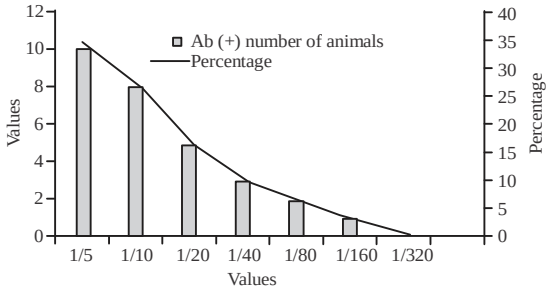

Fig. 1: Pestiviruses antibody titre values of seropositive animals

Table 1: Seropositivity rates of pestiviruses in goat populations and flocks

Agroecological sub-region	No. of samples	Positive	Negative	Percentage	Flock	Positive	Negative	Percentage
1	82	7	75	8.54	10	1	9	10.00
11	78	9	69	11.54	12	2	10	16.67
111	70	6	64	8.57	8	1	7	12.50
1V	44	7	37	15.91	4	1	3	25.00
Total	274	29	245	10.58	34	5	29	14.71

$\chi^2 = 5.70$; $p = 0.02<0.05$

Table 2: Positivity distribution of pestiviruses according to goat sex

Sex	No. of samples	Positive	Negative	Percentage
Male	64	2	62	3.13
Female	210	27	183	12.86
Total	274	29	245	10.58

$\chi^2 = 4.90$; $p = 0.026<0.05$

Table 3: Positivity distribution of pestiviruses according to goat age

Age	No. of samples	Positive	Negative	Percentage
06-24 monthly	83	2	81	2.41
24-48 monthly	138	23	115	16.66
48 monthly<	53	4	49	7.55
Total	274	29	245	10.58

$\chi^2 = 11.77$; $p = 0.002<0.05$

within countries from region to region. It was reported as 4.5% in Norway, 5.6% in Ireland, 27% in the Netherlands, 29.4% in Australia and 67% in Switzerland[23-29].

There is currently no vaccination and eradication program for pestiviruses in Turkey. Numerous studies on pestivirus infection in Turkey have shown that the infection is common in sheep populations. Fewer studies have been carried out in goat flocks than in sheep flocks. These studies, studies on the seroprevalence of pestivirusin Turkey have reported that it varies between 4.08-63.6% in goats and between 18.94-79% in sheep[14, 30-38].

This study is the first one to determine the seroprevalence of pestiviruses in different randomly sampled goat flocks in Denizli Province of Turkey. The individual seroprevalence in this study was found to be 10.58%. The seropositivity varies between 8.54% and 15.91% among the sub-regions. A significant correlation was found between seropositivity and the sex and age of animals ($p<0.05$). The study findings are similar to the findings of other researchers[37, 38]. These are the antibody titer ratios determined in seropositive animals in terms of the presence of specific antibodies. The majority of antibody titers were determined to be 34.48% (1:5) in goats. It is known that the results of seroepidemiological studies are affected by many factors such as the number of animals sampled, age, study time, care and feeding conditions of animals. Factors that lead to an increase in virus resistance such as overcrowding and inadequate nutrition and breeding of sheep, goats and cattle and the lack of knowledge of animal owners about preventive medicine, may cause the prevalence of the disease to increase.

CONCLUSION

As a result, due to the high prevalence trend in the pestivirus infections of small ruminants in Turkey, the training of enterprise owners and suggestions to breeders about the measures to be taken against the said infection, study results and other infections were provided. Although, abortions and births with anomalies due to the presence of ruminant pestivirus have not been reported in the sampled livestock enterprises, it would be useful to consider that it may cause economic losses in the future.

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REFERENCES

01. Heinz, F.X., M.S. Collet, R.H. Purcell, E.A. Gould and C.R. Howard *et al*., 2000. Family Flaviviridae. In: Virus Taxonomy: Classification and Nomenclature of Viruses; Seventh Report of the International Committee on Taxonomy of Viruses, Van Regenmortel, M.H.V., C.M. Fauquet, D.H.L. Bishop, E.B. Carsten and M.K. Estes *et al*. (Eds.)., Academic Press, Cambridge, Massachusetts, pp: 859-878.
02. Lindenbach, B., H.J. Thiel and C.M. Rice, 2003. Family Flaviviridae. In: Fields Virology, Knipe, D.M. and P.M. Howley (Eds.). Lippincott-Raven Publishers, Philadelphia, pp: 1101-1133.
03. Moening, V., 1990. Pestiviruses: A review. Vet. Microbiol., 23: 35-54.
04. Becher, P., R.A. Ramirez, M. Orlich, S.C. Rosales and M. Konig *et al*., 2003. Genetic and antigenic characterization of novel pestivirus genotypes: Implications for classification. Virology, 311: 96-104.
05. Rumenapf, T. and H.J. Thiel, 2008. Molecular Biology of Pestiviruses. In: Animal Viruses: Molecular Biology, Mettenleiter T.C. and F. Sobrino (Eds.)., Caister Academic Press, Norwich, England, pp: 39-96.
06. Depner, K., O.J.B. Hubschle and B. Liess, 1991. BVD-Virus Infection in Goats-Experimental Studies on Transplacental Transmissibility of the Virus and its Effect on Reproduction. In: Ruminant Pestivirus Infections, Liess, B., V. Moennig, J. Pohlenz and G. Trautwein (Eds.). Springer, Vienna, pp: 253-256.
07. Zaghawa, A., 1998. Prevalence of antibodies to bovine viral diarrhoea virus and/or border disease virus in domestic ruminants. J. Vet. Med. Ser. B., 45: 345-351.

08. Krametter-Froetscher, R., A. Loitsch, H. Kohler, A. Schleiner and P. Schiefer *et al*., 2006. Prevalence of antibodies to pestiviruses in goats in Austria. J. Vet. Med. Ser. B., 53: 48-50.
09. Loken, T., 1992. Pestivirus infections in ruminants in Norway. Int. Sci. Technique-Office Des Epizooties, 11: 895-895.
10. Stalder, H.P., P.H. Meier, G. Pfaffen, C. Wageck-Canal and J. Rufenacht *et al*., 2005. Genetic heterogeneity of pestiviruses of ruminants in Switzerland. Preventive Vet. Med., 72: 37-41.
11. De Mia, G.M., I. Greiser-Wilke, F. Feliziani, M. Giammarioli and A. De Giuseppe, 2005. Genetic characterization of a caprine pestivirus as the first member of a putative novel pestivirus subgroup. J. Vet. Med. Ser. B., 52: 206-210.
12. Burgu, I. and A. Ozkul, 1993. Detection by cultural isolation of Bovine Virus Diarrhoea (BVD) virus following field infections in cattle and their fetuses in Turkey. DTW. Deutsche Tierarztliche Wochenschrift, 100: 361-363.
13. Burgu, I., Y. Akca, F. Alkan, A. Ozkul and M.T. Karaoglu *et al*., 2001. [Investigation of Bovine Viral Diarrhea (BVD) virus infection in terms of serological, virological and pathogenesis in ewes before and after calving (In Turkish)]. Turk. J. Vet. Anim. Sci., 25: 551-557.
14. Hasircioglu, S., M. Kale and A. Acar, 2009. Investigation of pestivirus infections in aborted sheep and goats in burdur region. Kafkas Univ. Vet. Fak. Derg., 15: 163-167.
15. Albayrak, H. and E. Ozan, 2012. The investigation of pestivirus and Rift valley fever virus infections in aborted ruminant foetuses in the Blacksea region in Turkey. Kafkas Univ. Vet. Fak. Derg., 18: 457-461.
16. Frey, H.R. and B. Liess, 1971. [Multiplication kinetics and usability of a cytopathogenic VD-MD virus strain for diagnostic investigations with the microtiter method (In German)]. Zbl. Vet. Med. B., 18: 61-71.
17. Brako, E.E., R.W. Fulton, S.S. Nicholson and G.F. Amborski, 1984. Prevalence of bovine herpesvirus-1, BVD, PI-3, goat respiratory syncytial, bovine leukemia and bluetongue viral antibodies in sheep. Am. J. Vet. Res., 45: 813-816.
18. Radostits, O.M., C.C. Gay, K.W. Hinchcliff and P.D. Constable, 2008. Veterinary Medicine, A Textbook of the Diseases of Cattle, Horses, Sheep, Pigs and Goats. 10th Edn., W.B. Sounders, London, New York, Oxford.
19. Lindberg, A., J. Brownlie, G. Gunn, H. Houe and V. Moening *et al*., 2006. The control of bovine viral diarrhoea virus in Europe: Today and in the future. Plurithematic Issue Sci. Tech. Rev., 25: 961-979.
20. Mishra, N., K. Rajukumar, A. Tiwari, R.K. Nema, S.P. Behera, J.S. Satav and S.C. Dubey, 2009. Prevalence of Bovine Viral Diarrhoea Virus (BVDV) antibodies among sheep and goats in India. Trop. Anim. Health Prod., 41: 1231-1239.
21. OIE., 2020. Listed diseases. World Organisation for Animal Health, Paris, France.
22. Pratelli, A., V. Martella, F. Cirone, D. Buonavoglia, G. Elia, M. Tempesta and C. Buonavoglia, 2001. Genomic characterization of pestiviruses isolated from lambs and kids in Southern Italy. J. Virological Methods, 94: 81-85.
23. Loken, T., J. Krogsrud and I.L. Larsen, 1991. Pestivirus infections in Norway. Serological investigations in cattle, sheep and pigs. Acta Vet. Scand., 32: 27-34.
24. Nettleton, P.F., J.A. Gilray, P. Russo and E. Dlissi, 1998. Border disease of sheep and goats. Vet. Res., 29: 327-340.
25. Schaller, P., H.R. Vogt, M. Strasser, P.F. Nettleton, E. Peterhans and R. Zanoni, 2000. Seroprevalence of maedi-visna and border disease in Switzerland. Schweiz. Arch. Tierheilkunde, 142: 145-153.
26. O'Neill, R.G., M. O'Connor and P.J. O'Reilly, 2004. A survey of antibodies to pestivirus in sheep in the Republic of Ireland. Irish Vet. J., 57: 525-530.
27. Meer, F.J.U.M.V.D., 2009. [Seroprevalence of antibodies against pestiviruses in small ruminants in the Netherlands (In Turkish)]. Tijdschr Diergeneeskd, 134: 380-384.
28. Krametter-Froetscher, R., M. Duenser, B. Preyler, A. Theiner, V. Benetka, K. Moestl and W. Baumgartner, 2010. Pestivirus infection in sheep and goats in West Austria. Vet. J., 186: 342-346.
29. Cirone, F., S. Cirone, P. Trerotoli, R. Buono and G. Ciappetta *et al*., 2018. Cross sectional study for pestivirus infection in goats in Southern Italy. Small Ruminant Res., 166: 12-16.
30. Ataseven, V.S., L. Ataseven, T. Tan, C. Babur and T.C. Oguzoglu, 2006. Seropositivity of agents causing abortion in local goat breeds in Eastern and South-Eastern Anatolia, Turkey. Revue Med. Vet., 157: 545-550.
31. Okur-Gumusova, S., Z. Yazici and H. Albayrak, 2006. Pestivirus seroprevalence in sheep populations from inland and coastal zones of Turkey. Revue Med. Vet., 157: 593-596.
32. Gur, S., 2009. A investigation of border disease virus in sheep in Western Turkey. Trop. Anim. Health Prod., 41: 1409-1412.

33. Avci, O., 2010. [Investigation of border disease virus infection in sheep herds with abortion problem in Konya and its surroundings]. Ph.D. Thesis, Selcuk University, Konya, Turkey. (In Turkish)
34. Azkur, A.K., S. Gazyagci, M.E. Aslan and N. Unal, 2011. Molecular and serological characterization of pestivirus infection among sheep in Kirikkale, Turkey. Kafkas Univ. Vet. Fakultesi Dergisi, 17: 83-92.
35. Albayrak, H., S.O. Gumusova, E. Ozan and Z. Yazici, 2012. Molecular detection of pestiviruses in aborted foetuses from provinces in Northern Turkey. Trop. Anim. Health Prod., 44: 677-680.
36. Yilmaz, V., Y. Yildirim and N. Coskun, 2014. Molecular and serological investigation of border disease virus infection in sheep in the Kars District of Turkey. Acta Vet. Brno, 83: 175-179.
37. Albayrak, H., E. Ozan, H. Kadi, A. Cavunt and C. Tamer, 2016. Seroprevalence of pestiviruses in some goat breeds in Samsun province. Ataturk Univ. Vet. Bilimleri Dergisi, 11: 1-5.
38. Ural, Z.E. and E.R.O.L. Nural, 2017. [Serological and virological investigation of pestivirus infection in sheep and goats in Aydin and Izmir provinces (In Turkish)]. Harran Univ. Vet. Fakultesi Dergisi, 6: 63-68.