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Searching Akabane and Pestivirus Infections in Native Breed Sheep with Abortion History

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ABSTRACT

Background: Infectious abortions happen mostly by bacteria and viruses. First of all, it is necessary to determine the causative agents in order to prevent abortions. There are different views on the relationship and sensitivity between breed and diseases. Native breed sheep with abortion history, which were caused by microorganisms, were investigated in the paper.

Methods: Three hundred and eighty-four blood samples were collected from four different native breed sheep with abortion history. The samples were investigated in terms of some important bacterial, parasitary and viral agents by serological and virological tests.

Result: Akabane (AKAV) antibody (Ab) 3.90% seropositivity and pestivirus antigen (Ag) 0.26% positivity was found in aborted sheep. Not only was the highest seropositivity detected in age group of five in terms of AKAV infection but also the highest seropositivity was found in age group of five in terms of pestivirus (Ab) according to age while pestivirus (Ag) presence was detected in only one animal in age group of four. The highest seropositivity in terms of AKAV infection according to races was found in Kivircik sheep as 4.65% while as 56.40% in terms of pestivirus (Ab) and as 0.58% in terms of pestivirus (Ag) in Anatolian Merinos sheep. In terms of both infections, an increase of infections was seen in elder sheep, the highest AKAV seropositivity was found in Kivircik sheep and the highest pestivirus seropositivity was detected in Anatolian Merinos sheep.

Key words: Abortion, Akabane, Bacteria, Native sheep, Pestivirus.

INTRODUCTION

Native breed sheep with decreased productivity that are adapted to negative environment conditions they live in constitute 97% of sheep population in Turkey (Akçapınar, 1994). The fact that stock breeding in Turkey has an extensive character in general terms, sheep are able to benefit from low-order forages at their best, people demand sheep meat and dairy products and especially it is the means of living for breeders who have limited land areas reveal the importance of sheep breeding in the country's economy (Altınel *et al.,* 1998).

Cases of abortion in sheep occur due to infectious and non-infectious causes. Abortions happen as sporadic or enzootic. Owing to the increase in abortions in herds by more than 2%, factors or causes creating abortion cases should absolutely be searched. Non-infectious abortions are usually formed sporadically in any period of pregnancy. These kinds of abortions occur as a result of stress, housing conditions, transport, toxemia, metabolic disorders, nutripathy, hereditary factors, physical factors and toxins. Infectious abortions generally occured during the second half of pregnancy and can also be seen during the advanced periods. These abortions develop as generalized infections symptomatically as a result of pathological changes depending on specific factors in placenta. These specific factors are bacteria, rickettsia, chlamydia, fungi, protozoan and viruses (Vidic *et al.,* 2007).

In this study, it is aimed to detect important bacterial, parasitary and viral factors in some native breed sheep with abortion history raised around Western Mediterranean region in Turkey and to find their resources.

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MATERIALS AND METHODS

Animals, samplings, bacterial and parasitary tests

Between January 2008 and May 2010, 384 blood samples were collected from four different native breed sheep (Anatolian Merinos, Sakiz, Awassi, Kivircik) of 1-5 years of age with abortion history raised by pasture feeding and kept under barn conditions around central and district villages in Burdur region (29°-24′ and 30°-53′ East Longitudes and 36°-53′ and 37°-50′ North Latitudes). The sampled animals had not been vaccinated against AKAV, pestivirus and Brucella. Anatolian Merinos was obtained by interbreeding German mutton merinos with native Akkaraman sheep. Sakiz sheep takes its name after Sakiz Island in Greece, thus it is also called Çeşme sheep. The native land and feeding area of Awassi sheep is Mesopotamia region, where Fırat and Dicle rivers flow. Kivircik sheep is raised in Thrace, Marmara and Aegean regions (Garip, 2013). Blood samples were taken

from *Vena jugularis* of all animals into 10 ml. anticoagulant tubes with K_3EDTA. Samples were centrifuged for 30 minutes at +4°C under 3000 rpm and leukocyte and serum samples were obtained. Serums were inactivated in bain-marie for 30 minutes at 56°C and were stored after PBS washing of leukocytes and centrifugation at -80°C until testing. Blood serum samples taken from these aborted animals were examined with rose bengal plate test antigen (RBPTAntigen™, Seromed), *Brucella* spp., *Coxiella burnetii* (PrioCheck™Ruminant Q Fever Ab Plate Kit, Thermo Fisher Scientific), *Chlamydia* spp. (ID Screen® Chlamydophila abortus indirect Multi-species, IDvet), *Leptospira hardjo* (PrioCheck™L.Hardjo Ab Strip Kit, Thermo Fisher Scientific) and Neospora caninum (PrioCheck™BovineNeospora Ab 2.0 Serum/Milk Kit, Thermo Fisher Scientific).

Akabane (Ab) ELISA

In order to detect anti-g1 antibodies developed against AKAV in the collected serum samples, ID Screen® Akabane Competitive ELISA kit of IDVet company was used. The test was applied according to the procedure set by the firm.

BVDV (Ab) ELISA

In order to detect antibodies developed against BVDV in the collected serum samples, BVDV Total Antibody® Test kit of IDEXX Company was used. The test was applied according to the procedure set by the firm.

BVDV (Ag) ELISA

In order to detect BVDV antigens in the collected leukocyte samples, BVDV Ag/Serum Plus® Test kit of IDEXX Company. The test was applied according to the procedure set by the firm.

RESULTS AND DISCUSSION

Bacterial and parasitological tests

Test results of *Brucella* spp., *Coxiella burnetii*, *Chlamydia* spp., *Leptospira hardjo* and Neospora caninum applied on blood serum samples taken from these aborted animals were found negative.

Viral tests [Akabane (Ab), BVDV (Ab) and BVDV (Ag)]

As a result of the applied examinations, AKAV (Ab) seropositivity distribution according to age was found as 3.84% for 3-year-olds, 6.41% for 4-year-olds, 9.21% for 5-year-olds and BVDV (Ab) seropositivity distribution was detected as 39.47% for 1-year-olds, 46.05% for 2-year-olds, 48.72% for 3-year-olds, 53.85% for 4-year-olds and 65.79% for 5-year-olds. BVDV (Ag) positivity was found in only one 4-year-old (1.28%) sheep detected as BVDV seronegative (Table 1).

In the study, AKAV (Ab) seropositivity distribution according to race was found as 4.65% for Anatolian Merinos sheep, 2.61% for Sakiz sheep, 3.45% for Awassi sheep, 5.13% for Kivircik sheep and BVDV (Ab) seropositivity distribution was detected as 56.40% for Anatolian Merinos sheep, 39.13% for Sakiz sheep, 55.17% for Awassi sheep and 53.85% for Kivircik sheep. BVDV (Ag) positivity was found in only 1 (0.58%) BVDV seronegative Anatolian Merinos sheep (Table 2).

Sheep and goats have many microorganisms causing abortions (Dorsch *et al.,* 2021). For abortions or stillbirths of lambs or yeanlings over 5% in herds, bacterial factors should be considered and discussed before viruses. If no results could be obtained, examinations should be carried out in terms of viruses and other factors (Holler, 2012). During the serological screenings for healthy looking sheep raised in Middle Italy Alps, *Toxoplasma gondii* (78%),

Table 1: Distribution of AKAV (Ab) and BVDV (Ab and Ag) infections according to age in aborted sheep.

Ages	AKAV (Ab)			BVDV (Ab)			BVDV (Ag)		
(year)	n	+	%	n	+	%	n	+	%
1	76	-	-	76	30	39.47	76	-	-
2	76	-	-	76	35	46.05	76	-	-
3	78	3	3.84	78	38	48.72	78	-	-
4	78	5	6.41	78	42	53.85	78	1	1.28
5	76	7	9.21	76	50	65.79	76	-	-
Total	384	15	3.90	384	195	50.78	384	1	0.26

Table 2: Distribution of AKAV (Ab) and BVDV (Ab and Ag) infections according to race in aborted sheep.

Breeds	AKAV (Ab)			BVDV (Ab)			BVDV (Ag)		
	n	+	%	n	+	%	n	+	%
Anatolian Merino	172	8	4.65	172	97	56.40	172	1	0.58
Sakiz	115	3	2.61	115	45	39.13	115	-	-
Awassi	58	2	3.45	58	32	55.17	58	-	-
Kivircik	39	2	5.13	39	21	53.85	39	-	-
Total	384	15	3.90	384	195	50.78	384	1	0.26

Chlamydophlia spp. (20%), Pestivirus (90%), Bovine respiratory syncytial virus (82%) and *Mycoplasma conjuctivae* (81%) was found while no Brucella spp. could be detected (Gaffuri *et al.,* 2006). In a study carried out for sheep herds with abortion history, *Brucella* spp., *Camphylobacter* spp., *Salmonella* spp., *Chlamydia* spp., *Toxoplasma* spp., *Leptospira* spp. and Bluetongue virus was detected negative while both BVDV (Ag) and Ab presence could be found (Ural *et al.,* 2011). In our study, during the examinations on blood serum samples taken from sheep with abortion history, *Brucella* spp., *Coxiella burnetii*, *Chlamydia* spp., *Leptospira hardjo* and Neospora caninum test results were found negative.

AKAV infection progresses subclinically in mature small ruminants of various races and age in areas where it has an endemic progress and during this period, the unborn foetus gains an active immunity so as to protect itself from the virus. In various studies, in detecting AKAV (Ab), competitive AKAV ELISA was found more specific with faster methodology compared to serum neutralization test and was recommended to be used (Tsuda *et al.,* 2004). During AKAV infection ELISA (Ab) screenings for the aborted sheep or stillbirths, Kojouri *et al.* (2015) and Ahi *et al.* (2015) found it as 5.56% while Oluwayelu *et al.* (2016) detected 4.4% and Al-Behadili and Salman (2017) 2% seroprevalence rates during the screenings for healthy looking sheep. In our study, we found a seroprevalence at a rate of 3.9% for aborted sheep. Thus, research results found lower than ours were also obtained either in aborted sheep herds or healthy-looking ones (0.08%) by Pestil and Bulut, (2014). Besides, there is a study in which antibody presence against AKAV infection could not be detected in healthy looking sheep (Koç and Erol, 2014). There are also results found higher than ours (Sevik, 2017, 44.9%; Wang *et al.,* 2017, 11.9%).

Because the virus is at a very low level in pestivirus persistent infected sheep, virus isolation is commonly recommended. Therefore, if ELISA antigen test is to be used for screening or diagnosis in blood samples in herds, leukocyte samples need to be preferred. This condition has been reported to have caused wrong negative results for blood serum or clotted blood in persistent infected (PI) animals (Kirkland and MacKintosh, 2006). In this study, pestivirus (Ab) seropositivity rate was found as 50.78% in aborted sheep. The closest results to ours in blood samples from healthy looking sheep were those obtained by Ural and Erol (2017) and Seyfiabad Shapouri *et al.* (2007) (47.59% and 46.62% respectively). While pestivirus (Ab) seropositivity rates of some researchers (Avci and Yavru 2014. 79%; Feknous *et al.,* 2018, 68.20%) were found high, results of others (Tamadhir and Salman 2020, 26.67%; Mahmoud and Abulmagd 2019, 18.18%; Campbell *et al.,* 2021, 17.6%) were low. Nevertheless, in our study, PI pestivirus (Ag) rate for aborted sheep was 0.26%. In their studies, Krametter-Froetscher *et al.* (2010) found it as 0.32%; Valdazo-Gonzalez *et al.* (2008) 0.24%, which was close to the determined level. Besides, there are also researchers who found it higher than 1% (Yilmaz *et al.,* 2014, Hassan, 2021).

In this study, it was realized that AKAV infection increased from the age of three and the highest seropositivity (9.21%) was seen in age group of five. Similarly, Oluwayelu *et al.* (2016) detected AKAV (Ab) seropositivity as 4.4% in sheep of 2-3 age group and as 8.2% in those of 4-5 age group. These researchers confirmed that AKAV antibody prevalence progressed parallel to increasing age in sheep and cattle. It was reported that an increasing linear relation occurred between AKAV prevalence rates and age (between <6 months-3 years) (Elhassan *et al.,* 2014). Nevertheless, no relation could be found between infection and age in sheep herds with abortion history (Ahi *et al.,* 2015).

In present study, we realized that pestivirus-Ab level in sheep increased with increasing age and the highest rate was found in age group of five (65.79%). Seyfiabad Shapouri *et al.* (2007) stated that pestivirus-Ab prevalence in sheep increased with age and observed seropositivity as 25.2% for < 2-year-olds, 44.3% for elders, 45% for 3-year-olds and 53% for ≥4-year-olds. Berriatua *et al.* (2004) stated that pestivirus seroprevalence for sheep of over 1-year-old in herds not PI infected and seroprevalence in young ones was lower compared to older ones. Mishra *et al.* (2009) found that BDV seroprevalence in sheep showed parallelism with increasing age. However, there are various research reports stating that no connection could be detected between pestivirus seropositivity in sheep and age and gender (Azkur *et al.,* 2011). Also, the presence of pestivirus antigen (Ag) was detected in blood samples, brain and liver of months old kid, brain and spleen of two animals *viz*. six months and two months old (Hasircioglu *et al.,* 2017). PI animals in herds are the most crucial source for transmitting the infection to other animals (Nettleton, 2000). Detection of BDV PI-born lambs, increasing of BDV titre flowing in blood and detection from skin tissue could be easier. This condition might enhance the permanence of the virus within the herd and spreading of the infection within the susceptible animal population. Therefore, removing the PI infected animals out of the herd should decrease virus circulation in the herd and cause a decrease in antibody positive animal level gradually (Houe, 1999). In this study, pestivirus-Ag presence could be detected in only one 4-year-old animal (1.28%). It was also reported that detecting viral antigen presence might be possible in animals older than 4 years of age and some of these might have specific antibody against BDV (Nettleton *et al.,* 1992). In many studies, it was stated that BDV PI sheep presence progressed between 0.3% and 20% (Gur, 2009). As the other researchers also emphasized (Feknous *et al.,* 2018), we detected BDV PI level at a low rate in an old animal due to the fact that samplings were performed in small herds, lambs were sent for slaughtering frequently for food consumption, samplings were performed on animal populations between 1-5 years of age and animals were not imported frequently.

Ahi *et al.* (2015) found AKAV infection prevalence low in Arabian and mixed-race sheep and stated that exotic races were more susceptible to this disease than native ones. Nevertheless, Elhassan *et al.* (2014) stated that in cattle, AKAV prevalence in cross breed (39.9%) was a lot higher than native ones (8.9%). Anatolian Merinos we used in our study is a combined productive in terms of meat and wool. Sakiz is a sheep breed with high milk yield and fertility. Awassi is another breed with developed milk and meat yield. Kivircik is raised for meat and milk yield (Akçapınar, 1994). All the sheep used in our study were composed of native and native × merino crossbreed sheep (crossbreed of German mutton merino × native Akkaraman sheep). AKAV (Ab) seropositivity distribution in these sheep was found between 2.61% and 5.13% and the highest seropositivity was 5.13% for Kivircik sheep. In mature sheep, AKAV infection was seen as completely subclinical and endemic in animals of many races and age (Taylor and Mellor, 1994). Among the reasons why AKAV infection prevalence rate was found low were regional climate conditions, insect distribution and animal selection type (Kojouri *et al.,* 2015). In this sense, when pathogenic effects of AKAV infection spread around virus endemic areas, infected hosts and infected vectors might also be seen in these areas (Taylor and Mellor, 1994). The climate of Burdur Basin, where the study was carried out, has a transitional feature between Mediterranean and Continental climates. Accordingly, summer is hot and dry while winter is fairly cold. Annual average temperature is 13°C and annual average rainfall is 405mm (Yigitbaşıoglu and Uğur, 2010). It also has eight lakes and is also called Region of Lakes. Samplings were collected from barns and managements close to lake basins. Among these, especially Burdur Lake Basin, one of the largest of all, is a closed basin, that is, its waters do not reach the sea. Across southwest and northeast directions, there are salty marshes because of alluvial accumulation. Due to salt and arsenic in lake waters, living organisms such as plants and fish living in the lake and their diversity are scarce and endemic species live in this lake (Kaya *et al.,* 2015). Therefore, mosquito population in the region is quite high and probably the most important reservoir for transmitting AKAV factors to sheep. In our study, pestivirus (Ab) seropositivity distribution in sheep was detected between 39.13% and 56.40% and the highest seropositivity in terms of pestivirus (Ab) was found as 56.40% (97/172) and 0.58% in terms of (Ag) (1/172) in Anatolian Merinos sheep. Merinos breed, generally considered resistant to BDV disease, might be infected like others did and there is no report supporting that it is resistant to BDV (Azkur *et al.,* 2011). Same researchers found a significant relation ($p<0,05$) between sheep races and antibody response against pestivirus. The results obtained during BDV seroprevalence studies carried out in Northern Ireland in herds of native breed sheep previously (Graham *et al.,* 2001) were found lower than those (O'Neill *et al.,* 2004) in herds of non-native breed sheep. Thus, we attribute our results to this condition. In addition, despite studies (Alpay *et al.,* 2014, Gökçeada-İmroz sheep 1.8%; Yapici *et al.,* 2014, Jaydara sheep 7.32%) with low pestivirus seroprevalence results in native breed sheep, there are also studies with high seroprevalence results (Ural *et al.,* 2011, Sakiz sheep 68%; Gur, 2009, Akkaraman sheep 78.5%).

CONCLUSION

In this study, presence of AKAV and pestivirus infections was searched in some native breed aborted sheep around Western Mediterranean region of our country. Bacterial, parasitary and related viral factors were checked in blood serum and leukocyte samples taken from animals. Bacterial and parasitary factors were not seen in these samples and detection of AKAV and pestivirus seropositivity was performed. Pestivirus antigen presence was found at a low rate. A higher seropositivity presence was detected in elder animals in terms of both viral factors. According to races, AKAV (Ab) was found at a higher rate in Kivircik sheep and pestivirus (Ab) and Ag) in Anatolian Merinos sheep. In the study, AKAV was considered the more prominent primary agent in abortion cases. Therefore, we believe that detailed examination of vectors playing an important role in contamination in AKAV in terms of diagnosis (virus isolation) in the region, identification of types (molecular) and encouragement to develop vaccines that are not used in our country could be highly useful.

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Conflict of interest

On the behalf of all authors of the manuscript, we can determined that there is no conflict of interest.

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