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Detecting Toxoplasma, Listeria and Brucella antibodies in goitered gazelles in Turkey

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Summary

The aim of this study was to determine the prevalence of toxoplasmosis, listeriosis and brucellosis in goitered gazelles (Gazella subgutturosa) in Sanliurfa region, Turkey. A total of 82 sera were collected from healthy gazelles and tested for listeriosis, brucellosis and toxoplasmosis by the Osebold Agglutination Test (OAT), Serum Agglutination Test (SAT) and Sabin-Feldman Dye Test (SFDT), respectively. 82 gazelles 5 (6.09%) were seropositive for listeriosis, 23 (28.04 %) for toxoplasmosis and all of them were seronegative for brucellosis. No statistically significant differences were observed between male and female gazelles in the seroprevalences of toxoplasmosis and listeriosis. As a result, the presence of anti-Toxoplasma gondii and Listeria spp. specific antibodies in G. subgutturosa in the region of Sanliurfa was determined.

Keywords: gazelle, Toxoplasma, Listeria, Brucella

Epidemiological data of diseases from wild animals are often difficult to obtain, because the number of animals available for study are generally limited, and frequent and systematic sampling can disrupt the management and well-being of the animals (12).

Gazelles live in Mongolia, Syria, Iran, Saudi Arabia, Turkmenistan and Turkey. There are presently approximately 2000 gazelles (*Gazella subgutturosa*) world-wide (8). They are bred in a large enclosed area in Ceylanpinar, 141 km southeast of Sanliurfa, Turkey where they roam freely.

Toxoplasmosis, brucellosis, and listeriosis are important worldwide zoonotic infections affecting both domestic animals and human beings (15). Toxoplasmosis is observed in most mammalian species including humans, reptiles and birds. It is responsible for major economic losses in all classes of livestock through miscarriages, still births and neonatal losses. The disease is transmitted by the ingestion of oocysts in contaminated food and water or bradyzoits in the tissues of an infected animal (9, 15).

Brucellosis is caused by bacteria of the *Brucella* genus and characterized by misscarriage, retained placenta, and, to a lesser extent orchitis and infection of the accessory sex glands in males (4). The disease is prevalent in most countries of the world. It primarily affects cattle, buffalo, bison, pigs, sheep, goats, dogs, elk, and occasionally horses. It also can have great economic impact by limiting transport of infected animals and products. *Brucella* can be transmitted through unpasteurized milk and dairy products to the general public (1, 21).

Listeriosis results from *Listeria monocytogenes* infections and may be observed worldwide, more frequently in temperate and colder climates (16). Infected animals shed *L. monocytogenes* in faecal, milk and uterine discharges. Grazing animals ingest the organism and further contaminate vegetation and soil (7, 17, 20).

The seroprevalence of toxoplasmosis, brucellosis and listeriosis have been reported by many researches all around the world (4, 25). Prevalence of the diseases in gazelles has not been yet investigated in Turkey and, thus, this study was conducted to determine the seroprevalence of toxoplasmosis, brucellosis and listeriosis in *G. subgutturosa* in Turkey.

Material and methods

Study area. Sanliurfa (37°N, 38.8°E) is located in Southeastern Anatolia, Turkey. This area is in the central part of the Southeastern Anatolia Project, a regional development project comprising several irrigation and energy projects. It is approximately 600 m above sea level. The area supports a relict population of goitered gazelles (*G. subgutturosa*) and there is a breeding station at the state farm. Approximately 900 gazelles live in the Wild Life Reservation Unit of the State Production Farm, Ceylanpinar, Sanliurfa.

Animals and blood samples. A total of 82 sera were collected from healthy gazelles, consisting of 46 females (56.09%) and 36 males (43.91%) in Sanliurfa. The animal's age ranged from 1-3 years. Approximately 10 ml of blood samples were taken from the *vena jugularis* of each animal. The blood samples were allowed to clot, and then centrifuged at 4000 rpm for five minutes at room temperature and sera was harvested. Serum samples were decanted into 5 ml plastic bottles and stored at 20°C until they were used for serological analyses.

Serological testing. Sabin-Feldman dye Test (SFDT). Serum samples were tested for toxoplasmosis by using vital antigen and methylene-blue dying. An antibody titer of 1/16 and over was accepted to be positive (24).

Osebold Agglutination Test (OAT). An antibody titration test to detect antibodies for *L. monocytogenes* was carried out according to the method of Osebold et al. (22). The test antigen was prepared in the Laboratories of the Refik Saydam National Hygiene Center (Department of Communicable Diseases Research) and the assay was carried out in 3 steps. Firstly, whole cell antigens were prepared from *Staphylococcus aureus* (ATCC 29213) strains by the Osebold method. Secondly, *Listeria* antigens were prepared from *L. monocytogenes* 1/2a, 1/2b, 4b, 4c and 4d strains and were combined in the same suspension. In the last step an agglutination test was performed after the absorption of sera samples with *S. aureus* antigen.

Brucella Micro Agglutination Test (MAT). The MAT was performed as described by Baum et al. (5). Briefly, two-fold serial dilutions of sera, ranging from 1 : 2.5 to 1 : 40, were prepared in saline and 0.5% phenol in V-shaped microtiter plates. Fifty µl *B. abortus* S99 antigen solutions stained with safranin-O (0.02%) diluent were composed of 5% NaCl, and 0.5% phenol was added to each well containing 50 µl diluted serum and the plate was covered with a lid. The negative control wells contained phenol saline and the antigen. The results were recorded after 18 h of incubation at 37°C. The agglutination results were considered to be negative (compact red dot) or positive (large diffuse red mat). Positive and negative controls were run for each test. Antibody titers of 1 : 10, 1 : 16 and 1 : 100 were considered to be positive for *Brucella*, *Toxoplasma* and *Listeria*, respectively.

Statistical analysis. A chi-square (χ^2) test was used to detect significant differences between proportions, and a probability of less than 0.05 was considered to be statistically significant.

Results and discussion

The seroprevalence results of tested gazelles for brucellosis, listeriosis and toxoplasmosis are shown in tab. 1. Sera samples of 82 gazelle, 5 (6.09%) were found to be seropositive for listeriosis and 23 (28.04%) for toxoplasmosis. In four cases, antibody titers were detected in 1 : 100 and one case was 1 : 200 for listeriosis. In nineteen cases, antibody titers were detected 1 : 16 and four cases antibody titers were detected 1 : 64 for toxoplasmosis. No seropositive gazelles were tested for *Brucella* antibodies.

There was no significant difference between male and female gazelles in the seroprevalences of toxoplasmosis and listeriosis.

Wild animals play an important role in the epidemiology of a number of zoonotic diseases and actively distribute diseases amongst domestic animals and humans. Gazelle and deer are of interest to veterinarians as they may be sylvatic reservoirs of some important livestock diseases such as rinderpest, foot-and-mouth disease, brucellosis, leptospirosis, anaplasmosis and toxoplasmosis (15). Little information is available on the details of specific gazelle species disease in order to make a direct comparison with those in *G. subgutturosa*.

Toxoplasmosis is a zoonotic disease caused by *T. gondii* which is an obligate intracellular protozoon. The disease is observed in most mammalian species including humans, reptiles and birds (27). In different countries, the prevalence of *T. gondii* in humans has been reported from 10-93% (9) and 5-90% in Turkey (6, 25, 28). However, the prevalence of anti-*T. gondii* antibodies in sheep, goat and cattle have been ascertained as 22.6-72.9%, 11.6-96% and 59-66%, respectively (3, 14, 26). In the current study, seroprevalence of *T. gondii* was found to be 28.04% in *G. subgutturosa*.

The prevalence of antibodies to *T. gondii* in Arabian gazelles has been reported to be between 4.0-5.9% (19). The prevalence of antibodies to *T. gondii* in our study was higher than those of in Arabian gazelles. This may be dues to several reasons. Firstly, contamination of pasture fields by cat or *Felidae* feces may probably be the main source of toxoplasmosis in the region because the oocysts are extremely resistant to unfavorable conditions and can remain viable for long periods of time in the pastures (11). Another possible source of *T. gondii* infection could be coitus with infected males. *T. gondii*, in particular, in its tachyzoite stage may remain for a long time in the semen of infected males of susceptible species (9, 10).

In the present study there was no significant difference for the seroprevalence of *T. gondii* between males

Tab. 1. Serological results of *G. subgutturosa* tested for listeriosis and toxoplasmosis

Sex	Number of tested (%)	Toxoplasmosis			Listeriosis		
		Number of positives (%)	Antibody titers		Number of positives (%)	Antibody titers	
			1 : 16	1 : 64		1 : 100	1 : 200
Male	36 (43.9)	8 (22.2)	8	–	3 (8.3)	2	1
Female	46 (56.1)	15 (32.6)	11	4	2 (4.3)	2	–
Total	82 (100.0)	23 (28.0)	19	4	5 (6.1)	4	1

and females. Similarly, Mas Bakal et al. (18), and Mohammed and Hussein (19), reported no differences between sexes for seroprevalence of toxoplasmosis in Arabian gazelles.

Listeriosis is becoming increasingly prevalent in animals on a global scale. The disease generally has been reported in sheep feeding from silage in winter. Some epidemiological data for listeriosis has been reported for sheep in Turkey as 0.3-16.3% (23). In the present study, the prevalence of listeriosis in gazelles was found to be 6.09%.

Previously, serological studies of brucellosis in Turkey have been reported to be between 1.26-1.83% and 1.18-2.08% seropositivity in cattle and sheep, respectively (2, 13). In the current study, no seropositivity was found for brucellosis. The lack of antibodies against brucellosis in gazelles was probably due to the lesser susceptibility of the gazelles compared to other ruminants (21). Another explanation may be that the gazelle are isolated from any infected domestic animals which could potentially transmit the disease to them.

In conclusion, the results of this study confirm the presence of anti-*T. gondii* and *Listeria spp*. specific antibodies in *G. subgutturosa* in the Sanliurfa region of Turkey. To the best of the authors' knowledge, this is the first report which investigates the prevalence of zoonotic gazelle diseases in Turkey.

References

1. *Arda M., Minbay A., Leloglu N., Aydin N., Kahraman M., Akay O., Ilgaz A., Izgur M., Diker K. S.*: Ozel Mikrobiyoloji. Epidemiyoloji, Bakteriyel ve Mikotik Infeksiyonlar. Medisan Yayinevi, Ankara 1997.
2. *Aslantas O., Babur C.*: Kars yöresinde siğir ve koyunlarda Bruselloz ve Toksoplazmoz uzerine seroepidemiyolojik arastirmalar. Etlik Vet. Mik. Derg. 2000, 11, 47-55.
3. *Babur C., Esen B., Biyikoglu G.*: Yozgat'ta Toxoplasmosis gondii'nin seroprevalansi. Turk. J. Vet. Anim. Sci. 2001, 25, 283-285.
4. *Balows A. H.*: Manual of Clinical Microbiology. Amer Society for Microbiology, Washington 1991, p. 1364.
5. *Baum M., Zamir O., Bergman-Rios R., Katz E., Beider Z., Cohen A., Banal M.*: Comparative Evaluation of Microagglutination Test and Serum Agglutination Test as Supplementary Diagnostic Methods for Brucellosis. J. Clin. Microbiol. 1995, 33, 2166-2170.
6. *Berktas M., Baki I., Yilmaz H., Bozkurt H.*: Cesitli obstetrik sorunlari bulunan kadin hastalarda Toxoplasma antikorlarinin arastirilmasi. Turkiye Parazitol. Derg. 1997, 21, 360-362.
7. *Blenden D. C., Kampelmacher E. H., Torres-Anjel M. J.*: Listeriosis. JAVMA. 1987, 12, 1546-1551.
8. *Demirsoy A.*: Genel ve Turkiye Zoocografyasi „Hayvan Cografyası". Meteksan A. S., Ankara 1999, p. 304.
9. *Dubey J. P., Beattie C. P.*: Toxoplasmosis of Animals and Man. CRC Press Inc., Boca Raton, Florida 1988, 1-220.
10. *Dubey J. P., Sharma S. P.*: Prolonged execretion of Toxoplasma gondii in semen of goats. Am. J. Vet. Res. 1980, 41, 794-795.
11. *Frenkel J. K., Dubey J. P.*: Effect of freezing on the viability of Toxoplasma oocysts. Int. J. Parasitol. 1973, 59, 587-588.
12. *Hawkey C. M., Hart M. G., Fitzgerald A. K.*: Hematological values in mouflon (Ovis musimon): Influence of age, sex, season and Vitamin E status. Res. Vet. Sci. 1984, 36, 37-42.
13. *Inci A., Aydin N., Babur C., Cam Y., Akdogan C., Kuzan S.*: Kayseri yoresi siğir ve koyunlarda toksoplazmozis ve brusellozis uzerine seroepidemiyolojik arastirmalar. Pendik Vet. Mikrob. Derg. 1999, 30, 41-46.
14. *Karatepe B., Babur C., Karatepe M., Cakmak A., Nalbantoglu S.*: Seroprevalence of toxoplasmosis in sheep and goats in the Nigde province of Turkey. Ind. Vet. J. 2004, 81, 974-976.
15. *Krauss H., Weber A., Appel M., Enders B., Isenberg H. D., Schiefer H.G., Slenczka W., von Graevenitz A., Zahner H.*: Zoonoses; infectious diseases transmissible from animals to humans. ASM Press, Washington 2003, 456.
16. *Lorber B.*: Listeriosis. Clin. Infect. Dis. 1997, 24, 1-11.
17. *Low J. C., Donachie W.*: A review of L. monocytogenes and Listeriosis. Vet. J. 1997, 153, 9-29.
18. *Mas Bakal P., Karstad L., Veld N. I.*: Serologic evidence of toxoplasmosis in captive and free-living wild mammals in Kenya. J. Wildl. Dis. 1980, 16, 559-564.
19. *Mohammed O. B., Hussein S. H.*: Antibody prevalence of toxoplasmosis in Arabian gazelles and Oryx in Saudi Arabia. J. Wildl. Dis. 1994, 30, 560-562.
20. *Müller H. E.*: Listeriosis in animals. İnfect. Derg. 1988, 2, 505-521.
21. *Nicoletti P.*: Brucellosis in Animals. Monir Madkour (Ed), Madkour's Brucellosis. Springer-Verlag, Berlin 2001, 267-279.
22. *Osebold J., Aalund O., Chrisp C. E.*: Chemical and immunological composition of surface structures of Listeria monocytogenes. J. Bacteriol. 1965, 89, 84-86.
23. *Pasa S., Kilic S., Babur C., Ocal N., Gazyagci S.*: Seroprevalence of listeriosis and brucellosis on sheep in Aydin. Etlik Vet. Mikrob. Derg. 2003, 14, 11-17.
24. *Sabin A. B., Feldman H. A.*: Dyes as microchemical indicators of a new immunity phenomenon affecting a protozoon parasite (Toxoplasma). Science. 1948, 108, 660-663.
25. *Sevinc F., Birdane F. M., Sevinc M., Dik B., Altinoz F.*: Konya yöresi siğirlarinda Toxoplasma gondii'nin IHA (Indirekt Hemaglutinasyon) ve IFA (Indirekt Fluoresan Antikor) testleri ile seroprevalansi. Turkiye Parazitol. Derg. 2000, 24, 176-179.
26. *Sevinc F., Dik B., Gökcen A., Altinoz F., Uslu U.*: Konya yoresi kecilerinde Toxoplasma gondii'nin IHA (Indirekt Hemaglutinasyon) ve IFA (Indirekt Fluoresan Antikor) testleri ile seroprevalansi. Turkiye Parazitol. Derg. 2000, 24, 57-60.
27. *Tenter A. M., Heckeroth A. R., Weiss L. M.*: Toxoplasma gondii from animals to humans. Int. J. Parasitol. 2000, 30, 1217-1258.
28. *Yildiz K., Babur C., Kilic S., Aydenizoz M., Dalkilic I.*: Kirikkale mezbahasinda kesilen koyun ve sigirlar ile mezbaha calisanlarinda anti-Toxoplasma antikorlarinin arastirilmasi. Turkiye Parazitol. Derg. 2000, 24, 180-185.

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