

**OVIS ARIES AND FELIS SILVESTRIS CATUS TOXOPLASMIC INFECTION
IN TIMIȘ COUNTY, ROMANIA****INFECȚIA TOXOPLASMICĂ LA OVIS ARIES ȘI FELIS SILVESTRIS CATUS
ÎN JUDEȚUL TIMIȘ, ROMÂNIA**

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ABSTRACT | REZUMAT

Toxoplasmosis is a general protozoosis caused by parasites belonging *Toxoplasma* family. It parasitize in various organs and tissues to over 350 species of vertebrates. Economic losses are important due to declining birth rates and producing economic abortions in farm animals, especially in sheep.

The aim of this study was to determine the seroprevalence of toxoplasmosis in the definitive host for *T. gondii*, cat, and of most receptive intermediate host, sheep. So, blood samples were taken from 300 adult sheep, from 10 localities of the Timis county. Blood was collected individually by the research team. From each herd were harvested 30 samples. From cats were harvested 65 serological samples. Cats tested were from all over the county. Serum samples were processed by indirect ELISA for the determination of immunoglobulin G resulting from an older infection with *Toxoplasma gondii*. It used the ID-VET kit Screen Multi-species (ID-VET Innovative Diagnostics, France) and positive samples are highlighted by a color reaction.

Seroprevalence obtained was 39.33% (118/300) in sheep, with variations between 14 and 69% and in cats 63.07% (41/65) with two strongly positive samples, 23 - negative and an uncertain sample.

Increasing shared in the same farm, several species of animals, including cats, and placing forage deposits around animals shelters with feline access inside them were predisposing factors for infection with *Toxoplasma gondii* for small ruminants in Timiș county.

Toxoplasma infection of sheep is important because of lambs losses that may occur by abortions and because of the possibilities of disease transmission to people through poorly cooked meat or raw milk.

Keywords: *Toxoplasma gondii*, sheep, cat, Timiș county

Toxoplasmoza este o protozooză generală produsă de coccidii ce aparțin familiei *Toxoplasma*. Parazitează în diverse organe și țesuturi la peste 350 specii de vertebrate. Pierderile economice sunt importante datorită reducerii natalității și producerii avorturilor la animalele de interes economic, în special la ovine.

Scopul acestui studiu a fost stabilirea seroprevalenței toxoplasmozei la gazda definitivă pentru *T. gondii*, pisica, și la cea mai receptivă dintre gazdele intermediare, oaia. Probele de sânge au fost prelevate de la 300 de oi adulte, din 10 localități ale județului Timiș. Din fiecare turmă s-au recoltat câte 30 de probe. De la pisici, au fost recoltate 65 de probe serologice. Pisicile testate au provenit de pe toată raza județului. Probele de ser au fost prelucrate prin ELISA indirectă, pentru determinarea imunoglobulinelor G rezultate ca urmare a unei infecții mai vechi cu *Toxoplasma gondii*. S-a folosit kit-ul ID-VET Screen Multi-species (ID-VET Innovative Diagnostics, France), probele pozitive fiind evidențiate printr-o reacție de culoare.

Seroprevalența obținută a fost de 39,33% (118/300) la ovine, cu variații cuprinse între 14 și 69%, iar la pisici, de 63,07% (41/65) din care două puternic pozitive, 23 - negative și o probă incertă.

Creșterea la comun, în aceleași ferme, a mai multor specii de animale, printre care și pisici, precum și amplasarea depozitelor de furaje în preajma adăposturilor animalelor, cu accesul felinelor în interiorul lor au reprezentat factorii favorizanți pentru infecția cu *Toxoplasma gondii*, la rumegătoare mici din județul Timiș.

Infecția toxoplastică a ovinelor prezintă importanță atât datorită pierderilor de miei care pot să apară în urma avorturilor, cât și datorită posibilităților de transmitere a bolii la oameni, prin carne insuficient preparată termic sau prin lapte.

Cuvinte cheie: *Toxoplasma gondii*, ovine, pisică, județul Timiș

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Toxoplasmosis is one of the most common parasites in humans and animals, standing as the top three global spread. Toxoplasmosis causes significant economic losses in the livestock sector.

Intermediate hosts are many species of mammals and birds. In cats, the definitive hosts, *T. gondii* develop in the small intestine, while for intermediate hosts, the location is in the brain, muscles and other organs (12). At European level, the interest of specialists to toxoplasmosis is remarkable (2, 3, 12). In Romania, research related to this zoonosis are developing (1, 6, 13).

By serological surveillance it was found that *T. gondii* infection is very common in farm animals, sheep being the most susceptible species (3). The prevalence of toxoplasmosis vary depending on the living conditions of animals; is higher in cats bred freely, who can hunt small mammals or birds and is much lower at animals that grown only indoors without access to the outside. For farm animals, the prevalence is higher where animals have contact directly or indirectly, via feeds, with cats. Seroprevalence of *T. gondii* infection varies from one country to another, but also within the same country, from one region to another, or even within the same city. These differences depend on the degree of civilization and culture of the human population.

The aim of this study was to perform a seroepidemiologic array of infection with *Toxoplasma gondii* in cats, with the role of the definitive host for this parasite and the most receptive of the species of animals for human consumption (sheep) to highlight the multiple sources of infestation in toxoplasmosis. This information will help to control such parasites infections in animals and humans, the Romanian conditions.

MATERIALS AND METHODS

In Timis county were analyzed 300 samples from adult sheep, from 10 localities from the county. The sheep were between 2.5 and 5 years, were female sex and belonged to private farms. Blood was collected individually by the research team, shifted ground in each of the 10 localities. From each flock were collected every 30 samples; the flocks containing between 300 and 850 adult sheep.

To identify the prevalence of anti-*Toxoplasma gondii* antibody in cats in Timis county, 65 serological samples were collected, from cats aged between three months to 13 years. Cats were from both urban and rural areas. Cats who came from rural areas, were animals kept around the owners homes, along with several species of animals (sheep, pigs, horses, birds, dogs).

Serum samples were examined by indirect ELISA method using ID VET Screen Multi-species kits specific for IgG anti-*Toxoplasma* antibody, resulting due to an

infection with *Toxoplasma gondii*. The kit can be used for the determination of anti-*Toxoplasma* antibodies in the sera of ruminants, pigs and cats. 96-well plate is lined with *Toxoplasma gondii* P30 antigen, and the antigen-antibody complex is formed using peroxidase conjugate added subsequently. After adding substrate solution, antigen-antibody complex gets blue color and intensity of the color depends on the amount of antibodies in the serum. Adding stop solution, the color turns to yellow. The plate was then analyzed by the microplate reader at 450 nm wavelength.

RESULTS AND DISCUSSIONS

In Timis County were processed 300 samples from adult sheep, of which 118 (39.33%) were positive, with variations between 14 and 69% in the 10 studied localities (L1-L10) (Fig. 1).

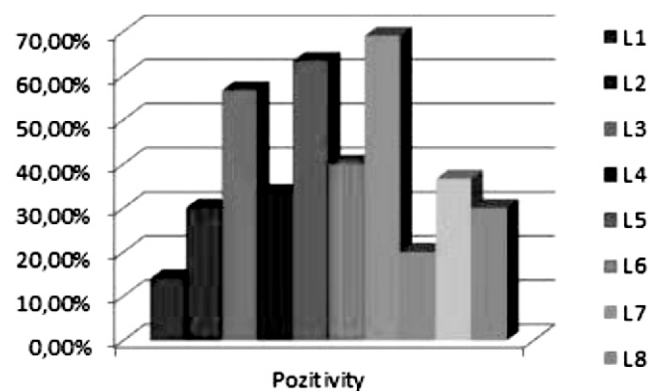


Fig. 1. Graphical representation of the prevalence of *Toxoplasma gondii* infection in sheep from Timiş County

In Timis County 65 samples were collected also from cats. Of these, 41 were positive for *Toxoplasma gondii* infection (63.07%), with two strongly positive samples, 23 were negative and one sample was uncertain (1.53%).

Influence of age on cats' positivity highlighted the following results: in the youth category, aged between three months and one year (n=15), 4 samples were positive (26.66%). The difference of 11 samples was negative (73.33%). Of the 50 adult cats aged between one year and three months to 13 years, 37 were positive (74%) two strongly positive, aged one and three months and one year and five months. 12 samples were found negative (24%) and a sample was uncertain (2%).

Depending on the area of origin, 29 samples were taken from cats that came from urban areas and 36 in

rural areas. In urban areas were identified 14 positive samples (48.27%) and 15 negative samples (51.73%). In rural areas, 27 samples were positive (75%) for *T. gondii* infection, 8 samples were negative (22.22%) and a sample was uncertain (2.78%).

The results obtained from this study can be explained primarily by the living environment of animals, but also by keeping and feeding conditions (Fig. 2). Cats that came from rural areas and were maintained around the homes or farms where they can hunt small animals showed an increased prevalence of *Toxoplasma gondii* infection. Free access of cats in Romania increase the risk of dispersion of oocysts eliminated and makes pastures, feed stores or animal shelters to be easily contaminated with *Toxoplasma gondii*.

Of journeys made on the field it can be observed the conditions for maintenance and feeding of sheep. In most localities, the sheep were kept in outside shelters even during the births. After calving were taken to graze with lambs. In winter stables, it was arranged special places where lambs were accustomed to consume concentrated feed in addition to mother's milk. Even these spaces were open, with indoor access of cats.

In three of the studied localities (L3, L5, L7), sheep were grazed as soon as they started calving, so most of the sheep have lambed on pasture. These sheep shelters were built between bushes and precarious maintenance.

In locality L1, sheep shelters and the pastures were in an area farthest from homes and, besides watchdogs, the owners had no other animals. In this locality, the prevalence of infection with *Toxoplasma gondii* showed the lowest value (14%).

In localities L2, L10 and L4, conditions for maintenance of sheep were good, with a corresponding state of hygiene. Feed stored were not near the sheep shelters but in facilities spaces and brought in farm animals only for feeding. In these settlements obtained prevalence was lower (30% and respectively 33.33%) than in other studied localities.

In winter, sheep were kept in shelters separate mothers sheep from rams. Feeding achieve was done in the stall with different categories of grass. Additionally, during pregnancy, sheep were receiving concentrated and grain. Forage deposits were outside the shelter, in construction provided only with roof, without side walls and no floor. Thus, feed could easily be contaminated with *T. gondii* oocysts, both through free access of cats in the area and through atmospheric factors (wind, rain) that can disperse oocysts into the environment.



Fig. 2. Maintenance conditions for sheep in Timiș county

The results obtained from this study can be explained primarily by the living environment of animals, but also their maintenance conditions and nutrition. We make this statement based on the results achieved in small ruminants' farms where cats' access is not controlled. In Romania, free access of cats increase the risk of eliminated oocysts dispersion and makes pastures, feed stores or animal shelters to be easily contaminated with *Toxoplasma gondii*.

Around the world, the results are the most diverse. Thus, in Serbia, sheep disease prevalence is 84.5%, of which 10% were diagnosed in the acute phase of infection (7). In Italy, infection seropositivity was 28.5% and from 117 farms examined, 91 of them had at least one infected sheep. Moreover, from the total examined sheep, 3.4% had milk-specific DNA (4). ELISA serological technique has been applied to small ruminants in Turkey and Zimbabwe, detecting a disease prevalence of 31 or 67.9% (5, 11). In Bulgaria, the indirect hemagglutination determined a toxoplasmosis incidence of 43.3% (12). Feline degree of seropositivity (ELISA) increases with age: 22% in cats under one year and 80 % in those over 10 years. Between 9 and 46% of pet cats in Europe and in US presents, on the serological test, a past exposure to the parasite, while seroprevalence of toxoplasmosis in Asia was estimated between 6 and 9% (2).

In Romania, Iovu et al., 2008, examined 105 samples of sheep slaughtered in Maramureș and Bihor. Samples were examined by ELISA (CHEKIT Toxotest) and identified a prevalence of 45.7% (45% in sheep aged 2 and 3 years and 46, 3% in sheep 7-8 years) (6).

In another study, Titilincu et al., 2009, 1570 serological sheep samples were examined (1453 ewes and 117 lambs), from the central and north-western Romania. The authors identified a prevalence of 64.34% in sheep, 25.23% of lambs, aged between 6 months and

1 year, and 50% (5/10) lambs aged one month were positive. The technique used was ELISA (Chekit Toxotest) (13). The same authors (Titilincu et al., 2009) tested 94 sera from sheep, by ELISA, using a kit prepared in the laboratory, with the RH strain of *Toxoplasma gondii*. The samples were retested using IFAT technique. A prevalence of 55.3% was obtained by ELISA, 50% by IFAT technique and 64% by commercial ELISA test - Chekit Toxotest. The correlation between the three tests was good ($k = 0.81$) (14).

Titilincu et al., 2009, tested 401 serum samples from goats (371 adult goats and 30 kid goats) from the central and north-western Romania (Transylvania, Crişana and Maramureş). Samples were examined by ELISA (Chekit Toxotest) and obtained a prevalence of 64.15% in adults and 3.33% in kid goats (13).

Serological, Mircean et al., 2008, identified a prevalence of 57.7% by ELISA ImmunoComb, in central and north-western Romania cats. The authors obtained a prevalence increasing with age so: in kittens under six months - 29.4%, in cats aged between six months and two years - 50%, in cats aged 2-10 years - 69.1% and cats over 10 years - 66.6% (8).

Petriceanu et al., 2007, identifying a prevalence of 45% in cats in southern Romania (9). In the same area of the country, Antoniu et al., 2008, tested 42 serological samples in cats. By indirect immunofluorescence method identified prevalence of 47.61% and by ELISA (ImmunoComb) - 54.76% (1). Popa et al., 2003, obtained a prevalence of 20% using the same assay - ELISA ImmunoComb (10).

Toxoplasma gondii infection is of importance due to economic losses that occur through abortions or death products. Also, knowledge of epidemiological data on *Toxoplasma gondii* infection in these species is important because of the risk of transmitting the disease to humans or carnivore animals by eating insufficiently cooked meat.

CONCLUSIONS

In Timiş county, the obtained seroprevalence was 39.33% (118/300) in sheep and 63.07% (41/65) in cats.

Sheep toxoplasmosis prevalence obtained in Timiş county were positively impacted by poor hygiene conditions in which animals were kept and cats access on pasture and feed storage.

Seroprevalence of *Toxoplasma gondii* infection in cats was influenced by age and background, being raised in adult cats and in those from rural areas.

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