Spatial epidemiology of *Toxoplasma gondii* infection in goats in Serbia

Vitomir Djokić[1], Ivana Klun[1], Vincenzo Musella[2], Laura Rinaldi[3], Giuseppe Cringoli[3], Smaragda Sotiraki[4], Olgica Djurković-Djaković[1]

[1]National Reference Laboratory for Toxoplasmosis, Centre for Parasitic Zoonoses, Institute for Medical Research, University of Belgrade, Belgrade, Serbia; [2]Department of Health Sciences, University Magna Græcia, Catanzaro, Italy; [3]Unit of Parasitology and Parasitic Diseases, Department of Veterinary Medicine and Animal Productions, University of Naples Federico II, Naples, Italy; [4]Veterinary Research Institute, National Agricultural Research Foundation, Ionia, Thessaloniki, Greece

Abstract. A major risk factor for *Toxoplasma gondii* infection is consumption of undercooked meat. Increasing demand for goat meat is likely to promote the role of this animal for human toxoplasmosis. As there are virtually no data on toxoplasmosis in goats in Serbia, we undertook a cross-sectional serological study, including prediction modelling using geographical information systems (GIS). Sera from 431 goats reared in 143 households/farms throughout Serbia, sampled between January 2010 and September 2011, were examined for *T. gondii* antibodies by a modified agglutination test. Seroprevalence was 73.3% at the individual level and 84.6% at the farm level. Risk factor analysis showed above two-fold higher risk of infection for goats used for all purposes compared to dairy goats (P = 0.012), almost seven-fold higher risk for goats kept as sole species *versus* those kept with other animals (P = 0.001) and a two-fold lower risk for goats introduced from outside the farm compared to those raised on the farm (P = 0.027). Moreover, households/farms located in centre-eastern Serbia were found to be less often infected than those in northern Serbia (P = 0.004). The risk factor analysis was fully supported by spatial analysis based on a GIS database containing data on origin, serology, land cover, elevation, meteorology and a spatial prediction map based on kriging analysis, which showed western Serbia as the area most likely for finding goats positive for *T. gondii* and centre-eastern Serbia as the least likely. In addition, rainfall favoured seropositivity, whereas temperature, humidity and elevation did not.

Keywords: *Toxoplasma gondii*, goats, seroprevalence, risk factors, geographical information systems, spatial analysis, Serbia.

Introduction

With its widest array of mammalian hosts including humans, *Toxoplasma gondii* may be the most successful parasite on Earth. This cosmopolitan protozoan, capable of infecting all warm-blooded animals and some cold-blooded animal species, has been estimated to infect one third of the global human population (Tenter et al., 2000). The organism's complex life cycle includes a sexual cycle completed in the intestines of members of the Felidae family (definitive hosts) resulting in the production of oocysts, while the asexual cycle involves encysted parasites circulating between prey and predator (the intermediate host). *T. gondii*

persists in the host by conversion from the proliferative tachyzoite stage into quiescent encysted bradyzoites, a mechanism controlled by the host immune response. Thus, although generally mild and self-limiting in immunocompetent individuals, *T. gondii* infection may cause life-threatening disease in the foetus and the immunosuppressed host.

Human infection occurs through ingestion of oocysts (via water, fruit and vegetables) or from tissue cysts present in infected meat. Goats, along with pigs and sheep, are the animal species mostly associated with human infection (Esteban-Redondo et al., 1999). Consumption of raw (unpasteurised) goat milk and milk products (e.g. cheese) has been linked to toxoplasmosis in humans and pigs (Skinner et al., 1990; Dubey 2009; Jones et al., 2009). Tachyzoites have been experimentally shown to survive in goat milk 3-7 days at +4 °C (Walsh et al., 1999).

One study showed that 33% of milk samples from seropositive goats are positive for *T. gondii* DNA, a rate that increases with age (Spišák et al., 2010). As obligate herbivores, goats become infected by inges-

Corresponding author:
Olgica Djurković-Djaković
National Reference Laboratory for Toxoplasmosis
Centre for Parasitic Zoonoses, Institute for Medical Research
University of Belgrade
Dr. Subotica 4, P.O. Box 102, Belgrade 11129, Serbia
Tel. +381 11 2685 788; +381 11 2685 788; Fax +381 11 2643 691
E-mail: olgicadj@imi.bg.ac.rs

tion of oocysts and toxoplasmosis is an important cause of foetal loss in this species. In pregnant goats, a primary infection may lead to placental and foetal infection resulting in foetal death and resorption, abortion or stillbirth (Dubey et al., 1985a). There is a body of data on the seroprevalence of toxoplasmosis in goats throughout the world with values ranging from 0% in Pakistan (Zaki, 1995) and 4% in Maranhao, Brazil (Moraes et al., 2011) to as high as 77% in France (Chartier et al., 1997) and 30-100% in Poland (Czopowicz et al., 2011). Interestingly, *T. gondii* infection in goats seems to be more prevalent in Europe than in Asia, Africa and South America with seroprevalence rates above 60% being reported in some European countries, including the Czech Republic, Spain and, more recently, Austria (Hejliček and Literak, 1994; Rodriguez-Ponce et al., 1995; Edelhofer and Prossinger, 2010).

Goats are traditionally an important source of meat and milk in many developing countries, but the last few decades have seen increased demand for goat products in developed countries as well. In the United States of America, some 850,000 goats are slaughtered annually for food and this number is expected to grow (Hill and Dubey, 2013). Likewise, in Serbia, as in Europe in general, the number of goats and goat farms is constantly increasing in the last decades. Currently, the number is estimated at between 80,000-100,000 goats.

The fact that goats constitute a significant infection reservoir, along with the increasing market share of goat products coupled with the presumption, common in some cultures, that goat milk should be consumed raw (unpasteurised) as well as current practices of "healthy eating", which often includes undercooking of meat, all contribute to the likely increased role of goats in human toxoplasmosis. However, there are virtually no data on toxoplasmosis in goats in Serbia. Therefore, a cross-sectional serological survey was conducted in order to: (i) determine the prevalence of *T. gondii* in Serbian goats; (ii) identify risk factors for infection; (iii) perform spatial analysis and prediction modelling using geographical information systems (GIS).

Materials and methods

Study area

The study covered all of Serbia, a land-locked country on the Balkan Peninsula, covering 77,512 km². According to the CORINE Land Cover (http://www.eea.europa.eu/publications/COR0-landcover) 34,473 km² is agricultural land, 29,682 km² covered by forests and semi natural areas, 2,606 km² built-up areas and the rest wetlands and water bodies. As seen in Fig. 1, Serbia can be divided into northern, western and centre-eastern geographical regions (CE) plus the Belgrade district. The province of Vojvodina in the North has a typical continental climate and is mainly

Fig. 1. Location map of Serbia with its four regions marked out.

made up of agricultural plains north of the Sava and Danube rivers. While both the western and CE regions have a milder continental climate and are mostly characterised by agricultural river plains and forests, the western region is hilly to mountainous, and has more rainfall and humidity. The Belgrade district, set on the estuary of the Sava River into the Danube, is an administrative rather than a geographical region.

Study population and sampling

The study was conducted on a total of 431 goats reared in 143 households/farms throughout Serbia (Fig. 2). This sample size was calculated based on both a previous study in ruminants, which showed a seroprevalence of 76% in cattle and 84% in sheep (Klun at al., 2006), and a pilot study on 194 goats, which showed a prevalence of 82% (Klun et al., 2010). Thus, the sample size for an expected prevalence of at least 70% at the 5% absolute precision level for a 95% confidence interval (CI) was calculated at 323 animals (Thrusfield, 2005). Sampling of households/farms was representative of the different geographical areas of Serbia in relation to their size as follows: northern Serbia (n = 51), western Serbia (n = 15), CE (n = 71) and the Belgrade district (n = 6).

According to the national goat and sheep database established by the Ministry for Agriculture, Forestry and Water Management (MAFWM), goats are kept on a total of 16,096 farms (Fig. 2). These include a multitude of households owning small flocks of goats (up to 10), which actually comprise 95% of all farms and are here defined as "household farms". A limited number of larger operations usually owning several dozen animals, representing 5% of all goat-rearing facilities, are here referred to as "farms". Most animals (64.3%, 277/431) in the study sample were from household farms (86.7%, 124/143 households), which accurately reflects the state of goat rearing in Serbia, i.e. households rear 67%, whereas the farms rear 33% of all goats in the country.

Blood samples were mostly collected between January and December 2010 (n = 362), whereas an additional 69 samples were collected from western and CE Serbia in September 2011. The 2010 samples were obtained from the serum collection for the national brucellosis-testing programme, whereas the additional samples were obtained at the households/farms through the help of local veterinarians. Once at the laboratory, blood samples were centrifuged (400 g for 20 min) and the obtained serum samples stored at -20 °C pending analysis.

Collection of epizootiological data

Data on potential risk factors including region, herd size, gender, age, origin, purpose, husbandry, presence of other livestock, presence of cats, feeding
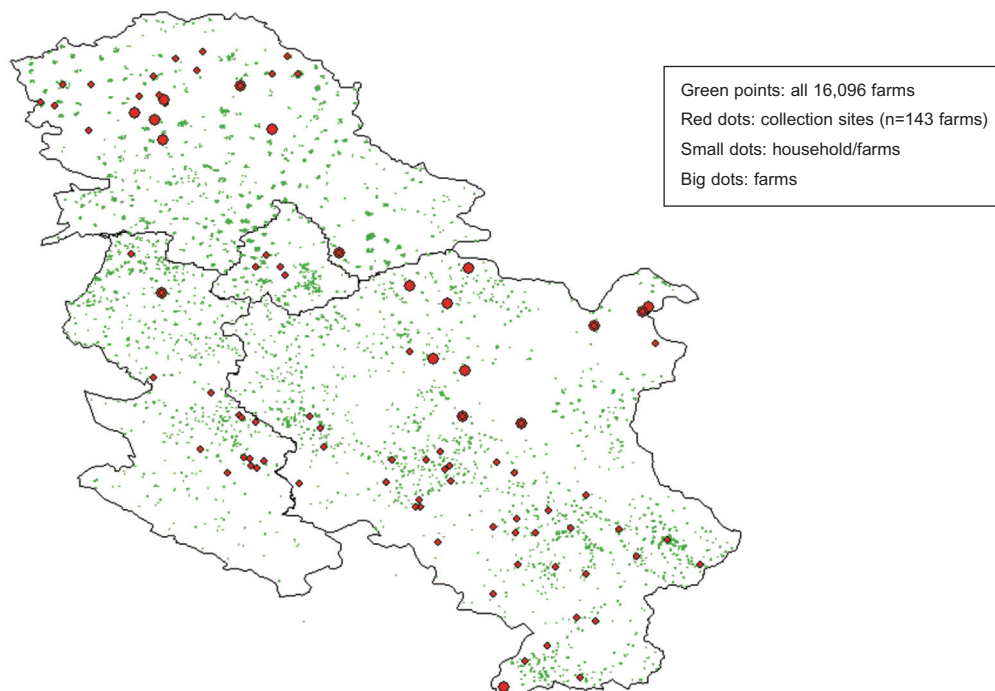

Fig. 2. Goat farm polulation and collection sites in the four regions of Serbia.

and watering habits, breeding, health status and last year's health history, were obtained by means of a standardised questionnaire, which was filled in for each animal through a telephone interview with the owner or the local veterinarian. Ownership of each animal was determined by the ear tag number through the MAFWM national goat and sheep database. Some animals could not be traced and a few owners were not cooperative, so completed questionnaires were only available for 372 (86.3%) animals from 100 (69.9%) households/farms from all four regions.

Animals were classified by gender and by age as either young (<1 year) or adults (>1 year). The origin of the animals was noted as those raised on a household/farm or introduced from elsewhere. According to purpose, goats were categorised into those used for milk and cheese production *versus* all other purposes (milk, cheese and meat production, breeding, companion/pet animals). The herd size was defined as previously described, either as small households (≤10 animals) or farms (>10 animals). The farms were categorised according to the type of housing into "stable only" (total confinement), "stable with access to outside pen" (partial confinement) and "stable with access to pasture" (with or without access to pen). According to the presence of other animals on the household/farm, categories included those that keep only goats, those that also keep other ruminants and/or horses, and those that also own pigs, dogs, chicken and other small animals. Presence of cats was noted dichotomously (yes/no), although the responses cannot be taken as absolutely reliable since cats are generally present in farms in Serbia and even when not owned by a particular goat-keeper, the presence of stray or neighbourhood cats cannot be ruled out. As to the feeding practices, goats were categorised into those are given food in a manger with or without trough, and those that are also fed by pasture. The watering practices were categorised as farms with regular tap water and farms supplied by a local well, river or stream. The questionnaire also contained information on whether animals had offspring and whether any had shown signs of clinical illness in the year prior to sample collection.

Serology

Detection of *T. gondii* antibodies was performed by the modified agglutination test (MAT) as described by Dubey and Desmonts (1987), which is considered the test of choice for the detection of *T. gondii* antibodies

in animals (Dubey et al., 1985b; Klun et al., 2006). The antigen was kindly provided by the Centre National de Référence de la Toxoplasmose, Reims, France (Isabelle Villena). The starting dilution was 1:25 and all sera positive at that level or higher were considered positive.

Statistical analysis

All statistics were performed using the SPSS version 11.5 statistical package (SPSS Inc.; Chicago, USA). First, a univariate analysis of the influence of the different independent categorical variables on the *T. gondii* serological status (positive/negative) was performed by a χ^2 test. Variables associated with positivity at P ≤0.1 at the 95% confidence level were tested for colinearity and included in a multivariate logistic regression model. Overall fit of the logistic regression model was assessed by the Hosmer-Lemeshow goodness-of-fit statistics (1989). Results are presented as adjusted odds ratios (OR) with 95% confidence intervals (CI). The level of significance was 5%.

All 16,096 farms in the country were first georeferenced (Fig. 2) according to the WGS 1984 system (http://www.oosa.unvienna.org/pdf/icg/2012/template/WGS_84.pdf). Next, a GIS database containing data on the study population (n = 431), including origin, serological test results, land cover and climatic data, was generated using ArcGIS version 10.0 GIS software (ESRI; Redlands, USA). Each animal was georeferenced individually. Meteorological data including average annual temperature, rainfall and humidity for 2010 were taken from the 2010 annual report published by the National Meteorological Service (RHSS, 2011). A buffer zone of 10 km in diameter (chosen as one which contained all the study animals) was constructed around each of the 92 meteorological stations in the country. Meteorological data from stations with buffers containing study samples that were also at the same approximate elevation as the neighbouring farms (n = 54) were used for the analysis. These data together with the serological results were interpolated on a raster dataset by kriging and the geostatistical analyst tool to assess the uncertainty of the predictions derived from the measures of relationship in the samples based on sophisticated weighted average techniques (Laslett, 1994). All examined animals were taken into account for the analysis, regardless of repetition of coordinates when occurred. The best model was fit with an ordinary type of prediction without transformations and no order of trend

removal. The final spatial prediction map was constructed based on a semivariogram calculated automatically with a major range among parameters of 1.946 degrees, with 12 lags and lag size of 0.24. Predicted values were extrapolated on the map of all goat farms/households in Serbia.

Results

Seroprevalence

The overall seroprevalence of *T. gondii* infection in the goats (Table 1) was 73.3% (316/431), the highest in the Belgrade district (93.7%) and the lowest in CE Serbia (62.6%). Considering a farm positive if a single animal tested positive, the overall seroprevalence at the farm level was 84.6%.

Risk factors

Risk factor analysis was performed on 372 animals for which the questionnaires were complete. Univariate analysis showed that a number of variables, such as age, husbandry, purpose, feeding practices, watering practices, origin, presence of other animals and the region of farm location, were associated with positive serology for *T. gondii* (Table 2). Since high colinearity was detected between "husbandry" and "feeding practices" (Cramer's V coefficient of 0.88), only the variable with the higher effect size ("husbandry") was included in the logistic regression analysis; thus the final multivariate model included seven variables. Hosmer-Lemeshow goodness-of-fit was 0.553 with 75.5% of correct prediction.

As seen in Table 3, the risk of infection was increased above two-fold (2.22) in goats used for all purposes *versus* those exclusively used for milk and cheese production (P = 0.012), and almost seven-fold (6.64) in goats kept as sole species on a farm, *versus*

those kept at a household/farm together with fowl, pigs, dogs and/or cats (P = 0.001). On the other hand, a protective effect was shown for household/farm location in CE compared with northern Serbia (P = 0.004), and for goats introduced from the outside compared to those raised on the farm (P = 0.027). Although age above 1 year was shown to have a protective effect (OR = 0.097, P = 0.030) compared to younger ages, this finding may be biased by the miniscule proportion (4%, 16/372) of kids *versus* older animals.

Spatial analysis

Association between meteorological data and seroprevalence is visualised in Fig. 3. It can be seen that seronegativity is associated with lower rainfall within CE Serbia, whereas seropositivity is associated with higher rainfall, particularly in western Serbia (Fig. 4). Regarding elevation, more seropositive animals were found at lower average elevations (hilly areas) stretching from western Serbia to the central and southern parts of CE Serbia. No influence was seen for humidity and temperature.

A spatial analysis of seroprevalence of toxoplasmosis in goats was performed in ArcGIS version 10.0 using ordinary kriging without the nugget effect, because of the high seroprevalence. The constant mean assumption for the spatial mean surface was selected based on visual inspection of the empirical semivariogram, which levelled out almost perfectly. Taking into account unequal region sizes, the analysis predicted, some intra-regional differences notwithstanding, the greatest chance of finding goats testing positive for *T. gondii* in western Serbia (61-100% probability), followed by northern Serbia (31-100% probability) and the Belgrade district (71-100% probability). CE Serbia had the lowest chance of seropositivity (0-100% probability), while the highest was seen in the

Table 1. Seroprevalence of *T. gondii* infection in goats at the individual and at the farm level in the four Serbian regions.

Region	Individual level			Farm level		
	Tested	Prevalence (%)	95% CI*	Tested	Prevalence (%)**	95% CI*
North	120	77.5	70.0-85.0	51	88.2	79.3 -97.0
West	73	87.7	80.2-95.2	15	100	100
Central/East	206	62.6	56.0-69.2	71	77.5	67.8-87.2
Belgrade	32	93.7	85.3-100	6	100	100
Total	431	73 .3	69.1- 77.5	143	84.6	78.7- 90.5

*95% confidence intervals; ** a farm was considered positive if at least one animal tested positive.

Table 2. Univariate analysis of the prevalence of *T. gondii*-specific antibodies in goats (n=372) in Serbia according to zoographic and farm management characteristics.

Variable	N	Prevalence (%)	95% CI[a]	OR[b]	95% CI[a]	P-value
Gender						0.440
Male	28	64.3	40.7-87.9	1.00		
Female	344	71.2	63.7-78.7	1.37	0.61-3.08	
Age (years)						0.071
<1	16	93.8	48.1-100	1.00		
>1	356	70.1	62.8-77.3	0.15	0.02-1.17	
Herd size						0.242
≤10	223	73.1	63.6-82.6	1.00		
>10	150	67.3	56.6-78.0	0.76	0.48-1.19	
Husbandry						0.002
Stable	131	59.5	49.4-69.6	1.00		
Access to outside pens	79	74.7	58.3-91.0	2.00	1.08-3.71	
Access to pasture	162	77.8	65.9-89.7	2.38	1.43-3.96	
Principal activity						<0.001
Dairy production	258	64.7	56.9-72.6	1.00		
Additional use[c]	114	84.2	68.8-99.6	2.91	1.65-5.11	
Feeding practice						0.009
Manger/manger and trough	203	65.0	56.1-73.9	1.00		
Manger and trough, pasture	169	77.5	65.9-89.2	1.85	1.17-2.94	
Watering practice						0.001
Public water distribution	232	64.2	56.0-72.4	1.00		
Public and/or well/lake/stream	140	81.4	68.0-94.8	2.44	1.48-4.04	
Origin						0.020
Born on farm	232	75.0	65.4-84.6	1.00		
Outside source	140	63.6	53.1-74.0	0.58	0.37-0.92	
Presence of other animals						0.003
Fowl/pigs/dogs/cats	306	66.7	59.3-74.1	1.00		
Ruminants, horses	47	84.2	60.3-100	2.67	0.76-9.36	
Exclusively goats	19	91.5	50.6-100	5.37	1.88-15.39	
Breeding (kid)						0.413
Yes	335	71.3	63.7-78.9	1.00		
No	37	64.9	44.1-85.6	1.35	0.66-2.76	
Clinically treated last year						0.357
Yes	15	60.0	29.9-90.1	1.00		
No	357	71.1	63.8-78.5	0.61	0.21-1.75	
Presence of cats						0.106
Yes	321	69.2	61.6-76.7	1.00		
No	51	80.4	58.5-100	0.55	0.26-1.14	
Land cover						0.166
Agricultural cover	149	75.8	63.7-87.9	1.00		
Forests/seminatural areas	126	69.1	57.1-81.0	0.71	0.42-1.21	
Built up areas	97	64.9	52.1-77.8	0.59	0.34-1.03	
Region						<0.001
North	102	74.5	60.1-88.9	1.00		
West	69	86.9	66.5-100	2.28	0.99-5.23	
Central/East	176	59.1	50.4-67.8	0.49	0.29-0.85	
Belgrade	25	92.0	56.1-100	3.93	0.87-17.84	
Total	372	70.7	67.1-74.3			

[a]95% confidence interval; [b]odds ratio; [c]dairy and meat production, breeding, companion animals or pet animals.

Table 3. Final logistic regression model of the risk factors for *T. gondii* infection in goats in Serbia.

Variable	Adjusted OR*	95% CI[a]	P-value
Animal age (years)			
<1	1.00		
>1	0.10	0.01-0.80	0.030
Principal farm activity			
Dairy production	1.00		
Additional uses	2.22	1.19-4.15	0.012
Animal origin			
Born on farm	1.00		
Outside source	0.56	0.34-0.94	0.027
Presence of other animals			
Fowl/pigs/dogs/cats	1.00		
Ruminants, horses	1.50	0.34-6.51	0.590
Goats only	6.64	2.24-19.68	0.001
Region			
North	1.00		
West	2.03	0.85-4.87	0.111
Central/East	0.43	0.24-0.76	0.004
Belgrade	4.19	0.88-20.02	0.073

*odds ratio; **95% confidence intervals.

centre of the country (Fig. 4). In western Serbia, the probability was constant throughout the region, but the probability of finding positive animals in northern Serbia increased in the north (31%) south direction to reach 100% at the boundary with western Serbia and the Belgrade district. Within CE Serbia, a decreasing trend in the eastern and southern directions was noted, so that the probability of seropositivity was only 0-30% in a whole third of the region. This agrees perfectly with the results of the risk factor analysis, which showed a protective effect for household/farm location in CE *versus* northern Serbia.
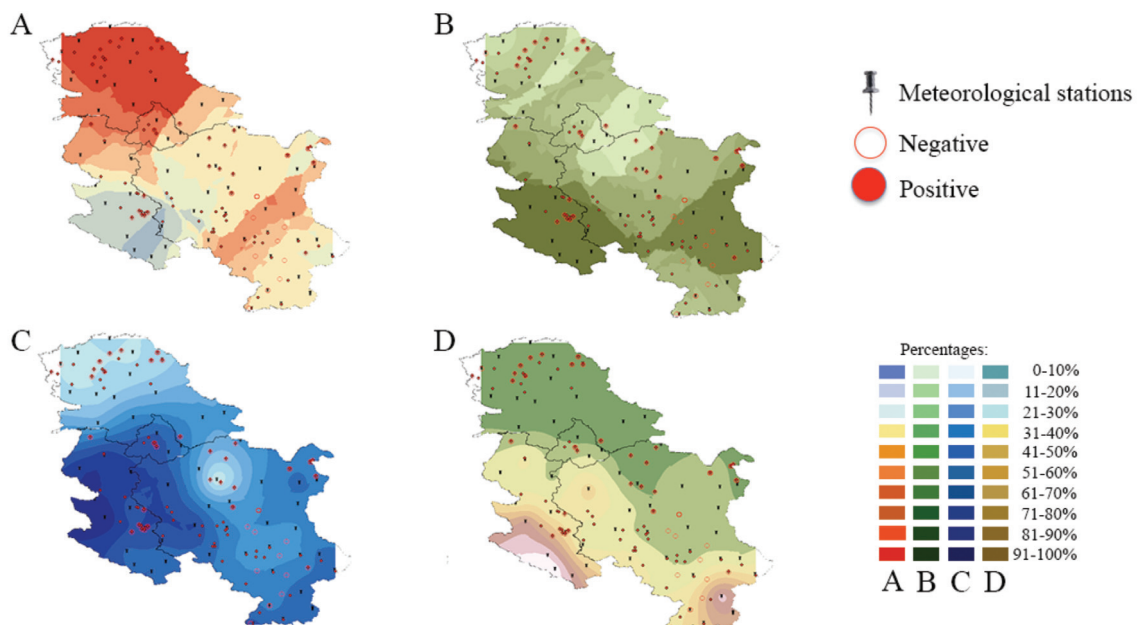

Fig. 3. Farm prevalence of *T. gondii* in goats in relation to meteorological and environmental data in Serbia for the year 2011. A = temperature; B = humidity; C = rainfall; D = elevation (0% = lowest and 100% = highest recorded value). Cut-off points of the colour shading are the 5%, 50% and 95% quantiles of the empirical distribution.
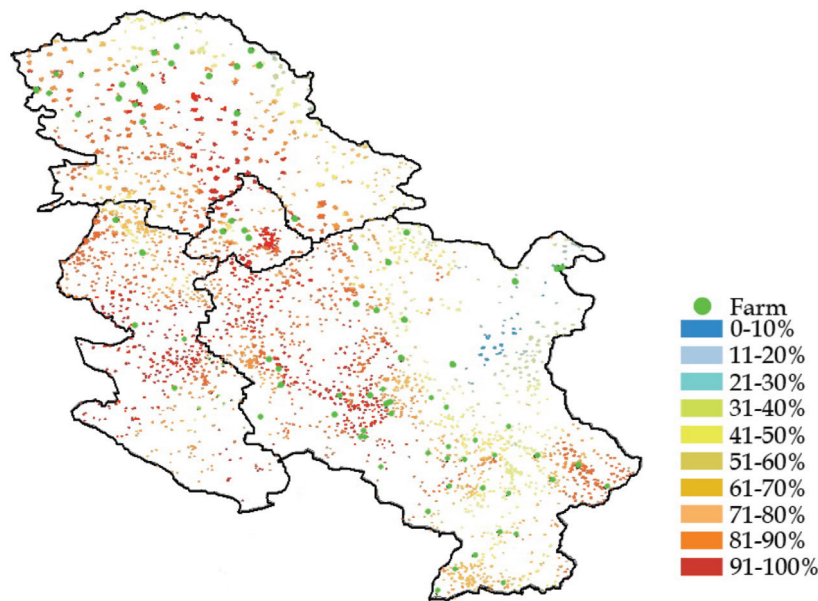
Fig. 4. Spatial prediction of seroprevalence of *T. gondii* infection in Serbian goat farms. Isopleth map based on kriging analysis (cut-off points of the colour scale are the 5%, 50% and 95% quantiles of the empirical distribution).

Discussion

The *T. gondii* prevalence in goats in Serbia detected in this study, both at the individual level (73.3%) and at the farm level (84.6%), is higher compared to recent data from other European countries. For example, a study in Portugal showed a prevalence of 18.5% at the individual level with the same agglutination test as we used (Lopes et al., 2013), whereas a survey in Greece based on enzyme - linked immunosorbent assay (ELISA), found a prevalence of 31% (Tzanidakis et al., 2012). The highest prevalence at the individual level (52.8%) outside Serbia was recently reported in Romania (Iovu et al., 2012). On the other hand, high farm prevalence rates are common. A study in Poland showed a 100% farm level with a 30-100% prevalence at the individual level (Czopowicz et al. 2011), while in Norway, despite a 17% prevalence at the individual level, the farm prevalence was 75% (Stormoen et al., 2012) and a prevalence of 25.1% for animals and 72.2% for farms has been reported from Spain (Garcia-Bocanegra et al., 2013).

In view of the findings mentioned above, it is surprising that we found some uninfected farms (Table 1) despite a very high prevalence at the individual level. However, our findings agree with the prevalence of *T. gondii* in other ruminants in Serbia; e.g. 76% and 84% in cattle and sheep, respectively, reported by Klun et al. (2006), a finding that might reflect environmental as well as zoohygienic and zootechnical conditions under which animals are reared for meat. In this context, it was very important to examine the risk factors for goat infection. Whereas a number of factors, including age, husbandry, type of farm (meat or dairy), feeding practices, watering practices, origin, presence of other animals and regional location, were all associated with *T. gondii* seropositivity, keeping goats for all purposes and keeping goats only, were *T. gondii* infection risk factors. Conversely, farm location in CE Serbia and introduction of outside animals were factors protective against infection.

An increased risk of *T. gondii* infection in all-purpose compared to dairy goats may be expected and has often been reported (García-Bocanegra et al., 2013; Santos et al., 2012; Zewdu et al., 2013). All-purpose animals tend to be kept in small household farms where zoohygienic vigilance is generally lower than on farms that exclusively rear dairy goats since the latter are generally commercially oriented, larger and run with higher vigilance. Although this explanation seems at first glance discrepant with the finding of an increased risk of infection in case of keeping goats only on the farm (as opposed to their cohabitation with other species), it is likely that if there is a source of infection in a large(r) commercial goat-breeding facility, more animals would be exposed. An increased risk of infection was shown in goat kids compared to animals older than 1 year, a result that could have arisen from the tiny proportion of young animals in the study sample (4%, 16/372). The perhaps surprising

protective effect shown for goats introduced from outside the farm *versus* those raised on the farm may be explained by the possibility the introduced goats originated from farms or regions of Serbia with a lower seroprevalence. Indeed, seropositivity related to a lack of replacements in the preceding year has been reported (Mainar et al., 1996).

The protective effect for household/farm located in CE *versus* northern Serbia was corroborated with the spatial analysis, which predicted the greatest chance of finding *T. gondii* positive goats in western Serbia and the lowest in CE Serbia, especially in its southeastern part. This prediction, based on kriging, also allowed us to predict the serological status of animals in unsampled areas, since this approach takes place within the borders of a spatial linear model (Berke, 2004). Considering this spatial correlation, our prediction model is valid for the geometrical rectangle formed among the most northern, eastern, southern, and western sampling points on the territory of Serbia.

The concordance between higher prevalence and rainfall in the western and CE regions could be explained by the fact that oocysts have a longer viability in damp soil, a fact that has been experimentally proven by Lèlu et al. (2012) and also observed in the field (Dumètre and Dardé, 2003). Additionally, the prediction map showed that the chances of finding *T. gondii* positive goats in northern Serbia near the border with Romania are between 51% and 60%, which perfectly agrees with the seroprevalence of 52.8% determined by Iovu et al. (2012) in the region of Romania bordering Serbia. Also, the chances of finding *T. gondii* positive goats decrease from western Serbia in the eastern and southern directions to values between 0% and 60%, whereas in northern Greece a seroprevalence of 30.7% has been reported (Tzanidakis et al., 2012). Unfortunately, these data are not available for the region in-between (the Former Yugoslav Republic of Macedonia.) Further field studies are needed to validate the presented prediction model, i.e. verify areas of lower environmental contamination.

Conclusion

The high prevalence of *T. gondii* infection in goats in Serbia reported here provides further evidence of extensive environmental contamination with this parasite. This requires strong preventive measures, including improvement of zootechnical and zoohygienic measures (e.g. specific rules for goat production aiming at better goat health). In addition, farmers should be recommended to frequently introduce unexposed new stock and encouraged to raise goats together with other species. The results of the spatial prediction model may serve as a recommendation for establishing new farms in the CE region.

Acknowledgement

References

Berke O, 2004. Exploratory disease mapping: kriging the spatial risk function from regional count data. Int J Health Geogr 3, 18.

Chartier C, Beziaud E, Buzoni-Gatel D, 1997. Enquete sero-epidemiologique sur les avortements infectieux des caprins en region Poitou-Charentes. Rev Med Vet (Toulouse) 148, 489-496.

Czopowicz M, Kabaa J, Szalus-Jordanowb O, Nowickia M, Witkowskia L, Frymusb T, 2011. Seroprevalence of *Toxoplasma gondii* and *Neospora caninum* infections in goats in Poland. Vet Parasitol 178, 339-341.

Dubey JP, 2009. Toxoplasmosis of animals and humans. CRC Press, 313 pp.

Dubey JP, Desmonts G, 1987. Serological responses of equids fed *Toxoplasma gondii* ocysts. Equine Vet J 19, 337-339.

Dubey JP, Desmonts G, Antunes F, McDonald C, 1985a. Serologic diagnosis of toxoplasmosis in experimentally infected pregnant goats and transplacentally infected kids. Am J Vet Res 46, 1137-1140.

Dubey JP, Desmonts G, McDonald C, Walls KW, 1985b. Serologic evaluation of cattle inoculated with *Toxoplasma gondii*: comparison of Sabin-Feldman dye test and other agglutination tests. Am J Vet Res 46, 1085-1088.

Dumètre A, Dardé ML, 2003. How to detect *Toxoplasma gondii* oocysts in environmental samples? FEMS Microbiol Rev 27, 651-661.

Edelhofer R, Prossinger H, 2010. Infection with *Toxoplasma gondii* during pregnancy: seroepidemiological studies in Austria. Zoonoses Public Health 57, 18-26.

Esteban-Redondo I, Maley SW, Thomson K, Nicoll S, Wright S, Buxton D, Innes EA, 1999. Detection of *Toxoplasma gondii* in tissues of sheep and cattle following oral infection. Vet Parasitol 86, 155-171.

García-Bocanegra I, Cabezón O, Hernández E, Martínez-Cruz MS, Martínez-Moreno A, Martínez-Moreno J, 2013. *Toxoplasma gondii* in ruminant species (cattle, sheep, and goats) from Southern Spain. J Parasitol 99, 438-440.

Hejliček K, Literak I, 1994. Incidence and prevalence of toxoplasmosis among sheep and goats in southern and western Bohemia. Acta Vet Brno 63, 145-150.

Hill DE, Dubey JP, 2013. *Toxoplasma gondii* prevalence in farm animals in the United States. Int J Parasitol 43, 107-113.

Hosmer D, Lemeshow S, 1989. Applied logistic regression. A much-cited recent treatment utilized in SPSS routines. New York: John Wiley and Sons.

Jones JL, Dargelas V, Roberts J, Press C, Remington JS, Montoya JG, 2009. Risk factors for *Toxoplasma gondii* infection in the United States. Clin Infect Dis 49, 878-884.

Iovu A, Györke A, Mircean V, Gavrea R, Cozma V, 2012. Seroprevalence of *Toxoplasma gondii* and *Neospora caninum* in dairy goats from Romania. Vet Parasitol 186, 470-474.

Klun I, Bradonjić S, Djokić V, Djurković-Djaković O, 2010. *Toxoplasma gondii* infection in goats in Serbia (preliminary results). CAPARA 2nd working groups workshop and management committee meeting, 29 Sep - 1 Oct 2010, Abstract book, p. 3.

Klun I, Djurković-Djaković O, Katić-Radivojević S, Nikolić A, 2006. Cross-sectional survey on *Toxoplasma gondii* infection in cattle, sheep and pigs in Serbia: seroprevalence and risk factors. Vet Parasitol 135, 121-131.

Laslett M, 1994. Kriging and splines: an empirical comparison of their predictive performance in some applications. J Am Stat Assoc 89, 391-409.

Lélu M, Villena I, Dardé ML, Aubert D, Geers R, Dupuis E, Marnef F, Poulle ML, Gotteland C, Dumètre A et al., 2012. Quantitative estimation of the viability of *Toxoplasma gondii* oocysts in soil. Appl Environ Microbiol 78, 5127-5132.

Lopes AP, Dubey JP, Neto F, Rodrigues A, Martins T, Rodrigues M, Cardoso L, 2013. Seroprevalence of *Toxoplasma gondii* infection in cattle, sheep, goats and pigs from the north of Portugal for human consumption. Vet Parasitol 193, 266-269.

Mainar RC, de la Cruz C, Asensio A, Domínguez L, Vázquez-Boland JA, 1996. Prevalence of agglutinating antibodies to *Toxoplasma gondii* in small ruminants of the Madrid region, Spain, and identification of factors influencing seropositivity by multivariate analysis. Vet Res Commun 20, 153-159.

Moraes LM, Raimundo JM, Guimarães A, Santos HA, Macedo Junior Gde L, Massard CL, Machado RZ, Baldani CD, 2011.

Occurrence of anti-*Neospora caninum* and anti-*Toxoplasma gondii* IgG antibodies in goats and sheep in western Maranhão, Brazil. Rev Bras Parasitol Vet 20, 312-317.

RHSS, 2011. Annual report 2010. Republic Hidrometeorological Service of Serbia, 20 pp.

Rodriguez-Ponce E, Molina JM, Hernandez S, 1995. Seroprevalence of goat toxoplasmosis on Grand Canary Island (Spain). Prev Vet Med 24, 229-234.

Santos Cde S, de Azevedo SS, Soares HS, Higino SS, Pena HF, Alves CJ, Gennari SM, 2012. Risk factors associated with *Toxoplasma gondii* seroprevalence in goats in the State of Paraíba, Brazil. Rev Bras Parasitol Vet 21, 399-404.

Skinner LJ, Timperley AC, Wightman D, Chatterton JMW, Ho-Yen DO, 1990. Simultaneous diagnosis of toxoplasmosis in goats and goatowner s family. Scand J Infect Dis 22, 359-361.

Spišák F, Turceková L, Reiterová K, Špilovská S, Dubinsky P, 2010. Prevalence estimation and genotypization of *Toxoplasma gondii* in goats. Biologia 65, 670-674.

Stormoen M, Tharaldsen J, Hopp P, 2012. Seroprevalence of *Toxoplasma gondii* infection in Norwegian dairy goats. Acta Vet Scand 54, 75-77.

Tenter AM, Heckeroth AR, Weiss LM, 2000. *Toxoplasma gondii*: from animals to humans. Int J Parasitol 30, 1217-1258.

Thrusfield M, 2005. Veterinary epidemiology. Blackwell Science, 610 pp. (third edition).

Tzanidakis N, Maksimov P, Conraths FJ, Kiossis E, Brozos C, Sotiraki S, Schares G, 2012. *Toxoplasma gondii* in sheep and goats: seroprevalence and potential risk factors under dairy husbandry practices. Vet Parasitol 190, 340-348.

Walsh CP, Hammond SE, Zajac AM, Lindsay DS, 1999. Survival of *Toxoplasma gondii* tachyzoites in goat milk: potential source of human toxoplasmosis. J Eukaryot Microbiol 46, 73-74.

Zaki M, 1995. Seroprevalence of *Toxoplasma gondii* in domestic animals in Pakistan. J Pak Med Assoc 45, 4-5.

Zewdu E, Agonafir A, Sisay Tessema T, Tilahun G, Girmay Medhin G, Vitale M, Di Marco V, Cox E, Vercruysse J, Dorny P, 2013. Seroepidemiological study of caprine toxoplasmosis in East and West Shewa zones, Oromia Regional state, Central Ethiopia. Res Vet Sci 94, 43-48.