Neosporosis is a disease caused by a protozoan parasite closely related to Toxoplasma gondii. As intermediate hosts, it affects mainly bovines causing abortion and birth of chronically infected calves that implies important economic losses mainly in dairy industry. The abortion can take place from the third month until the last month of pregnancy but mostly occurs at five-six months of gestation (Moore, 2005). The economical losses are determined by the pregnancy loss, lower production of milk, rebreeding of the animal and medical assistance and costs determined by the eventual replace of the infected animal (Dubey et al., 2007). Other intermediate hosts can be represented by ovines, goats, dears, buffalos, and many others animals. As definitive hosts dogs, coyotes and Australian dingoes have been identified (McAllister et al., 1998; Gondim et al., 2004; King et al., 2010). Worldwide the seroprevalence of neosporosis in cattle varies and different values were obtained: 5.7 % in Japan (Koiwai et al., 2006), 13.9 % in Uruguay (Banales et al., 2006), 11.3 % in Venezuela (Lista-Alves et al., 2006), 20.1 % in Slovakia (Reiterova et al., 2009), 24.1 % in Spain (Panadero et al., 2010) and 2.8 % in Sweden (Loobuyck et al., 2009).

In Romania the first report regarding Neospora caninum infection in cattle was in 2002 (Ionescu et al., 2002) showing a prevalence of 20 % (30/150 animals). The next year a larger study was in conducted and bovine sera were tested giving a seroprevalence of 33.71 % (Ionescu et al., 2003).

In this study a larger number of samples were taken under study and they were collected from a larger...
number of counties providing a much more reliable result regarding the spread of neosporosis in cattle from Romania.

**MATERIALS AND METHODS**

Between 2008 and 2010, a total number of 901 sera samples were collected from 16 dairy farms. These farms were located in six different counties from central and northwestern parts of Romania: Alba, Cluj, Maramureș, Mureș, Sibiu and Satu Mare. The lowest number of samples collected from a farm was six and the highest was 188. All the sampled farms had vaccination schemes against brucellosis, leptospirosis, infectious bovine rhinotracheitis and bovine viral diarrhea.

The blood was collected by puncture from jugular or caudal vein and, incubated at 37 °C and then centrifuged for serum collection. Sera were kept under -20 °C until further use. All sera were tested for antibodies against *N. caninum* by using a commercial enzyme immuno-assay kit Neospora caninum Antibody Test Kit produced by IDEXX Laboratories, Switzerland. Briefly, each serum sample (diluted 1:100) was added to the Neospora antigen-coated microplates and incubated for 30 minutes at room temperature. Then, the antigen provided in the kit was added in each well and incubated at room temperature for another 30 minutes. The reaction was revealed by adding the chromogen and incubating for 15 minutes at room temperature. The color reaction was stopped by adding stop solution. Plates were read at 650 nm and the results were expressed as optical density (OD) values and were analyzed using the formulas provided by the manufacturer. Using a statistical package (EpiInfo Version 5) the p-value was also calculated.

**RESULTS**

Antibodies against *N. caninum* were detected in 312 (34.6 %) serum samples from 901 pure breed cows. No results were considered as being doubtful. The average within-heard seroprevalence was 31.11 %, with a range between 11.1 and 60.0 % in seropositive farms (Table I). In adult cows and calves the seroprevalence were 34.8 % (300/862) and 30.8 % (12/39) respectively.

Out of the 901 animals tested, 137 (15.2 %) had at least one abortion in their history. The seroprevalence of *N. caninum* infection in these animals reached a value of 40.9 % (95 % CI 32.6-49.6). Amongst the rest of the animals that had no previous reproduction problems the seroprevalence was 33.5 % (256/764). The difference between these two animal categories was statistically significant: p < 0.004.

Also the tested animals were divided into two other categories: animals, which had no pregnancy, and animals that had at least one pregnancy in their history. From the first group, (176 animals), 77 presented antibodies against *N. caninum*, reaching a percentage of 43.8. From the second group (725 animals), 32.4 % were positive. These results were also statistically significant: p < 0.004.

![Table](image)

* overall prevalence.

* Table I. – Seroprevalence of *N. caninum* in cattle form different counties of Romania.

* Table II. – Seroprevalence of *N. caninum* infection in cattle that had at least one abortion in their history.
DISCUSSION

The seroprevalence of *N. caninum* infection in cattle was studied in many countries. It varied between countries and regions. In Romania, some other studies regarding neosporosis were performed and the prevalence obtained was of 56.2 % (Gavrea et al., 2008), 19.3 % for cattle raised in extensive system (Gavrea & Cozma, 2009) and 55.95 % in cattle with reproductive failure (Gavrea & Cozma, 2010). The overall seroprevalence obtained in dairy cattle in six counties from Romania was 34.6 % similar to that obtained in Thailand (34 %) (Kyaw et al., 2004), Costa Rica (43.3 %) (Romero et al., 2005) and Mexico (42 %) (Garcia-Vazquez et al., 2005). In the present herd seroprevalences varied between 11.1 and 60.0 % and the differences were statistically significant between herds. The fact that all the farms taken under study had cattle positive for *N. caninum* infection indicates that this infection in dairy cattle is widespread in Romania. There have been many studies that analysed and demonstrated the association between seroprevalence of neosporosis and abortion. Although the association between abortion and *N. caninum* seropositivity was not strong (odds ratio, 0.72) there was some evidence of neosporosis-associated abortions. A relatively high seroprevalence of *N. caninum* was recorded in animals that aborted. There was also a significant difference between animals that had no recorded pregnancies and the ones that had at least one pregnancy in their history.

ACKNOWLEDGEMENTS

This study was supported by the Executive Unit for Financing Higher Education, Research, Development and Innovation from Romania, Grant PNII PC 52-177/2008, director Prof. Dr. Vasile Cozma.

REFERENCES

Banales P., Fernandez L., Repiso M.V., Gil A., DargaTz D.A. & Osaka T. A nationwide survey on seroprevalence on *Neospora caninum* infection in beef cattle in Uruguay. *Vet Parasitol*, 2006, 139, 15-20.

Dubey J.P., Schares G. & Ortega-Mora L.M. Epidemiology and control of neosporosis and *Neospora caninum*. *Clin Microbiol Rev*, 2007, 20, 323-367.

King J.S.K., Slapeta J., Jenkins D.J., Al-Qassab S.E., Ellis J.T. & Windsor P.A. Australian dingoes are definitive hosts of *Neospora caninum*. *Int J Parasitol*, 2010, 40, 945-950.

Kopwai M., Hamaoka T., Haritani M., Shimizu S., Zeniya Y., Eto M., Yokoyama R., Tsutsui T., Kimura K. & Yamane I. Nationwide seroprevalence of *Neospora caninum* among dairy cattle in Japan. *Vet Parasitol*, 2006, 135, 175-179.

Kyaw T., Virakul P., Muangyai M. & Suwimonteerabutr J. *Neospora caninum* seroprevalence in dairy cattle in central Thailand. *Vet Parasitol*, 2004, 121, 255-263.

Garcia-Vazquez Z., Rosario-Cruz R., Ramos-Aragon A., Cruz-Vazquez C. & Maps-Sanchez G. *Neospora caninum* seropositivity and association with abortions in dairy cows in Mexico. *Vet Parasitol*, 2005, 134, 61-65.

Gavrea R., Gherman C., Tittincu A., Mircean V. & Cozma V. Seroepidemiology of neosporosis in cattle from a dairy farm in Mureş county. *Sci Parasitol*, 2008, 2, 26-30.

Gavrea R. & Cozma V. Seroprevalence of neosporosis in cattle raised in extensive system in a village from Cluj country. *Buletin USAMV*, 2009, 66, 99-102.

Gavrea R. & Cozma V. Seroprevalence of *Neospora caninum* in cows with reproductive failure in center and northwest of Romania. *Sci Parasitol*, 2010, 11, 67-70.

Gondim L.F., McAllister M.M., Pitt W.C. & Zemlicka D.E. Coyotes (Canis latrans) are definitive hosts of *Neospora caninum*. *Int J Parasitol*, 2004, 34, 159-161.

Ionescu A., Bildaru M. & Ionescu V. Screening privind infestaţia cu *Neospora caninum* la caprine şi bovine. *Rev Rom Parasitol*, 2002, XII, 28-29.

Ionescu V., Popa E. & Moldoveanu D. Neosporoza la bovine. *Rev Rom Parasitol*, 2005, XIII, 125.

Kyaw T., Virakul P., Muangyai M. & Suwimonteerabutr J. *Neospora caninum* seroprevalence in dairy cattle in central Thailand. *Vet Parasitol*, 2004, 121, 255-263.

Lista-Alves D., Palomares-Naveida R., Garcia F., Obando C., Arrieta D. & Hoet A.E. Serological evidence of *Neospora caninum* in dual-purpose cattle herds in Venezuela. *Vet Parasitol*, 2006, 136, 347-349.

Loobuyck M., Frosslin J., Lindberg A. & Bjorkman C. Seroprevalence and spatial distribution of *Neospora caninum* in a population of beef cattle. *Prev Vet Med*, 2009, 92, 116-122.

McAllister M.M., Dubey J.P., Lindsay D.S., Jolley W.R., Wills R.A. & McGuire A.M. Dogs are definitive hosts for *Neospora caninum*. *Int J Parasitol*, 1998, 28, 1473-1478.

Moore D.P. Neosporosis in South Africa. *Vet Parasitol*, 2005, 2, 87-97.

Panadero R., Painceira A., Lopez C., Vazquez L., Paz A., Diaz P., Dacal V., Cienfuegos S., Fernandez G., Lago N., Diez-Banos P. & Morbondo P. Seroprevalence of *T. gondii* in wild and domestic ruminants sharing pastures in Galicia (Northwest Spain). *Res Vet Sci*, 2010, 89, 111-115.

Reiterova K., Spilovska S., Antolova D. & Dubinsky P. *Neospora caninum*, potential cause of abortion in dairy cows: the current serological follow-up in Slovakia. *Vet Parasitol*, 2009, 159, 1-6.

Romero J.J., Van Breda S., Vargas B., Dolz G. & Franken K. Effect of neosporosis on productive and reproductive performance of dairy cattle in Costa Rica. *Theriogenology*, 2005, 64, 1928-1939.

Received on June 17th, 2011
Accepted on September 13th, 2011