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Short communication

Seroprevalence and risk factors of *Neospora* spp. in donkeys from Southern Italy

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ABSTRACT

In some European countries there is an increasing interest on donkey. Despite there are few data regarding the donkey's parasitic diseases especially those with a protozoal etiology as neosporosis. Samples used in the study were collected from 238 domestic donkeys during year 2010 in Southern Italy from 207 females and 31 males of five breeds (Martina-Franca, Amiata, Sicilian-Grey, Ragusano, Sardinian) and crossbreeds with the average age 9 years (1 month – 24 year). Sera were tested by a competitive-inhibition enzyme-linked immunosorbent assay for antibodies against *Neospora caninum*; the sera were marked positive, if more than 30% inhibition was found. Out of a total 238 donkeys, 28 (11.8%) were found positive for *Neospora* antibodies with 12% in females and 6% in males. Different seroprevalence 15.4%, 16%, 12% and 8.8% were found in age categories <1 year, 1–4 years, 5–9 years and ≥10 years, respectively. The seroprevalence ranged in different breeds from 36% (Sicilian-Grey) to 0% (Sardinian) and in different use from 17% (for breeding) to 0% (for meat production).

Logistic regression analysis demonstrated evidence of a significant ($P < 0.05$) association between crossbreed origin of samples and risk of protozoan infection; age of donkeys was also significant risk factor for protozoan infection. No statistical significant difference ($P > 0.05$) was found among genders and use of donkeys and risk of *N. caninum* infection. This is the first serological survey for *Neospora* spp. performed in donkeys.

1. Introduction

The donkeys (*Equus asinus*) were traditionally used as working animal for transport and riding and for farm activities. In the recent years, an increasing interest have been shown for donkeys and in particularly for their use as a pet animal, in leisure activities, for onotherapy and especially for the rediscovery of donkey milk as a food source for children affected with cow milk allergy. Equine neosporosis is a protozoal disease caused by a cyst-forming coccidian of the phylum Apicomplexa. Two species, *Neospora caninum* and *Neospora hughesi*, have been identified as infecting the horse and were associated with neurological disease and fetal loss (Dubey and Porterfield, 1990; Pitel et al., 2003). Based on experimental studies, domestic dogs (*Canis lupus*), Australian dingoes (*Canis domesticus*) and coyotes (*Canis latrans*) were confirmed as definitive hosts of *N. caninum* while the definitive host of *N. hughesi* remains to be

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elucidated. Seroprevalence of *Neospora* spp. in asymptomatic horses were reported from North America (Dubey, 2003), South America (Villalobos et al., 2006; Dangoudoubiyam et al., 2011) and Europe (Pronost et al., 1999; Pitel et al., 2001; Ciaramella et al., 2004; Jakubek et al., 2006; Kligler et al., 2007; Kilbas et al., 2008; Piantedosi et al., 2009; Bártová et al., 2010).

In Southern Italy, were the population of donkey is going to increase, *Neospora* infection has been reported in horses (Ciaramella et al., 2004), cattle (Guarino et al., 1998), water buffaloes (Guarino et al., 2000) and in dogs (Cringoli et al., 1996). Since, the presence of donkeys is in promiscuity with these species in many farms, this condition let us to assume a possible spreading of *Neospora* infection also in donkeys. Until now, no reports are available about the prevalence of this protozoal disease in donkeys.

The aim of the present study is to improve the information about the seroprevalence of *Neospora* spp in animals from southern Italy.

2. Materials and methods

2.1. Sample size and donkeys sampled

The survey was conducted on 238 clinically healthy donkeys bred and raised in Southern Italy. This sample size was calculated by using the formula proposed by Thrusfield (1995) inserting the following values: study population (9991 donkeys, data supplied by Italian Association of Breeders) expected prevalence of neosporosis (20%, data reported in horses in Southern Italy by Ciaramella et al., 2004), confidence interval (95%), and desired absolute precision (5%). During autumn 2010 blood samples were collected in 20 donkey farms (5 large farms ≥ 10 donkeys and 15 small farms < 10 donkeys) of 16 municipalities of Southern Italy. General data including gender, age, breed, use and period of grazing during year, presence of dogs and ruminants in the farms were obtained through a questionnaires completed during sample collection. The donkeys were divided into four age categories: <1 year, 1–4 years, 5–9 years, ≥10 years; the average age of donkeys was 8 years and 11 month (1 month – 24 year). Furthermore, a complete clinical examination was done on each donkey.

2.2. Sera preparation and serological test (cELISA)

All blood samples were obtained from the jugular vein by using a vacuum tube without anticoagulant. Sera were obtained from clotted blood samples by centrifugation and stored at −20 °C until analysis. Sera were tested for the presence of antibodies to *N. caninum* using a competitive-inhibition enzyme-linked immunosorbent assay (cELISA, VMRD, Inc., Pullman, WA, USA). Serological examination and evaluation was done according to the manufacturer's instructions; samples associated with percent inhibition value ≥ 30% were considered positives. The optical density values were obtained using an automatic plate reader (Dynex Technology MRXII, Prague, Czech Republic).

Table 1
Risk factors for *Neospora* spp. infection in donkeys as a result of the logistic regression multivariate analysis.

	Coefficient	Std error	*z*	*P*
Intercept	−1.98	0	0.9998	
Gender (male)	−17.0	−0.002	0.9980	
Age (year)	−0.709	−2.011	0.0443	0.01
Breed				
Crossbreed	−1.37	−2.277	0.0228	0.01
Sicilian-Grey	1.15	1.569	0.1167	
Martina-Franca	−381	−0.667	0.5047	
Amiata	−18.0	−0.004	0.9964	
Ragusano	0.176	0.192	0.8479	
Sardinian	−18.5	−0.005	0.9963	
Use				
Milk	1.19	−0.004	0.9999	
Pet	1.58	−0.002	0.9998	
Breeding	17.9	−0.007	0.9944	
Presence of dog	−0.28	1.05	0.1750	
Presence of ruminants	−0.31	1.21	0.0980	

2.3. Statistical methods

Statistical analyses were performed on the basis of the individual animal as the unit. A multivariate analysis was used to evaluate the contribution of each variables involved in infection risk. A logistic regression (general linear models, GLM) was used to predict seropositivity according to additive and linear relationship between variables. Statistical analysis was performed using GraphPad Prism version 6.00 for Mac OS X, GraphPad Software, La Jolla, California, USA. $P < 0.05$ was considered statistically significant.

3. Results

Out of a total 238 donkeys, 28 (11.8%) were found positive for *Neospora* spp. antibodies with inhibition ranging from 30.07% to 44.34%. *Neospora* spp. antibodies were found in 12% (26/207) females and 6% (2/31) males. The following seroprevalences 15.4% (4/26), 16% (8/50), 12% (7/60) and 8.8% (9/102) were found in age categories <1 year, 1–4 years, 5–9 years and ≥10 years, respectively. We also found different seroprevalence in breeds with 36% (5/14), 25% (2/8), 20% (9/46), 13% (7/53), 5% (5/110) and 0% (0/7) in Sicilian-Grey, Ragusano, Amiata, Martina-Franca, crossbreeds and Sardinian, respectively. The following seroprevalences 17% (2/12), 13% (23/183), 11% (3/27) and 0% (0/16) were detected in donkeys used for breeding, milk production, as a pet animal and for meat production, respectively. In case of 28 donkeys positive for *Neospora* spp., 82% came from large farms (≥10 donkeys), 64% donkeys grazed during whole year, in 29% cases, the dogs had access to food and water used for feeding donkeys, in 100% and 21% cases horses and ruminants were bred on the same farms.

Logistic regression analysis involving all considered variables demonstrated evidence of a significant ($P = 0.01$) association between crossbreed origin of samples and risk of protozoan infection (Table 1). Age of donkeys was also significant ($P = 0.01$) risk factor for protozoan infection. It is evident that the donkeys older than two years can highlight *N. caninum* positivity (Fig. 1). No statistical significant

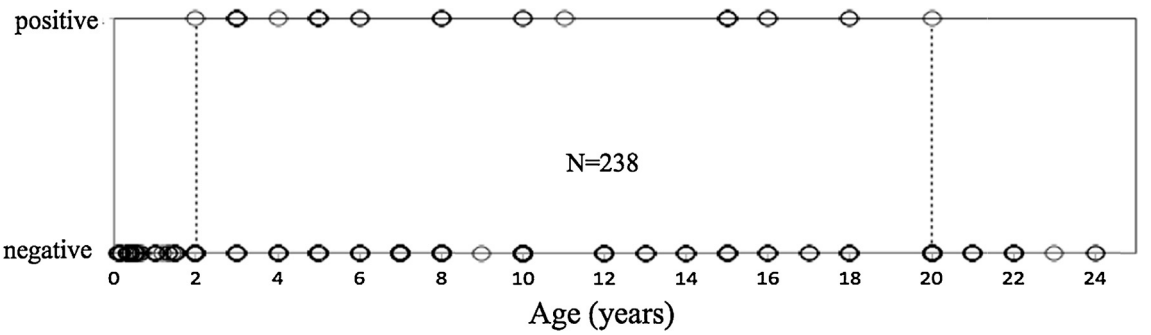

Fig. 1. Association between age of donkeys and their positivity to Neospora antibody. It is evident that the donkeys older than two years can highlight positivity (broken line on the left). This arrangement seems to end at the age of 20 years (broken line on the right), although the number of samples older than this age is low.

difference was found between genders and different use of donkeys and risk of *N. caninum* infection.

The clinical examination of all the seropositive donkeys did not show any neurological signs, reproductive disorders and abortion.

4. Discussion

This is the first serologic survey for *Neospora* spp. antibodies performed on donkeys. As regards to the clinical investigations, the absence of fetal loss and neurological signs, it indicates that a substantial degree of *Neospora* spp. infection occurs sub-clinically in these animals. We found *Neospora* spp. antibodies in 11.8% donkeys. Since there are no similar studies on donkeys, we can compare our results with seroprevalence in horses. Considering ELISA as diagnostic test used in Europe, similar prevalence 9% was found in horses from Turkey (Kilbas et al., 2008) and Sweden (Jakubek et al., 2006), while a higher prevalence 24% was found in horses from the Czech Republic (Bártová et al., 2010). Indirect fluorescent antibody test (IFAT) was used for surveys in Italy and Israel. The prevalence of 28% (Ciaramella et al., 2004) and 10% (Piantedosi et al., 2009) was found in horses coming from different Italian regions, while 12% was found in horses from Israel (Kligler et al., 2007). In France, 23% prevalence was obtained in horses tested by direct agglutination test (Pitel et al., 2001).

In our study, no statistical significant difference was found between genders and use of donkeys and their positivity, while age of donkeys was significant risk factor for protozoan infection. Since the majority of donkeys became infected until the age of 2 years, it let us to assume that donkeys were exposed to the parasite infection at the early stages of their life. We found two young donkeys (2 and 1 months old) positive for *N. caninum* antibodies; their mothers were also positive. Recently, Pusterla et al. (2011) confirmed that *N. hughesi* can persist in horse population via endogenous transplacental infection. Considering this fact and positivity of she-ass of young donkeys leads us to consider possible vertical transmission of *Neospora* infection in donkeys in further studies. The crossbreed seems to be more resistant to *Neospora* spp. infection since four times difference was found between positivity of crossbreeds and breeds.

It is difficult to explain what is the critical points for the *Neospora* infection, considering that a limitation of the present study is the impossibility to discriminate *N. hughesi* versus *N. caninum* we can only postulate some hypothesis: the possible source of *Neospora* spp. infection for donkeys in farms could be water, food or pastures contaminated with *Neospora* spp. oocysts. In 29% donkeys positive for *Neospora* spp., the dogs had access to food and water used for feeding donkeys. Although the presence of dogs in farms has not been confirmed as a risk factor for *Neospora* spp. infection, The donkeys as well as horses in Southern Italy could be exposed to *Neospora* spp. infection. Further studies should focus on concrete farms to find the potential risk factors and routes for infection transmission.

Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

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