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Research article
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Neosporosis and toxoplasmosis are two prevalent and important protozoses in dairy cows in small farms from Thessaly, Central Greece

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ABSTRACT: Neosporosis and toxoplasmosis are two significant protozoan parasitoses that can cause infertility and abortions in dairy cows worldwide. Those parasites, in contrary of other coccidia, are adapted to infect hosts by several routes of transmission. These include the consumption of sporulated cysts that have contaminated the environment, transplacental infection and accidental consumption of row meat infected with bradyzoites. Infertility, abortions, neonatal mortality and other clinical symptoms in cattle in both parasitoses can cause severe financial losses, especially when these diseases acquire an endemic or epidemic manner.

The aim of this study was to report the prevalence of neosporosis and toxoplasmosis in dairy cattle as detected by ELISA technique, under the correlation with the age of infected animals. Blood samples were collected by venipuncture of the tail vein of 627 Holstein–Friesian dairy cows raised in 7 small farms (ranging from 55 the smaller to 137 the larger farm) from the region of Thessaly, central Greece. All farms have recorded a variety of infertility problems in the past. Animals were separated in three age groups; under 3 years old, between 3 and 6 years and over 6 years old. In a total of 627 cows from which the samples were taken and examined, 131 (20.89 %) of them were found positive for *N. caninum* while 51 (8.13 %) were positive for *T. gondii*. The prevalence in the three different age groups was recorded at 16.36%, 23.51% and 26.17% respectively for neosporosis and in the same age groups it was 5.20%, 9.16% and 13.08% respectively for toxoplasmosis. From the above results it becomes clear that preventative measures should be suggested to and adapted by the farmers, in order to decrease their financial losses.

Keywords: Neosporosis, toxoplasmosis, cattle, prevalence, economic impact, zoonosis

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INTRODUCTION

Neosporosis and toxoplasmosis, are two significant protozoan parasitoses that can cause infertility and abortions in dairy cows worldwide (Dubey and Shares, 2011; Dubey, 2003). These parasites, unlike other coccidia, are adapted to infect hosts by several routes of transmission.

Neospora caninum (*Sarcocystidae*) is an apicomplexan protozoan parasite which is the etiological agent of neosporosis (Dubey and Linsday, 1996). In its life cycle, dogs and other related canids are the only definitive hosts that shed through their feces the unsporulated oocysts into the environment. Concurrently, many other species such as large and small ruminants, horses, rabbits, mice and others are intermediate hosts of the parasite (Dubey et al., 2007). Despite the large number of species that can be infected, to the present neosporosis has emerged as a serious disease only in cattle and dogs worldwide (Dubey at al., 2007 ; Dubey and Schares, 2011).

Toxoplasmosis is caused by *Toxoplasma gondii* (*Sarcocystidae*), a zoonotic parasite of great importance and global distribution (Robert et al., 2012). Toxoplasmosis is a protozoosis, in which the cat and other Felidae serve as final hosts, whereas nearly all warm-blooded animals and human can be infected and serve as intermediate hosts (Sukthana, 2006.; Dubey, 2009). Infected cats are capable of shedding millions of oocysts with their faeces (Dabritz and Conrad, 2010). These oocysts mature in the environment, where they can survive for 12-18 months, depending on climatic conditions, thereby serving as an important source of infection for grazing animals (Sukthana, 2006; Baxton, 1998).

Infertility, abortions, neonatal mortality and other clinical symptoms in cattle in both parasitoses can cause severe financial losses, especially when these diseases acquire an endemic or epidemic character. The economic impact of these parasitoses is directly related to the costs associated with abortions and subsequent loss of the calf and indirectly with the cost of veterinary services, rebreeding, loss of milk yield and possible replacement of the cows that aborted (Ansari-Lari and Rowshan-Ghasrodashi, 2017). Moreover, toxoplasmosis is also a disease of zoonotic importance (Sukthana, 2006).

The aim of this study was to report the prevalence of neosporosis and toxoplasmosis in dairy cattle as detected by an ELISA technique, in combination with the age of infected animals, in order to suggest preventative measures to the farmers of this area as well as other areas with similar problems.

MATERIALS AND METHODS

Blood samples were collected by tail vein venipuncture from 627 Holstein–Friesian dairy cows raised in 7 small farms (ranging from 55 the smaller to 137 the larger farm) from the region of Thessaly, central Greece. All farms have recorded infertility problems in the past.

The samples were separated in three groups according to the age of the cows. The first group included cows aged less than three years, the second between 3 to 6 years and the third those above 6 years old with 269, 251 and 107 cows respectively.

All blood samples were retrieved from the tail vein at the time of the examination procedure for gestation and collected in 5ml plastic test tubes containing EDTA. Each test tube was marked with the date, the animal's ear tag number and its age group. The collected samples were stored at 4oC and transferred to the laboratory, where they were centrifuged at 2000rpm for 15 minutes. The sera were tested for antibodies against *N. caninum* and *T. gondii* using the commercial ELISA test kits, ID Screen, according to the manufacturer's instructrions.

The data from the serological results and the age of the cows tested in this research were edited and analyzed statistically using the chi-square (χ^2) test .The statistical significance level used was $P < 0.05$.

It should be pointed that in all examined farms infertility problems had been recorded and free ranged dogs and cats that did not receive appropriate veterinary care where present.

RESULTS

A total of 627 cows were examined, from which 131 (20.89 %) were found positive for *N. caninum* and 51 (8.13 %) for *T. gondii*.

The prevalence in the three different age groups was recorded at 16.36%, 23.51% and 26.17% for *N. caninum* infection (Table 1), while for *T. gondii* infection it was 5.20%, 9.16% and 13.08% respectively (Table 2).

Table 1. Prevalence of neosporosis in dairy cattle in relation to their age

Age of cattle	Number of seropositive	Number of seronegative	Prevalence of neosporosis %
<3 years n=269	44	225	16.36
3-6 years n=251	59	192	23.51
>6 years n=107	28	79	26.17
Total n=627	131	496	20.89

Table 2. Prevalence of toxoplasmosis in dairy cattle in relation to their age

Age r of cattle	Number of seropositive	Number of seronegative	Prevalence of toxoplasmosis %
<3 years n=269	14	255	5.20
3-6 years n=251	23	228	9.16
>6 years n=107	14	93	13.08
Total N=627	51	576	8.13

DISCUSSION

According to data from previous studies worldwide, the prevalence of *N. caninum* in cattle has been reported to show a wide range; in South America it is reported to be between 7.6% and 76.9% (Cedeρo and Benavides, 2013; Sousa et al., 2012), in Asia between 5.7% and 43% (Nazir et al., 2013; Koiwai et al., 2006), while in Africa it ranges only between 10.7% and 19.6% (Ghalmi et al.,2012; Ibrahim et al., 2012) and finally in Australia the prevalence is found to be 10.2% (Hall et al., 2005). Moreover, the prevalence of *N. caninum* in cattle in some European countries (Germany, The Netherlands, Spain, Romania and Sweden) ranges from 16% to 76 % (Imre et al., 2012; Bartels et al., 2006; Ortega-Mora, 2006).

There are studies from Balkan countries that have reported higher prevalence of neosporosis in cattle than our findings. Cvetojević et al., (2018) recorded prevalence 25% in Serbia, Mitrea et al., (2012) reported (41.7%) and Gavrea et al., (2008, 2010) recorded overall prevalence 55.95% from dairy cows from Romania. Moreover there are also similar surveys which have described prevalence of *N. caninum* antibodies at 27%, 24.4% and 22% in Germany, Italy and Denmark, respectively (Magnino et al, 1999; Jensen et al 1999; Schares et al, 1998). On the contrary, there are several studies which reported lower prevalence than our findings in Europe. They have recorded prevalences of 12.2% in Belgium (De Meerschman et al, 2000), 4.1% in Germany (Conraths et al, 1996), 3.3% in Hungary (Hornok et al, 2006), 12.6% in Ireland (McNamee et al, 1996), 11.2% in Spain (Caetano-da-Silva et al, 2004) and 3.1% in Czech Republic (Václavek et al, 2003).

N. caninum causes abortions in both dairy and beef cattle. The abortions can occur from the third month of gestation until parturition (Reiterova et al., 2009; Dubey et al., 2007; Wouda et al., 1999). This parasite can also cause fetal viability disorders or neurological birth defects in newborn calves (Lassen et al., 2012; Malaguti et al., 2012) and those younger than 2 months of age (Dubey, 2003). The infected young calves may present neurological signs, low birth weight (Dubey and Schares, 2011), difficulty to rise and stand, flexed or hyperextended hind and/or forelimbs, and in some cases exophthalmia or asymmetrical appearance of the eyes. However, most of the calves born congenitally-infected remain clinically free of neosporosis signs (Dubey 2003; Bielanski and Phipps-Todd 2002).

Toxoplasmosis in cattle is usually asymptomatic or causes mild symptoms while a primary infection in pregnant animals is capable of establishing a placental and fetal infection, which may result in fetal death and resorption, abortion, or stillbirth (Dubey and Jones, 2008; Dubey, 1992). Although cattle are considered as poor hosts for *T. gondii* and good hosts for *N. caninum*, *T. gondii* infections could also cause economic losses and an increased potential for transmission to other animals and humans (Dubey and Jones, 2008).

There are studies worldwide that report the prevalence of *T. gondii* in cattle in some regions of the world showing a large variation in the reported proportions of positive findings.

Compared to our results, some researchers record lower prevalence of infection in cattle, such as in Poland 3.15% (Lucyna et al., 2013), 0% in Iran (Sharif et al, 2007), 2.3% in China (Yu et al, 2007) and 6.6% in Central Ethiopia (Bekele and Kasali, 1989) while higher seroprevalence values were found in Serbia (76.3%) (Klun I, et al., 2006), France (7.8%) (Gilot-Fromont et al., 2009), Italy 11.5% (Vesco et al., 2006), eastern (23.33%) and central parts (22.73%) of Iran (Asgari et al, 2010), China (9.5%) (Deng et al., 2018), South Asia (27.9%) (Khan et al., 2017), Afri-

ca (12%) (Tonouhewa et al., 2017) and Brazil (71%) (Santos et al., 2009).

With regard to the age of infected cows with *N. caninum* in our research, which was obtained in small farms, we recorded a significant increase of the prevalence ($p<0.05$) after the age of 3 years. A positive relation between the age of the cows and the infection with *N. caninum* has also been reported in other studies, from the northwestern America (Sanderson et al, 2000, Dyer et al., 2000), Spain (Bartels et al., 2006) and Denmark (Jensen et al, 1999). On the other hand, studies from Canada and Sweden (Waldner et al., 1998, Bartels et al., 2006) have reported a negative age effect on the prevalence of seropositive animals in dairy cattle.

With respect to the age of infected cows with *T. gondii,* our results showed over the double percentage of prevalence (13.08% compared to 5.2%) in the group of cows aged more than 6 years compared to these aged less than 3 years. These findings are similar to those of Jittapalapong et al., (2005), Teshale et al., (2007), and Tilahun et al., (2019), who reported a low prevalence in young animals and a high one in adults. This could be easily justified by the longer exposure of adults to *T. gondii* infection (Dubey, 2019). The longer animals live the more likely they are to be exposed to *T. gondii* sources (Rouatbi et al., 2019; Klun et al. 2006). A fact that we also agree on, is that in small farms the most important source of *T. gondii* for animals is the consumption of oocysts from the environment and the frequent presence of rodents.

Some researchers report that beef, especially when it is raw or undercooked, is considered to be the most important source of human infections with *T. gondii* (Baril et al., 1999; Cook et al., 2000; Jones et al., 2009 ; Opsteegh et al., 2011). Congenital toxoplasmosis in humans may cause abortion, neonatal death, or fetal abnormalities mainly in the neuromuscular system and eyes (Hayde and Pollak, 2000).

Cows can be infected via the environmental cyst reservoir or by accidental consumption of a rodent or other intermediate host that carries tissue cysts. Appropriate rodent control is therefore one of the measures that helps in the prevention of the infection of farm animals and cats. (Jones and Dubey, 2012). Moreover calves may acquire the infection prenatally, transplacentally (Canada et al., 2002; Costa et al., 2011), or postnatally or from other feed, water or the environment (Dubey, 2003).

In small dairy farms there are some risk factors such as the lack of proper hygienic measures, the easier access of dogs and cats in the facilities, the environment and feeding materials of cows. All these reasons lead to a higher prevalence of neosporosis and toxoplasmosis than expected. One other important risk factor is that animals in Greece are more frequently grazing pastures rather than kept in feed-lots and farmed intensively. This point has also been mentioned in reviews by Tenter et al, (2000), Klun et al. (2006), and Dubey et al, (2019). Moreover, epidemiological studies have concluded that pastures may be the most common source of these/parasitic infections (Rouatbi,et al, 2019; Gebremedhin Ortega-Mora et al., 2007).

Veterinarians' duty is the continuous consulting and training of farmers on the modes of transmission of these parasitoses as well as on the methods and measures that can be utilized to prevent exposure of uninfected animals and transmission to the entire herd, highlighting the zoonotic potential of toxoplasmosis. Moreover, veterinarians should inform the farmers about the increased cost of losses caused by neosporosis and toxoplasmosis and the additional costs that include the veterinary services and everything that is associated with establishing diagnosis, rebreeding, possible loss of milk yield and possible replacement costs of cows with fertility issues due to these parasitoses.

Under the scope of one-health, animal well-being and profit maximization, *N. caninum* and *T. gondii* infections of dairy cows in small farms should not be neglected. The most important factor in their control is to prevent the contamination of feed, water, bedding and pastures with canine and feline feaces respectively. To this direction, dogs must be prohibited to consume aborted fetuses, afterbirths and dead calves, supplies (water, feeds and bedding) and the facilities must be periodically examined for specific pathogens, in combination with rodent control and veterinary monitoring of dogs and cats that live in close proximity with the herd. These are some key-point measures that should be adopted by farmers, in combination with their own appropriate behavior of good hygiene. In case of neosporosis, in every way seropositive calves have to be excluded from breeding.

CONFLICT OF INTEREST

None declared by the authors.

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