First Report of *Neospora caninum* Seroprevalence in Dairy Cattle in Jiangxi Province, Southeastern China

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Abstract: *Neospora caninum* is one of the major causes of abortion and neonatal mortality among livestock. Although, *N. caninum* infection in dairy cattle has been investigated in many countries around the world, little information was available in China. In the present study, the seroprevalence of *N. caninum* infection in dairy cattle were investigated in Jiangxi Province, Southeastern China between May 2012 and February 2013. A total of 640 serum samples collected from nine administrative cities/regions in Jiangxi Province were examined for antibodies against *N. caninum* using Enzyme-Linked Immunosorbent Assay (ELISA) and Indirect Fluorescent Antibody Test (IFAT). The overall *N. caninum* seroprevalence in dairy cattle was 15.63% (95% CI = 12.81-18.44%) (100/640) ranging from 11.76% (95% CI = 4.11-19.42%) to 18.33% (95% CI = 8.54-28.12%). The seroprevalence of *N. caninum* in aborting cattle (20.59%; 95% CI = 15.78-25.39%; 56/272) was significantly higher than that in non-aborting cattle (11.96%; 95% CI = 8.64-15.27%; 44/368) (p>0.05). The 5 years old dairy cattle had the highest seroprevalence (25.53%; 95% CI = 16.72-34.35%) followed by those that were 6 years old (19.17%; 95% CI = 12.12-26.21%) (p>0.05). The results of the present survey indicate that *N. caninum* infection is highly prevalent in dairy cattle in Jiangxi Province, China. The results of the present study have implications for the prevention and control of *N. caninum* infection in dairy cattle in Jiangxi Province, China and elsewhere.

Key words: Seroprevalence, dairy cattle, *Neospora caninum*, Jiangxi Province, China

INTRODUCTION

Neospora caninum is an obligate intracellular protozoan parasite that is distributed worldwide and infects a wide range of animal hosts (Almeria and Lopez-Gatius, 2013). Neosporosis is an emerged disease caused by *N. caninum* and is a major problem in livestock operations due to abortions and neonatal mortality (Dubey *et al.*, 2007). The important route of transmission of *N. caninum* is transplacental (or vertical) in cattle within many herds but it is not yet known whether other factors are also infectious (Goodswen *et al.*, 2013).

In cattle, *N. caninum* is well recognized as the major cause of abortions and stillbirths causing significant economic losses (Reichel *et al.*, 2013). *N. caninum* infection in dairy cattle has been recently investigated in many countries and regions of the world (Eiras *et al.*, 2011; Nematollahi *et al.*, 2011; Ibrahim *et al.*, 2012; Sengupta *et al.*, 2012; Asmare *et al.*, 2013; Bruhn *et al.*, 2013). *N. caninum* infection in dairy cattle was also reported in some provinces and regions in China (Wang *et al.*, 2010; Shi *et al.*, 2011; Xia *et al.*, 2011; Xu *et al.*, 2012). However, the majority of these literatures were published in local Chinese journals which are often not readily accessible to the international readers. Moreover, there has not yet been survey of *N. caninum* infection in dairy cattle in Jiangxi Province, China.

The objective of the present study was to determine the seroprevalence of *N. caninum* infection in dairy cattle in Jiangxi Province, China. The results should provide a solid foundation for the execution of control strategies against *N. caninum* infection in dairy cattle in this province and elsewhere.

MATERIALS AND METHODS

All of the blood samples were collected from 640 animals that were raised in various administrative cities/regions in Jiangxi Province, China between May 2012 and February 2013 (Table 1). All the sampled animals were adult females, aged between 2 and 7 years old (Table 2). All the animals were regularly dewormed and

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Table 1: Seroprevalence of *Neospora caninum* in dairy cattle in Jiangxi Province, Southeastern China

Locations	No. examined	No. positive	Prevalence (%)
Nanchang	58	9	15.52
Shangrao	83	13	15.66
Jiujiang	68	8	11.76
Pingxiang	64	11	17.19
Xinyu	60	11	18.33
Yingtan	98	15	15.31
Ganzhou	66	9	13.64
Yichun	56	10	17.86
Fuzhou	87	14	16.09
Total	640	100	15.63

Table 2: Seroprevalence of *Neospora caninum* infection in dairy cattle of different ages in Jiangxi Province, Southeastern China

Age (years)	No. examined	No. positive	Prevalence (%)
2	108	13	12.04
3	105	15	14.29
4	97	9	9.28
5	94	24	25.53
6	120	23	19.17
7	116	16	13.79
Total	640	100	15.63

none of the animals were vaccinated against *N. caninum*. All animals were randomly selected and one blood sample was collected from each animal. Blood samples were centrifuged and sera were harvested and stored at -20°C until assayed.

Antibodies (IgG) against *N. caninum* were detected using commercially available indirect Enzyme-Linked Immunosorbent Assay (ELISA) kits (*N. caninum* antibody ELISA kits, IDEXX Laboratories, Inc., Westbrook, Maine, USA) according to the manufacturer's instructions. Samples positive by ELISA were subsequently confirmed by an Indirect Fluorescent Antibody Test (IFAT). *N. caninum* tachyzoites were used as antigen and a polyclonal rabbit anti-cattle IgG labelled with fluorescein isothiocyanate (F5137; Sigma, St. Louis, USA) was used as conjugate. A test was considered positive if cut off of 1:50 or higher. Overall, samples were considered positive if both the ELISA and IFAT results were positive.

Frequency, prevalence and its 95% confidence interval of *N. caninum* infections were established. The statistic was performed using the PASW Statistics 18 (IBM Corporation, Somers, NY, USA) and the Win Episcope 2.0 (University of Zaragoza, Spain) Software. Differences were considered statistically significant when the value of $p < 0.05$.

RESULTS

Antibodies against *N. caninum* were detected in 100 (15.63%; 95% CI = 12.81-18.44%) of 640 dairy cattle (Table 1). The seroprevalence of *N. caninum* varied in various administrative cities/regions, ranging from 11.76% (95% CI = 4.11-19.42%) to 18.33% (95% CI = 8.54-28.12%)

(Table 1). However, there were no statistically significant differences in the seroprevalence among various administrative cities/regions ($p > 0.05$). The *N. caninum* seroprevalence in aborting dairy cattle (20.59%; 95% CI = 15.78-25.39%; 56/272) was significantly higher than that in non-aborting dairy cattle (11.96%; 95% CI = 8.64-15.27%; 44/368) ($p > 0.05$). The *N. caninum* seroprevalence varied in different age groups, ranging from 9.28% (95% CI = 3.51-15.05%) to 25.53% (95% CI = 16.72-34.35%) (Table 2) ($p > 0.05$) (Table 2).

DISCUSSION

The overall seroprevalence of *N. caninum* was higher than that reported in other countries (Nematollahi *et al.*, 2011; Sengupta *et al.*, 2012; Asmare *et al.*, 2013) and Xinjiang (Wang *et al.*, 2009), China, similar to that reported in Guangdong (Xu *et al.*, 2012) but lower than those of reported in Brazil (Bruhn *et al.*, 2013), Pakistan (Nazir *et al.*, 2013), Australia (Nasir *et al.*, 2012) and Romania (Mitrea *et al.*, 2012). This is likely due to difference in animal welfares, climates, category and animal husbandry practices. Another important reason for differences in seroprevalence may be due to different investigative methods.

The *N. caninum* seroprevalence in aborting dairy cattle was significantly higher than that in non-aborting dairy cattle ($p > 0.05$), suggesting that *N. caninum* infection may significantly increase the risk of abortion in the dairy cattle. These findings indicate that neosporosis pose a potential threat to pregnant dairy cattle. The *N. caninum* seroprevalence varied in different age groups, indicating that *N. caninum* is prevalent all age groups which is consistent with that of a recent study (Xia *et al.*, 2011). The present study showed that seroprevalence of *N. caninum* peaks in 5 and 6 years old. However, previous study has indicated that seroprevalence increased with ages (Jensen *et al.*, 1999).

The previous (Wang *et al.*, 2010; Shi *et al.*, 2011; Xia *et al.*, 2011; Xu *et al.*, 2012) and present studies provide strong evidence that *N. caninum* infection is prevalent in dairy cattle in China and can cause major economic impact on these animals. This finding may be explained by the presence of dogs on farms. Researchers observed that dogs were present on these farms and had free access to the storehouses during the present investigation. This may represent a major risk factor for *N. caninum* infection in dairy cattle in these farms because dogs play significant roles in the transmission of this parasite (Dubey and Schares, 2011) although, researchers have not investigated the *N. caninum* seroprevalence in dogs in this province. Therefore, further

studies are necessary to further investigate the seroprevalence of *N. caninum* in dogs in this province.

CONCLUSION

The results of the present survey indicate that *N. caninum* infection is highly prevalent in dairy cattle and integrated control strategies should be implemented to prevent and control *N. caninum* infection in dairy cattle. This is the first report of *N. caninum* seroprevalence in dairy cattle in Jiangxi Province, China.

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