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Seroprevalence of *Chlamydia* infection in dairy cattle in subtropical southern China

Qing-You Liu[1,2], Min-Jun Xu[1]*, Jing-Hua Fu[1,3], Xian-Hui He[1,4], De-Shun Shi[3], Kui-Qing Cui[3], Shang-Song Guan[1] and Ya-Nan Wang[4]

[1]State Key Laboratory of Veterinary Etiological Biology, Lanzhou Veterinary Research Institute, CAAS, Lanzhou, Gansu Province 730046, People's Republic of China.
[2]Animal Reproduction Institute, Guangxi Key Laboratory of Subtropical Bioresource Conservation and Utilization, Guangxi University, Nanning, Guangxi Zhuang Autonomous Region 530005, People's Republic of China.
[3]College of Animal Sciences, South China Agricultural University, Guangzhou, Guangdong Province 510642, People's Republic of China.
[4]College of Veterinary Medicine, South China Agricultural University, Guangzhou, Guangdong Province 510642, People's Republic of China.

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A total of 875 serum samples from dairy cattle in six districts in Zhuang Autonomous Region (GZAR) were collected between June 2009 to March 2010, and *Chlamydia* antibodies were assayed by indirect hemagglutination test (IHA). The overall seroprevalence of *Chlamydia* in dairy cattle was 17.83% (156/875) in GZAR. The seroprevalence of *Chlamydia* in aborting dairy cattle (18.18%) was higher than that in non-aborting dairy cattle (16.69%) (not statistically significant). Dairy cattle of seven year old had the highest seroprevalence (35.42%), and dairy cattle with seven calves had the highest positive level (100%, 1/1). Statistically significant differences were observed between *Chlamydia* prevalence and ages ($P<0.05$), especially in dairy cattle of 5 to 7 year old ($P<0.01$). Moreover, statistically marked differences were also found between *Chlamydia* prevalence and numbers of pregnancies ($P<0.01$). These results indicated that infection of *Chlamydia* in dairy cattle is widespread in GZAR and is closely related to abortion of dairy cattle, therefore integrated strategies should be carried out to prevent the outbreak of *Chlamydia* infection.

Key words: *Chlamydia*, seroprevalence, dairy cattle, Southern China, indirect hemagglutination assay (IHA).

INTRODUCTION

The obligate intra-cellular bacteria *Chlamydia* is implicated in a wide variety of clinically and economically important diseases of livestock animals and humans. Chlamydial seroprevalence ranged from 45 to 100% at a herd level worldwide in the last two decade s (Reinhold et al., 2011). Seroprevalence investigations showed 45% prevalence in Austria, 47.1% in Switzerland, and 100% in Germany (Biesenkamp-Uhe et al., 2007; Teankum et al., 2007; Petit et al., 2008). In China, prevalence of *Chlamydia* in cattle was 32% in Ningxia, 19.23% in Qinghai, and 28.3% in Xi'an (Ma et al., 2004; Zhou and Qiu, 2007).

Dairy cattle are one of the most important economic animals raised in subtropical Guangxi Zhuang Autono-

*Corresponding author. E-mail: mjxu@yahoo.cn. Tel: +86-931-8342837 Fax: +86-931-8340977.

mous Region (GZAR), China, producing nearly 50 thousand tons of milk a year. However, it is yet to know whether *Chlamydia* infection is present in dairy cattle in GZAR. Giving the impact of *Chlamydia* infection on the performance of dairy cattle, therefore, the objective of the present survey was to investigate the prevalence of *Chlamydia* antibodies in dairy cattle in GZAR using the indirect hemagglutination (IHA) technique.

MATERIALS AND METHODS

Serum preparation

The present study was conducted in GZAR, subtropical southern China (104º 26'-112º 04' E, 20º 54'-26º 24' N). From June 2009 to March 2010, a total of 875 blood samples were collected from the tail vein of dairy cattle from randomly selected dairy herds in four administrative cities in six districts, including four intensive farms and nine smallholder dairy farms where abortions had occurred. After the collection, the blood samples were put at 4°C for 5 h for solidification, followed by centrifugation at 4, 000×*g* for 10 min, and supernatants were transferred to new centrifuge tubes and kept at -20°C until use. Information about dairy cattle, including age, abortion, and number of pregnancies was obtained from the owners.

Detection procedure

The IHA technique was used to assay *Chlamydia* antibodies in dairy cattle in GZAR using a commercially marketed kit purchased from Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Sciences (Batch No. 100823). Briefly, 75 µl of IHA dilution solution was used to mix with 25 µl of each serum sample. Then, the mixed sample was 4-fold gradually diluted for 2 times with another 75 µl IHA dilutions to form 1:4, 1:16 and 1:64 grades. 25 µl *Chlamydia* antigen were added into each grade, and then the mixtures were incubated at 37°C for 2 h followed by checking the results. Under the condition that positive, negative and blank controls were all successful, sample with layer of agglutinated erythrocytes formed in wells of 1:16 or 1:64 was considered as positive. The positive and negative controls were all provided by the kits.

Statistical analysis

Statistical analysis of *Chlamydia* prevalence in dairy cattle of different ages, with or without abortion, and pregnancies were performed by chi-squared test. The linear regression was applied to study the association between *Chlamydia* and abortion, age or number of pregnancies. All analyses were performed by using the software of SPSS (Statistical Analysis System, Version 11.5, Chicago, Illinois). The differences were considered statistically significant if $P < 0.05$.

RESULTS

Of the 875 dairy cattle examined, antibodies to *Chlamydia* were found in 156 (17.83%), with percentages ranging from 11.36 to 20% (Table 1).

Of the 875 investigated dairy cattle, 688 had pregnancy history records and were used for association analysis between abortion and *Chlamydia* infection. The seroprevalence of *Chlamydia* in dairy cattle with abortion (18.18%) was slightly higher than that in non-abortion dairy cattle (16.69%), but the difference was not statistically significant ($P = 0.83$, $R^2 = 0.63$). Among the 4 dairy cattle with abortion in Guilin, two dairy cattle aborted three and two times, respectively, but both of them were *Chlamydia* negative.

Of the 875 cattle examined, 341 had age records and were used for the analysis of the association between *Chlamydia* seroprevalence and ages (Table 2). The highest level was found in dairy cattle of 7 years old with prevalence of 35.42%, followed by dairy cattle of 5 years old (20.41%). The *Chlamydia* seroprevalence in different ages ranged from 0 to 35.42%, but the differences were not statistically significant among all of the age groups ($P = 0.08$, R2 = 0.41). No prevalence of *Chlamydia* was found in dairy cattle of one year old. However, for dairy cattle < 8 years old, the *Chlamydia* seroprevalence was statistically significant among different age groups ($P = 0.04$, $R^2 = 0.86$), especially in dairy cattle of 5 to 7 years ($P = 0.002$, $R^2 = 0.54$).

Of the 875 cattle examined, 378 had pregnancy record and were used for the analysis of association between *Chlamydia* seroprevalence and numbers of pregnancies (Table 3). The highest prevalence was found in dairy cattle with 7 calves (100%, 1/1), followed by dairy cattle having 5 (38.46%) and 6 (37.50%) pregnancies. The *Chlamydia* seroprevalence in dairy cattle with different pregnancies ranged from 7.78 to 100%, with statistically significant differences ($P = 0.002$, $R^2 = 0.26$).

DISCUSSION

Chlamydia psittaci was also classified as *Chlamydophila psittaci*. The 'mammalian' *C. psittaci* of abortion, feline and Guinea pig strains have been moved to three new species, including *C. abortus*, *C. felis* and *C. caviae* (http://en.wikipedia.org/wiki/Chlamydophila_psittaci).

Some *Chlamydia* species cause pneumonia (*C. pneumoniae*), abortion and subfertility (*C. abortus*) in humans, resulting in spontaneous abortion in pregnant women (Borel et al., 2006; Bebear and de Barbeyrac, 2009; Reinhold et al., 2011). Disease syndromes caused by Chlamydia infection in cattle including abortion, mastitis, infertility, pneumonia, hepatitis, conjunctivitis, encephalomyelitis, as well as subclinical infections have been reported globally (Wehrend et al., 2005; Borel et al., 2006; Biesenkamp-Uhe et al., 2007). In dairy farms with such problems, milk production was significantly decreased, and abortion, pre-mature calving and peri-natal calf death were often observed, especially in smallholder farms (Kemmerling et al., 2009; Banda et al., 2011). For calves, *Chlamydia* infection is also related to significantly high body temperature, lower body weight

Table 1. Seroprevalence of *Chlamydia* infection in dairy cattle in different cities in Guangxi Zhuang Autonomous Region (GZAR), subtropical southern China.

City	District of the city	Examined number	Positive number	Prevalence (%)
Baise	Youjiang	44	5	11.36
Guilin	Qixing	100	18	18.00
Nanning	Xixiangtang	281	47	16.73
	Yongning	280	53	18.93
Liuzhou	Liunan	50	9	18.00
	Liubei	120	24	20.00
Total	6	875	156	17.83

Table 2. Seroprevalence of *Chlamydia* infection in dairy cattle of different ages in Guangxi Zhuang Autonomous Region (GZAR), subtropical southern China.

Age (year)	Examined number	Positive number	Prevalence (%)
1	9	0	0.00
2	17	2	11.76
3	26	5	19.23
4	31	5	16.13
5	41	7	17.07
6	43	4	9.30
7	48	17	35.42
8	49	10	20.41
>8	77	12	15.58
No record	534	94	17.60

and lower hematocrit (Reinhold et al., 2008).

The seroprevalence of *Chlamydia* in dairy cattle with abortion (18.18%) was slightly higher than that in non-abortion dairy cattle (16.69%) in subtropical southern China's GZAR, and dairy cattle with high frequency of abortion (2 and 3 times for each) were *Chlamydia* negative. There are a lot of pathogens that might result in cattle abortion, such as virus (Infectious Bovine Rhinotracheitis, Bovine Viral Diarrhea-Mucosal Disease, Akabane Disease, Aino Disease, Chuzan Disease, Ibaraki Disease, Rfit Valley Disease, Blue Tongue), bacteria (*Brucella abortus*, *Campylobacter fetus*, *Salmonella enterica serovar Dublin*, *Leptospira interrogans serovar Pomona*, *Listeria monocytognes*, and *L. ivanovii*), rickettsia (*C. psittaci*), fungus (*Aspergillus fumigatus*, and *Absidia ramosa*) and protozoa (*Tritrichomonas foetus*, and *Neospora caninum*) (Xu et al., 2012): that might be the reason why dairy cattle with high frequency of abortion were *Chlamydia* negative.

The *Chlamydia* seroprevalence in GZAR was much lower than in other countries (Biesenkamp-Uhe et al., 2007; Teankum et al., 2007; Petit et al., 2008) and other provinces of China including Ningxia, Qinghai, and Xi'an (Ma et al., 2004; Zhou and Qiu, 2007). Besides, although the average seroprevalence (17.83%) of *Chlamydia* in dairy cattle in GZAR was lower than the average prevalence in other countries and other provinces of China, some dairy cattle had high *Chlamydia* seroprevalence, such as dairy cattle with five and six pregnancies and dairy cattle of seven years old. In *Chlamydia*-positive dairy herds, carrier dairy cattle are the most likely source for the infection of calves and working with small ruminants in dairy herds or contact with infected animals directly is among the routes of human chlamydial infections, especially for smallholder dairy farmers to whom milk production in many tropical areas still rely on hand-milking (Millogo et al., 2012; VanLeeuwen et al., 2012). Therefore, the integrated strategies and measures should be executed to prevent the outbreak of *Chlamydia* infection.

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Table 3. Seroprevalence of *Chlamydia* infection in dairy cattle with different numbers of pregnancies in Guangxi Zhuang Autonomous Region (GZAR), subtropical southern China.

Number of pregnancy	Examined number	Positive number	Prevalence (%)
0	45	5	11.11
1	73	10	13.70
2	90	7	7.78
3	75	13	17.33
4	57	12	21.05
5	13	5	38.46
6	24	9	37.50
7	1	1	100.00
No record	497	94	18.91

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