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Full Length Research Paper

The initial report of Mycoplasma bovis infection in dairy Cattles in Guangzhou, subtropical southern China

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Mycoplasma bovis **is a major bacterial pathogen causing mastitis in dairy cattle, pneumonia and arthritis, and reduced weight gain in calves and reproductive problems in both dairy cattle and bulls, resulting in significant economic losses. The objective of the present investigation was to examine the *M. bovis* seroprevalence in dairy cattle in Guangzhou, subtropical Southern China by using an enzyme-linked immunosorbent assay (ELISA). A total of 370 serum samples of dairy cattle were collected between July, 2009 and March, 2010 from 5 different farms and examined for *M. bovis* antibodies using a commercially available ELISA kit. The overall seroprevalence of *M. bovis* infection in dairy cattle was 5.95% (22/370). One year-old dairy cattle had the highest seroprevalence (10.5%), followed by dairy cattle of 3 year-old (9.61%). Dairy cattle without pregnancy had the highest seroprevalence (9.26%), followed by dairy cattle with 2 pregnancies (8.62%). However, no statistically significant association was found between *M. bovis* infection and ages or numbers of pregnancies (P>0.05). These results indicate that *M. bovis* infection was present in dairy cattle in Guangzhou, subtropical Southern China, and integrated strategies and measures should be executed to control and prevent *M. bovis* infection and disease outbreak in the study region.**

Key words: *Mycoplasma bovis*, seroprevalence, dairy cattle, China, enzyme-linked immunosorbent assay (ELISA).

INTRODUCTION

Mycoplasma bovis (formerly *Mycoplasma agalactiae* subsp. bovis) is a significant but sometimes neglected bacterial pathogen of adult dairy cattle, intensively reared beef and dairy calves (Maunsell et al., 2009, 2011; Rérat et al., 2012). *M. bovis* was first isolated in 1961 from a cow with severe mastitis in USA and described as a cause of respiratory disease in 1976 (Nicholas and Ayling, 2003; Caswell and Archambault, 2008). This pathogen can cause mastitis in cows, pneumonia, arthritis and reduced weight gain in calves and reproductive problems in both cows and bulls (Tenk et al., 2004; Tenk et al., 2006; Caswell and Archambault, 2008; Maunsell et al., 2011; Punyapornwithaya et al., 2010, 2011). *M. bovis* has also been associated with abortion and infertility, tenosynovitis, endometritis, otitis media, decubital abscesses, kerato conjunctivitis, oophoritis, salpingitis and seminal

Table 1. Seroprevalence of *M. bovis* infection in dairy cattle by ELISA in Guangzhou, subtropical southern China.

Farm code	Number examined	Number positive	Prevalence (%)
A	74	2	2.70
B	73	5	6.85
C	60	9	15.0
D	80	3	3.75
E	83	3	3.61
Total	370	22	5.95

vesiculitis (Nicholas and Ayling, 2003; Caswell and Archambault, 2008; Maunsell et al., 2011; Soehnlen et al., 2011). Clinical diseases caused by *M. bovis* in dairy cattle is often chronic, debilitating and no response to antibiotic treatment, whereas acute infection tends to become chronic leading to poor performance, culling and death loss (Maunsell et al., 2009, 2011), causing significant economic losses to both the dairy and meat industries (Nicholas and Ayling, 2003; Kauf et al., 2007; Maunsell et al., 2011).

M. bovis infection can be definitively diagnosed by a variety of methods, including isolation of the causative agent from individual animals or herds, serological tests and specific polymerase chain reaction (PCR) methods. In recent years, enzyme-linked immunosorbent assays (ELISA) and PCR-based methods have gradually replaced culture as the method of choice for detecting *M. bovis*. In particular, ELISA has been demonstrated as more reliable method for the herd diagnosis of *M. bovis* infection, as antibody levels detected by ELISA remain high for many months (Nicholas and Ayling, 2003; Tenk et al., 2004; Tenk et al., 2006; Ball and Nicholas, 2011; Maunsell et al., 2011).

Dairy cattle are one of the most important economic animals raised and farmed for milk production in Guangzhou, subtropical southern China. However, little is known about the infection of *M. bovis* in dairy cattle in Guangzhou. The objective of the present investigation was to determine the seroprevalence of *M. bovis* infection in dairy cattle in Guangzhou using ELISA, which may provide base-line data for the implementation of effective strategies and measures for the control and prevention of *M. bovis* infection in dairy cattle in this region.

MATERIALS AND METHODS

The investigated city

The survey was conducted in Guangzhou city which is the capital of Guangdong Province, China and has a subtropical monsoon climate. Crossed by the Tropic of Cancer, it is located between longitude 112° 57' to 114° 3' east and latitude 22° 26' to 23° 56' north, bordering on the South China Sea. It is the biggest cosmopolitan city in South China, and also China's Southern Gateway to the world. The annual average temperature is 22.8°C, the average relative h umidity is about 68%, and the annual rainfall at the urban area is over 1600 mm. The city covers a total area of 7434.4 km^2 and has a population of approximately 12 millions.

Collection of serum samples

Blood samples were collected from 370 dairy cattle on 5 farms between July, 2009 and March, 2010 in Guangzhou city, Guangdong Province, China. The dairy cattle populations represented a local breed (Chinese Holstein) and introduced breed (American/Australian Holstein-Friesian and British Jersey). The animals of each herd were randomly selected, and 1 blood sample was collected from each animal. All the blood samples were immediately transported to the laboratory at College of Veterinary Medicine, South China Agricultural University, Guangzhou, China. Blood samples were centrifuged at 3,000 rpm for 10 min, and serum was obtained, frozen, and stored at -20°C until teste d for assessing antibodies to *M. bovis*. Biometric data for dairy cattle, including age, breed and numbers of past pregnancies were obtained through a questionnaire at the time of blood collection.

Serological examination

Antibodies to *M. bovis* were examined by ELISA using a commercially available kit (Bovine *Mycoplasma* antibody ELISA kit, Huijia BioTech, Ltd, Xiamen, China) according to the manufacturer's recommendations and protocols reported previously (Fu et al., 2011). Positive and negative sera were provided in the kit. Briefly, the *M. bovis* specific antigen was coated on a 96-well ELISA plate. After incubation of the diluted serum sample in the test well and subsequent washing, a conjugate was added. The plate was washed again, and then a chromogenic enzyme substrate was added. The optical density (OD) at 450 nm was read using a photometer (BIO-RAD, Hercules, California, USA).

Statistical analysis

Differences in seroprevalence of *M. bovis* infection among dairy cattle of different age groups and numbers of pregnancies were analyzed using a Chi square test in SPSS for Windows (Release 18.0 standard version, SPSS Inc., Chicago, Illinois). The differences were considered statistically significant when $P<0.05$.

RESULTS

A total of 370 serum samples from dairy cattle in Guangzhou, Southern China were examined by ELISA for *M. bovis* antibodies. Twenty-two (22) of 370 (5.95%) examined dairy cattle were seropositive for *M. bovis* infection by ELISA (Table 1). Different levels of seropositivity were

Table 2. Seroprevalence of *M. bovis* in dairy cattle of different ages in Guangzhou, subtropical southern China.

Age (year)	Number examined	Number positive	Prevalence (%)
1≤ yr<2	38	4	10.5
2≤ yr<3	50	4	8.0
3≤ yr<4	52	5	9.61
4≤ yr<5	25	2	8.0
5≤ yr<6	76	2	2.63
6≤ yr<7	48	1	2.08
7≤ yr<8	55	3	5.45
≥ 8	26	1	3.85
Total	370	22	5.95

Table 3. Seroprevalence of *M. bovis* in dairy cattle with different numbers of pregnancies in Guangzhou, subtropical southern China.

Number of pregnancy	Number examined	Number positive	Prevalence (%)
0	54	5	9.26
1	70	6	8.57
2	58	5	8.62
3	40	2	5.0
4	59	1	1.69
5	33	1	3.03
6	42	2	4.76
≥ 7	14	0	0
Total	370	22	5.95

detected among the 5 different farms, namely 2.70, 6.85, 15.0, 3.75 and 3.61% of the examined samples from farms A, B, C, D and E were *M. bovis* antibody-positive, respectively (Table 1).

The ages of the examined dairy cattle ranged between 1 and 8 years, seroprevalence varied in different age groups, ranging from 2.08 to 10.5% (Table 2). One-year-old dairy cattle had the highest seroprevalence of 10.5% (4/38), followed by 3-year-old dairy cattle (9.61%, 5/52) (Table 2).

The numbers of parturition of dairy cattle ranged between 0 and 7 pregnancies, seroprevalence varied in dairy cattle with different numbers of pregnancies, ranging from 0 to 9.26% (Table 3). The highest seroprevalence was found in dairy cattle with 0 pregnancy (9.26%), followed by dairy cattle having 2 pregnancies (8.62%), although there were no statistically significant differences among different groups (P>0.05). No *M. bovis* antibodies were detected in dairy cattle with 7 pregnancies.

DISCUSSION

M. bovis is a highly contagious pathogen which can infect all ages of cattle and cause serious diseases, and it may be spread from dairy cattle to cattle by the hands of milkers and fomites, such as the milk claw, in the milking parlor (Kauf et al., 2007). *M. bovis* has been highly adapted to cattle but occasionally it was isolated from buffalos, small ruminants, chickens and even humans (Nicholas and Ayling, 2003; Ongor et al., 2008). Because no effective antibiotics and vaccine are commercially available for the treatment or prevention of diseases caused by *M. bovis*, the present measures for controlling *M. bovis* are mainly to limit infection and the culling of infected animals (Pinnow et al., 2001; Kauf et al., 2007; Maunsell et al., 2011).

In the present survey, *M. bovis* antibodies were detected in 22 (5.95%) of 370 dairy cattle by ELISA (Table 1). The overall seroprevalence was lower than that reported in Chongqing, Hubei and Guangxi Zhuang Autonomous Region in China (Shi et al., 2008; Ran et al., 2010; Fu et al., 2011), and some other countries or regions such as Canton du Jura, Ireland and Pennsylvania (Burnens et al., 1999; Byrne et al., 2001; Soehnlen et al., 2012), but higher than that documented in France (Arcangioli et al., 2011). These differences may due to different diagnostic methods used, cattle of different sources and surveyed, and samples from different regions.

As shown in Table 2, the seroprevalence varied in different age groups (2.08 to 10.5%), with dairy cattle of 1 year old having the highest seroprevalence of 10.5%, followed by dairy cattle of 3 years old (9.61%). However, there were no statistically significant differences between different age groups (P>0.05). The varied seroprevalence in different age groups suggests the possibility of horizontal transmission in the investigated herds. The highest *M. bovis* seroprevalence in dairy cattle of 1 year old may be due to ingestion of infected milk which is an important means of *M. bovis* transmission. Higher *M. bovis* seroprevalence in dairy cattle of 3 years old may increase the risk of milk contamination with *M. bovis*, because 3 to 4 years old dairy cattle are the main producer of milk.

The association between *M. bovis* seroprevalence and numbers of pregnancies was also analyzed in the present study (Table 3), and varied *M. bovis* seroprevalence was detected. The seroprevalence in dairy cattle without births was the highest (9.26%), followed by dairy cattle with 2 pregnancies, but the differences were not statistically significant (P>0.05).

Conclusion

The results of the present survey indicated that *M. bovis* infection is prevalent in dairy cattle in Guangzhou, subtropical southern China, which may represents one of the causes of bovine abortion, pneumonia and arthritis. Therefore, integrated strategies and measures should be performed to control and prevent *M. bovis* infection and disease outbreak in dairy cattle in the study region.

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