African Journal of Biotechnology Vol. 10(54), pp. 11313-11316, 19 September, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.1237
ISSN 1684–5315 © 2011 Academic Journals

*Full Length Research Paper*

# Seroprevalence of *Mycoplasma bovis* infection in dairy cows in subtropical southern China

**Jing-Hua Fu[1,2,3], Qing-You Liu[2], Min-Jun Xu[1], De-Shun Shi[2], Xian-Hui He[4], Yan Pan[2,5], Rui-Bin Guo[4], Qi Gao[4], Sheng-Xian Yi[4], Hua-Sheng Si[4] and Xing-quan Zhu[1,6]***

[1]State Key Laboratory of Veterinary Etiological Biology, Lanzhou Veterinary Research Institute, CAAS, Lanzhou 730046, People's Republic of China.
[2]Animal Reproduction Institute, Guangxi Key Laboratory of Subtropical Bioresource Conservation and Utilization, Guangxi University, Nanning 530005, People's Republic of China.
[3]College of Animal Science, South China Agricultural University, Guangzhou 510642, People's Republic of China.
[4]College of Veterinary Medicine, South China Agricultural University, Guangzhou 510642, People's Republic of China.
[5]Guangxi Veterinary Research Institute, Nanning 530001, People's Republic of China.
[6]College of Animal Science and Technology, Yunnan Agricultural University, Kunming 650201, People's Republic of China.

Accepted 7 July, 2011

**The seroprevalence of *Mycoplasma bovis* infection in dairy cows in Guangxi Zhuang Autonomous Region (GZAR) in subtropical southern China was surveyed between June 2009 and March 2010. A total of 455 serum samples of dairy cows were collected from 6 districts in 4 different cities, and examined for *M. bovis* antibodies with the indirect enzyme-linked immunosorbent assay (ELISA) using a commercially available kit. The overall seroprevalence of *M. bovis* infection in dairy cows was 7.69% (35/455). Three year-old dairy cows had the highest seroprevalence (15.0%), followed by dairy cows of 4 year-old (11.1%). Dairy cows with the history of 5 pregnancies had the highest seroprevalence (33.3%). However, no statistically significant association was found between *M. bovis* infection and age or number of pregnancies ($p > 0.05$). All the aborting dairy cows were negative for *M. bovis* antibodies, suggesting that bovine abortion may have no association with *M. bovis* infection in GZAR. These results indicate that *M. bovis* infection in dairy cows was widespread in GZAR, and integrated strategies and measures should be performed to control and prevent *M. bovis* infection and disease outbreak.**

**Key words:** *Mycoplasma bovis*, seroprevalence, dairy cows, Guangxi Zhuang Autonomous Region (GZAR), China, enzyme-linked immunosorbent assay (ELISA).

## INTRODUCTION

*Mycoplasma bovis* is one of major bovine pathogens and is associated with a number of diseases including respiratory diseases and severe otitis, and most commonly implicated in mastitis and pneumonia (Nicholas and Ayling, 2003; Foster et al., 2009; Ball and Nicholas, 2010). *M. bovis* infection is chronic and nonresponsive to antimicrobial treatment, and it usually co-exists with other bacteria. Therefore, disease outbreaks usually have high morbidity rates, which cause great economic loss and can be economically devastating to the affected farms, especially in developing countries (Butler et al., 2000; Stipkovits et al., 2000; Gagea et al., 2006; Caswell and Archambault, 2009; Maunsell et al., 2009; Wiggins et al., 2011). However, despite the widespread socioeconomic impact of the pathogen, knowledge on the seroprevalence of *M. bovis* in China is scarce and there has been only one epidemiological investigation of *M. bovis* in dairy cows infection in Chongqing, China conducted so far, which revealed a seroprevalence of 20 to 80% (Ran et al., 2010).

Dairy cow is one of the most important farm animals for milk production in Guangxi Zhuang Autonomous Region (GZAR), in subtropical southern China. The objective of

*Corresponding author. E-mail: xingquanzhu1@hotmail.com . Tel: +86 931 8342837. Fax: +86 931 8340977.

**Table 1.** Seroprevalence of *M. bovis* infection in dairy cows in different cities, in Guangxi Zhuang Autonomous Region (GZAR), China.

| City | Region of city | Examined number | Positive number | Prevalence (%) |
|---|---|---|---|---|
| Baise | Youjiang | 44 | 4 | 9.09 |
| Guilin | Qixing | 91 | 8 | 8.79 |
| Nanning | Xixiangtang | 91 | 3 | 3.30 |
| | Yongning | 91 | 9 | 9.89 |
| Liuzhou | Liunan | 47 | 7 | 14.89 |
| | Liubei | 91 | 4 | 4.40 |
| Total | 6 | 455 | 35 | 7.69 |

**Table 2.** Seroprevalence of *M. bovis* infection in dairy cows of different ages in Guangxi Zhuang Autonomous Region (GZAR), China.

| Age (year) | Examined number | Positive number | Prevalence (%) |
|---|---|---|---|
| 1 | 8 | 0 | 0 |
| 2 | 25 | 1 | 4 |
| 3 | 20 | 3 | 15 |
| 4 | 36 | 4 | 11.11 |
| 5 | 38 | 2 | 5.26 |
| 6 | 28 | 1 | 3.57 |
| 7 | 20 | 0 | 0 |
| 8 | 18 | 1 | 5.56 |
| >8 | 18 | 2 | 11.11 |
| No record | 244 | 21 | 8.61 |

this study was to investigate the seroprevalence of *M. bovis* infection in GZAR. The results would provide baseline data for the implementation of effective strategies for the control of *M. bovis* infection in dairy cows in China.

## MATERIALS AND METHODS

The study was conducted between June 2009 and March 2010 in 6 main cow-rearing districts of 4 administrative cities in GZAR. These 4 cities are the main cow breeding and milk producing areas in GZAR. Cows were randomly selected and blood samples were collected from tail vein of 455 dairy cows (Table 1). The serum was removed by centrifugation at 4,000 ×*g* for 10 min and stored at -20℃ until testing.

Serum samples were tested by the indirect enzyme-linked immunosorbent assay (ELISA) using a commercially available kit (Bovine *Mycoplasma* antibody ELISA kit, Jiahui BioTech, Ltd, Xiamen, China). Positive and negative controls were provided in the kit. Optical density of the samples was read with a photometer (BIO-RAD, Hercules, California, USA). The testing was performed according to the manufacturer's instructions. Basic biometric data on dairy cows including age (records from 211 cows), number of pregnancies (from 207 cows) and history of abortions (from 268 cows) were obtained from the owners by personal interview.

### Statistical analysis

Statistical analysis of *M. bovis* prevalence in dairy cows of different age (in years), with abortion or without abortion, and different numbers of pregnancies were performed by chi-square test using the SPSS software (Statistical Analysis System, Version 11.5, Chicago, Illinois). The differences were considered statistically significant when $p < 0.05$.

## RESULTS

Antibodies to *M. bovis* were found in 35 out of 455 dairy cows yielding the overall seroprevalence of 7.69%. *M. bovis* seroprevalence in dairy cows from different geographical locations ranged from 3.3 to 14.89% (Table 1).

The highest seroprevalence of 15% (3/20) was found in 3-year-old dairy cows followed by 11.11% in 4-year-old cows (4/36). No seropositive samples were found in 1 year-old animals and 7 year-old cows (Table 2). No relationship between the seroprevalence and the age could be revealed.

The highest prevalence was found in dairy cows with 5 pregnancies (33.33%), followed by dairy cows having 1 and 3 pregnancies (6.12%). The *M. bovis* seroprevalence in dairy cows with different numbers of pregnancies ranged from 0 to 33.33%, but there were not statistically significant differences among different groups ($p > 0.05$). No *M. bovis* antibodies were found in dairy cows with 0, 6 and 7 pregnancies (Table 3).

**Table 3.** Seroprevalence of *M. bovis* infection in dairy cows with different numbers of pregnancies in Guangxi Zhuang Autonomous Region (GZAR), China.

| Number of pregnancy | Examined number | Positive number | Prevalence (%) |
|---|---|---|---|
| 0 | 11 | 0 | 0 |
| 1 | 49 | 3 | 6.12 |
| 2 | 51 | 3 | 5.88 |
| 3 | 49 | 3 | 6.12 |
| 4 | 30 | 1 | 3.33 |
| 5 | 6 | 2 | 33.33 |
| 6 | 10 | 0 | 0 |
| 7 | 1 | 0 | 0 |
| No record | 248 | 23 | 9.27 |

The seroprevalence of *M. bovis* in non-abortion dairy cows was 8.33% (22/264). There were 4 cows with abortion, including 2 dairy cows that aborted 3 and 2 times, respectively, but they were all seronegative for *M. bovis* antibodies.

## DISCUSSION

*M. bovis* is a major cause of calf pneumonia, mastitis and arthritis, and a comprehensive understanding of *M. bovis* seroprevalence in dairy cows is essential for the control and prevention of *M. bovis* infection. *M. bovis* infection have been reported globally, with prevalence of 13 to 23% in Ireland, 25 to 50% in UK, 70% in USA, and 60 to 100% in France (Byrne et al., 2001; Nicholas et al., 2003; Arcangioli et al., 2008). It is estimated that up to 1.9 million cows become infected annually in UK, with 157,000 calves lost due to pneumonia and related diseases (Nicholas and Ayling, 2003). However, there was only one survey of *M. bovis* seroprevalence in Chongqing, China.

This study indicates that the overall *M. bovis* seroprevalence in GZAR was lower than that in other countries (Byrne et al., 2001; Nicholas and Ayling, 2003; Arcangioli et al., 2008) and in Chongqing, China (Ran et al., 2010), but close to the lowest level in Ireland (Byrne et al., 2001). However, it was also found that there were particular groups which had high *M. bovis* seroprevalence, such as those of 3 and 4-year-old as well as those with a history of 1, 3 and 5 pregnancies. Cows of 3 to 4 years old were the main producers of milk. Thus, high *M. bovis* seroprevalence in these ages will increase the risk of milk contamination with *M. bovis*. In addition, cows of 3 or 4 years old and those with a history of a few pregnancies were the main part of a herd, therefore, carrier dairy cows are the most likely source for the infection for calves in *M. bovis*-positive dairy herds.

The seroprevalence of *M. bovis* in GZAR in dairy cows without abortion was 8.33%, and no *M. bovis* antibodies were found in aborting dairy cows, including those with high frequency of abortions (2 and 3 times for each). This suggests that no association between bovine abortion and *M. bovis* infection exists in GZAR and abortions might be caused by factors other than *M. bovis* infection.

Results of this study indicate that infection of dairy cows with *M. bovis* was widespread in GZAR. It was reported that healthy dairy cows can be infected with *M. bovis* by clinically healthy calves or young cows shedding the *Mycoplasma*, and the pathogen is then very difficult to be eradicated once introduced into a herd, and there are no commercial vaccines for *M. bovis* at present (Nicholas and Ayling, 2003; Nicholas et al., 2009). Therefore, integrated strategies and measures should be executed to prevent and control *M. bovis* infection.

## ACKNOWLEDGEMENTS

Project support was provided in part by the State Key Laboratory of Veterinary Etiological Biology, Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Sciences (Grant Nos. SKLVEB2009KFKT014 and SKLVEB2010KFKT010) and Yunnan Provincial Program for Introducing High-level Scientists (Grant No. 2009CI125) to XQZ, the National Natural Science Foundation of China (Grant No. 31001117) to JHF, and the National Natural Science Foundation of China (Grant No. 30960251) to QYL.

### REFERENCES

Arcangioli MA, Duet A, Meyer G, Dernburg A, Bézille P, Poumarat F, Le Grand D (2008). The role of *Mycoplasma bovis* in bovine respiratory disease outbreaks in veal calf feedlots. Vet. J. 177: 89-93.

Ball HJ, Nicholas RA (2010). *Mycoplasma bovis*-associated disease: here, there and everywhere. Vet. J. 186: 280-281.

Butler JA, Sickles SA, Johanns CJ, Rosenbusch RF (2000). Pasteurization of discard *mycoplasma* mastitic milk used to feed calves: thermal effects on various *mycoplasma*. J. Dairy Sci. 83: 2285-2288.

Byrne WJ, McCormack R, Brice N, Egan J, Markey B, Ball HJ (2001). Isolation of *Mycoplasma bovis* from bovine clinical samples in the Republic of Ireland. Vet. Rec. 148: 331-333.

Caswell JL, Archambault M (2009). *Mycoplasma bovis* pneumonia in

cattle. Anim. Health Res. Rev. 8: 161-186.
Foster AP, Naylor RD, Howie NM, Nicholas RA, Ayling RD (2009). *Mycoplasma bovis* and otitis in dairy calves in the United Kingdom. Vet. J. 179: 455-457.
Gagea MI, Bateman KG, Shanahan RA, van Dreumel T, McEwen BJ, Carman S, Archambault M, Caswell JL (2006). Naturally occurring *Mycoplasma bovis*-associated pneumonia and polyarthritis in feedlot beef calves. J. Vet. Diagn. Invest. 18: 29-40.
Maunsell FP, Donovan GA, Risco C, Brown MB (2009). Field evaluation of a *Mycoplasma bovis* bacterin in young dairy calves. Vaccine 27: 2781-2788
Nicholas RA, Ayling RD, McAuliffe L (2009). Vaccines for *Mycoplasma* diseases in animals and man. J. Comp. Pathol. 140: 85-96.
Nicholas RA, Ayling RD (2003). *Mycoplasma bovis*: disease, diagnosis, and control. Res. Vet. Sci. 74: 105-112.
Ran ZG, Xie JH, Luo L, Yang ZL, Ling HQ, Su CZ, Xiong ZL, Fan WX, Mi ZY (2010). Etiological diagnosis of *Mycoplasma bovis* pneumonia and arthritis in some provinces in China. Chinese J. Prev. Vet. Med. 32: 40-43 (in Chinese).
Stipkovits L, Ripley P, Varga J, Pálfi V (2000). Clinical study of the disease of calves associated with *Mycoplasma bovis* infection. Acta Vet. Hung. 48: 387-395.
Wiggins MC, Woolums AR, Hurley DJ, Sanchez S, Ensley DT, Donovan D (2011). The effect of various *Mycoplasma bovis* isolates on bovine leukocyte responses. Comp. Immunol. Microbiol. Infect. Dis. 34: 49-54.