Prevalence, Diagnosis, and Vaccination Situation of Animal Chlamydioidis in China

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Since the first case of Chlamydia infection in duck had been reported in 1956 and the first case from domestic animal had been reported in 1979 in China, the chlamydia prevalence in China was heavily according to the published data. The Chlamydia in avian prevalence has been reported at least 11 provinces, Chlamydia in sheep and goats at least 11 provinces, in swine at least 15 provinces, in cows at least 13 provinces and in yaks at least 5 provinces with result of IHA detection. Different diagnostic method such as CFT, ELISA and ABC-ELISA (avidin-biotin-complex ELISA) had been established besides IHA. The inactivated vaccines have been developed with isolated strains from sheep, goats, swine and cows. These inactivated vaccines have been used since 1980s and Chlamydia prevalence in China has been successfully controlled in domestic animal. However, the inactivated vaccines of Chlamydia isolated from avian species have not been successful, although a series of experimental vaccine have been done. Due to the unsustainable eradication plan of Chlamydia in China, sporadic outbreak in animal would happen if the vaccinations were suspended and economy lose in some farmers. Although Chlamydia prevalence in China has a long history, however, almost all published studies are in Chinese, which, in some degree, blocked scientists in other countries to understand the prevalence situation and control measures of Chlamydia in China.

Keywords: prevalence, diagnosis, vaccine, Chlamydia, China

INTRODUCTION

Chlamydiae, zoonotic and obligate intracellular Gram-negative bacteria, have a worldwide distribution and cause a wide range of diseases in human hosts, livestock, companion animals, wildlife, exotic species (1), and poultry (2). Chlamydioidis, a disease caused by Chlamydia, has been found in many countries around the world where sheep-rearing is practiced, ranging from Europe to Africa, North America (3), and Asia (4). These include France (5), Poland (6), Spain (7), Australia (8), the United Kingdom (9), Ireland (10), China (11) and Switzerland (12).

Vaccination is one of the best ways to control Chlamydioidis. Formalin-inactivated, egg-grown vaccine of Chlamydia abortus was developed (13) in the U.K. The inactivated vaccine of C. abortus was used in sheep although the efficacy of the vaccine against EAE was unsatisfactory (14). After the first reported cases of avian Chlamydia in China in 1959 (15) and reports of infection in domestic animals in Qinghai province in 1981 (4), China began developing inactivated vaccines using different isolates from sheep, swine, and cow, which successfully controlled the prevalence...
of *Chlamydia* in China. However, almost all these achievements were reported only in Chinese, rather than in English, which presents a challenge for scientists all over the world to understand the situation of *Chlamydia* prevalence and control measures in China.

**CHLAMYDIA PREVALENCE**

Following the first report of *Chlamydiosis* in China from a duck in Beijing in 1959 (15), prevalence of avian *Chlamydia* has been reported in 10 provinces, including Beijing, Tianjin, Jiangsu, and Guangdong due to lack of effective vaccines (16).

The first instance of livestock *Chlamydiosis* was found in ruminants in the Qinghai province by scientists from the Lanzhou Veterinary Research Institute (LVRI), Chinese Academy of Agricultural Sciences (CAAS), in 1979 (17). *Chlamydia* was detected in sheep and goats in at least 11 provinces, in swine in 15 provinces, in bovines in 13 provinces, and in yaks in 5 provinces, using the indirect hemagglutination assay (IHA) test; this prevalence if the disease caused a huge economic loss in China (Figure 1). Although the seropositive rate was high in different animals in China, similar seropositive rates existed in other countries. For example, a German study reported *Chlamydia* antigen prevalence in sheep to range between 5 and 71% (18) and a Polish study reported the prevalence of *Chlamydial* infection in birds in Europe to range between 6.3
and 19.2% (19). There was uncertainty about the accuracy of the results of *Chlamydia* detected in animals using the IHA technique in China. However, compared with the results from other countries, which used McCoy cell culture isolation, the IHA technique may reflect the real prevalence of *Chlamydia* in China.

Using IHA, *Chlamydia* was found to be prevalent in chicken, ducks, pigeons, geese, and other avian species in 11 provinces, with seropositive rates of 10 to 60% in last three decades (20–22) (Table 1). Likewise, *Chlamydia* was prevalent in cows, yaks, sheep, goats, and pigs with seropositive rates of 2–40% in goats and sheep (4, 33–38) (Table 2), 5–53% in swine (50–55) (Table 3), 3–35% in bovine (67–71) (Table 4), and 2–30% in yak (11, 76–79) (Table 5). The disease was found in almost the entire country through IHA detection.

| Year | Positive rate | Area | Reference (published in Chinese) |
|------|---------------|------|----------------------------------|
| 1981 | 6.9%          | Qinghai | Yang et al. (1981:13–15) (4) |
| 1987–1989 | 2.96% | Hubei | Wang et al. (1992:34) (43) |
| 1998 | 5.18%         | Guanxi | Wu et al. (2000:465–6) (42) |
| 1996–1998 | 1.98% | Yunnan | Wang et al. (2000:41) (38) |
| 1991–1995 | 26.12% | Hubei | Qi et al. (1999:4) (41) |
| 2003 | 19.3%         | Ningxia | Bao et al. (2003:13–14) (43) |
| 2009 | 7.57%         | Neimeng | Wang et al. (2009:154) (44) |
| 2010–2011 | 36.12% | Guizhou | Hong et al. (2012:127–9) (47) |
| 2012 | 1.4%          | Xinjiang | Lei et al. (2012:29–9) (45) |
| 2013 | 20.9%         | Xizang | Huang et al. (2013:243–5) (46) |
| 2014 | 57.78%        | Yunnan | Zhang et al. (2014:38–9) (47) |
| 2015 | 30.56%        | Sichuan | Cheng et al. (2015:472–4) (49) |
| 2016 | 67.74%        | Ningxia | Cheng et al. (2015:472–4) (49) |
| 2017 | 90%           | Gansu | Cheng et al. (2015:472–4) (49) |
| 2012–2015 | 13.09% | Qinghai | Su (2016:29–30) (35) |
| 2015–2016 | 12.5% | Henan | Gao et al. (2017:66–9) (49) |
| 2013–2017 | 11.87% | Qinghai | Zha et al. (2017:60–1) (38) |

### DIAGNOSTIC METHOD

Before 1984, antibodies to *Chlamydia* were detected by using the complement fixation (CF) method in China. However, this technique was cumbersome and time consuming (88). Thus, a new technique, IHA, was developed. The IHA technique had a higher sensitivity and specificity than CF, and has been used to determine the prevalence of *Chlamydia* in domestic animals in China since the 1980s (88). It has also been used as a high throughput method of seroprevalence detection. Positive results are detectable within 2 h, but false positive and negative readings are possible, since scoring is subjective to the researcher’s observation (88). Besides

| Year | Positive rate | Area | Reference (published in Chinese) |
|------|---------------|------|----------------------------------|
| 1988 | 26.81%        | Hubei | Yang et al. (1988:5–6) (72) |
| 1997–1998 | 16.13% | Shandong | Zhou et al. (2000:14–15) (67) |
| 1997–1998 | 23.1% | Hubei | Zhou et al. (2000:14–15) (67) |
| 1998–1999 | 43.18% | Shaanxi | Zhou et al. (2000:14–15) (67) |
| 1998–1999 | 25.71% | Henan | Zhou et al. (2000:14–15) (67) |
| 1998–1999 | 20.68% | Gansu | Zhou et al. (2000:14–15) (67) |
| 1999–2000 | 25.6% | Shaanxi | Zhou et al. (2000:14–15) (67) |
| 2000–2001 | 16.8% | Ningxia | Zhou et al. (2000:14–15) (67) |
| 2001–2002 | 15.49% | Qinghai | Zhou et al. (2000:14–15) (67) |
| 2012 | 24.14% | Ningxia | Xie et al. (2012:102–4) (68) |
| 2013 | 26.8% | Guizhou | Hong et al. (2012:127–9) (47) |
| 2013 | 21.3% | Chongqing | Hua (2013:67) (73) |
| 2014 | 26.4% | Shaanxi | Ma et al. (2013:213–5) (31) |
| 2013 | 17.71% | Gansu | Tan et al. (2015:1283–7) (74) |
| 2013 | 36.97% | Ningxia | Tan et al. (2015:1283–7) (74) |
| 2014 | 36.8% | Gansu | Zhang et al. (2014:38–9) (47) |
| 2014 | 8.25% | Qinghai | Su (2016:29–30) (35) |
| 2015 | 26.31% | Qinghai | Wang et al. (2016:16–17) (69) |
| 2015 | 37.43% | Henan | Li et al. (2017:22–4) (71) |
| 2015–2016 | 1.75% | Qinghai | Chen et al. (2017:33–5) (75) |
| 2013–2017 | 9.13% | Qinghai | Zha et al. (2017:60–1) (38) |
TABLE 5 | Yak Chlamydia seroprevalence in China.

| Year | Positive rate | Area          | Reference (published in Chinese) |
|------|---------------|---------------|----------------------------------|
| 1988 | 29.0%         | Qinghai       | Shuai et al. (1988:76–81) (76)   |
| 1993 | 21.03%        | Qinghai       | Dong et al. (1993:25) (93)       |
| 1996 | 2.1%          | Xinjiang      | Wang et al. (1996:49) (77)       |
| 2000 | 20.69%        | Gansu         | Zhou et al. (2000:14–15) (67)    |
| 2000 | 15.49%        | Gansu         | Zhou et al. (2000:14–15) (67)    |
| 2000 | 25.6%         | Sichuan       | Zhou et al. (2000:14–15) (67)    |
| 2004 | 19.23%        | Qinghai       | Ma et al. (2004:14) (61)         |
| 2009 | 9.81%         | Qinghai       | Zhang et al. (2009:14–15) (62)   |
| 2010 | 17.39%        | Qinghai       | Hou(2011:10) (73)                |
| 2010–2012 | 2.8% | Qinghai | Kang et al. (2012:51–51) (83) |
| 2012 | 4.13%         | Li et al.     | (2013:128) (64)                 |
| 2013 | 27.7%         | Xie(2013:33)  | (85)                            |
| 2012–2013 | 15.9% | Gansu | Qin(2015:8) (in English) (79) |
| 2014 | 25.08%        | Gansu         | Yin(2014:281–289) (86)           |
| 2009–2014 | 7.68% | Qinghai | Fu et al. (2016:50–51) (67)     |
| 2015 | 23.81%        | Qinghai       | Li et al. (2015:1–6) (in English) (11) |

IHA, enzyme linked immunosorbent assay (ELISA) (89) and avidin-biotin-complex ELISA (ABC-ELISA) (90) techniques were developed. Although the ABC-ELISA and ELISA methods have higher sensitivity than the IHA method, they have not been used in clinical studies due to high cost.

For pathogen diagnosis, chicken egg isolation and Giemsa staining were combined to detect *Chlamydia*. The yolk sac membranes from dead chicken embryos were spread on slides and fixed with methanol or through heating, and Giemsa stain was used to stain them for half an hour or overnight. *Chlamydia* to stain them for half an hour or overnight.

Fixed with methanol or through heating, and Giemsa stain was used to stain them for half an hour or overnight.

Chlamydia elementary body (EB) and inclusion were detected by light microscopy (91). The yolk sac membranes were disrupted with a body (EB) and inclusion were detected by light microscopy (91).

Simply, the process for developing inactivated vaccine is as follows: *Chlamydia* seeds were isolated from the yolk sac membranes of 7-day-old chicken embryos between the 4 and 8th days after inoculation. The titer of *Chlamydia* used for vaccines was at least $10^{12}$ ELD$_{50}$/0.4 ml. The yolk sac membrane was disrupted with a homogenizer and filtered through a mesh strainer. Formalin (0.3%) used to inactivate *Chlamydia* with an equal volume of 206 adjuvant (SEPPIC, France) and mixed under 40 Mpa of pressure. After 7 days of inactivation with formalin, the safety and efficacy of the vaccine were tested using specific pathogen free (SPF) embryos and mice (100–102).

*Chlamydia* inactivated vaccines for sheep and goat have been used since 1981–1986 (100). During that time, 120,000 sheep and goats in Qinghai, Gansu province, and Xinjiang Uygur Autonomous region were vaccinated and the abortion rate declined sharply. No abortion happened due to vaccination in pregnant sheep and goats. A total of 2,000,000 ml (about 700,000 doses) of inactivated vaccine was produced and used in 1988 (100). The duration of immunity was at least 2 years, but 75% of the sheep and goats were protected from infection in the 4th year after vaccination (100, 103). Besides, the inactivated vaccine could be used after a 2 year storage period at 4°C (100). A similar inactivated vaccine of *Chlamydia* isolated from goat was studied by Zhang in the Inner Mongolia Autonomous Region (33). According to this report, 5,099 goats belonging to 51 groups were vaccinated with inactivated vaccine and at least 90% of the goats were protected. Other regions, such as Huachi County, Gansu province, showed similar results of very high prevalence of *Chlamydia* in goats during 1981–1986, with abortion rate in goats being 20–40%. The disease was controlled when inactivated vaccine was used, reducing the abortion rate of the vaccinated groups to 3.3–6.5%, compared with the control group abortion rate of 14.03–14.3%, during 1986–1988 (102).

*Chlamydia* of swine was also detected and isolated in Qinghai province and the GuangXiZhuang Autonomous Region (55, 56, 88). According to these reports, abortion happened among sows and the highest positive rate of abortion was 56.1% in one of the groups in which *Chlamydia* was detected using the complement fixation test (CFT). Two strains were isolated using 7-day-old SPF eggs, and the inactivated vaccine was produced and tested (101). A total of 1,080 sows were vaccinated in two farms and each sow was immunized subcutaneously with 3 ml of inactivated vaccine (101). After 3 months, 482 sows that had been vaccinated and 439 sows that were not vaccinated were studied; only 1.45% abortion rate was observed in the vaccinated group, while 29.53% abortion rate was observed in the non-vaccinated group (101). These results showed that the inactivated vaccine provided good infection protection (101). Subsequently, 10,594 sows were vaccinated in Qinghai, Shanxi province, and GuangxiZhuang Autonomous regions. The duration of immunity was at least 1 year when 2 ml of vaccine was injected (104). The vaccine remained active after storage at 4–8°C for 1 year (101).

*Chlamydia* in bovines was reported firstly in China in 1986 (70). In 1990, 2 strains (CCS10 and CCS15) were isolated from cows in a farm in Shaanxi province (105). However, the strain used for the inactivated vaccine was isolated in 2006 (102). This isolated strain (SX5) from a farm in Shaanxi province was tested and the LD$_{50}$/0.4 ml remained at $10^{-12}$ after at least 5 times propagation in SPF eggs. The minimal effective dosages for the vaccine was 3 ml for adult dairy cow and 1.5 ml for calf. The average protection was about 94.4%, while the duration of immunity was 10 months (102).

Although formalin-inactivated *C. abortus* vaccines have been used in China since 1985, their production was discontinued because of lack of good manufacturing practices (GMP), causing...
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sporadic outbreaks in sheep (46), yaks (106), and other animals. However, there is no information about Chlamydia prevalence in recent years from Dulan county (100), Delingha (56), Qinghai province, and Huachi county (101), Gansu province, where Chlamydia was first isolated and animals were vaccinated with inactivated vaccine. On the contrary, farms near the original places, such as in Wulan county, Guinan county, Haiyan county, Gonghe county, Huzhu county, Huangyuan county, and Tianjun County, Qinghai province, reported that Chlamydia caused huge abortion among sheep, goats, and yaks in recent years (11, 35, 38, 68, 75, 84, 87, 107–109). Interestingly, during the investigation, shepherds reported that dead Tibetan antelopes were found and their eyes were obviously blind (personal communication). They also reported that Chlamydia spread among different groups of sheep that had never been in contact with each other, suggesting that wild animals may play a very important role to spread Chlamydia to domestic animal. Therefore, based on this information, we can conclude that the original source of Chlamydia infections in China is wild animals in Qinghai province.

Besides inactivated vaccine, the subunit vaccine for Chlamydia in ewes, which has three different doses of major outer-membrane protein from genetically engineered Chlamydia psittaci, was developed by the State Key Laboratory of Pathogen and Biosecurity, Institute of Microbiology and Epidemiology, Academy of Military Medical Sciences, China. The study analyzed the antibody responses in ewes vaccinated with the subunit vaccine of rCps-MOMP. The sera of ewes were detected before vaccination and at different times post-vaccination. Experimental results indicated that multivalent intramuscular injection in the neck region with a dose of 0.5 mg per ewe could stimulate good immune response (110). Because of the good security and immunity protection, a new veterinary drug certificate was awarded by the Ministry of Agriculture of the People’s Republic of China (111). However, there are few reports of the promotion and application of the subunit vaccine in China (112). Although some successes were obtained for controlling Chlamydia in livestock in China since the inactivated vaccines were introduced, each year huge economic losses are caused by avian Chlamydia in chicken production (28). A subunit vaccine and a recombinant adenovirus live vector vaccine have been developed (16, 113). However, the recombinant adenovirus vector vaccine has not been approved by the government due to potential biological concerns, and no further data about the subunit vaccine has been published. Avian Chlamydia is still serious in China due to a lack of effective and safe vaccine. Moreover, the disease is a potential risk for human health. Therefore, further investigation into the development of vaccines is necessary.

TREATMENT

Antibiotics such as tetracycline, oxytetracycline, and penicillin sodium are used to treat Chlamydirosis in animals in China. However, since 2014, to regulate the use of veterinary antibiotics in China, the government established a veterinary prescription drug management system, including measures for administration of veterinary prescription and over-the-counter drugs. The quality of antibacterial drug products has been improving every year. The quality of sampling inspection is maintained at more than 95%, whereas the rate of residues of veterinary drugs in livestock and poultry products remains stable at more than 97%. Thus, the use of antibiotics has been greatly reduced.

FUTURE PERSPECTIVE

Although IHA plays a very important role in detecting Chlamydia in animals in China, it does not reflect the real situation of Chlamydirosis prevalence in animals. However, Hagemann JB (114) reported that aborting sheep exhibited a strong antibody response to surface (MOMP, MIP, and Pmp13G) and virulence-associated (CPAF, TARP, and SINC) antigens. While the latter disappeared within 18 weeks following abortion in majority of the animals, antibodies to surface proteins persisted beyond the duration of the study. In contrast, experimental non-abortion infected sheep developed antibodies mainly to surface antigens (MOMP, MIP, and Pmp13G), all of which did not persist. This indicates that new diagnostic methods need to be established to improve the accuracy of disease diagnosis and provides scientific basis for controlling animal Chlamydiosis.

CONCLUSION

Since the first case of avian Chlamydia was reported in China, Chlamydia infection has been observed in different animals in most areas of China, which causes serious economic losses each year. Several diagnostic techniques, including CF, IHA, ELISA, ABC-ELISA, egg isolation, and PCR, have been studied and used in China. Formalin inactivated vaccines of Chlamydia from sheep, goat, swine, and cow were developed and have been used since 1981 in those areas where animals are threatened by Chlamydia. Because Chlamydirosis is considered an unimportant disease in animals by the Chinese government and no eradication plan has implemented, there are sporadic outbreaks of Chlamydia in domestic animals in some areas, especially where vaccination has been suspended. However, the abortion rate was down sharply when inactivated vaccines for Chlamydia were used in domestic animals. This may have contributed to the lack of large-scale outbreak of Chlamydirosis in domestic animals in the last 30 years. The most important problem now is avian Chlamydiosis, which has a seropositive rate of 10–50% with IHA and easily spreads from birds to humans. Due to lack of effective vaccines, avian Chlamydiosis may become a public health problem in China.

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