frontiers in Veterinary Science

REVIEW
published: 30 May 2018
doi: 10.3389/fvets.2018.00088

Prevalence, Diagnosis, and Vaccination Situation of Animal Chlamydiosis 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 *Chlamydi*a 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

OPEN ACCESS

Edited by:
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Specialty section:
This article was submitted to Veterinary Infectious Diseases, a section of the journal Frontiers in Veterinary Science

Received: *20 December 2017*
Accepted: *06 April 2018*
Published: *30 May 2018*

Citation:
Zhou J, Li Z, Lou Z and Fei Y (2018) Prevalence, Diagnosis, and Vaccination Situation of Animal Chlamydiosis in China. Front. Vet. Sci. 5:88. doi: 10.3389/fvets.2018.00088

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). Chlamydiosis, 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 Switerland (12).

Vaccination is one of the best ways to control Chlamydiosis. 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 unstisfatory (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

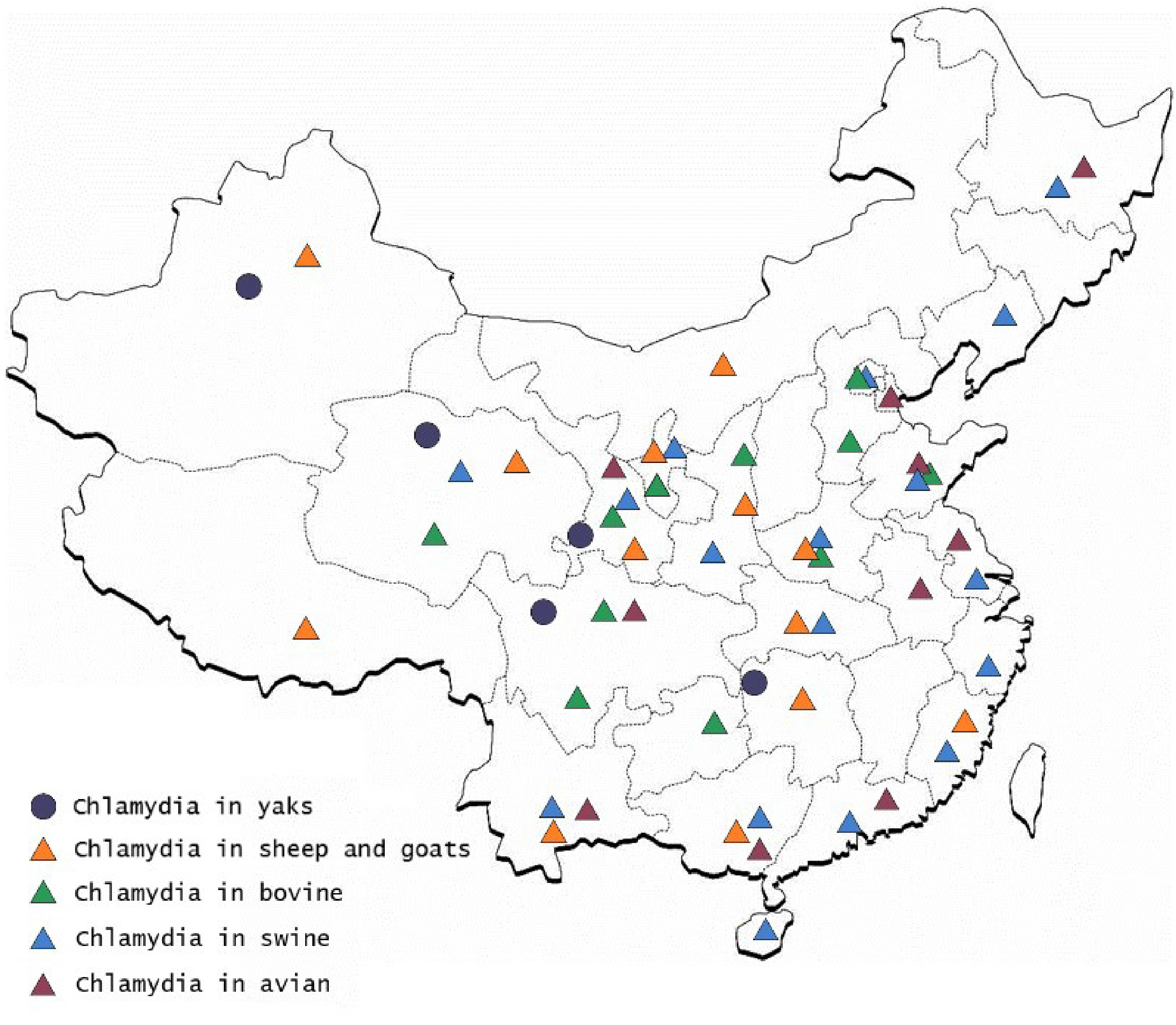

FIGURE 1 | Distribution of *Chlamydia* prevalence in different animals and areas in China.

TABLE 1 | Avian *Chlamydia* seroprevalence in China.

Year	Positive rate	Area	Reference (published in Chinese)
1991	21.8%	Shandong	Wu *et al.* (1990:61) (23)
1988–1989	39.2%	Sichuan	Xu *et al.* (1991:18–19) (24)
1991	26.91%	Gansu	Wang *et al.* (1991:18) (25)
1994	20–40%	Jiangsu	Yu *et al.* (1994:13–15) (20)
1994	22.7%	Yunnan	Wang *et al.* (1997:10–12) (26)
1998	5.1%	Heilongjiang	Jiang *et al.* (1998:25–6) (27)
2001	20.66%	Guangxi	Liu *et al.* (2001:13) (28)
2003	10–30%	Beijing Tianjin	Shi *et al.* (2003:217–21) (21)
2003	59.9%	Guangdong	Zhang *et al.* (2003:29) (29)
2012	20–45.4%	Anhui	Ou *et al.* (2012:61-3) (30)
2013	3.9%	Qinghai	Ma *et al.* (2013:213–5) (31)
2012–2014	36.97%	Tianjin	Zhu *et al.* (2016:148–50) (32)
2016	43.2%	Sichuan	Ouyang *et al.* (2016:46–51) (22)

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.

TABLE 2 | Sheep and goat *Chlamydia* seroprevalence in China.

Year	Positive rate	Area	Reference (published in Chinese)
1981		Qinghai	Yang *et al.* (1981:13–15) (4)
1987–1989	6.9%	Zhejiang	Wang *et al*.(1990:11) (39)
1988–1989	2.95%	Hubei	Zhang *et al.* (1992:34) (40)
1998	5.78%	Hunan	Qiu et al.(1998:3-5) (41)
1996–1998	1.98%	Guangxi	Wu *et al.* (2000:41) (36)
1991–1995	26.12%	Yunan	Wang *et al.* (2000:465–6) (42)
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) (37)
2012	1.4%	Xinjiang	Lei *et al.* (2012:28-9) (45)
2013	20.9%	Xizang	Huang *et al.* (2013:243–5) (in English) (46)
2014	40.3%	Qinghai	Zhang *et al.* (2014:38–9) (47)
2014	60%	Hubei	Cheng *et al.* (2015:472–4) (48)
2014	42.86%	Anhui	Cheng *et al.* (2015:472–4) (48)
2014	52.75%	Shandong	Cheng *et al.* (2015:472–4) (48)
2014	40.13%	Xinjiang	Cheng *et al.* (2015:472–4) (48)
2014	51.91%	Jilin	Cheng *et al.* (2015:472–4) (48)
2014	30.56%	Sichuan	Cheng *et al.* (2015:472–4) (48)
2014	67.74%	Ningxia	Cheng *et al.* (2015:472–4) (48)
2014	90%	Gansu	Cheng *et al.* (2015:472–4) (48)
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)

TABLE 3 | Swine *Chlamydia* seroprevalence in China.

Year	Positive rate	Area	Reference (published in Chinese)
1983–1984	29.72%	Hubei	Jiang *et al.* (1985:32–4) (50)
1985	33.3%	Qinghai	Diao *et al.* (1990:21–2) (56)
1991	20.4%	Guangxi	Yi *et al.* (1991:6–9) (55)
1997	13.65%	Shandong	Ji *et al.* (2003:39) (57)
1998–2000	34.91%	Gansu	Gao *et al.* (2001:13–14) (58)
2003	2.3%	Henan	Lang *et al.* (2004:29) (59)
2003	5.16%	Liaonin	Wang (2004:26) (60)
2005	49.49%	Hainan	Suo *et al.* (2005:31–2) (51)
2005	41.41%	Shanghai	Jin *et al.* (2005:23) (61)
2005	6.82%	Zhejiang	Jin *et al.* (2005:23) (61)
2004–2006	14.97%	Guangdong	Zhu *et al.* (2007:26–7) (62)
2006–2007	27.71%	Fujian	Zhou *et al*. (2008:30–5) (63)
2011	18.5%	Yunnan	Bi *et al.* (2011:134–6) (52)
2012	7.6%	Shaanxi	Wang *et al.* (2013:9–10) (64)
2013	53.30%	Qinghai	Ma *et al.* (2013:213–5) (31)
2014	58.59%	Jiangxi	Jang *et al.* (2014:27–28) (65)
2014	57.0%	Qinghai	Zhang *et al.* (2014:38–9) (47)
2014	18.88%	Yunnan	Li *et al.* (2014:29–30) (66)
2015	18.4%	Yunnan	Su *et al.* (2015:155–6) (53)
2013–2015	11.3%	Henan	Ma *et al.* (2016:119–22) (54)

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

TABLE 4 | Bovine *Chlamydia* seroprevalence in China.

Time	Positive rate	Area	Published in original journal
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%	Hebei	Zhou *et al.* (2000:14–15) (67)
1997–1998	43.18%	Shaanxi	Zhou *et al.* (2000:14–15) (67)
1997–1998	25.71%	Henan	Zhou *et al.* (2000:14–15) (67)
1997–1998	20.68%	Gansu	Zhou *et al.* (2000:14–15) (67)
1997–1998	25.6%	Sichuan	Zhou *et al.* (2000:14–15) (67)
1997–1998	16.8%	Ningxia	Zhou *et al.* (2000:14–15) (67)
1997–1998	15.49%	Qinghai	Zhou *et al.* (2000:14–15) (67)
2012	24.14%	Ningxia	Xie *et al.* (2012:102–4) (68)
2010–2011	2.86%	Guizhou	Hong *et al.* (2012:127–9) (37)
2013	21.3%	Chongqing	Huo (2013:67) (73)
2013	26.4%	Qinghai	Ma *et al.* (2013: 213-5) (31)
2013	17.71%	Gansu	Tan *et al.* (2015:1283–7) (74)
2013	38.97%	Ningxia	Tan *et al.* (2015:1283–7) (74)
2014	36.8%	Qinghai	Zhang *et al.* (2014:38–9) (47)
2012–2015	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) (80)
1996	2.1%	Xinjiang	Wang *et al.* (1996:46) (77)
2000	20.69%	Gansu	Zhou *et al.* (2000:14–15) (67)
2000	15.49%	Qinghai	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) (81)
2009	9.81%	Qinghai	Zhang *et al.* (2009:14–15) (82)
2010	17.39%	Qinghai	Hou(2011:10) (78)
2010–2012	2.8%	Qinghai	Kong *et al.* (2012:51–51) (83)
2012	4.13%	Qinghai	Li *et al.* (2013:126) (84)
2013	27.7%	Qinghai	Xie(2013:33) (85)
2012–2013	15.9%	Gansu	Qin(2015:8) (in English) (79)
2014	25.08%	Gansu	Yin(2014:281–285) (86)
2009–2014	7.68%	Qinghai	Fu *et al.* (2016:50–51) (87)
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* elementary body (EB) and inclusion were detected by light microscopy (91).

The PCR and real-time PCR tests, although highly sensitive and used to detect *Chlamydia* in different animals in other countries (92–96) have seldom been used on a large scale due to high cost. The *omp1* gene, which is very conservative, was used as a target gene to detect *Chlamydia* in different animals when IHA results were ambiguous (16, 97). In China, the PCR-RFLP method was developed and used only to identify *Chlamydia* species isolated from animals (11), while recombinase polymerase amplification (RPA) was used to identify *C. abortus* (98). However, IHA is considered as a simple, safe, and reliable means of testing *C. abortus* antibodies, and has been employed in previous serological investigations (79, 99).

PREVENTION AND CONTROL OF CHLAMYDIOSIS

Vaccination

Vaccination is one of the most important methods of disease prevention in animals. The inactivated vaccine of *C. abortus* plays a huge role to control the spreading of the disease in China. 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%) was 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 Guangxi Zhuang 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 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 multilocus 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 submit 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 Chlamydiosis 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 Chlamydiosis 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 Chlamydiosis was considered an unimportant disease in animals by the Chinese government and no eradiation 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 Chlamydiosis 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.(Reorder)

AUTHOR CONTRIBUTIONS

Substantial contribution to the references of the work: ZLi, ZLou, YF. Draft and/or critical revision of the manuscript: JZ. Final approval of the version to be published: JZ, ZLi, ZLou, YF.

FUNDING

This work was supported by the National Key Research and Development Plan (2017YFD0500905).

ACKNOWLEDGMENTS

Special thanks to Xueli Yang, Yingcai Li, Yongyu Shuai, and Changqing Qiu who devoted their life to researching *Chlamydia* and controlling its prevalence in China.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.