Chlamydial infections in Chinese livestock

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Summary

The occurrence and impact of chlamydial infections in Western livestock is well documented in the international literature, but less is known about these infections in livestock in the People's Republic of China (hereafter referred to as China). China's livestock production and its share in the global market have increased significantly in recent decades. In this review, the relevant English and Chinese literature on the epidemiology of chlamydial infections in Chinese livestock is considered, and biosecurity measures, prophylaxis and treatment of these infections in China's livestock are compared with Western practices. Chlamydial infections are highly prevalent in Chinese livestock and cause important economic losses, as they do in the rest of the world. Surveillance data and diagnostic results of abortion outbreaks in cattle, sheep and goats highlight the importance of virulent chlamydial infections in China's major ruminant species in many of China's provinces, autonomous regions and municipalities. Data from many of China's provincial divisions also indicate the widespread presence of chlamydial infections in industrially reared swine across the country. Less is known about chlamydial infections in yak, buffalo and horses, but available reports indicate a high prevalence in China's populations. In these reports, chlamydiosis was related to abortion in yak and pneumonia in horses. In Western countries, chlamydial infections are principally treated with antibiotics. In China, however, traditional medicine is often used in conjunction with antibiotics or used as an alternative treatment.

Keywords

Antibiotic treatment – Biosecurity – China – *Chlamydiaceae* – Livestock – Traditional medicine.

Introduction

The *Chlamydiaceae* are a family of Gram-negative obligate intracellular bacteria with a unique biphasic developmental cycle in which the pathogen differentiates intracellularly between the elementary body and the reticular body. Elementary bodies are the extracellular infectious form, whereas reticular bodies are the non-infectious form only present inside the living cell. Zoonotic transmission occurs by inhalation or accidental ingestion of elementary bodies in animal excretions and discharges.

In this paper, 'livestock infection' refers to infection in ruminants, pigs and equines raised in agricultural settings and does not include infection in poultry, rabbits, game or farmed fish. Livestock *Chlamydiaceae* include *Chlamydia abortus*, *C. pecorum*, *C. suis*, *C. psittaci* and equine *C. pneumoniae*. *Chlamydia abortus* is a major abortigenic agent in ruminants and causes abortion in pregnant women through zoonotic transmission (1). Infection in swine and horses may also result in abortion, but does not result in abortion storms as seen in affected flocks of sheep and goats (1, 2). Infection with *C. pecorum* in ruminants, swine and horses has been associated with urogenital symptoms, reproductive failure, conjunctivitis, respiratory distress, polyarthritis and pericarditis (2). Until now, *C. suis* infections have been thought to be restricted to swine. However, preliminary data indicate the possibility of zoonotic transfer (Vanrompay *et al.*, unpublished data). In swine, virulent *C. suis* strains may cause reproductive failure and respiratory and intestinal signs (3). The zoonotic chlamydial species *C. psittaci*, for which birds are the natural host, also infects pigs and cattle (4, 5, 6). Equine *C. pneumonia* is currently confined to one respiratory isolate, for which experimental infection in horses remains asymptomatic (7).

Global importance of Chinese livestock production

The changing political orientation of the People's Republic of China (hereafter referred to as China) over the last 30 years has facilitated its agricultural growth rate (8). Consequently, China has become a major livestock producer, exporting meat and dairy products globally. Major export destinations are within the Asian region; in particular, Japan, Kyrgyzstan, Jordan and the United Arab Emirates. China's most important meat export product is pork and the country is the fifth-largest exporting nation worldwide. According to FAOSTAT 2010 data, China's total annual meat production is 80.74 Mt, of which 64% is pork, 13% ruminant meat and 0.5% equine meat. This represents 47.3%, 13.2% and 43.7% of the global pork, ruminant meat and equine meat production, respectively. The meat of sheep, goats and buffalo, which accounts for merely 2.6%, 2.3% and 0.4% of China's total meat production, contributes 24.3%, 36.2% and 9.1% of global production, respectively. Equine meat production in China consists of 46.8% horsemeat, 40.1% ass meat and 13% mule meat, accounting for 27.4%, 90.1% and 98.8% of global production, respectively (9).

In 2000, China's share of global milk production was only 2.1%. However, during the past decade, China's explosive increase in dairy production (+232.6%) has resulted in a current global share of 5.7% and amounts to 41.15 Mt of milk annually. Milk from sheep and goats represents only 2.3% and 1.4% of dairy products globally, of which China produces only a minor fraction. Similarly, Chinese production of buffalo and camel milk is only a tiny percentage of global production of these products. Nevertheless, despite contributing only 3.4% of global buffalo milk production, China is considered the third-largest producer worldwide after India and Pakistan (9). Lastly, although not included in FAOSTAT statistics, yaks are an important ruminant species in China. The yak population is estimated at 13 million animals, representing 92.8% of the global population (10). These sturdy but low milk-yielding ruminants are perfectly adapted to the high altitude and related extreme environmental conditions of the Qinghai-Tibet Plateau (which includes the Tibetan Autonomous Region and the Qinghai, Gansu, Sichuan and Yunnan provinces), such as low temperature, low oxygen and high solar radiation (11, 12).

Diagnosis of chlamydial infection in Chinese livestock

Seroprevalence

The World Organisation for Animal Health (OIE) recommends the use of the complement fixation test (CFT) and enzyme-linked immunosorbent assay (ELISA) for detecting chlamydial antibodies (13). However, few publications on detection of chlamydial antibodies in Chinese livestock report the use of these tests. Most serological data have been obtained using a commercially available indirect haemagglutination assay (IHA) developed at the Lanzhou Veterinary Research Institute. The Lanzhou IHA uses antigen prepared from the Chinese ovine *C. abortus* strain B11001. The vast quantity of serological data on ruminants demonstrates that chlamydiae are endemic in small and large ruminants across China, except in the provinces of Jiangsu and Shaanxi and the Province-level Municipality of Shanghai (Tables I and II). Similarly, prevalence data from many of China's provinces, autonomous regions and municipalities demonstrate that chlamydial infections are widespread in industrially produced swine across the country (Table III). Seroprevalence data on horses indicate the presence of chlamydial antibodies but are limited to four Chinese provinces (Table IV).

Whole chlamydial organisms are used as target antigens in IHAs, CFTs and ELISAs and thus make no distinction between chlamydial species, because the lipopolysaccharides of *Chlamydiaceae* are family specific. These serological methods were compared in two studies on Chinese swine of unknown status. In one trial, the German ovine abortion strain B394 was used in a Dot-ELISA and a CFT, finding seropositivity rates of 47.3% (52/110) and 29.1% (32/110), respectively (14). In a similar trial, comparison was made between the widely used Lanzhou IHA kit and an in-house Dot-PPA (protoplasmic antigen) ELISA based on strain B394. The highest seroprevalence rates were recorded with the Dot-PPA ELISA: 35.8% (43/120) compared with 21.7% (26/120) with the IHA (15). In both trials it was concluded that the ELISA had the higher sensitivity. However, each of these tests is prone to false positives resulting from cross-reactivity of target antigen with antibodies against, for example, lipopolysaccharides of other Gram-negative bacteria (16). This was indicated in a comparison of the Lanzhou IHA kit with the ID Screen® *Chlamydia abortus* indirect ELISA (ID-VET Innovative Diagnostics, Montpellier, France), where a synthetic *C. abortus*-specific fragment of the major outer membrane protein (MOMP) is used. Results revealed that the seropositivity rate detected with the IHA kit (4.14%; 21/507) was almost twice that detected with the ID-VET ELISA (2.17%; 11/507) (17). At present, an ELISA using recombinant protein fragments of the *C. abortus* polymorphic outer membrane protein POMP90 provides superior sensitivity and specificity for species-specific detection of *C. abortus* antibodies (18). Chinese researchers are currently also focusing on ELISAs using recombinant fragments of the *C. abortus* POMPs and MOMP variable domains (VDI and/or VDII), as those sequences reveal a great deal of diversity between *C. abortus* and *C. pecorum* strains. However, to the best of the authors' knowledge, such tests have not yet been applied to livestock.

Diagnosis in Chinese livestock: culture, antigen detection and gene detection

As in the rest of the world, diagnosis of current chlamydial infection in Chinese livestock was initially achieved by culturing the bacteria in the yolk sacs of specific-pathogen-free (SPF) chicken embryonated eggs at six to seven days of age (Table V). However, rapid methods of antigen detection have recently been introduced. For example, experiments with IMAGEN™ (DAKO) direct immunofluorescence (DIF) staining gave a positivity rate of 37.5% (6/16) for boar sperm samples and 27.5% (11/40) for sow vaginal swabs from intensive pig farms in Beijing (17). In two studies, an in-house indirect ELISA using a monoclonal antibody against a bovine *C. abortus* isolate was used to determine the prevalence of chlamydial antigen in ruminant sera. The first study reported a positivity rate of 25.8% (33/128) in goat sera and 32.2% (29/90) in calving cow sera in Ningxia (an autonomous region in north-west China) (19). In the second study, all examined sheep farms ($n = 10$) and cattle farms ($n = 13$) in Ningxia had seropositive animals: the average positivity rate was 22.1% (154/698) in sheep and 9.2% (107/1,161) in cattle (20). Unfortunately, no information is available on the species-specificity of the monoclonal antibody or the health status of the examined flocks.

As antigen detection methods often lack sensitivity and specificity, nucleic acid amplification assays have also been developed recently by Chinese scientists. Several Chinese chlamydial isolates have been characterised using sequencing of the *ompA* gene (Table V). During an epizootic outbreak of cattle and caprine abortion in Chinese Taipei, *ompA*-based polymerase chain reaction (PCR) with *C. abortus*-specific primers was used for direct detection of the gene (21). Only 8.3% (1/12) of aborted calves and 33.3% (3/9) of aborted kids (young goats) tested positive in PCR, whereas seropositivity rates were as high as 71.4% and 58% in the respective maternal serum samples (Tables II and III). Moreover, PCR assays were more often positive in vaginal swabs of healthy cows (45.2%; 14/31) and does (38.9%; 7/18) compared with cows (34.9%; 22/63) and does (21.4%; 24/112) that recently aborted. Nevertheless, in contrast to successful isolation in 22.7% (5/22) and 33.3% (8/24) of PCR-positive vaginal swabs from affected cows and does respectively, chlamydiae could not be isolated from any of the PCR-positive vaginal swabs of healthy animals. Two consistent point variations were found in all isolates in Chinese Taipei, which shared 98.9% to 100% sequence identity, and bovine *C. abortus* strain LW508 (21). Recently, however, it has become clear that *ompA* sequencing does not allow unambiguous identification of *C. psittaci* and *C. abortus*, which are phylogenetically highly related (22). It would be interesting to re-examine all characterised Chinese *C. abortus* and *C. psittaci* isolates using multilocus sequence typing and/or multilocus variable number of tandem repeat analysis in order to confirm the originally assigned species name.

Like investigators in other regions of the world, Chinese researchers have also developed *Chlamydiaceae*-specific 23S rRNA-based real-time PCR assays using SYBR® Green fluorescence and the Lightcycler™ system (Roche Applied Sciences). Development of a highly sensitive test gave a detection limit as low as 250 femtograms of chlamydial DNA (*C. trachomatis*, *C. abortus*, *C. psittaci*, *C. pecorum*) (23). Specificity was evaluated from melting curve analysis and by analysis of PCR products on agarose gels, together with nucleotide sequencing. Each of these analyses confirmed the specificity of the real-time PCR (23).

Clinical disease resulting from chlamydial infection in Chinese livestock

Abortion is the most frequently observed clinical manifestation of chlamydiosis in Chinese ruminants. In Gansu province, Tibet and Inner Mongolia, 20% to 25% of does in affected goat flocks aborted after approximately four months of gestation, which was often followed by placental retention in the aborting animal (24). Abortion rates in nine farms with outbreaks of chlamydiosis in Chinese Taipei ranged between 27% and 87% of pregnancies. Does did not show clinical signs before aborting, which they generally did in the last two months of gestation (25). In sheep, interstitial pneumonia and polyarthritis in lambs was concomitantly observed with abortion and stillbirth in ewes (26). In Hubei province, dairy cattle aborted at four to seven months of pregnancy and developed mastitis; surviving calves showed symptoms of pneumonia, polyarthritis and conjunctivitis (27). In bovine abortion cases in Ningxia and Shaanxi province, abortion typically occurred between seven and nine months of gestation and aborted fetuses were oedematous (28). None of these reports mentioned the presence of uterine discharge around the time of abortion, although this is a common symptom in chlamydial abortion; however, the timing of the abortions and lack of previous overt clinical signs is consistent with *C. abortus* infection. In Beijing, newborn calves developed polyarthritis with symptoms including fever and tarsal and carpal swellings (29). Symptoms observed in swine include polyarthritis in piglets (30) and pneumonia in suckling piglets (31); in one study piglets died from pneumonia and enteritis (32). In addition, metritis, pneumonia, arthritis and diarrhoea have been observed in aborting sows (33). In horses, chlamydiosis was suspected to be present in mares producing weak foals with pneumonia, joint swellings and diarrhoea (34).

Prevention and treatment

Biosecurity

In China, farmers usually disinfect stables with a 2% to 3% weight/volume (w/v) solution of sodium hydroxide, a 0.5% w/v solution of sodium hypochlorite, 2% to 5% Lysol® or quaternary ammonium surfactants. This approach, together with other basic hygiene measures, such as the use of disinfectant foot baths and the prevention of contact with susceptible animals alien to the group, is feasible and is used in the closed environment of China's large-scale vertically integrated pig farms. For grazed or herded ruminants under extensive management conditions, these measures are, at best, limited to disinfection of equipment and prevention of contact between different herds or flocks. Similarly, acquisition of new stock from *C. abortus*-free flocks or herds is also not feasible for small-scale farmers in rural Chinese areas, where access to veterinary services is limited.

Antibiotics and Chinese traditional medicine

In Western countries, chlamydial infections in livestock are principally treated with antibiotics. In China, however, traditional medicines based on plant extracts are often used in conjunction with antibiotics or are used as alternative therapies. The latter is common in rural areas, where access to veterinary services and antibiotic treatment is limited. These traditional medicines are also used prophylactically. Chinese traditional medicines are centuries old and are often, but not exclusively, prescribed to treat observed symptoms rather than as aetiological treatments.

Chlamydiae are sensitive to the broad-spectrum antibiotics tetracyclines, quinolones and macrolides (35). In China, the antibiotics of choice for treatment of chlamydial infections in livestock are the tetracyclines, followed by quinolone antibiotics. The Chinese government has set maximum residue limits (MRLs) for tetracyclines and promulgated a government standard (GB/T 21317-2007) establishing a method for its determination in animal tissues. The Chinese MRL for tetracycline in meat is 100 µg/kg (36). Moreover, prophylactic use of these antibiotics is strongly discouraged, to prevent the emergence of resistant, potentially zoonotic, strains of chlamydiae. These regulations are similar to those in many other areas of the world.

Chinese herbal remedies for chlamydial kerato-conjunctivitis in cattle and horses include Xiao Huang San (clearing yellow swelling powder) and Jue Ming San (haliotis powder) (Table VI). Ingredients are ground into a fine powder or prepared as a decoction followed by drying. Decoction refers to the filtered liquid after three consecutive cycles of boiling in water (37). The remedies are administered twice daily as top-dressing on feed at a dose of 15 g to 60 g Xiao Huang San for one month, or up to 50 g Jue Ming San for one to two months. Historically, all ingredients of Jue Ming San were ground into a fine powder and mixed with honey to form a paste for topical administration. Yu Benyuan and Yu Benheng, two noted veterinarians in the Ming Dynasty, first described the use of these herbal prescriptions in 1608 in Yuan Heng’s *Therapeutic Treatise of Horses*. Xiao Huang San was originally developed for the management of lameness in horses, but is currently regarded as the fundamental veterinary formula to treat painful swellings, including mastitis. Jue Ming San was developed to treat chronic ophthalmic inflammation. A Chinese report in 2009 describes successful treatment of contagious kerato-conjunctivitis in cattle with the use of either of these two herbal prescriptions (38). However, concurrent topical treatment with ofloxacin, a second generation fluoroquinolone antibiotic, called into question the benefit of the herbal prescriptions.

Chinese herbal prevention and treatment of chlamydiosis in swine includes oral administration of a 1:1 mixture of plantain (*Plantago asiatica* L., Cheqian) and white eclipta (*Eclipta alba*, Han Lian Cao) at a dose of 10 g per kg bodyweight for 14 days (39). In Chinese medicine, bactericidal and expectorant properties are ascribed to plantain (40). A major active compound in white eclipta is wedelolactone, for which an immunomodulating activity has been demonstrated. In Swiss albino mice, oral administration of methanol-extracted wedelolactone from white eclipta significantly increased:

– the phagocytotic index after intravenous injection of carbon ink

– total white blood cell count after cyclophosphamide-induced myelosuppression

– humoral antibody titre after intra-peritoneal injection of sheep red blood cells (41).

A matter of concern inherent to Chinese traditional medicine is that it largely relies on tradition instead of on scientifically sound efficacy and safety trials as required in Western medicine. Although centuries of tradition are no proof of product efficacy and safety, Chinese medicine should not be repudiated without investigation. It is indisputable that many herbs contain bioactive substances that can be associated with beneficial effects, and several of these secondary plant metabolites are synthetically produced for use in conventional medicine. However, herbal medicine lacks standardised dosing and involves a risk of co-administration of toxic plant compounds. Moreover, in contrast to conventional drugs, traditional medicines are not subjected to strict regulations and a mandatory quality control policy. The latter poses an additional safety risk that must not be underestimated. Contamination with heavy metals, adulteration with undeclared synthetic drugs and cases of mistaken plant identity have all been repeatedly associated with severe adverse effects following the use of Chinese herbal remedies (42).

Vaccination

Currently, commercial vaccines are available only for prevention of *C. abortus* infection. Moreover, the vaccines available in Europe and the United States are licensed only for use in sheep, immunisation in other ruminants being off-label usage. At present, the preferred strategy for control of epizootic ovine abortion is vaccination and keeping flocks closed, but implementing these measures does not guarantee exclusion of infection and ensuing disease (43). These vaccines are not available in China; however, the Lanzhou Veterinary Research Institute markets its own formalin-inactivated whole-organism vaccine for use in both sheep and goats.

Public health significance of chlamydiosis in China

Zoonotic transmission of chlamydial species has been described for *C. psittaci* from poultry and less frequently for *C. abortus* from small ruminants (44). Globally, human acquisition of *C. psittaci* from mammals is yet to be substantiated. Similarly, although zoonotic transfer of *C. pecorum* or *C. suis* has never been officially reported, preliminary results indicate that *C. suis* can be transmitted to humans (Vanrompay *et al*., unpublished results).

From a search of the literature it appears that a confirmed case of zoonotic transfer of *C. abortus* has not been reported in China. However, a possible transfer of *C. abortus* from swine to man has been described in Beijing, based on DIF positivity of throat swabs from pig farmers (23.5%; 4/17) concomitant with DIF positivity of boar sperm (37.5%, 6/16) and sow vaginal swabs (27.5%, 11/40), as analysed using the IMAGEN™ Chlamydia test. At the same time, *C. abortus* antibodies were also detected in sera of sampled swine using the ID Screen® *Chlamydia abortus* ELISA (17). Nevertheless, confirmed transmission of *C. abortus* from swine to humans has yet to be demonstrated.

Conclusion

Virulent chlamydial strains are highly prevalent in Chinese livestock, causing disease, abortion and economic losses, as in the rest of the world. Stringent surveillance is warranted in view of the spread of Chinese isolates through the export of live animals. A general lack of biosecurity measures in extensive management systems in rural China is a matter of concern with respect to the control of chlamydial infections in livestock and the ensuing risk of zoonotic transfer. Another critical concern is the widespread Chinese practice of substituting antibiotic therapy with herbal medicines that lack scientifically sound trials of efficacy/safety and standardised dosing of active compounds. Checks on the possible co-administration of toxic secondary plant metabolites and a general mandatory quality-control policy are also lacking. Notwithstanding the inherent risks of inadequate treatment and even accidental poisoning following herbal treatment, it is indisputable that many herbs contain bioactive substances that can be associated with beneficial effects. Herbal remedies should therefore not be repudiated without investigation.

References

1. Aitken I.D. (2000). – Chlamydial abortion. *In* Diseases of sheep (W.B. Martin & I.D. Aitken, eds), 3rd Ed. Blackwell Science, 81–86.

2. Everett K.D. (2000). – *Chlamydia* and *Chlamydiales*: more than meets the eye. *Vet. Microbiol.*, **75** (2), 109–126.

3. Schautteet K. & Vanrompay D. (2011). – *Chlamydiaceae* infections in pig. *Vet. Res.*, **42**, 29.

4. Eggemann G., Wendt M., Hoelzle L.E., Jager C., Weiss R. & Failing K. (2000). – Prevalence of *Chlamydia* infections in breeding sows and their importance in reproductive failure [in German]. *Dtsch. tierärztl. Wochenschr.*, **107** (1), 3–10.

5. Vanrompay D., Geens T., Desplanques A., Hoang T.Q., De Vos L., Van Loock M., Huyck E., Mirry C. & Cox E. (2004). – Immunoblotting, ELISA and culture evidence for *Chlamydiaceae* in sows on 258 Belgian farms. *Vet. Microbiol.*, **99**, 59–66.

6. Reinhold P., Sachse K. & Kaltenboeck B. (2011). – *Chlamydiaceae* in cattle: commensals, trigger organisms, or pathogens? *Vet. J.*, **189** (3), 257–267.

7. Mair T.S. & Wills J.M. (1992). – *Chlamydia psittaci* infection in horses: results of a prevalence survey and experimental challenge. *Vet. Rec.*, **130** (19), 417–419.

8. Windhorst H.W. (2008). – Asia's changing role in the global egg industry: an analysis of past, present and foreseeable future dynamics. *World Poult. Sci. J.*, **64** (4), 533–552.

9. Food and Agriculture Organization of the United Nations (2010). – FAOSTAT database. Available at: faostat.fao.org/site/339/default.aspx (accessed in July 2012).

10. Huang W.X. (1996). – Conservation and management of yak genetic diversity in the Tibetan Autonomous Region. *In* Conservation and management of yak genetic diversity: proceedings of workshop (D.J. Miller, S.R. Craig & G. Rana, eds), 29–31 October, Kathmandu, 93–104.

11. Zhaoli Y., Guangrong L. & Ning W. (2002). – A probe into the pastoral production system in Hongyuan, eastern Qinghai-Tibetan plateau. *In* Yak production in central Asian highlands (H. Jianlin, C. Richard, O. Hanotte, C. McVeigh & J.E.O. Rege, eds). Proceedings of the third international congress on yak, Lhasa, 4–9 September 2000. International Livestock Research Institute, Nairobi, 120–127.

12. Dong S., Long R. & Kang M. (2007). – Milking performance of China yak (*Bos grunniens*): a preliminary report. *Afr. J. agric. Res.*, **2** (3), 52–57.

13. World Organisation for Animal Health (OIE) (2012). – Chapter 2.3.1.: Avian chlamydiosis. *In* Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (mammals, birds and bees), 7th Ed. Available at: http://www.oie.int/fileadmin/Home/fr/Health_standards/tahm/2.03.01_AVIAN_CHLAMYD.pdf (accessed on 27 January 2013).

14. Chen H., Wen X. & Liao D. (1995). – Studies on detection of anti-chlamydial antibodies in pig by Dot-ELISA [in Chinese]. *Chin. J. prev. vet. Med.*, **95**, 10–13.

15. Zhang H., Wen X. & Cao S. (2007). – Detection of chlamydial antibodies in porcine sera by dot horseradish peroxidase enzyme-linked staphylococcus protein A immunosorbent assay (Dot-PPA-ELISA) [in Chinese]. *Chin. J. prev. vet. Med.*, **29**, 637–640.

16. Brade H., Brade L. & Nano F.E. (1987). – Chemical and serological investigations on the genus-specific lipopolysaccharide epitope of *Chlamydia*. *Proc. natl Acad. Sci. USA*, **84** (8), 2508–2512.

17. Tian D., Liu Z., Li J., Yang J., Xin X., Wang M. & He C. (2012). – Preliminary investigation of *Chlamydophila abortus* in intensive pig farms [in Chinese]. *Chin. J. anim. Hus. vet. Med.*, **39**, 210–213.

18. Wilson K., Livingstone M. & Longbottom D. (2009). – Comparative evaluation of eight serological assays for diagnosing *Chlamydophila abortus* infection in sheep. *Vet. Microbiol.*, **135** (1–2), 38–45.

19. Xie Q., He C., Shi J. & Yan H. (2001). – Diagnosis of chlamydiosis in sheep and cattle sera by monoclonal antibody [in Chinese]. *Chin. J. vet. Sci. Technol.*, **31**, 21–22.

20. Qiu H. & Xie Q. (2006). – Survey on chlamydiosis and brucellosis in sheep and cattle in Ningxia [in Chinese]. *Chin. J. Zoonoses*, **22**, 1004–1005.

21. Wang F.I., Shieh H. & Liao Y.K. (2001). – Prevalence of *Chlamydophila abortus* infection in domesticated ruminants in Taiwan. *J. vet. med. Sci*., **63** (11), 1215–1220.

22. Van Loock M., Vanrompay D., Herrmann B., Vander Stappen J., Volckaert G., Goddeeris B.M. & Everett K.D. (2003). – Missing links in the divergence of *Chlamydophila abortus* from *Chlamydophila psittaci*. *Int. J. syst. evolut. Microbiol*., **53** (3), 761–770.

23. Yang J., Hao Y., Zhao D. & He C. (2006). – Development of a real-time PCR assay for detection and quantification of chlamydiae using SYBR green and the light cycler [in Chinese]. *Acta vet. Zootech. Sinica*, **41**, 84–90.

24. Yang X., Zhang Y., Xu D., Yin S., Shuai Y. & Huang M. (1981). – Study of abortigenic chlamydiae in sheep and goats [in Chinese]. *Vet. Sci. China*, **15**, 16.

25. Liao Y., Chain C., Lu Y., Li N., Tsai H. & Liou P. (1997). – Epizootic of *Chlamydia psittaci* infection in goats in Taiwan. *J. basic Microbiol*., **37** (5), 327–333.

26. Shuai Y., Li Y., Dan K., Duan Y., Cheng G., Yang Z., Yang X., Diao Y., Yu G. & Li X. (1988). – Diagnosis of enzootic abortion in yak [in Chinese]. *Sci. agric. Sinica*, **21**, 76–81.

27. Yang Y., Jiang T., Fang Y., Meng Q., Tan H. & Zhou Y. (1986). – Diagnosis of chlamydiosis and brucellosis mixed infection in cow [in Chinese]. *Hubei J. anim. vet. Sci*., **4**, 9.

28. Qiu C., Zhou J., Cheng S. & Cao X.A. (2006). – Identification and immunogenicity of pathogen causing chlamydial abortion in dairy cows [in Chinese]. *Vet. Sci. China*, **36**, 270–273.

29. Xiao D., Jiang H., Li L., Huang Y., Wang Z., Zheng J., Wang Y. & Yu H. (2006). – Study on newborn calf chlamydial arthritis [in Chinese]. *China Dairy Cattle*, **34**, 37.

30. Yang Y., Jiang T., Meng Q., Fang Y. & Han S. (1984). – Isolation and identification of chlamydiae in pigs [in Chinese]. *Chin. J. vet. Med.*, **10**, 4–9.

31. Ren Y., Lu H., Liang H. & Zhang Z. (1994). – Survey on chlamydiosis in pigs in Guangdong province [in Chinese]. *Chin. J. vet. Med.*, **20**, 37.

32. Qiu C. (2002). – Progress in the study of avian chlamydiosis [in Chinese]. *Chin. J. anim. Hus. vet. Med.*, **8**, 12–15.

33. Yang Y., Jiang T., Fang Y., Li F. & Zhou Y. (1991). – *Chlamydia psittaci* isolated from faeces in six pig farms [in Chinese]. *Hubei J. anim. vet. Sci.*, **22**, 14.

34. Bao G. & Guo H. (1992). – Serological survey on chlamydiosis in a stud farm in Menyuan [in Chinese]. *Chin. J. Prev. vet. Med.*, **24** (2), 22.

35. Mohamad K.Y. & Rodolakis A. (2010). – Recent advances in the understanding of *Chlamydophila pecorum* infections, sixteen years after it was named as the fourth species of the *Chlamydiaceae* family. *Vet. Res.*, **41** (3), 27.

36. Chinese National Standard (2007). – GB 21317-2007: Determination of tetracyclines residues in food of animal origin – LC-MS/MS method and HPLC method. Standardization Administration of the People's Republic of China, Beijing.

37. Xie H. & Preast V. (2010). – Xie's Chinese Veterinary Herbology. Wiley-Blackwell, Oxford.

38. Luo S. & Abulaitepu K. (2009). – Cattle infectious keratoconjunctivitis treated by integrated traditional and Western medicines [in Chinese]. *Chin. J. trad. vet. Sci.*, **3**, 34–35.

39. Lin Z. (2002). – Chlamydiosis in pigs treated by integrated traditional and Western medicines [in Chinese]. *Chin. J. anim. Sci. vet. Med.*, **21**, 42.

40. Ferris H. & Zheng L. (1999). – Plant sources of Chinese herbal remedies: effects on *Pratylenchus vulnus* and *Meloidogyne javanica*. *J. Nematol.*, **31**, 241–263.

41. Jayathirtha M.G. & Mishra S.H. (2004). – Preliminary immunomodulatory activities of methanol extracts of *Eclipta alba* and *Centella asiatica*. *Phytomedicine*, **11** (4), 361–365.

42. Rodolakis A. & Souriau A. (1983). – Response of ewes to temperature-sensitive mutants of *Chlamydia psittaci* (*var. ovis*) obtained by NTG mutagenesis. *Ann. Rech. vét.*, **14** (2), 155–161.

43. Longbottom D., Entrican G., Wheelhouse N., Brough H. & Milne C. (2013). – Evaluation of the impact and control of enzootic abortion of ewes. *Vet. J.*, **195** (2), 257–259.

44. Longbottom D. & Coulter L. (2003). – Animal chlamydioses and zoonotic implications. *J. comp. Pathol.*, **128** (4), 217–244.

45. Shuai Y., Huang M., Ge W., Cheng X. & Yang X. (1981). – Study of chlamydial abortion in sheep and goats [in Chinese]. *Vet. Sci. China*, **11**, 3–7.

46. Wang G. & Liu R. (1986). – Investigation of chlamydial abortion in sheep [in Chinese]. *Xinjiang agric. Sci.*, **38**, 39.

47. Yang Y., Jiang T., Fang Y., Xu T., Li G., Gan Ge., Li F., Qin W., Zhou Y. & Xia B. (1992). – Serological investigation of chlamydiosis in livestock in Hubei province [in Chinese]. *Hubei J. anim. vet. Sci.*, **18**, 22.

48. Ge W., Shuai Y., Dan K., Li Y., Yang Z. & Yang X. (1986). – Detection of antibodies against chlamydial abortion of ewes by ELISA (enzyme-linked immunosorbent assay) [in Chinese]. *Chin. J. vet. Sci. Technol.*, **16**, 16–20.

49. Han Y. & Chen G. (2010). – Monitoring chlamydiae in cattle and sheep in Gangcha County [in Chinese]. *Shandong J. anim. Sci. vet. Med.*, **31**, 105–106.

50. Liang J. & Xiao C. (1989). – Serological investigation of chlamydiosis in sheep in Jinlin with complement fixation test [in Chinese]. *Jilin anim. Sci. vet. Med.*, **11**, 45–46.

51. Wang X. & Zhao G. (1990). – Investigation of chlamydial infection in sheep in Zhejiang [in Chinese]. *Chin. J. vet. Sci. Technol.*, **20**, 13.

52. Liu B., Yang Y., Pei T., Bai J., Bie Q. & Wang Y. (1991). – Serological investigation of sheep chlamydiosis in Gansu and Qinghai pasture lands where troops are stationed [in Chinese]. *Chin. J. vet. Sci. Technol.*, **21**, 24–25.

53. Lin Z., Huang M., Zhang Z. & Liang D. (1991). – Survey on chlamydiosis in livestock in Shanxi Province [in Chinese]. *Chin. J. vet. Sci. Technol.*, **21**, 19–21.

54. Du C., Jin Z., Guo Z., Zhang D., Ma J., Li H., Lian C., Kong X., Zhan F. & Zhang Z. (1991). – Serological survey on sheep abortion pathogens in Gansu Province [in Chinese]. *Chin. J. vet. Sci. Technol.*, **21**, 15–16.

55. Jin G., Huang X., Zhao H., Ren D., Wang Z., Yan S., Shi C. & Feng S. (1993). – Investigation and pathogen isolation of chlamydial infection in humans and livestock in Xinjiang [in Chinese]. *Chin. J. Zoonoses*, **9**, 38–40.

56. Feng G., Chen X., Pan Y., Qi X., Mi F., Yang B. & Song W. (2007). – Serological surveillance of ovine chlamydiosis and comparison of two indirect haemagglutination assays [in Chinese]. *Chin. J. vet. Med.*, **43**, 19–20.

57. Qiu L., Fu S., Qin S., Hu S., Chen H. & Shu X. (1998). – Serological survey on six epidemic diseases of cattle and sheep in Huaihua, Hunan [in Chinese]. *Hunan J. anim. Sci. vet. Med.*, **17**, 18.

58. Wang Q., Zhang J. & Zhang R. (2000). – Survey of chlamydiosis in livestock in Honghe, Yunnan [in Chinese]. *Chin. J. prev. vet. Med.*, **22**, 66–67.

59. Wu L., Liu Q., Zhen L., Chen Z. & Teng B. (2000). – Serological survey of goat chlamydiosis in Guangxi [in Chinese]. *Chin. J. vet. Sci. Technol.*, **30**, 41.

60. Han F. (2001). – Serological investigation of brucellosis and chlamydiosis in goats in Xunhua, Qinghai [in Chinese]. *Chin. Qinghai J. anim. vet. Sci.*, **31**, 34.

61. Huang X., Wei T., Huang S., Li H., Zhou L. & Feng J. (2001). – Serological survey on bluetongue and chlamydiosis of goats in Guangxi [in Chinese]. *Sichuan anim. vet. Sci.*, **28**, 22.

62. Liu B., Liu Y., Wang Q., Wang Z., Guo J. & Yang W. (2003). – Investigation of the cause of caprine abortion in Honghe Prefecture of Yunnan province [in Chinese]. *Chin. J. vet. Sci. Technol.*, **33**, 30–34.

63. Yang H. (2004). – Serological survey of goat chlamydiosis in Dunan, Qinghai [in Chinese]. *Chin. J. anim. Health. Inspect.*, **21**, 32.

64. Lu Y., Li X. & Ma S. (2005). – Serological survey on chlamydiosis in a stud farm in Qinghai [in Chinese]. *Chin. Qinghai J. anim. Vet. Sci.*, **35**, 26.

65. Yao S., He Y., Ren X., Hang B., Wang C. & Liu X. (2008). – Epidemiological investigation of chlamydiosis in swine and sheep [in Chinese]. *Chin. J. vet. Med.*, **44**, 36–37.

66. Yuan Y. & Ma L. (2007). – Serological investigation of chlamydiosis in small-tailed Han sheep in Qinghai [in Chinese]. *Chin. J. anim. Health Inspect.*, **24**, 37.

67. Fan X., Wang S. & Ma S. (2008). – Survey of sheep chlamydiosis in Haiyan County, Qinghai province [in Chinese]. *Anim. Breed. Feed.*, 52–53.

68. Wang C. & Wei Y. (2009). – Serological investigation of sheep chlamydiosis in alpine pastures [in Chinese]. *Chin. J. anim. Health Inspect.*, **31**, 80–81.

69. He C., Bao S., Kong X. & Zhu J. (2010). – Serological investigation of chlamydiosis in sheep and cattle in Haibei pasturing area surrounding Qinghaihu Lake [in Chinese]. *Chin. J. anim. Health Inspect.,* **27**, 48.

70. Ran S., Jin W., Li C., Tian X., Ya H. & Dong T. (2010). – Serological survey and study of three epidemic diseases in Yingde'er breeding sheep farm of Qinghai province [in Chinese]. *Chin. J. anim. Health Inspect.*, **27**, 54–55.

71. Tie F. (2010). – Serological survey of chlamydiosis in sheep [in Chinese]. *Chin. anim. Husb. vet. Med.*, **38**, 234–235.

72. Min Y., Li W., Li F. & Zhang X. (2010). – Serological detection of chlamydiosis of Native Tibetan sheep in Tianjun County [in Chinese]. *Chinese Qinghai J. anim. Vet. Sci.*, **40**, 15.

73. Wang L., Sun C., Sui L., Tang K., Wang X., Wang G., Qiu K. & Si H. (2010). – Epidemiological survey on sheep chlamydiosis in a part of Hulun Buir City [in Chinese]. *Anim. Husb. Feed Sci.*, **31**, 154.

74. Zhao G.H., Zhang M.T., Lei L.H., Shang C.C., Cao D.Y., Tian T.T., Li J., Xu J.Y., Yao Y.L., Chen D.K. & Zhu X.Q. (2011). – Seroprevalence of *Toxoplasma gondii* infection in dairy goats in Shaanxi province, Northwestern China. *Parasit. Vectors*, **4** (1), 47.

75. Jiang W., Xiao K. & Xiang X. (1989). – A serological study of chlamydial infection in cattle and pigs in Hunan [in Chinese]. *J. Hunan agric. Univ.*, **15**, 97–101.

76. Zuo X., Tong Q., Hou D. & Hu S. (1991). – Serological investigation of chlamydiosis in cattle and rabbits in Santan County in Sichuan province [in Chinese]. *Sichuan anim. vet. Sci.*, **4**, 30.

77. Yuan C., Yuan W., Shuai Y., Li Y. & Yang X. (1994). – Chlamydial abortion pathogen isolation and immunisation in yak in Yushu, Qinghai [in Chinese]. *Chin. J. vet. Sci. Technol.*, **24**, 20–21.

78. Wang G., Shi B. & Ma X. (1996). – Serosurvey on chlamydiosis in yak [in Chinese]. *Cao Shi Jia Chu* (*Grass-Feeding Livestock*), **3**, 46.

79. Lizhong M. (2002). – A diagnosis study of brucellosis and *Chlamydia* infection in yak. *In* Yak production in central Asian highlands (H. Jianlin, C. Richard, O. Hanotte, C. McVeigh & J.E.O. Rege, eds). Proceedings of the third international congress on yak, Lhasa, 4–9 September 2000. International Livestock Research Institute, Nairobi, 463–464.

80. Zhou J., Qiu C., Cheng S., Gao S., Zhang X. & Li Z. (2000). – Serological investigation of chlamydiosis in meat cattle in parts of China [in Chinese]. *Chin. J. vet. Sci. Technol.*, **30**, 14–15.

81. Qiu C., Cheng S., Gao S. & Zhou J. (2001). – Seroprevalence of *Chlamydia* infection in buffaloes in China. *Indian J. anim. Sci.*, **71**, 27.

82. Geilhausen H. (2002). – Serological survey on infectious diseases of a White yak herd in the Gansu Province. *In* Yak production in central Asian highlands (H. Jianlin, C. Richard, O. Hanotte, C. McVeigh & J.E.O. Rege, eds). Proceedings of the third international congress on yak, Lhasa, 4–9 September 2000. International Livestock Research Institute, Nairobi, 445–449.

83. Changqing Q., Yongxin H., Jizhang Z., Xiao-An C. & Fangfang Z. (2006). – Epidemiological investigation of chlamydiosis in dairy cattle in China. *Indian J. anim. Sci.*, **76** (12), 985–987.

84. Ma S., Li X., Lu Y., Yang Q., Ben X. & Ma D. (2004). – Serological investigation of chlamydiosis in yak [in Chinese]. *China Qinghai J. anim. vet. Sci*, **34**, 14.

85. He C., Zhang H. & Zhang G. (2007). – Pathogen isolation and serosurvey of chlamydial abortion in dairy cows [in Chinese]. *Gansu anim. vet. Sci.*, **37**, 10–12.

86. Yang D., Ye Z., Yang Z. & Zhang J. (2008). – Diagnosis and treatment of chlamydial abortion in cows [in Chinese]. *Chin. J. vet. Med.*, **44**, 72–73.

87. Tie F. (2010). – Serosurvey on chlamydiosis of yak in Haiyan County [in Chinese]. *Chin. J. anim. Health Inspect.*, **27**, 49.

88. Jiang T., Yang Y., Meng Q., Fang Y. & Han S. (1985). – Serological survey on chlamydiosis in swine [in Chinese]. *Chin. J. vet. Sci. Technol.*, **15**, 25–27.

89. Qiu C., Gao S., Zhou J., Cheng S. & Shuai Y. (2003). – Investigation on chlamydiosis in pigs [in Chinese]. *Prog. vet. Med*, **24**, 88–91.

90. Meng Q., Jiang T., Fang Y. & Yang Y. (1987). – An indirect complement fixation (ICF) test for detection of anti-chlamydial antibodies in pigs [in Chinese]. *Chin. J. Zoonoses*, **3**, 21–24.

91. Jiang W., Xiao K. & Xiang X. (1989). – A serological study of chlamydial infection in cattle and pig in Hunan [in Chinese]. *J. Hunan agric. Univ.*, **15**, 97–101.

92. Chen H., Wen X. & Liao D. (1995). – Studies on detection of anti-chlamydial antibodies in pig by Dot-ELISA [in Chinese]. *Chin. J. Prev. vet. Med.*, **95**, 10–13.

93. Jin A., Gu B., Wei X. & Fang G. (2005). – Serological study of pig chlamydiosis in Shanghai and other provinces [in Chinese]. *Shanghai J. anim. Husb. vet. Med.*, **6**, 23.

94. Lu W. (2007). – Serological investigation of swine chlamydiosis in Guangxi district and isolation and identification of *Chlamydia psittaci* [in Chinese]. Thesis, Nanjing Agricultural University.

95. Zhang H., Wen X. & Cao S. (2007). – Detection of chlamydial antibodies in porcine sera by dot horseradish peroxidase enzyme-linked staphylococcus protein A immunosorbent assay (Dot-PPA-ELISA) [in Chinese]. *Chin. J. Prev. vet. Med.*, **29**, 637–640.

96. Zhou P., Zhen Z., Wang T., Luo C., Qiu S. & Zhang H. (2008). – Epidemiological investigation of porcine chlamydiosis sera in several regions of Longyan City [in Chinese]. *Prog. vet. Med.*, **29**, 30–35.

97. Xu M., He Y., Liang R., Zhou D., Lin R., Yin C., He X., Liang M. & Zhu X. (2010). – Seroprevalence of *Chlamydia* infection in pigs from intensive farms in Southern China. *J. anim. vet. Adv.*, **9** (7), 1143–1145.

98. Bi J., Yang B., Gao L., Yang G., Tai X. & Yin G. (2011). – Serological investigation of porcine chlamydial infection in Yunnan Province [in Chinese]. *Prog. vet. Med.*, **32**, 134–136.

99. Deng C., Wang B. & Chen E. (1983). – Enzootic abortion in sheep: isolation, identification and pathogenicity of chlamydial strain [in Chinese]. *Chin. J. Prev. vet. Med.*, **1**, 23–27.

100. Song L., Li Y., Liu G., He J., Zhu H. & Duan Q. (2009). – Genotyping of *Chlamydophila psittaci* strains derived from avian and mammalian species. *Vet. Res. Commun.*, **33** (6), 577–580.

101. Xu W., Wang J., Qin J., Shi S. & Zhou X. (1987). – Isolation and diagnostic studies of chlamydiosis in sheep in Yunan province [in Chinese]. *Chin. J. Prev. vet. Med.*, **3**, 4–5.

102. Lin Z., Huang M., Zhang Z., Liang D., Li Y., Duan Y., Gao S. & Yang X. (1992). – Isolation and identification of the pathogen of chlamydial abortion in dairy cows [in Chinese]. *Chin. J. vet. Sci. Technol*., **22**, 28–31.

103. Qiu C., Zhou J., Gao S., Chen S., Duan Y., Li Y., Chen G. & Yang X. (1998). – Monitoring of pig chlamydiosis in large-scale pig farms in Hubei and five other provinces (regions) [in Chinese]. *Chin. J. vet. Sci. Technol*., **28**, 3–5.

104. Li J., Du H., Zhu C., Xu G. & Huang W. (2000). – Isolation and identification of pseudorabies virus and *Chlamydia psittaci* [in Chinese]. *J. Sichuan Inst. anim. Husb. vet. Med*., **3**, 9–13.

Table I
Prevalence of chlamydial antibodies in Chinese sheep and goats

Year	Province	Seroprevalence		Test	Species	Symptoms	Ref. no
1981	Qinghai	27.7%	(47/176)	CFT	Goat	n/a	(45)
1981	Gansu	36.7%	(4/11)	CFT	Goat	n/a	(45)
1983	Xinjiang	25.5%	(27/106)	CFT	Sheep/goat	Abortion	(46)
1985	Hubei	6.8%	(324/4,753)	CFT, IHA	Sheep/goat	n/a	(47)
1985	Hubei	29.8%	(25/84)	CFT,IHA	Sheep/goat	Abortion, conjunctivitis	(47)
1986	Yunnan	60.7%	(17/28)	ELISA[a]	Goat	Abortion	(48)
1986	Xinjiang	20.0%	(14/70)	ELISA[a]	Goat	Abortion	(48)
1989	Qinghai	15.4%	(53/344)	IHA	Sheep/goat	n/a	(49)
1989	Jilin	6.0%	(138/2,291)	CFT	Sheep	n/a	(50)
1990	Zhejiang	3.2%	(28/872)	IHA	Sheep	n/a	(51)
1991	Gansu	6.6%	(13/198)	IHA	Sheep	n/a	(52)
1991	Shaanxi	1.6%	(103/6,615)	IHA	Sheep/goat	n/a	(53)
1991	Gansu	34.4%	(360/1,047)	IHA	Sheep/goat	Abortion	(54)
1993	Xinjiang	8.48%	(126/1,486)	IHA	Sheep	n/a	(55)
1993	Xinjiang	27.1%	(23/85)	IHA	Sheep	Abortion	(55)
1993–1995	Chinese Taipei	82.0%	(246/300)	ELISA[b]	Goat	Abortion	(25)
1997–2002	Beijing	21.0%	(713/3,398)	IHA	Sheep	n/a	(56)
1998	Hunan	5.9%	(29/496)	IHA	Goat	n/a	(57)
1999–2000	Chinese Taipei	16.7%	(4/24)	ELISA[b]	Goat	All clinically healthy	(21)
1999–2000	Chinese Taipei	58.0%	(65/112)	ELISA[b]	Goat	All recently aborted	(21)
2000	Yunnan	26.1%	(1,024/3,917)	IHA	Sheep/goat	n/a	(58)
2000	Guangxi	2.0%	(43/2,168)	IHA	Goat	n/a	(59)
2001	Qinghai	2.9%	(20/686)	IHA	Goat	n/a	(60)
2001	Guangxi	1.1%	(69/6,273)	IHA	Goat	n/a	(61)
2003	Ningxia	19.3%	(113/586)	IHA	Sheep	Cough, wheezing, thin	(62)
2003	Yunnan	32.3%	(1,268/3,925)	IHA	Goat	n/a	(62)
2004	Qinghai	11.2%	(20/179)	IHA	Goat	n/a	(63)
2005	Qinghai	4.8%	(8/165)	IHA	Sheep	n/a	(64)
2006	Sichuan	5.6%	(15/268)	IHA	Sheep	n/a	(28)
2007	Beijing	18.0%	(116/646)	IHA	Sheep	n/a	(56)
2007	Henan	18.4%	(7/38)	IHA	Goat	Abortion	(65)
2007	Qinghai	4.9%	(11/224)	IHA	Sheep	n/a	(66)
2008	Qinghai	5.5%	(13/237)	IHA	Sheep	n/a	(67)
2009	Gansu	26.3%	(172/654)	IHA	Sheep	Abortion, orchitis, arthritis	(68)
2010	Qinghai	2.6%	(8/303)	IHA	Sheep/goat	n/a	(49)
2010	Qinghai	7.4%	(91/1,223)	IHA	Sheep	n/a	(69)
2010	Qinghai	19.0%	(33/174)	IHA	Goat	Abortion	(70)

2010	Qinghai	3.9%	(7/179)	IHA	Sheep	n/a	(70)
2010	Qinghai	8.6%	(27/314)	IHA	Sheep	Abortion	(71)
2010	Qinghai	9.2%	(29/316)	IHA	Sheep	n/a	(72)
2010	Inner Mongolia	7.6%	(103/1,360)	IHA	Sheep	Abortion, orchitis	(73)
2010	Shaanxi	2.9%	(21/729)	IHA	Goat	n/a	(74)

CFT: complement fixation test (developed in-house)

ELISA: enzyme-linked immunosorbent assay

IHA: indirect haemagglutination assay (Lanzhou Veterinary Research Institute, China)

n/a: information on health status not available

a) ELISA developed in-house

b) commercial ELISA (ImmunoComb)

Table II
Prevalence of chlamydial antibodies in Chinese large ruminants

Year	Province	Seroprevalence		Methods	Species	Symptoms	Ref. no.
1985	Hubei	8.7%	(382/4386)	CFT, IHA	Cattle (dairy)	n/a	(47)
1986	Hubei	26.8%	(37/138)	CFT, IHA	Cattle (dairy)	Abortion, pneumonia, conjunctivitis	(27)
1988	Qinghai	29.0%	(45/155)	CFT	Yak	Abortion	(26)
1989	Qinghai	26.7%	(24/90)	IHA	Cattle	n/a	(49)
1989	Hunan	8.1%	(33/410)	IHA	Cattle (dairy)	n/a	(75)
1991	Shaanxi	28.3%	(107/378)	IHA	Cattle (dairy)	n/a	(53)
1991	Sichuan	6.5%	(7/107)	IHA	Cattle	n/a	(76)
1993	Xinjiang	4.9%	(35/711)	IHA	Cattle	n/a	(55)
1994	Qinghai	42.7%	(236/553)	IHA	Yak	Abortion	(77)
1996	Xinjiang	2.1%	(6/288)	IHA	Yak	n/a	(78)
1996	Xinjiang	1.8%	(3/171)	IHA	Cattle (dairy)	n/a	(78)
1996	Hainan	2.9%	(15/526)	IHA	Yak	n/a	(79)
1999–2000	Chinese Taipei	51.3%	(377/735)	ELISA	Cattle	All clinically healthy	(21)
1999–2000	Chinese Taipei	71.4%	(45/63)	ELISA	Cattle	All recently aborted	(21)
2000	Shandong	16.1%	(10/62)	IHA	Cattle (meat)	n/a	(80)
2000	Henan	25.7%	(9/35)	IHA	Cattle (meat)	n/a	(80)
2000	Ningxia	16.8%	(16/95)	IHA	Cattle (meat)	n/a	(80)
2000	Hebei	23.1%	(3/13)	IHA	Cattle (meat)	n/a	(80)
2000	Shaanxi	42.2%	(57/132)	IHA	Cattle (meat)	n/a	(80)
2000	Gansu	10.8%	(4/37)	IHA	Cattle (meat)	n/a	(80)
2000	Gansu	20.7%	(18/87)	IHA	Yak	n/a	(80)
2000	Gansu	25.5%	(24/47)	IHA	Yak	n/a	(81)
2000	Qinghai	15.5%	(22/142)	IHA	Yak	n/a	(80)
2000	Sichuan	25.6%	(10/39)	IHA	Yak	n/a	(80)
2000	Yunnan	34.6%	(823/2,378)	IHA	Cattle	n/a	(58)
2000	Guanxi	20.0%	(10/50)	IHA	Buffalo	n/a	(82)
2000	Anhui	18.7%	(28/150)	IHA	Buffalo	n/a	(82)
2000	Jiangsu	14.0%	(20/143)	IHA	Buffalo	n/a	(82)
2002–2004	Guangdon	49.1%	(27/55)	IHA	Cattle (dairy)	n/a	(83)
2002–2004	Jiangsu	0.0%	(0/20)	IHA	Cattle (dairy)	n/a	(83)
2002–2004	Shanghai	0.0%	(0/49)	IHA	Cattle (dairy)	n/a	(83)
2002–2004	Henan	20.8%	(10/48)	IHA	Cattle (dairy)	n/a	(83)
2002–2004	Ningxia	25.4%	(177/698)	IHA	Cattle (dairy)	n/a	(83)
2002–2004	Gansu	43.2%	(19/44)	IHA	Cattle (dairy)	n/a	(83)
2002–2004	Shaanxi	10.3%	(25/243)	IHA	Cattle (dairy)	n/a	(83)
2002–2004	Shanxi	10.0%	(1/10)	IHA	Cattle (dairy)	n/a	(83)
2002–2004	Inner Mongolia	15.7%	(11/70)	IHA	Cattle (dairy)	n/a	(83)

2002–2004	Heilongjiang	17.2%	(20/116)	IHA	Cattle (dairy)	n/a	(83)
2004	Qinghai	19.2%	(30/156)	IHA	Yak	Abortion	(84)
2007	Ningxia	28.4%	(108/380)	IHA	Cattle (dairy)	Abortion	(85)
2008	Ningxia	53.3%	(16/30)	IHA	Cattle (dairy)	Abortion	(86)
2010	Qinghai	2.0%	(6/300)	IHA	Cattle	n/a	(48)
2010	Qinghai	5.9%	(19/321)	IHA	Yak	n/a	(87)
2010	Qinghai	2.5%	(25/1,410)	IHA	Cattle	n/a	(69)

CFT: complement fixation test (developed in-house)

ELISA: enzyme-linked immunosorbent assay (CHEKIT®)

IHA: indirect haemagglutination assay (Lanzhou Veterinary Research Institute, China)

n/a: information on health status not available

Table III
Prevalence of chlamydial antibodies in Chinese swine

Year	Province	Seroprevalence		Seropositive farms		Methods	Symptoms	Ref. no.
1985	Hubei	29.7%	(244/821)	91.2%	(31/34)	IHA	n/a	(88)
1986	Qinghai	33.3%	(101/333)	n/a		IHA	n/a	(89)
1987	Sichuan	11.5%	(462/2,989)	n/a		IHA	n/a	(89)
1987	Xinjiang	22.2%	(303/1428)	n/a		IHA	n/a	(89)
1987	Hubei	29.9%	(256/857)	n/a		ICFT	n/a	(90)
1988	Shanxi	38.0%	(19/50)	n/a		IHA	n/a	(89)
1989	Hunan	9.0%	(10/111)	n/a		IHA	Abortion, stillbirth	(91)
1990	Guanxi	21.2%	(21.2/1428)	n/a		IHA	n/a	(89)
1991	Yunnan	24.5%	(1,342/5,477)	n/a		IHA	n/a	(89)
1992	Guangdong	50.2%	(542/1,080)	n/a		IHA	n/a	(89)
1992	Gansu	42.2%	(1,604/3,709)	n/a		IHA	n/a	(89)
1993	Henan	35.1%	(10/28)	n/a		IHA	n/a	(89)
1994	Guangdong	50.2%	(542/1,080)	100%	(23/23)	IHA	n/a	(31)
1995	Henan	29.1%; 47.3%	(32/110; 52/110)	n/a		CFT, ELISA[(a)]	n/a	(92)
1999	Sichuan	80.0%	(48/60)	n/a		IHA	n/a	(89)
1999	Ningxia	15.1%	(18/119)	n/a		IHA	n/a	(89)
2000	Qinghai	42.9%	(6/4)	n/a		IHA	n/a	(89)
2000	Henan	18.2%	(6/33)	n/a		IHA	n/a	(89)
2000	Hainan	38.2%	(21/55)	n/a		IHA	n/a	(89)
2000	Hubei	37.5%	(3/8)	n/a		IHA	n/a	(89)
2000	Hunan	16.7%	(6/36)	n/a		IHA	n/a	(89)
2001	Jianxi	50.0%	(4/8)	n/a		IHA	n/a	(89)
2001	Liaoning	43.2%	(32/74)	n/a		IHA	n/a	(89)
2001	Heilongjiang	77.1%	(27/35)	n/a		IHA	n/a	(89)
2001	Jilin	20.5%	(8/39)	n/a		IHA	n/a	(89)
2005	Shanghai	41.4%	(65/157)	55.6%	(5/9)	IHA	n/a	(93)
2005	Jiangsu	1.7%	(3/174)	25.0%	(1/4)	IHA	n/a	(93)
2005	Zheijiang	6.8%	(12/176)	62.5%	(5/8)	IHA	n/a	(93)
2007	Guangxi	51.0%	(197/386)	66.7%	(14/21)	IHA	Abortion	(94)
2007	Sichuan	21.7%; 35.8%	(26/120; 43/120)	n/a		IHA, ELISA[(a)]	n/a	(95)
2008	Fujian	27.7%	(641/2,313)	100%	(15/15)	IHA	n/a	(96)
2010	Guangdong	30.8%	(313/1,017)	93.3%	(14/15)	IHA	n/a	(97)
2011	Yunnan	19.4%	(232/1,257)	n/a		IHA	n/a	(98)
2012	Bejing	4.1%; 2.2%	(21/507; 11/507)	n/a		IHA, ELISA[(b)]	n/a	(17)

ELISA: enzyme-linked immunosorbent assay
ICFT: indirect complement fixation test (developed in-house)
IHA: indirect haemagglutination assay (Lanzhou Veterinary Research Institute, China)
n/a: no data available
a) ELISA developed in-house
b) ID Screen® *Chlamydia abortus* ELISA

Table IV

Prevalence of chlamydial antibodies in Chinese horses

Year	Province	Seroprevalence		Methods	Symptoms	Ref. no.
1985	Hubei	9.3%	(70/754)	CFT, IHA	n/a	(47)
1992	Qinghai	6.9%	(6/87)	IHA	Pneumonia, diarrhoea, swollen joints	(34)
1993	Xinjiang	2.0%	(5/251)	IHA	n/a	(55)
2000	Yunnan	35.4%	(953/2,691)	IHA	n/a	(58)

CFT: complement fixation test (developed in-house)

IHA: indirect haemagglutination assay (Lanzhou Veterinary Research Institute, China)

n/a: information on health status not available

Table V

Molecular characterisation using *ompA* sequencing of chlamydial isolates from Chinese ruminants and swine

Strain	Year	Tissue	Species	Clinical symptoms	*OmpA* sequencing	Ref. no.
N	1981	Gastric content, organs (aborted kid)	Goat	Abortion	n/a	(45)
N	1983	Liver (aborted lamb)	Sheep	Abortion	n/a	(99)
N	1984	Aborted piglet	Swine	Abortion	n/a	(30)
N	1984	Synovial fluid (piglet)	Swine	Polyarthritis	n/a	(30)
CW1	1986	Aborted calf	Cattle	Abortion	n/a	(27)
CW2	1986	Colostrum	Cattle	Abortion, mastitis	*C. psittaci* genotype C	(27, 100)
CW3	1986	Milk	Cattle	Abortion, mastitis	*C. psittaci* genotype C	(27, 100)
N	1987	Synovial fluid (lamb)	Sheep	Polyarthritis	n/a	(101)
N	1988	Aborted calf (gastric content)	Yak	Abortion	n/a	(26)
CCS-5 / CCS-10	1992	Aborted calf	Cattle	Abortion	n/a	(102)
CYY1 / CYY2	1992	Aborted calf	Yak	Abortion	n/a	(102)
n/a	1997	Aborted kid, placenta, vaginal swab	Goat	Abortion	n/a	(25)
HB1	1998	Aborted piglet	Swine	Abortion	*C. psittaci*	(103)
HB2	1998	Vaginal swab (sow)	Swine	Abortion	*C. psittaci*	(103)
HB3	1998	Piglet	Swine	Pneumonia, enteritis	*C. psittaci*	(103)
CR99	2000	Aborted piglet	Swine	Abortion	n/a	(104)
N	n/a	n/a	Cattle/goat	Abortion	*C. abortus* bovine strain LW508	(21)
LZ1	2006	Milk	Cattle	Abortion	*C. psittaci*	(28)
SX5	2006	Liver (aborted calf)	Cattle	Abortion	*C. psittaci*	(28)
NX	2006	Gastric content (aborted calf)	Cattle	Abortion	*C. psittaci*	(28)
N	2006	Gastric content (aborted calf)	Cattle	Abortion	n/a	(85)
CG1	n/a	Lung	Sheep	Pneumonia	*C. psittaci*	(100)
CE1 / CE9	n/a	n/a	Sheep	Enteritis	*C. psittaci*	(100)

N: No given name
n/a: no available data

Table VI
Ingredient composition of Xiao Huang San (clearing yellow swelling powder) and Jue Ming San (haliotis or abalone powder) (37)

Common name	Scientific name	Chinese name	Concentration (%)	
			Xiao Huang San	Jue Ming San
Glauber's salt (mirabilite)	$Na_2SO_4.10H_2O$	Mang Xiao	22.5	–
Abalone shell (haliotis shell)	*Concha haliotidis*	Shi Jue Ming	–	14.1
Chinese senna (sicklepod)	*Senna obtusifolia*	Jue Ming Zi	–	14.1
Chinese rhubarb	*Rheum palmatum*	Da Huang	7.5	9.4
Myrrh	*Commiphora myrrha*	Mo Yao	–	6.3
Goldthread	*Coptis chinensis*	Huang Lian	7.5	6.3
Dioscera tuber	*Dioscorea bulbifera*	Hung Yao Zi	6.25	9.4
Stephania	*Stephania cephalantha* Hayata	Bai Yao Zi	6.25	9.4
Weeping golden bells	*Forsythiae suspensae*	Lian Qiao	6.25	–
Anemarrhena	*Anemarrhena asphodeloides*	Zhi Mu	6.25	–
Thunberg fritillaria bulb	*Fritillaria thunbergii*	Zhe Bei Mu	5	–
Membranous milk vetch	*Astragalus membranaceus*	Huang Qi	5	9.4
Gardenia jasmin	*Gardenia jasminoides*	Zhi Zi	5	9.4
Chinese skullcap	*Scutellaria baicalensis*	Huang Qin	5	6.3
Curcuma	*Curcuma* spp.	Yu Yin	5	6.3
Siler root	*Ledebouriella divaricata*	Fang Feng	5	–
Cicada moulting	*Cryptotympana atrata*	Chan Tui	3.75	–
Liquorice root	*Glycyrrhiza uralensis*	Gan Cao	3.75	–