Little is known about *Coxiella burnetii* infection among cattle farmers and farm residents in China. Thus, the present study was conducted to detect the seroprevalence of *C. burnetii* infection and estimate associated risk factors among cattle farmers and farm residents in China. A cross-sectional study was designed, and sera of 362 people living or working on 106 cattle farms were tested for *C. burnetii* IgG and IgM antibodies by immunofluorescence assay. Overall *C. burnetii* seroprevalence was 35.6% (129/362, 95% CI: 30.70–40.57), and 112 participants had experienced a past infection and seventeen (4.7%) had experienced a relatively recent infection. In the final combined multilevel model, the following activities were significantly associated with presence of antibodies against *C. burnetii*: milking cattle, providing general healthcare to cattle, providing birth assistance, contact dead-born animals, urbanization, and presence of mice and/or rats in the stable. Moreover, presence of disinfection equipment was a significant protective factor. This is the first study addressing the seroprevalence and risk factors of *C. burnetii* infection in cattle farmers and farm residents in three northeastern provinces and Inner Mongolia Autonomous Region, China.
2. Materials and Method

2.1. Study Population and Data Collection. This study was approved by the Animal Ethics Committee of Jilin Agriculture University, China. All cattle farms in three northeastern provinces and Inner Mongolia Autonomous Region with at least 50 cattle that were not vaccinated for Q-fever were selected from the register in the census of the zone. As an important cattle and sheep breeding base in China, with the development of economy, farms with different sizes were settled up quickly in Inner Mongolia Autonomous Region. The three northeastern provinces (Jilin, Liaoning, and Heilongjiang provinces) are comprehensive agricultural bases. Poultry, pigs, cattle, sheep, and deer are the main breeding animals in these areas. On eligible farms, we approached cattle farmers and one or two of their household members aged 12 years and older, and in some cases, other persons working or living on the farm such as farm employees. A maximum of five participants were included per farm. Nonresponders received a reminder 3 weeks after the initial invitation. After providing informed consent on farm and individual level, all participating farms were visited by professional laboratory assistants, who collected sera from October 2013 through July 2014. Each participant completed a questionnaire about personal characteristics (e.g., age, medical history, farm-related activities, contact with livestock and companion animals, and use of personal protective equipment). The farm owner or manager completed a questionnaire about herd size, cattle housing, presence of other livestock and companion animals, farm facilities, and hygiene measures.

2.2. Serological Method. An immunofluorescence assay (IFA) (Focus Diagnostics, Cypress, CA, USA) was used to test serum samples for *C. burnetii* phases I and II IgM and IgG. All samples were screened at an initial dilution of 1:32; those with negative results were considered negative. Positive samples were further classified as indicative of relatively recent infections (IgM phase II titer >32) or past infections (IgG phase II titer >32 and IgM phase II titer <32). Samples with all other outcomes were considered negative. The term relatively recent was chosen because phase II IgM is commonly found up to 1 year after infection in acute Q fever cases, but it may persist up to 3 years [16]. Phases I and II IgG end point titers were determined for all seropositive persons. In agreement with chronic Q fever diagnostic criteria used in the Netherlands [17], phase I IgG titers ≥1,024 in samples in the past infection group were considered indicative of possible chronic infection.

2.3. Statistical Analysis. Results were analyzed with SPSS 19.0 software package. For comparison of the frequencies among the groups, the Mantel-Haenszel test and when indicated the Fisher exact test were used. Bivariate, multivariate, and multilevel analyses were used to assess the association between participant- and farm-based characteristics of the subjects and the *C. burnetii* infection. Variables were included in the multivariate analysis if they had a P value of equal or less than 0.20 in the bivariate analysis. Adjusted odd ratio (OR) and 95% confidence interval were calculated by multivariate analysis using multiple, unconditional, and logistic regression. A P value less than 0.05 was considered statistically significant.

3. Results

3.1. Descriptive Characteristics. Of all 197 invited eligible cattle farms, 106 (53.8%) farms participated in this study. The number of cattle farms from Heilongjiang, Jilin, Liaoning, and Inner Mongolia was 21, 28, 22, and 35, respectively. The mean herd size was 95 cattle (range 50–327) in participating farms. From the 106 participating farms, 362 persons provided a blood sample (mean age 46.0 years (12–68), 45.3% male) (Table 1). All of the farm-based and participant-based questionnaires were completed by the 106 farmers and 362 persons.

3.2. Seroprevalence of *C. burnetii*. Overall *C. burnetii* seroprevalence was 35.6% (129/362, 95% CI: 30.70–40.57), and seroprevalence among farmers, spouses, children, and others was 38.3%, 31.5%, 31.1%, and 37.1%, respectively (Table 1). Of the 129 seropositive participants, 112 participants had experienced a past infection and seventeen (4.7%) had experienced a relatively recent infection, as demonstrated by presence of IgM phase II antibodies. IgG phase II end titers were known for the 76 participants with a past infection with IgG phase I ≥1:32: 1:32 (n = 25), 1:64 (n = 14), 1:128 (n = 13), 1:256 (n = 13), 1:512 (n = 10), and ≥1:1024 (n = 1). For the 36 participants with a past infection with IgG phase I ≥1:32, 3 persons had “possible chronic Q fever” with IgG phase I titers ≥1:1024 according to diagnostic standard used in the Netherlands [8]. We could not confirm that these truly were chronic Q fever cases due to lack of clinical information.
Table 2: Bivariate logistic regression analysis of participant-based characteristics associated with *Coxiella burnetii* positivity among cattle farmers and farm residents in three northeastern provinces and Inner Mongolia Autonomous Region, China.

| Variable                                      | Category          | Freq. (N) | Seroprevalence (%) | OR (95% CI)       | P value   |
|-----------------------------------------------|-------------------|-----------|--------------------|-------------------|-----------|
| Work and/or live on farm                     | Work and live     | 245       | 40.41              | 2.03 (0.98–4.22)  | 0.023     |
|                                               | Work, but not live| 73        | 26.03              | 1.06 (0.45–2.49)  |           |
|                                               | Not working, but live | 44   | 25.00              | Reference         |           |
| Hours working on farm                         | Fulltime          | 174       | 37.36              | 1.32 (0.65–2.66)  | 0.853     |
|                                               | Halftime          | 109       | 35.78              | 1.23 (0.59–2.59)  |           |
|                                               | Quarter week      | 34        | 32.35              | 1.06 (0.41–2.76)  |           |
|                                               | Sometimes/never   | 45        | 31.11              | Reference         |           |
| How often in stable                           | Every day         | 189       | 38.62              | 1.39 (0.70–2.79)  |           |
|                                               | Every week        | 104       | 35.58              | 1.22 (0.58–2.58)  |           |
|                                               | Every month       | 38        | 28.95              | 0.90 (0.35–2.32)  | 0.426     |
|                                               | Less than once a month/never | 31 | 25.81 | Reference | |
| Feeding cattle                                | Yes               | 299       | 38.45              | 2.19 (1.16–4.14)  | 0.014     |
|                                               | No                | 63        | 22.22              | Reference         |           |
| Milking cattle                                | Yes               | 203       | 43.84              | 2.32 (1.48–3.65)  | <0.001    |
|                                               | No                | 159       | 25.16              | Reference         |           |
| General healthcare of cattle                  | Yes               | 241       | 42.32              | 2.56 (1.53–4.21)  | <0.001    |
|                                               | No                | 121       | 22.31              | Reference         |           |
| Remove manure                                 | Yes               | 286       | 35.66              | 1.01 (0.59–1.71)  | 0.982     |
|                                               | No                | 76        | 35.53              | Reference         |           |
| Spread manure                                 | Yes               | 258       | 40.31              | 2.13 (1.28–3.57)  | 0.002     |
|                                               | No                | 104       | 24.04              | Reference         |           |
| Clean stables                                 | Yes               | 269       | 39.03              | 1.84 (1.09–3.11)  | 0.022     |
|                                               | No                | 93        | 25.81              | Reference         |           |
| Birth assistance                              | Yes               | 216       | 42.59              | 2.19 (1.38–3.46)  | <0.001    |
|                                               | No                | 146       | 25.34              | Reference         |           |
| Administration                                | Yes               | 219       | 38.81              | 1.43 (0.91–2.23)  | 0.076     |
|                                               | No                | 143       | 30.77              | Reference         |           |
| Wear overalls or boots                        | Yes               | 233       | 38.63              | 1.45 (0.92–2.30)  | 0.110     |
|                                               | No                | 129       | 30.23              | Reference         |           |
| Having a dog                                  | Yes               | 294       | 35.03              | 0.87 (0.51–1.50)  | 0.619     |
|                                               | No                | 68        | 38.24              | Reference         |           |
| Having a cat                                  | Yes               | 278       | 35.25              | 0.93 (0.56–1.55)  | 0.782     |
|                                               | No                | 84        | 36.90              | Reference         |           |
| Direct contact with cattle in their own or other farms | Yes | 287       | 38.68              | 2.00 (1.12–3.57)  | 0.018     |
|                                               | No                | 75        | 24.00              | Reference         |           |
| Direct contact with horses in their own or other farms | Yes | 210       | 39.52              | 2.56 (1.61–4.07)  | <0.001    |
|                                               | No                | 152       | 23.68              | Reference         |           |
| Contact with raw milk                         | Yes               | 243       | 43.21              | 3.01 (1.80–5.04)  | <0.001    |
|                                               | No                | 119       | 20.17              | Reference         |           |
| Contact with cattle manure                    | Yes               | 225       | 44.00              | 2.80 (1.73–4.54)  | <0.001    |
|                                               | No                | 137       | 21.90              | Reference         |           |
| Contact with dead-born animals                | Yes               | 196       | 45.41              | 2.62 (1.67–4.12)  | <0.001    |
|                                               | No                | 166       | 24.30              | Reference         |           |
| Contact with placenta/birth material          | Yes               | 188       | 38.30              | 1.27 (0.83–1.96)  | 0.272     |
|                                               | No                | 174       | 32.76              | Reference         |           |
Table 3: Bivariate logistic regression analysis of farm-based characteristics associated with *Coxiella burnetii* positivity among cattle farmers and farm residents in three northeastern provinces and Inner Mongolia Autonomous Region, China.

| Variable                              | Category                     | Number of humans tested | Positive (%) | OR (95% CI)        | P value |
|---------------------------------------|------------------------------|-------------------------|--------------|--------------------|---------|
| Region                                |                              |                         |              |                    |         |
|                                       | Heilongjiang                 | 98                      | 30.61        | Reference          |         |
|                                       | Jilin                        | 76                      | 27.63        | 0.87 (0.45–1.68)   | 0.667   |
|                                       | Liaoning                     | 92                      | 31.52        | 1.04 (0.56–1.93)   | 0.892   |
|                                       | Inner Mongolia               | 96                      | 51.04        | 2.36 (1.31–4.25)   | 0.004   |
| Urbanization                          | Moderate or minor urban area  | 149                     | 20.81        | Reference          | <0.001  |
|                                       | Rural area                   | 213                     | 46.01        | 3.24 (2.01–5.24)   |         |
| Herd size                              |                              |                         |              |                    |         |
|                                       | Small (50–100)               | 197                     | 32.48        | Reference          | 0.393   |
|                                       | Medium (100–150)             | 104                     | 39.42        | 1.35 (0.83–2.22)   |         |
|                                       | Large (>150)                 | 61                      | 39.34        | 1.35 (0.74–2.44)   |         |
| Beef cattle on the farm               | Yes                          | 202                     | 37.13        | 1.16 (0.75–1.79)   | 0.505   |
|                                       | No                           | 160                     | 33.75        | Reference          |         |
| Number of stables                     | >3 stables                   | 117                     | 32.48        | 0.81 (0.51–1.30)   | 0.386   |
|                                       | ≤3 stables                   | 245                     | 37.14        | Reference          |         |
| Use of artificial insemination        | Yes                          | 84                      | 46.43        | 1.81 (1.10–2.98)   | 0.018   |
|                                       | No                           | 278                     | 32.37        | Reference          |         |
| Laying hens on farm                   | Yes                          | 243                     | 40.33        | 1.92 (1.18–3.11)   | 0.008   |
|                                       | No                           | 119                     | 26.05        | Reference          |         |
| Presence of cat(s) in cattle stable   | Present                      | 198                     | 36.36        | 1.07 (0.70–1.65)   | 0.751   |
|                                       | Absent                       | 164                     | 34.76        | Reference          |         |
| Use of silage                         | Yes                          | 276                     | 40.22        | 2.54 (1.43–4.51)   | 0.001   |
|                                       | No                           | 86                      | 20.93        | Reference          |         |
| Use of maize                          | Yes                          | 288                     | 38.39        | 1.79 (1.01–3.17)   | 0.045   |
|                                       | No                           | 74                      | 25.68        | Reference          |         |
| Feeding method                        | Fodder mixer or automatic    | 241                     | 32.37        | 0.66 (0.42–1.03)   | 0.067   |
|                                       | Hand/wheelbarrow             | 121                     | 42.15        | Reference          |         |
| Presence of mice and/or rats in the stable | Present                   | 199                     | 45.23        | 2.63 (1.67–4.14)   | <0.001  |
|                                       | Absent                       | 163                     | 23.93        | Reference          |         |
| Farm visitors                         | Yes                          | 143                     | 37.76        | 1.17 (0.75–1.81)   | 0.495   |
|                                       | No                           | 219                     | 34.25        | Reference          |         |
| Farm boots for staff                  | Yes                          | 277                     | 36.46        | 1.17 (0.70–1.95)   | 0.553   |
|                                       | No                           | 85                      | 32.94        | Reference          |         |
| Presence of hygienic locker room      | Yes                          | 269                     | 31.97        | 0.55 (0.34–0.88)   | 0.013   |
|                                       | No                           | 93                      | 46.24        | Reference          |         |
| Presence of disinfection equipment    | Yes                          | 224                     | 29.46        | 0.50 (0.32–0.77)   | 0.002   |
|                                       | No                           | 138                     | 45.65        | Reference          |         |
| Birds in stable                       | Yes                          | 90                      | 45.56        | 1.75 (1.08–2.85)   | 0.023   |
|                                       | No                           | 272                     | 32.35        | Reference          |         |
| Veterinary service                    | Yes                          | 301                     | 31.62        | 1.40 (0.78–2.54)   | 0.273   |
|                                       | No                           | 61                      | 29.51        | Reference          |         |
| Type of farm management               | Closed herd                  | 298                     | 34.89        | 0.84 (0.48–1.46)   | 0.528   |
|                                       | Purchase of cattle           | 64                      | 39.06        | Reference          |         |

(e.g., presence of vascular infection, endocardial involvement, or other clinical risk factors).

3.3. Risk Factors for *C. burnetii* Infection. All individual and farm-based variables, which were tested in the bivariate analysis for relationship with human *C. burnetii* seropositivity, are shown in Tables 2 and 3.

In the multivariate analyses, from 14 individual variables which were associated in the bivariate analysis, six were independently associated with *C. burnetii* seropositivity (Table 4). Moreover, 5/18 farm-based variables included in the multilevel analyses were significantly independent risk or protective factors and together were used as the full multilevel start model (Table 5).
Table 4: Multivariate logistic regression analysis of participant-based characteristics associated with Coxiella burnetii positivity among cattle farmers and farm residents in three northeastern provinces and Inner Mongolia Autonomous Region, China.

| Variable          | Category | Adjusted odds ratio | 95% confidence interval | P value |
|-------------------|----------|---------------------|-------------------------|---------|
| Milking cattle    | Yes      | 1.88                | 1.21–2.94               | 0.005   |
|                   | No       | Reference           |                         |         |
| General healthcare of cattle | Yes  | 2.40                | 1.46–3.93               | <0.001  |
|                   | No       | Reference           |                         |         |
| Birth assistance  | Yes      | 2.07                | 1.31–3.27               | 0.002   |
|                   | No       | Reference           |                         |         |
| Contact raw milk  | Yes      | 2.47                | 1.50–4.06               | <0.001  |
|                   | No       | Reference           |                         |         |
| Contact cattle manure | Yes  | 3.23                | 1.92–5.44               | <0.001  |
|                   | No       | Reference           |                         |         |
| Contact dead-born animals | Yes  | 3.45                | 2.16–5.50               | <0.001  |
|                   | No       | Reference           |                         |         |

a The variables included were those with a P ≤ 0.20 obtained in the bivariate analysis.

b Adjusted by age and the rest of characteristics included in this table.

Table 5: Multivariate logistic regression analysis of farm-based characteristics associated with Coxiella burnetii positivity among cattle farmers and farm residents in three northeastern provinces and Inner Mongolia Autonomous Region, China.

| Variable          | Category                | Adjusted odds ratio | 95% confidence interval | P value |
|-------------------|-------------------------|---------------------|-------------------------|---------|
| Urbanization      | Rural area              | 3.66                | 2.25–5.96               | <0.001  |
|                   | Moderate or minor urban area | Reference          |                         |         |
| Laying hens on farm | Yes        | 2.04                | 1.25–3.32               | 0.004   |
|                   | No                      | Reference           |                         |         |
| Use of silage     | Yes                     | 1.99                | 1.15–3.44               | 0.013   |
|                   | No                      | Reference           |                         |         |
| Presence of mice and/or rats in the stable | Present | 2.49                | 1.58–3.91               | <0.001  |
|                   | Absent                  | Reference           |                         |         |
| Presence of disinfection equipment | Yes  | 0.58                | 0.37–0.90               | 0.015   |
|                   | No                      | Reference           |                         |         |

a The variables included were those with a P ≤ 0.20 obtained in the bivariate analysis.

b Adjusted by age and the rest of characteristics included in this table.

In the final combined multilevel model, significant risk factors were milking cattle, general healthcare of cattle, birth assistance, contact dead-born animals, urbanization, and presence of mice and/or rats in the stable. Moreover, presence of disinfection equipment was a significant protective factor (Table 6).

4. Discussion

This is the first study exploring the seroprevalence in cattle farmers and farm residents in China, and one of few risk factor studies on human C. burnetii infections in farm populations worldwide [18–20], suggesting that living and or working on cattle farm has a high lifetime risk for acquiring C. burnetii infection. Farmers and other household members are usually at highest risk for acquiring C. burnetii infection due to close contact with infected cattle and contaminated stables on farms.

The detected seroprevalence was high not only for the farmers (38.3%), as expected, but also among spouses (31.5%), children (31.1%), and others (37.1%) who lived and often also worked at the farm. The present seroprevalence obviously overs the estimates of 10.2% in the people studied to date in China [14]. The seroprevalence was also lower than those in other studies focusing on, nonfurther specified, farm populations, such as 49% among farmers from Northern Ireland [21], 72.1% in dairy cattle farmers in the Netherlands [18], but was comparable to the 27% seroprevalence in a farm cohort in the United Kingdom [10]. However, it is complicated to compare these seroprevalences due to some differences, including geographical conditions, the different study populations, diagnostic methods, and living styles. Moreover, in the present study, females have a higher seroprevalence than males. It is contrary to other places in the world where studies have suggested that males are more susceptible to C. burnetii infection [2]. Routine activities of women in rural areas including taking care of livestock and cleaning stables result in the high prevalence in females in China [14].

Several independent individual and farm-based risk factors for C. burnetii seropositivity were found such as working
Table 6: Combined multilevel analysis of participant- and farm-based characteristics associated with Coxiella burnetii seropositivity among cattle farmers and farm residents in three northeastern provinces and Inner Mongolia Autonomous Region, China.

| Variable                        | Category         | Adjusted odds ratio<sup>b</sup> | 95% confidence interval | P value |
|---------------------------------|------------------|---------------------------------|-------------------------|---------|
| Milking cattle                  | Yes              | 2.09                            | 1.33–3.27               | 0.001   |
|                                 | No               | Reference                        |                         |         |
| General healthcare of cattle    | Yes              | 2.25                            | 1.38–3.67               | 0.001   |
|                                 | No               | Reference                        |                         |         |
| Birth assistance                | Yes              | 1.86                            | 1.18–2.92               | 0.005   |
|                                 | No               | Reference                        |                         |         |
| Contact dead-born animals       | Yes              | 2.67                            | 1.69–4.45               | <0.001  |
|                                 | No               | Reference                        |                         |         |
| Urbanization                    | Rural area       | 2.34                            | 1.41–3.45               | <0.001  |
|                                 | Moderate or minor urban area | Reference                       |                         |         |
| Presence of mice and/or rats in the stable | Present | 1.95                            | 1.38–3.01               | 0.002   |
|                                 | Absent           | Reference                        |                         |         |
| Presence of disinfection equipment | Yes            | 0.37                            | 0.20–0.49               | <0.001  |
|                                 | No               | Reference                        |                         |         |

<sup>a</sup>The variables included were those with a P ≤ 0.20 obtained in the bivariate analysis.<br>
<sup>b</sup>Adjusted by age and the rest of the characteristics included in this table.

and/or living in farm, milking cattle, general healthcare of cattle, birth assistance, contacting raw milk, contacting cattle manure, contacting dead-born animals, urbanization, laying hens on farm, use of silage, presence of mice and/or rats in the stable, presence of disinfection equipment, and birds in stable. The individual risk factor involving direct contact with cattle or dust-producing activities in the cattle stable, such as milking, general healthcare of cattle, clean stables, birth assistance, contacting raw milk, contacting cattle manure, and contacting dead-born animals, reflects the stable environment contact [22]. Under these circumstances the risk of inhalation of contaminated aerosols is high, with a potential increased risk for acquiring an infection. The degree of total farm animal contact has been reported to seem more important than particular animal exposure, suggesting that risk of C. burnetii exposure is largely connected with farm environment contact [6, 22].

Two farm-related risk factors were identified to be associated with human seropositivity among cattle farm residents/staff: urbanization and presence of mice and/or rats in the stable. The concentration and management of cattle farming in the rural area of the study regions possibly promoted transmission between farms. The presence of mice and/or rats in the stable was observed as risk factor for human seropositivity, suggesting C. burnetii introduction or facilitation of spread by infected wild animals [23–25]. Moreover, presence of disinfection equipment was observed as protective factor for human seropositivity.

It is worth noting that Q fever is out of notifiable diseases in China and thus it is not easy to get test facilities. Most cases are diagnosed through retrospective and epidemiological studies which implies that misdiagnosis often occurred for acute cases, resulting in the greater possibility of chronic infections which have a poor prognosis and high mortality [14]. Thus, routine serological follow-up is helpful for prevention as well as basic biological safety rules, such as hygiene measures and the use of protection clothes.

To conclude, high C. burnetii seroprevalences demonstrate that cattle farmers and farm residents have a substantial lifetime risk for acquiring this zoonotic infection. We recommend reinforcing routine biosecurity measures to avoid indirect spread, avoiding access of companion and wild animals to the stable, and offer advice on eliminating nuisance animals in the cattle stables. Clinicians should strengthen their awareness to consider Q fever in this occupational group presenting with compatible symptoms or relevant sequelae to allow diagnosis and therapy in time.

Conflict of Interests

The authors declare that there is no conflict of interests in this paper.

Acknowledgment

Project support was provided by the project National Natural Science Foundation of China (Grant no. 31272566).

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