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Risk factors associated with *Salmonella* in dairy cattle farms in Henan and Hubei provinces, China

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Abstract

A cross-sectional study was conducted to evaluate the risk factors associated with the *Salmonella* infection status of dairy herds in Henan and Hubei provinces, China. Herds were assigned a *Salmonella* status based on the isolation of *Salmonella* from fecal samples obtained from adult lactating dairy cows. Information on potential biosecurity risk factors was collected using a questionnaire template via a 15-min face-to-face interview with dairy cattle producers from May 2020 to March 2021. The questionnaire consisted of 23 questions across two broad categories of potential biosecurity risk factors: farm and biosecurity managemental factors. A total of 48 farms were surveyed. In all (100%, 48/48) of the surveyed farms, although sick animals were separated from healthy animals using fencing, there were no strict quarantine protocols for newly introduced animals. Mixed species rearing was practiced in 35.4% (17/48) of the farms. Feces were removed more than once a day in 45.8% (22/48) of the farms, whereas the remainder (54.2%, 26/48) only removed animal feces once a day. A total of 29.2% (14/48) of the farms were located < 2 km from other livestock farms. The use of personal protective equipment was consistently performed on all farms, with a majority of the workers on most farms (81.3%, 39/48) always disinfecting footwear before entering production areas. A significant association between *Salmonella*-positive status and the high frequency of the presence of wildlife (birds and rodents in sheds and feed storage places) was recorded (OR: 11.9, 95% CI: 1.7, 84.1, $p = 0.013$). Fortunately, no farms shared farm equipment with other farms. The study highlights the occurrence of wildlife as a risk factor for the presence of *Salmonella* in investigated dairy herds. There is a need to institute appropriate on-farm control measures for wild birds and rodents to control the potential spread of *Salmonella* in dairy production systems.

Keywords Cattle, Management practices, Risk factors, *Salmonella*

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Introduction

Implementing optimal management and husbandry practices on farms not only improves the health and welfare of farm animals but also enhances productivity and food safety by potentially controlling foodborne pathogens, such as *Salmonella* (Agren et al. 2016) and *Escherichia coli* (Farrokh et al. 2013). In China, in 2006, the government published the national technical standards on sanitation for interventions (Standardization Administration of the People's Republic of China 2006). These guidelines specified the requirements relevant to several aspects of dairy farming, including feeding animals, hygiene of the environment and facilities, the health of staff, and surveillance programs. However, the standards have not been widely adopted (Fu et al. 2018). At the same time, several international studies have reported that the implementation of voluntary biosecurity programs is primarily driven by farmers (Sayers et al. 2013; Jones et al. 2015; Aleri and Laurence 2020).

Salmonella, an enteric microorganism, can cause diseases in adult cattle and calves, and result in cases of human foodborne infections (Fossler et al. 2005). Recently, *Salmonella* was detected in half of the dairy farms sampled in Western Australia (Aleri et al. 2022). In the USA, Nagoette et al. (2019) reported that 8.1% of bulk tank milk samples were positive for *Salmonella*. In China, Yang et al. (2014) reported that 2.0% of infant formula was also contaminated with *Salmonella*. Foodborne disease data collected from China in 2015 highlighted that *Salmonella* was the second leading cause of bacterial foodborne diseases (Fu et al. 2019).

There is a continued need for surveillance and control programs in animal production systems to ensure food safety (Cerva et al. 2014; McAuley et al. 2014; Ramos et al. 2020). Henan and Hubei provinces are located in central China, and although the dairy industry is important in these two provinces, no previous surveillance for *Salmonella* has been undertaken within the industry. This study was designed to evaluate potential biosecurity risk factors associated with *Salmonella* status in dairy herds from Henan and Hubei provinces. The results of this study have potential benefit in the control and eradication of *Salmonella* from dairy cattle farms in these, and potentially other, Chinese provinces.

Results

Response proportion

All 48 dairy farms contacted agreed to participate in the study (100%, 48/48). The main dairy breed on all farms (100%, 48/48) was Holstein–Friesian.

Farm demographics

Of the 48 farms, 20.8% (10/48) were located in Hubei Province, and 79.2% (38/48) were located in Henan Province. No significant differences between demographic variables were recorded between the two provinces ($p < 0.05$) (Tables 1 and 2 for the demographic variables**).** A total of 20.8% (10/48) of the dairy farms were owned by dairy companies, and 79.2% (38/48) were privately employed (Table 3). The medians of the total herd size, number of lactating cows, number of workers caring for sick animals, and farm area (hectare) were 689 (range 89- 4826), 372 (range 50- 2560), 2 (range 1- 6), and 7.7 ha (range 1.1- 66.6), respectively (Table 4). The mean of the operational years was 10.1 ± 4.3 years (standard deviation) (Table 4).

Rearing practices

There was no significant difference ($p = 0.83$) between the stocking density (available spaces per lactating cow) in the two provinces, with a median of 25.1 (range 9.7- 53.3) m^2 per cow (Table 2).

Free-ranging in a shed was practiced on 97.9% (47/48) of the farms, of which 41.7% (20/48) farmers housed lactating cows in a shed with an open-air yard to increase the activity space (Table 3). Animals were tethered in cow sheds on one (2.1%) farm.

A majority (79.2%, 38/48) of the farms utilized underground water for animals to drink, 18.8% (9/48) of the farms used tap water, and 2.1% (1/48) of the farms used stream water. Tap water was the primary source of animal drinking water in Hubei Province (Table 1). Mixed species rearing occurred in approximately one-third (35.4%, 17/48) of the farms (Table 3).

Food troughs were cleaned routinely (every time after feeding) on only 18.8% (9/48) of the farms and occasionally (once or less per day) on 81.3% (39/48) of the farms. The frequency of cleaning water troughs was more than once per two days in more than half (54.2%, 26/48) of the farms, whereas 45.8% (22/48) of the farms cleaned the water troughs every three days or less frequently. The feces in pens were removed more than once daily in 45.8% (22/48) of the farms and once every day in the remaining 54.2% (26/48) of the farms (Table 3).

In most (97.9%, 47/48) farms, workers regularly added bedding material on top of existing bedding, with 2.1% (1/48) of the farms replacing the whole bedding material every six months. In all (100%, 48/48) of the farms, milking parlors were cleaned every time after milking.

Biosecurity management practices

In 18.8% (9/48) of the farms, the interviewees mentioned the high frequency of the presence of wildlife (birds or

Table 1 Description and analysis of categorical variables assessing risk factors associated with *Salmonella* in dairy farms

Categories	Variables	Categories	Henan	Hubei	*p* value[a]
Farm demographics	Dairy company's farm	Yes No	7 31	3 7	0.414
	Salmonella status	Positive Negative	5 33	2 8	0.625
Rearing practices	Water source for animal drinking	Tap water others	2 36	7 3	0.000
	Cleaning adult cow's food troughs every time after feeding	Yes No	5 33	4 6	0.075
	Mixed species rearing	Yes No	11 27	6 4	0.134
	Frequency of cleaning adult cow's water troughs	> once per two days ≤ once per two days	23 15	3 7	0.152
	Housing with the open-air yard	Yes No	17 21	3 7	0.488
	Removing feces in the cow sheds more than once per day	Yes No	18 20	4 6	0.735
Biosecurity management	Proximity to other livestock farms (within a 2-km radius)	Yes No	13 25	1 9	0.242
	Cleaning shoes every time (workers)	Yes No	32 6	7 3	0.370
	Disinfection of visitors	Yes No	34 4	8 2	0.591
	The high frequency of observation of wildlife in the farms	Yes No	8 30	1 9	0.661
	Introduced cattle in the 12 month period prior to the survey	Yes No	8 30	2 8	1.000

[a] Compared between Hubei and Henan with Fisher's exact tests

Table 2 Description and analysis of continuous variables assessing risk factors associated with *Salmonella* in dairy farms

Categories	Variables	Location	Median (IQR[a])	*p* value[b]	Statistical test
Farm demographics	Farm size	Henan Hubei	769.0 (523.0–1469.5) 297.5 (131.0–2559.0)	0.077	Kruskal–Wallis test
	Number of milking cows	Henan Hubei	395.0 (253.0–755.0) 181.5 (95.9–1375.0)	0.103	Kruskal–Wallis test
	Years had been in operation	Henan Hubei	8.0 (6.0–13.0) 11.5 (9.0–13.0)	0.105	t test
	Number of workers cared for sick animals	Henan Hubei	2.0 (1.0–3.0) 2.0 (1.0–3.5)	0.903	Kruskal–Wallis test
	Farm area (hectares)	Henan Hubei	7.7 (5.3–14.2) 8.3 (4.0–20.0)	0.945	Kruskal–Wallis test
Rearing practices	Stocking density (m^2/cow)	Henan Hubei	24.0 (16.5–32.2) 27.9 (11.6–38.1)	0.832	Kruskal–Wallis test

[a] Interquartile range

[b] Comparison between Hubei and Henan

rodents) in the shed or feed storage area and moderate or rare presence in 81.3% (39/48) of the farms. A total of 29.2% (14/48) of the farms had another livestock farm within 2 km of their farms (Table 3).

A total of 79.2% (38/48) of the farms kept closed dairy herds, with the remaining (20.8%, 10/48) introducing some cattle in the 12-month period prior to the survey (Table 3). Sick animals were separated from healthy animals through the use of fencing in all (100%, 48/48) of the farms. However, in all (100%, 48/48) of the farms, no designated areas were reserved for quarantine/isolation of any new stock or sick animals before

Table 3 Description and analysis of categorical variables assessing risk factors associated with *Salmonella* in dairy farms

Categories	Variables	Categories	Positive	Negative	*p* value[a]
Farm demographics	Region	Hubei Henan	2 5	8 33	0.588
	Dairy company's farm	Yes No	2 5	8 33	0.588
Rearing practices	Housing with an open-air yard	Yes No	5 2	15 26	0.102
	Water source for animal drinking	Tap water others	2 5	7 34	0.477
	Mixed species rearing	Yes No	3 4	14 27	0.657
	Cleaning adult cow's food troughs every time after feeding	Yes No	1 6	8 33	0.745
	Frequency of cleaning adult cow's water troughs	> once per two days ≤ once per two days	4 3	22 19	0.864
	Removing feces in the cow sheds more than once per day	Yes No	3 4	19 22	0.864
Biosecurity management	The high frequency of observation of wildlife in the farms	Yes No	4 3	5 36	0.012
	Proximity to other livestock farms (within a 2-km radius)	Yes No	3 4	11 30	0.395
	Introduced cattle in the 12 month period prior to the survey	Yes No	2 5	8 33	0.588
	Cleaning shoes every time (workers)	Yes No	6 1	33 8	0.745
	Disinfection of visitors	Yes No	6 1	36 5	0.877

[a] Using univariable logistic regression analyses

Table 4 Description and analysis of management practices assessing risk factors associated with *Salmonella* in dairy farms

Categories	Variables	*Salmonella* status	Average	Median	Min–max	*p* value[a]
Farm demographics	Number of workers cared for sick animals	Positive Negative	2.1 2.5	2 2	1- 6 1- 5	0.566
	Number of milking cows	Positive Negative	505.0 612.8	287.0 375.0	73- 1400 50- 2560	0.664
	Year had been in operation	Positive Negative	9.6 10.2	10 10	3- 15 4- 23	0.703
	Farm size	Positive Negative	925.7 1127.3	623.0 698.0	89- 2200 125- 4826	0.710
	Farm area (hectares)	Positive Negative	16.0 13.8	10.0 6.7	1.1- 53.3 3.0- 66.6	0.736
Rearing practices	Stocking density (m^2/cow)	Positive Negative	32.3 23.8	33.5 21.1	15.1- 40.0 9.7- 53.3	0.012

[a] Using univariable logistic regression analyses

being introduced/reintroduced into the main herd. In all (100%, 10/10) open dairy farms, quarantine of new stock was performed in a different shed located within 50 m of the existing herd, which was commonly also used to keep lactating cows.

Most farms (87.5%, 42/48) required every visitor to use personal protective equipment (PPE), including gumboots and overalls, and to be disinfected prior to entry, with the remaining 12.5% (6/48) of the farms occasionally practicing this. Workers on every sampled farm were required to wear overalls provided by the farms, and in 81.3% (39/48) of the farms, workers followed the rules of disinfecting gumboots every time before entering a production area (Table 3). No farms shared vehicles or machinery with other farms (100%, 48/48).

Multivariable analysis

Details on the potential biosecurity risk factors (variables) are presented in Table 3 and Table 4. Three variables, “stocking density”, “housing with an open-air yard”, and “high frequency of the presence of wildlife”, had p values < 0.2 in the univariable analyses and were included in the initial saturated multivariable logistic regression model (Tables 3 and 4). The explanatory variable “stocking density” was not included in the final multivariable analyses due to collinearity with the variable “housing with an open-air yard” ($p < 0.01$). A significant association between *Salmonella*-positive status and “the high frequency of the presence of wildlife” on the farms was recorded (odds ratio (OR): 11.9, 95% confidence interval (CI): 1.7, 84.1, $p = 0.013$) (Table 5). After inputting “housing with an open-air yard” in the multiple analysis, the results of Nagelkerke's pseudo-R^2 increased from 0.22 to 0.323, the -2Loglikehood of the logistic regression decreased from 33.53 to 30.22, and the p value of the Hosmer–Lemeshow test was 0.803. Consequently, these two variables were included in the final model (Table 5).

Discussion

Outbreaks of foodborne diseases arising from contaminated milk have been traced back to pathogens carried by cows (Holschbach and Peek 2018), cross-contamination arising from the environment on dairy cattle farms (Qamar et al. 2020) and the production process (Hayman et al. 2020). The objective of this study was to evaluate the potential biosecurity risk factors associated with *Salmonella* in the feces of adult lactating dairy cows in Henan and Hubei provinces.

The present study showed that the presence of wildlife (birds or rodents) might play a vital role in spreading *Salmonella* on farms. As the excreta or tissues of wildlife were not collected in this study, there was no conclusive evidence from this study that *Salmonella* transmission was occurring between wildlife and dairy cattle, and the direction of transmission of *Salmonella* between wildlife and domestic animals could not be determined; however, it has been verified that wildlife can act as a source of *Salmonella* infection on some farms (Medhanie et al. 2014). Mathys reported that wild birds (including migratory and resident birds) could serve as reservoirs for extended-spectrum β-lactamase (ESBL)-resistant enteric bacteria (Mathys et al. 2017). At the same time, “housing with an open-air yard” may increase exposure to wildlife excreta in the cattle environment. Consequently, taking measures to reduce wildlife intrusion and recording animal activity have been suggested in regulatory standards (Topalcengiz et al. 2020). In contrast, in another study, the presence of wildlife in the feed storage location was not associated with the *Salmonella* antibody-positive status of bulk milk (Agren et al. 2017).

In this study, no farms shared vehicles used for transferring manure with other farms. However, vehicles used for transferring manure are a potential biosecurity risk factor for the spread of *Salmonella* between sheds within an individual farm. *Salmonella* can survive in feces at ambient temperature for more than a year and spread via fomites such as clothes, boots and vehicles. The presence of organic matter on equipment enhances the likely transmission of fecal pathogens to cattle and humans (Holschbach and Peek 2018). Hence, training all workers on animal waste management (identification, removal and recording) is essential to minimize this potential risk (Topalcengiz et al. 2020).

In the current study, no association was found between herd size and *Salmonella* status. However, Ruzante and Nielsen reported that *Salmonella* was more likely to be present in large dairy herds than in smaller dairy herds (Ruzante et al. 2010; Nielsen and Dohoo 2012). Similar to the results of Agren et al. (2017), in the univariable models of this study, high stocking density was a risk factor for spreading *Salmonella* ($p = 0.012$).

Salmonella can spread via aerosols. High-power washing can cause splashing and aerosolization of contaminated material, and weather conditions impact the propagation of microbial aerosols across some distance (Bemis et al. 2007; Wu et al. 2018). These findings were

Table 5 Multivariable analysis for the association between management practices and *Salmonella* status in dairy farms

Variables	β	BSD	Wald test	OR (95% CI)	*p* value
The high frequency of observation of wildlife in the farms	2.5	1.0	6.2	11.9 (1.7, 84.1)	0.013
Housing with an open-air yard	1.7	1.0	2.9	5.6 (0.8, 41.6)	0.092
Constant	-3.5	1.0	12.6		

β: Coefficient, BSD: Standard error of the coefficient

OR: Odds ratio, CI: Confidence intervals for the odds ratios substantiated by the findings that a nearby positive farm was a risk for *Salmonella* within herds (Nielson and Dohoo 2012; Agren et al. 2017). However, in the present study, the "presence of other livestock farms within a 2-km radius" was not a risk factor for *Salmonella*. Potential sources of *Salmonella* contamination in the environment include contaminated feed, water, soil, bedding, feeder, manure, and parlor (Qamar et al. 2020). A ten-month study monitoring the water source in dairy farms showed that a high burden of *Salmonella* was related to the source of water (Rodriguez et al. 2012). Herds without a broadcast manure spreader or with a lower frequency of feces removal have also been shown to have an increased risk for *Salmonella* (Habing et al. 2012). A high frequency of cleaning and disinfection in barns might decrease the infection (Habing et al. 2012; Tarazi and Abo-Shehada 2015). However, in our study, a high frequency of removing feces and cleaning food and water troughs was not protective factors for *Salmonella*-free status in sampled dairy herds.

The introduction of adult cattle and open farms poses a risk for the introduction of *Salmonella* (van Schaik et al. 2002; Davison et al. 2006) along with other pathogens and diseases (Khurana et al. 2021). However, in the present study, there was no association with such practices. The practice of screening and isolating new stock (45 days) is important and a critical biosecurity practice (Standardization Administration of the People's Republic of China 2006). However, this study showed that all of the open herds (100.0%, 10/10) failed to quarantine new stock. Routine screening should be undertaken for endemic and potential contagious diseases such as bovine tuberculosis and salmonellosis (Agren et al. 2017; Khurana et al. 2021).

In this study, several important variables, such as location, farm size, introduction of new cattle, and the frequency of removing feces in barns, were observed not to have associations with the herd status for *Salmonella*. Contrary to our results, it was observed that "herds with test-positive neighbors", "large herd size", "purchase from test-positive cattle herds", and "not using a broadcast manure spreader" were associated with *Salmonella* in dairy farms in other studies (Ruzante et al. 2010; Nielson and Dohoo 2012; Agren et al. 2016). In the present study, the failure to find such associations may be based on the low sample size, a limiting factor to this study. Only lactating dairy herds were included in the study, and the absence of younger age groups may underestimate the prevalence of *Salmonella* at the farm level. There is a potential benefit for cohort studies and case–control studies.

Conclusions

The study highlights the occurrence of wildlife as a risk factor for the presence of *Salmonella* in investigated Chinese dairy herds. There is a need to institute appropriate on-farm control measures on wild birds and rodents to control the spread of *Salmonella* in the dairy production systems of Henan and Hubei provinces and other areas of China.

Materials and methods

Study area

The study was conducted from May 2020 to March 2021. Data were collected in Henan and Hubei provinces. The methods and results of the detection of *Salmonella* in the selected dairy farms have been published (Wang et al. 2022, 2023). These two provinces had a human population of approximately 160 million in 2020 (National Bureau of Statistics of China 2021a). The primary dairy breed is Holstein–Friesian, producing approximately 2.2 million tonnes of milk in 2020 (National Bureau of Statistics of China 2021b).

Study design

The source population included all dairy farms registered with the dairy herd improvement (DHI) in Henan (99 farms) and Hubei (10 farms) provinces. The selection criteria included $\geq$ 50 adult milking cows. Cross-sectional studies have been conducted in Henan and Hubei provinces to estimate the herd prevalence of *Salmonella* in dairy farms (Wang et al. 2022, 2023). Participation in those studies was voluntary without any incentives provided. Assuming 95% confidence, 10% desired precision and an expected herd prevalence of 20% with a population size of 109, a total of 40 farms was needed. Ten (including all eligible farms) located in Hubei Province and 38 dairy farms in Henan Province based on convenience were contacted by telephone and invited to participate in the study. All 48 contacted farmers agreed to participate. A questionnaire was administered via a face-to-face interview to collect farm-level information on potential biosecurity risk factors for the presence of *Salmonella* and later matched to the *Salmonella* status of the farm. Individual farms were the unit of concern. Herds with at least one positive sample for *Salmonella* in the feces of sampled animals were classified as positive, and herds with all negative samples were classified as negative. The numbers of samples in each farm in Hubei and Henan provinces were described in previous studies (Wang et al. 2022, 2023).

Sample collection, isolation, and identification of *Salmonella*

Briefly, fecal samples from selected apparently healthy adult lactating dairy cows were obtained by inserting two sterile cotton swabs into the rectum and rotating against the rectal wall during milking, and each fecal sample was placed in an individual sterile tube containing 5 mL of buffered peptone water (BPW) (Hopebio Co., Ltd, Qingdao, China). Samples were transported at 4 ℃ within 24 h of collection to our laboratory, and then samples were examined according to the international standard method EN-ISO 6579:2002/A1: 2007: Amendment 1: Annex D for *Salmonella*. Finally, the presumptive *Salmonella* colonies were confirmed by performing polymerase chain reaction (PCR) and matrix-assisted laser desorption ionization-time-of-flight mass spectrometry (MALDI-TOF MS). As a result, a total of 14.6% (7/48) of the farms were positive for *Salmonella* (Table 1) (Wang et al. 2022, 2023).

Questionnaire design

A questionnaire template was designed and used to obtain data on potential biosecurity risk factors for the presence of *Salmonella* in the surveyed dairy farms. A three-stage process was used to develop and select questions for the final questionnaire (Aleri and Laurence 2020). First, 56 questions on potential risk factors for *Salmonella* in farms were drawn from published surveys (Nielson and Dohoo 2012; Tarazi et al. 2015; Agren et al. 2017). Thereafter, questions were prioritized and selected to fit into a 15-min face-to-face interview. Before the actual data collection, the questionnaire was trialed on five farms and then modified slightly based on feedback. The final questionnaire contained 23 questions across two broad categories of risk factors: farm and biosecurity managemental factors (see Supporting file 1).

Description of variables

Category 1 variables included farm demographics and animal rearing practices. Farm demographics were general descriptive questions on (1) region, (2) farm area (hectare), (3) farm size (total herd size), (4) number of milking animals, (5) years the farm had been in operation, (6) whether it was owned privately or by dairy companies, and (7) number of workers that attended to or cared for the sick animals. Rearing practices included (1) mixed species rearing, (2) stocking density (average area and size of the sheds used to house the lactating cows), (3) housing system, (4) water source for animal drinking, (5) several questions on the frequency of cleaning food/water troughs, removal of feces, changing/adding bedding material for lactating animals, and (6) frequency of cleaning the parlor.

Category 2 variables focused on essential biosecurity management practices. These included (1) the presence of other livestock farms within two kilometers of the surveyed farms, (2) the introduction of new cattle in the preceding 12 months, (3) quarantine of new stock, (4) isolation of sick animals, (5) the frequency of observation of wild animals on farms, sharing of farm machinery with/within herds, (6) the use of PPE including gumboots and overalls for visitors and workers, respectively, and (7) the frequency of the use of any other disinfection measures for visitors.

Data analysis

Data were collated and sorted with Microsoft Excel (Microsoft Office, version 16.41; 2020). The questionnaire responses were classified as binary (yes/no) or continuous variables, and all variables were analyzed using IBM-SPSS-26 (SPSS Inc. Chicago 111). Continuous data were checked for normality, means or medians were calculated, and proportions and percentages to determine farm management and rearing practices were calculated for categorical variables. Thereafter, univariable and multivariable logistic regression analyses were performed to investigate the associations between *Salmonella* status and potential biosecurity risk factors at the farm level. All associations with a $p < 0.2$ in the univariable analyses were included in the saturated multivariable logistic model. The final model was developed using a backward approach. Nagelkerke's pseudo-R^2, the Hosmer–Lemeshow test and -2Loglikehood were used to assess the goodness of fit of the final multivariable logistic analyses. ORs were estimated with 95% confidence intervals. The collinearity of variables ($p < 0.2$ in the univariable analyses) was analyzed, and the mediator was removed from the final analyses. For all analyses, $p < 0.05$ was considered significant.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s44149-023-00085-9.

Additional file 1.

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Authors' contributions

J.W. wrote the main manuscript. J.W. and X.Z. prepared and analyzed the data. Y.Z., Y.X., Z.Z. and L.Y. collected farms' information. Y.C. and A.G. provided funding. A.G., I.R. and J. supervised this study. All authors reviewed and approved the final manuscript.

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Availability of data and materials
The data used to support the findings of this study are included within the article.

Declarations

Ethics approval and consent to participate
The study was conducted following the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes and with the approval of the Animal Ethics Committee and Human Research Ethics Committee of Murdoch University (approval numbers R3201/19 and 2018/118, respectively). The study was also sanctioned by the Animal Ethics Committee of Huazhong Agriculture University.

Consent for publication
Not applicable.

Competing interests
The author Ian Robertson was not involved in the journal's review or decisions related to this manuscript. The authors declare no conflicts of interest.

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