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A case study investigating the effects of emergency vaccination with *Brucella abortus* A19 vaccine on a dairy farm undergoing an abortion outbreak in China

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Abstract

Brucellosis is an important zoonosis that results in substantial economic losses to the livestock industry through abortions and reduced milk yield. This study investigated an abortion outbreak in a dairy herd and then explored the effects of emergency vaccination with *Brucella abortus* A19 vaccine on the incidence of abortion and milk yield. A full dose of vaccine (6×10^{10}—12×10^{10} colony forming units, CFU) was administered subcutaneously to calves and non-pregnant heifers, and a reduced dose (6×10^{8}—12×10^{8} CFU) to adult cows and pregnant replacement heifers. Rose Bengal Test was used to screen *Brucella* infection status and then positive samples were tested with a C-ELISA. Animals that tested positive for both tests were considered positive to *Brucella spp*. The animal-level seroprevalence of brucellosis was 23.1% (95% CI: 17.0, 30.2), and the attributable fraction of abortions in seropositive animals was 89.1% (95% CI: 64.3, 96.7). The odds of seropositivity were significantly higher in cows that aborted compared to cows that calved normally (OR = 21.4, 95% CI: 4.4, 168.4). Cows in sheds A2 and C1 were 10.2 (95% CI: 1.4, 128.0) and 17.0 (95% CI: 2.8, 190.3) times more likely to be seropositive than cows in shed B1. Antibodies were not detectable in most heifers 12 months post-vaccination. The effectiveness of the vaccine in preventing abortions was estimated to be 56.8% (95% CI: 15.8, 77.8) for the entire herd, but increased to 86.7% (95% CI: 4.4, 98.1) when only primiparous heifers were considered. Furthermore, a significant increase in the average herd 305-day milk yield one-year after vaccination was also observed relative to that in the previous three years. It is concluded that emergency vaccination of a dairy herd undergoing an abortion outbreak with the A19 vaccine effectively reduced the incidence of abortion and indirectly increased milk yield one-year after vaccination.

Keywords: Brucellosis, Outbreak investigation, Emergency vaccination, *Brucella abortus* A19 vaccine, Abortion, 305-day milk yield

Introduction

Brucellosis is a significant zoonotic disease that is a severe health hazard to humans and can result in substantial economic losses to the livestock industry (Wang et al. 2021; Peng et al. 2020). *Brucella abortus* (*B. abortus*), the primary causative agent of bovine brucellosis, affects dairy cows' reproductive performance and productivity (Seleem et al. 2010; Godfroid et al. 2011).

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Infected cattle commonly display abortion, retained placenta, orchitis or epididymitis, and a reduced milk yield. However a subset of infected cattle are asymptomatic and latent, seroconverting only after calving or aborting (Bercovich 1998), making the early diagnosis of infected animals challenging. Following calving or abortion, infected cattle shed large numbers of *B. abortus* resulting in heavy environmental contamination, especially in intensively housed animals, with subsequent rapid transmission to susceptible animals leading to an abortion outbreak (Bercovich 1998). Although the infectious secretions gradually decrease 2–3 months after calving or abortion, some cattle can intermittently excrete *B. abortus* lifelong (Bercovich 1998; Godfroid et al. 2011). Therefore, once an outbreak of brucellosis occurs on a dairy farm, it is difficult to control and eliminate *B. abortus* from that farm.

Many countries have successfully controlled or eliminated *B. abortus* by implementing intervention measures, including test-and-slaughter, mass vaccination, and improved farm biosecurity (Zhang et al. 2018; Robertson 2020). However, test-and-slaughter of cattle in China is not practical or economical due to the large number of infected animals and the high cost of culling. Although farm biosecurity is generally poor on farms in China, this is one area that could be significantly improved in the future (Chen et al. 2021); however this alone cannot eliminate the disease from infected herds. The *B. abortus* A19 vaccine was introduced to China from the former USSR in the 1950s and has been identified to be 99.9% homologous with the internationally accepted and widely used *B. abortus* S19 Strain (Wang et al. 2020a, b; Cheng et al. 2021; WOAH 2019). The primary difference between these two strains is that the A19 strain is erythritol-resistant compared with the erythritol-sensitive S19 strain (Thomas et al. 1981; Wang et al. 2020a, b). Although the A19 vaccine is the bovine *Brucella* vaccine used in China, information on the exact origin of this strain has not been published and its protective effect and efficacy have never been thoroughly investigated. Notwithstanding this, vaccination of calves between 6 and 12 months of age with the A19 vaccine has been adopted in China to prevent infection and abortion, although the overall vaccination coverage in Chinese farms is reportedly low (Chen et al. 2021). This low vaccination coverage is probably attributable to the current veterinary policies in China, which discourage vaccination on dairy farms, and only allow vaccination if the within-herd seroprevalence is more than 1% (Ministry of Agriculture and Rural Affairs of the People's Republic of China 2016). Vaccination of adult cows is also generally not adopted in China as this may induce abortions post-vaccination for pregnant cows, especially given the erythritol-resistant nature of A19 (Hou et al. 2019). However, vaccination of adult animals with a reduced dose of the S19 vaccine was adopted in England and the United States of America (USA) in the early stage of their respective brucellosis control programs when culling infected animals was not economically feasible and test-and-slaughter alone was proved to be inadequate to eliminate the disease from infected herds (Lawson 1950; Barton and Lomme 1980). To date, vaccination with a reduced dose of A19 has not been practised in China.

In the face of an outbreak of brucellosis, rapid removal of all infected animals from the population is desirable, but again this is often not feasible or practical because of economic reasons and the lack of accurate diagnostic assays with perfect diagnostic sensitivity (Gall and Nielsen 2005; McDermott, Grace, and Zinsstag 2013). The alternative is to conduct emergency vaccination of the entire cattle population and gradually cull infected individuals. This control strategy using the S19 vaccine has been successfully adopted in high incidence areas in Portugal compared to only adopting a test-and-slaughter measure (Caetano et al. 2016). Herrera et al. (2008) reported that implementing a S19 vaccination program also increased the milk yield of dairy cows on a farm in Mexico where brucellosis was endemic. Although previous studies reported the role of A19 vaccination in the control of brucellosis (Liu et al. 2019; Tang 2017), its quantitative effects on abortion rate and milk production remain unknown in Chinese dairy cows.

The current study was designed to (1) identify the associated risk factors for brucellosis seropositivity during an abortion storm on a dairy farm in Hubei Province, China; (2) evaluate the effects of emergency vaccination with A19 on subsequent abortions and milk production on this dairy farm one-year after vaccination.

Results

Outbreak investigation

Of the 169 serum samples tested for antibodies against brucellosis in December 2018, 41 were positive on the RBT of which 39 were positive to the C-ELISA, resulting in an animal-level seroprevalence of 23.1% (95% confidence intervals (CI): 17.0, 30.2) when tests were interpreted in series. The monthly numbers of normal calving and aborting events from January 2017 to December 2020 are displayed in Fig. 1. Cows that aborted had a median pregnancy duration of 220 days (Inter Quartile Range (IQR): 145.5, 241.5), compared with 275 days (IQR: 271.0, 277.0) for those that calved normally in 2018. The majority of abortions in 2018 occurred during the last trimester of gestation (63.3%, 19/30) prior to implementing vaccination. The annual incidence of abortions in 2018 (pre-vaccination) and 2019 (post-vaccination) were 22.1% (95% CI: 15.4, 30.0, $p < 0.001$) and 34.0%

(95% CI: 24.6, 44.5, $p<0.001$), respectively, which were both significantly higher than that in 2017 (5.7%, 95% CI: 2.3, 11.4), with corresponding RRs of 3.9 (95% CI: 1.8, 8.5) and 6.0 (95% CI: 2.8, 13.0), respectively compared to 2017. Thirty-two females aborted within one-year after vaccination, and the abortion rate in the low-dose immunised group (32.9%, 25/76) was not significantly different from that in the standard-dose group (41.2%, 7/17) ($p=0.52$).

Of 84 females in 2018 prior to emergency vaccination, 14 had abortions and 24 were seropositive to *Brucella*. Notably, most aborting animals (78.6%, 11/14) were seropositive against *Brucella*. Seropositive animals were 9.2 times (95% CI: 2.8, 30.0, $p<0.001$) more likely to have aborted than seronegative animals. The attributable fraction (AF) for abortion arising from being seropositive to *Brucella* was estimated to be 89.1% (95% CI: 64.3, 96.7). Three factors, including specific sheds, animals purchased from outside the herd, and the presence of abortion, had a $p<0.20$ in the univariable analyses and were included in the saturated multivariable logistic regression model (Table 1). Only the presence of abortion and specific sheds were significantly associated with seropositivity at the animal level in the final model. Cows that aborted had a greater odds of being seropositive (Odds ratios (OR) = 21.4, 95% CI: 4.4, 168.4) than those that calved normally. Cows in sheds A2 and C1 were significantly more likely to be seropositive compared to those from shed B1 (OR = 10.2, 95% CI: 1.4, 128.0, OR = 17.0, 95% CI: 2.8, 190.3, respectively, Table 2). The Akaike information criterion (AIC) value of the final model was 78.76 with a Hosmer–Lemeshow goodness-of-fit value ($\chi^2 = 1.36$, df = 8, $p = 0.995$), indicating that the model was a good fit of the data. The area under the receiver operating characteristic (ROC) curve was 0.827 (95% CI: 0.727, 0.927), suggesting that the model had a good predictive ability.

Emergency vaccination

In Table 3 the extra serological results from one week pre-vaccination to 24 months post-vaccination (standard-dose vaccine group) are summarised for 33 heifers that received the full dose of the A19 vaccine. Two of these 33 heifers (6.1%) tested positive one week before vaccination. Forty-two percent tested positive at 8 months post-vaccination, and then only one heifer (3.0%) tested positive at 12 months post-vaccination (Table 3). The percentage of test positive increased to 24.2% (8/33) at 20 months post-vaccination and then decreased to 15.2% (5/33) at 24 months post-vaccination. Interestingly, six of the eight animals that tested positive at 20 months post-vaccination were seronegative at 24 months post-vaccination. Only one animal was test-positive at all sampling time points. One of the 33 cattle aborted in the first trimester but remained seronegative against *Brucella* throughout the observation period.

After implementing emergency vaccination against brucellosis in the herd in January 2019, the incidence of abortion remained high and declined from eight months post-vaccination before returning to levels comparable to

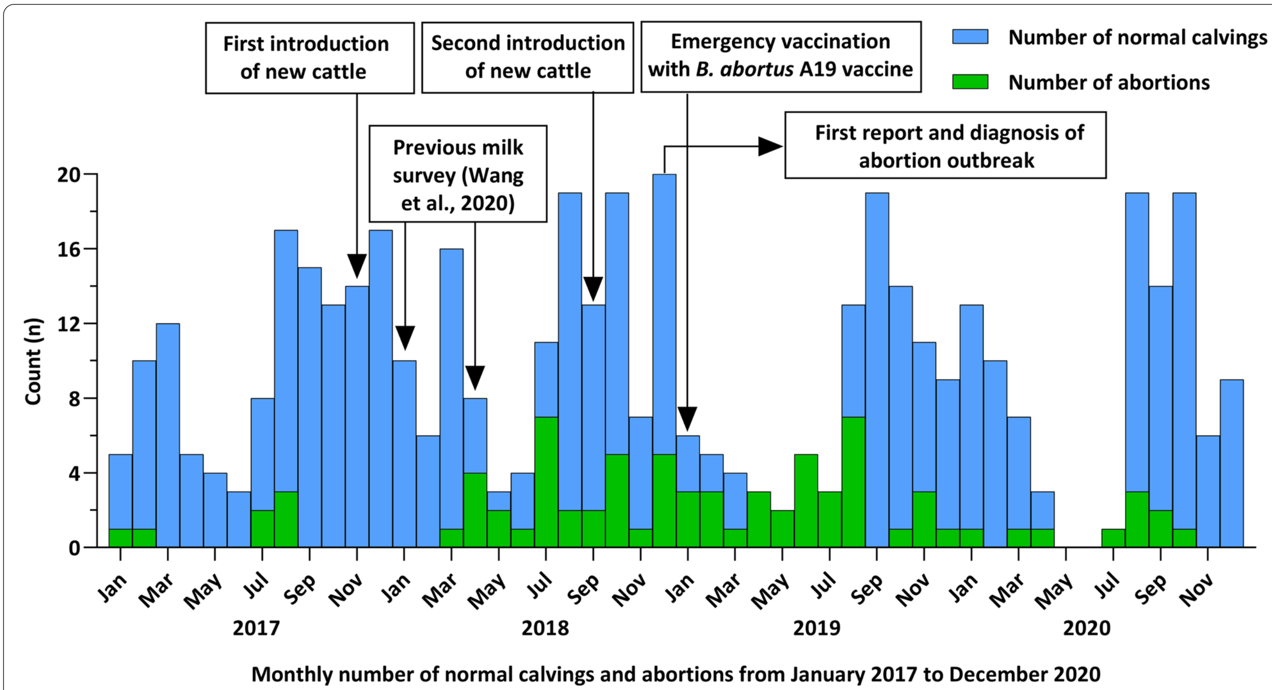

Fig. 1 An epidemiological curve for the monthly number of normal calvings and abortions from 2017 to 2020

Table 1 Univariable analysis of animal level risk factors associated with brucellosis seropositivity

Variables	Categories	Total	No. seropositive	Prevalence, 95% CI (%)	Odds ratio, 95% CI	*p* value [‡]
Parity						
	1st Parity	52	15	28.9 (17.1, 43.1)	1.0 (reference)	0.983
	2nd Parity	17	5	29.4 (10.3, 56.0)	1.0 (0.3, 3.4)	
	≥ 3rd Parity	15	4	26.7 (7.8, 55.1)	0.9 (0.3, 3.3)	
Season the animal calved/aborted						
	Spring	13	3	23.1 (5.0, 53.8)	1.0 (reference)	0.363
	Summer	20	3	15.0 (3.2, 37.9)	0.6 (0.1, 3.5)	
	Autumn	30	11	36.7 (19.9, 56.1)	1.9 (0.4, 8.6)	
	Winter	21	7	33.3 (14.6, 57.0)	1.7 (0.3, 8.1)	
Shed code [†]						
	A2	16	5	31.3 (11.0, 58.7)	4.5 (0.8, 27.4)	0.002
	B1	22	2	9.1 (1.1, 29.2)	1.0 (reference)	
	B2	21	3	14.3 (3.0, 36.3)	1.7 (0.2, 11.1)	
	C1	25	14	56.0 (34.9, 75.6)	12.7 (2.4, 66.6)	
Animal source						
	Introduced	11	5	45.5 (16.7, 76.6)	2.4 (0.6, 8.7)	0.18
	Self-breeding	73	19	26.0 (16.5, 37.6)	1.0 (reference)	
Presence of abortion						
	Yes	14	11	78.6 (49.2, 95.3)	16.1 (3.9, 66.0)	< 0.001
	No	70	13	18.6 (10.3, 29.7)	1.0 (reference)	

[†] shed A1 (calf shed) was excluded from the risk factor analysis

[‡] likelihood ratio test

those in 2017 (Fig. 1). Only 10 of 101 pregnant animals aborted in 2020 (annual incidence of abortion = 9.9%; 95% CI: 4.9, 17.5). This annual incidence in 2020 was significantly lower than that reported in 2018 (RR(relative risk) = 0.45, 95% CI: 0.23, 0.88, $p = 0.014$) and 2019 (RR = 0.29, 95% CI: 0.15, 0.56, $p < 0.001$) but was comparable to that of 2017 (RR = 1.74, 95% CI: 0.69, 4.41, $p = 0.237$) prior to the introduction of replacement heifers from northern China. The overall herd protection effectiveness against abortion was 56.8% (95% CI: 15.8, 77.8) (Table 4). When stratified by parity, the estimates increased to 86.7% (95% CI: 4.4, 98.1) and 63.3% (95% CI: 0.0, 87.3) for 1st parity and 2nd parity females, respectively (Table 4). However, vaccination did not offer sufficient protection against abortion for cows that were ≥ 3rd parity, as the incidence of abortions for this latter group did not significantly change between 2017 and 2020 ($p = 0.686$, Table 4).

During 2018 (pre-vaccination), although 150 cows were being milked, only half of them ($n = 76$) were still on the farm and available for sampling in December 2018. The mean 305-day milk yield of the seropositive group was slightly, but not significantly, lower than that of the seronegative group in 2018 (5215.3 vs 5578.0 kg, respectively—Table 5 and Fig. 2 Panel A) (Kruskal–Wallis χ^2 = 1.152, df = 1, p = 0.283). After implementing emergency vaccination in January 2019, the 305-day milk yield in 2019 for the whole milking herd was similar to that in 2017 and 2018 (Table 5 and Fig. 2 Panel B). However the milk yield in 2020

Table 2 Multivariable logistic regression analysis of risk factors associated with brucellosis seropositivity

Risk factors	Category levels	Estimate	Standard error	Odds ratio, 95% CI	*p* value [‡]
Constant		-3.30	0.97		< 0.001
Presence of abortion	Yes	3.06	0.90	21.36 (4.37, 168.42)	< 0.001
	No			1.00 (reference)	
Shed code	A2	2.32	1.12	10.22 (1.39, 128.02)	0.037
	B1			1.00 (reference)	
	B2	0.95	1.14	2.59 (0.29, 31.69)	0.405
	C1	2.83	1.04	16.99 (2.81, 190.26)	0.006

[‡] likelihood ratio test

Table 3 Serological follow-up results post-vaccination for 33 heifers vaccinated with a standard dose of A19

Time	No. positive	Seroprevalence (95% CI), %
1 week pre-vaccination	2	6.1 (0.7, 20.2)
8 months post-vaccination	14	42.4 (25.5, 60.8)
12 months post-vaccination	1	3.0 (0.1, 15.8)
20 months post-vaccination	8	24.2 (11.1, 42.3)
24 months post-vaccination	5	15.2 (5.1, 31.9)

was significantly higher than that in 2018 and 2019 ($p < 0.05$), and marginally higher than that in 2017 ($p = 0.063$) (Table 5). No significant difference in milk yield was identified between other years.

Discussion

Although the A19 strain has been registered for use in dairy cattle in China, few studies quantify its effects on reducing abortions and increasing milk production (Liu et al. 2019; Tang 2017). The current study is a case report highlighting the value of administering emergency A19 vaccination against *Brucella spp* on a Chinese dairy herd undergoing an abortion storm.

Most abortions occurred in the last trimester of pregnancy in this study, consistent with other reports for brucellosis (Seleem et al. 2010; Ducrotoy et al. 2017). Abortions result from the tropism of *B. abortus* in the uterus and placenta, especially during late gestation, due to the high concentrations of erythritol and steroid hormones in these organs, which facilitates the growth and multiplication of *B. abortus* (Neta et al. 2010). Furthermore the A19 strain used here is resistant to erythritol, in contrast to the S19 strain (Wang et al. 2020a, b), thus raising concerns that the A19 may induce abortions in pregnant cows. Unfortunately it was not possible to undertake bacteriological culture of aborted tissues to confirm or refute this hypothesis because of limited sample acquisition in this study. Future studies should, if at all possible, include collection of aborted material for bacteriological culture to confirm the exact identity of the causative agent.

Many studies have reported that cattle infected with *B. abortus* intermittently shed the bacteria for life (Lapraik and Moffat 1982; Capparelli et al. 2009), which is a significant problem in controlling and eliminating *Brucella* from infected herds. Vaccination with a reduced dose (10^9 CFU—1/10 dilution) of S19 has been shown to provide considerable protection against a challenge dose of 9.4×10^6 CFU, but did not afford satisfactory protection against challenge at a higher dose of 5.2×10^7 CFU (Confer et al. 1985). We hypothesise that this lack of protectiveness with low dose S19 also is likely to apply to the A19 vaccine since these two vaccine strains are highly homologous (Wang et al. 2020a, b). Given the high incidence of abortions, the large numbers of bacteria routinely shed after abortions, and their survivability in the environment, the exposure to vaccinated animals from

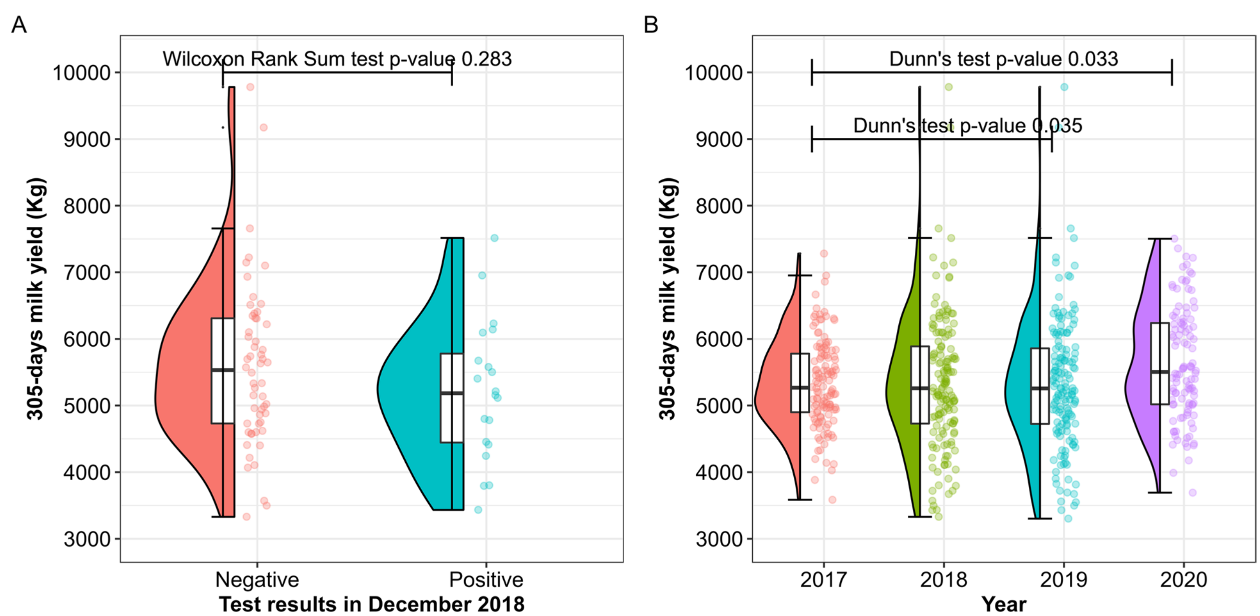

Fig. 2 305-day milk yield within the investigated dairy herd before and after implementing emergency vaccination with A19 vaccine against brucellosis. (A) 305-day milk yield in seropositive and seronegative groups in 2018 prior to vaccination (January 2019) (B) whole herd 305-day milk yield 2017–2020

Table 4 The influence of parity on estimates of vaccine (*Brucella abortus* A19) effectiveness for preventing abortions

Parity	Year	Incidence of abortion (95% CI), %	Relative risk (95% CI)	*p* value[†]	Percent vaccine effectiveness (95% CI), %
1st parity					
	2018	22.8 (14.1, 33.6)	1.00 (reference)	0.012	86.7 (4.4, 98.1)
	2020	3.0 (0.1, 15.8)	0.13 (0.02, 0.96)		
2nd parity					
	2018	30.3 (15.6, 48.7)	1.00 (reference)	0.072	63.3 (0.0, 87.3)
	2020	11.1 (3.1, 26.1)	0.37 (0.13, 1.06)		
≥ 3rd parity					
	2018	8.3 (1.0, 27.0)	1.00 (reference)	0.686	Not applicable
	2020	15.6 (5.3, 32.8)	1.88 (0.40, 8.85)		
All parities					
	2018	22.1 (15.4, 30.0)	1.00 (reference)	0.013	56.8 (15.8, 77.8)
	2020	9.9 (4.9, 17.5)	0.43 (0.22, 0.84)		

[†] Fisher's exact test; Relative risk, incidence of abortion compared to that in each category

a contaminated environment is likely significant. It is necessary to further identify and cull infected animals through testing with sensitive tests, such as a PCR, or by culturing samples (WOAH 2019; Blasco, Moreno, and Moriyón 2021). The safety and efficacy of A19 also need confirmation by conducting more rigorous clinical trials involving pregnant cattle to determine whether subsequent abortions are caused by the inoculated A19 strain or from circulating field strains. The maximum challenge dose of virulent strains that A19-vaccinated animals can resist is also worth further study.

In this outbreak investigation based on serological responses, 89.1% of abortions could be attributed to *Brucella* exposure, and the remaining may be caused by other agents (e.g. *Neospora caninum* and *Coxiella burnetii*), husbandry or management practices such as animal density or breed, false-negative results from the serological assays used, or heat stress (Gädicke and Monti 2013). Although abortions might be caused by co-infection with other pathogens, this was negligible in this study because only 10% of abortions were attributable to factors other than *Brucella* seropositivity. A hot environment may result in abortions and reduced milk yield in high-yielding Holstein cows (Mellado et al. 2016), coinciding with the abortion peak in the summer in 2017–2020 (Fig. 1). It may also be related to the mating and calving cycle of Chinese dairy cows and the biological features of *Brucella* infection. The peak calving season of Chinese dairy cows is usually from September to December each year, with artificial insemination or natural mating usually undertaken two months postpartum (Wang et al. 2021). If pregnant animals were exposed to *Brucella spp*., an abortion peak would likely be observed in the last trimester between June and August of the following year (Fig. 2).

Although only the housing of cattle in particular sheds and the presence of abortion were identified as

Table 5 Effects of brucellosis seropositivity and emergency vaccination with A19 vaccine on 305-day milk yield during 2017–2020

Year	Category levels	No. animals	305-day milk yield (Kg)				Statistics	
			Median	IQR	Mean	SD	*p* value [‡]	*p* value [§]
2018	Seropositive	20	5185.0	4444.3, 5779.3	5215.3	1055.1	0.283	-
	Seronegative	56	5533.0	4730.3, 6308.5	5578.0	1234.7		-
2017	All lactating animals	122	5268.5	4899.8, 5778.3	5333.4	673.2	0.019	0.063
2018	All lactating animals [†]	150	5259.0	4728.8, 5887.8	5339.6	1014.2		0.035
2019	All lactating animals	152	5256.5	4724.3, 5857.8	5332.1	1002.4		0.033
2020	All lactating animals	110	5505.0	5018.3, 6239.3	5629.5	840.2		-

[†] The difference between the total number of lactating animals and the total number of animals tested were caused by the removal of low milk yield and aborted animals before the sampling activity of December 2018

[‡] Wilcoxon Rank Sum test for two groups, Kruskal–Wallis Rank Sum test for three or more groups

[§] The Dunn's test with Holm-Bonferroni correction (only comparisons between 2020 and other individual years were shown, and other comparisons were not significant) associated with brucellosis seropositivity in the final model (Table 2), introducing new animals from outside is recognised as a risky practice for entry of brucellosis to herds (Musallam et al. 2015; Wang et al. 2020c; Robertson, et al. 2020; Li et al. 2021). The seven animals introduced in November 2017 were pregnant replacement heifers. Two of these aborted at the beginning of the abortion 'storm' in April 2018. These abortions were likely to cause heavy environmental contamination with *Brucella spp*., resulting in subsequent clustering of abortions and infections within the specific sheds where these introduced animals were housed. Another essential management practice was that dry cows were mixed with replacement heifers in shed A2 on this farm and then were randomlly transferred to milking sheds after calving, potentially facilitating the transmission between sheds. This hypothesis was supported by the significant association between brucellosis seropositivity and being housed in A2 and C1 sheds (Table 2). Mixing and shifting animals should not be practised to reduce the within-herd transmission of infectious diseases.

In China, the demand for meat and genetically superior replacement animals has led to frequent movement of animals between provinces, especially from northern to southern provinces (Li et al. 2020a, b). In conjunction with ineffective biosecurity and quarantine measures, this has facilitated the rapid transmission of many diseases between farms throughout China. Recent studies have shown that *Brucella* isolates from humans and livestock in southern China are closely related to *Brucella* isolates from northern China (Li et al. 2020b; Wang et al. 2020a, b). To effectively control the inter- and intra- provincial transmission of *Brucella spp*. and other infectious diseases, monitoring animal movement, pre- and post- movement testing and on-farm quarantine should be adopted (Robertson 2020). Another solution is to "accredit" more *Brucella*-free farms and allow them to trade animals without quarantine.

It is worth noting that two heifers tested positive prior to vaccination, probably due to maternal antibodies or vertical transmission. During the follow-up period in this study these two heifers calved normally and their titers had decreased to below detectable levels 12 months post-vaccination, which is consistent with other studies for the A19 vaccine (Anniwaer et al. 2020; Qiao et al. 2019). The subsequent significant increase in test prevalence 20 months post-vaccination likely resulted from transient exposure to a *Brucella*-contaminated environment. Of the eight animals that tested positive 20 months post-vaccination, six recovered to seronegative 24 months post-vaccination, indicating that they most likely experienced a transient exposure but successfully resisted infection through an anamnestic immune response (Bercovich 1998; Dorneles et al. 2015). Only one of the 33 heifers (3.0%) subsequently aborted in their first pregnancy; however, this aborted heifer consistently tested negative in the follow-up period, suggesting that this case was unlikely to have been caused by *Brucella* infection. This highlighted that administering a standard dose of A19 to calves could effectively reduce abortions and false-positive serological reactions when these animals reached maturity, as has been documented for S19 (Confer et al. 1985).

Emergency vaccination with A19 resulted in a reduction in the number of abortions and increased milk yield one-year post-vaccination. Although the overall protection effectiveness estimated in this study (56.8%) was lower than those reported for S19 and RB51 (Dorneles et al. 2015; de Oliveira et al. 2022), this could be related to the potentially high degree of exposure to the pathogen in the two years preceding vaccination. Notably, the effectiveness of protection against abortion (86.7%) was comparable to the S19 vaccine reported in other studies when only 1st parity cows were considered (Dorneles et al. 2015; de Oliveira et al. 2022). The confidence intervals of these estimates were, however, very wide (Table 4), indicating considerable variability in the estimates of protection effectiveness. This large variability may be due to the low incidence of abortion in 2020. Unfortunately, it was impossible to follow a classical clinical trial design with a non-vaccinated control group. The lack of protection against abortion in cows 'older' than 2nd parity was likely due to the lower immunity induced by the reduced dose and the high degree of environmental contamination as has been reported for S19 (Confer et al. 1985). These findings indicate that virulent strains of *Brucella* were likely still circulating within the dairy herd, and vaccination with a reduced dose of A19 may not provide sufficient immunity to prevent infection and hence abortion from the considerable environmental challenge, as has been reported by others (Confer et al. 1985). On the other hand, other measures (e.g. environmental cleaning and disinfection) would also reduce the spread of *Brucella*, possibly resulting in an overestimation of the protective effect of the A19 vaccine.

There was no significant difference between the herd 305-day milk yield in 2017 and that in 2018 and 2019 (Fig. 2). This may have resulted from the presence of *Brucella spp*. on the farm already in 2017. According to the previous milk sample survey on this farm (Wang 2020c; Robertson, et al. 2020), the animal-level lactoprevalence was 9.3% (95% CI: 1.8, 20.4) in January 2018, while the apparent lactoconversion rate was 10.3% per 3 months between January 2018 and April 2018 (Wang 2020c; Robertson, et al. 2020). Therefore, it is highly likely that *Brucella* had been circulating within the farm at least eight months before the current investigation. In addition, the remarkable increase in the whole herd 305-day milk yield in 2020, relative to the previous three years, could be associated with the decline in the incidence of abortions and the actual infection status being reduced one-year post-vaccination. It should be noted, however, that we could not exclude the influence of the natural course of the disease, environmental disinfection and the replacement of milking cows due to the unavailability of a parallel control group in this case study. Herrera et al. (2008) identified a direct relationship between a continuous increase in milk yield and a reduced prevalence resulting from a 6-year brucellosis vaccination control program. But these studies also lacked a positive control population, which is probably because the positive animals were eliminated much earlier than the negative ones. Many economic evaluation models have used values ranging from 10 to 25% reduction in total milk yield in infected animals (Bernués et al. 1997; Singh et al. 2015). In areas where brucellosis is endemic, vaccination interventions have been demonstrated to reduce not only the economic losses from the disease but also impacts on public health (Roth et al. 2003). However, a robust clinical trial to observe *Brucella*-exposed, non-exposed and vaccinated cows is needed to quantify its impact on milk production and abortion as many confounders (such as exposure earlier than vaccination) existed in the current study.

This study contained some limitations. Firstly, the overall vaccination effectiveness was likely overestimated since the effects of other measures implemented (e.g. environmental cleaning and disinfection) could not be excluded from the analysis, but through stratified analysis, it was found that A19 vaccination significantly reduced the abortion rate in primiparous and 2nd parity cows, suggesting the efficacy of the vaccine. Secondly, the lack of bacterial culture resulted in the inability to confirm the etiological cause of abortion pre- and post-vaccination. This deficiency is expected to be fixed in future clinical trials as this study cannot exclude the abortion caused by the previous exposure or the subsequent vaccination because of the long exposure time before vaccination. Existing sequencing technologies and PCR assays provide important tools for distinguishing between A19 and virulent strains (Wang, et al. 2020a, b; Nan et al. 2016). Since this farm had not been vaccinated against *Brucella* before, these animals (39/169) can be diagnosed with *Brucella* infection through combined serological tests recommended by WOAH (2019). *Brucella* infection accounted for 90% of abortions, and abortions from other causes were minimal. Finally, a few animal-level risk factors were identified, and more herd-level management practices and biosecurity measures should be investigated in future studies. These factors, such as the live cattle movement, are commonly considered to be one of the critical drivers of infectious disease transmission between farms, and risk-based control measures can only be developed if these are thoroughly understood.

To control or eradicate the disease in this dairy herd, vaccination alone is unlikely to be sufficient, and a combination of identification and removal of infected animals based on culture is needed, along with regular environmental disinfection. However this study highlights the value of using emergency vaccination with A19 in containing an abortion storm.

Conclusions

In conclusion, this study first demonstrated that the most likely cause of the abortion storm was *Brucella* infection with an attributable fraction of 89.1%. Administering emergency vaccination with A19 on this farm yielded good outcomes with: protection effectiveness of 56.8% and 86.7% for the entire population and 1st parity cows, respectively; reduced incidence of abortions; and increased milk yield one-year after vaccination. A rigorous clinical trial should be conducted to confirm the safety and efficacy of A19 to promote its broader use. In conjunction with vaccination, improved management practices and biosecurity levels should be coordinated and included in all future disease control programs.

Materials and methods

Dairy farm background, intervention and sampling design

A dairy farm located in the Yichang administrative area of Hubei Province participated in a cross-sectional study on brucellosis between January 2018 and April 2018 (Wang et al. 2020c). The initial herd size was 169, comprising 106 milking cows, seven dry cows, 38 replacement heifers and 18 calves. The cattle were confined to five sheds, namely A1 ($n=18$), A2 ($n=45$), B1 ($n=34$), B2 ($n=35$) and C1 ($n=37$). Calves were located separately in A1, while replacement heifers and dry cows were co-housed in A2. Milking cows were distributed in the remaining three sheds (B1, B2, C1). When the heifer calves reached six months of age, they were transferred to shed A2. After calving, replacement heifers and dry cows were allocated to one of the three sheds housing milking cows, depending on available space. This farm adopted an intensive management system with no grazing of pasture or fodder. This privately operated commercial dairy farm purchased seven and six replacement heifers in November 2017 and September 2018, respectively, from northern provinces of China where brucellosis is endemic (Wang et al. 2021). The owner reported that the introduced cattle had not been tested for any diseases before introduction and incorporation into the replacement heifer population in shed A2. Abortions were first observed in March 2018.

These abortions occurred in the middle and last trimester of gestation (Fig. 1).

At the end of December 2018, 169 serum samples were collected from all animals and sent to the State Key Laboratory of Agricultural Microbiology at Huazhong Agricultural University. These animals had not been vaccinated against brucellosis. Given that our previous study reported a true prevalence increased from 9.3% to 16.4% with a lactoconversion rate of 10.3% per three months between January and April 2018 using a commercial milk I-ELISA kit (Wang et al. 2020c), infection with *Brucella spp*. was initially suspected and investigated. Despite the high lactoconversion rate of brucellosis in milk samples, it cannot be excluded that the dairy farm had been infected prior to this study (Wang et al. 2020c). Following the National Standard Diagnostic Techniques for Animal Brucellosis (GB/T 18646–2018) and World Organisation for Animal Health (WOAH) manual of diagnostic tests and vaccines for terrestrial animals (WOAH 2019), combined serological tests were performed to detect antibodies against *Brucella spp*. This diagnostic strategy was chosen due to its extensive use in epidemiological studies of brucellosis and only serum samples available (Zhang et al. 2018). Unfortunately no tissues were cultured for *Brucella spp* or tested for DNA as only serum samples were available at that time. The serological test results (39/169), the typical clinical signs of abortions described by the farmer and the high lactoconversion rate in the early of 2018 from a previous study (Wang et al. 2020c), indicate that this abortion outbreak was highly likely caused by infection with *Brucella spp*. The dairy farm lacked facilities to separate seropositive and seronegative animals and could not afford to cull the large number of seropositive animals (39/169) in a short time. Emergency vaccination of all animals (seropositive and seronegative animals) with A19 was undertaken (outlined below), in conjunction with environmental cleaning and disinfection with 0.02% sodium hypochlorite solution. Subsequently seropositive animals that had aborted were removed from the herd over the animal's next production or lactation year.

Emergency vaccination of animals

In the first week of January 2019 all cattle on the farm were vaccinated with the A19 vaccine. Due to the ongoing abortions on this dairy farm (Fig. 1), having a non-vaccinated control group was not practical or ethical. Calves and non-pregnant replacement heifers (up to 15 months of age) were inoculated with 6×10^{10}—12×10^{10} colony forming units—CFU (a standard dose of A19), and the adults (≥ 2 years of age) or pregnant replacement heifers with a reduced dose of A19 (6×10^{8}—12×10^{8} CFU, 1/100 dilution). The rationale of this reduced dose is to minimise vaccine side effects, referring to studies reporting that a reduced dose of immunisation of adult and pregnant heifers does not induce abortion (Chand et al. 2015; Geong and Robertson 2000). A19 and S19 are highly homologous strains, but no studies have reported the safety and efficacy of a reduced dose of A19 in pregnant cows. Therefore, this study used a 100-fold reduced dose of A19 to immunize pregnant adults and heifers, as was done in the S19 studies (Chand et al. 2015; Geong and Robertson 2000). The vaccine was inoculated subcutaneously behind the shoulder. The vaccine (Reference number: CVCC 70202) was purchased from Spirit Jinyu Biological Pharmaceutical Co., LTD, China, and stored at -20℃ until use. Sterile vaccine diluent (phosphate-buffered-saline) was used to prepare the standard and reduced dose vaccine according to the manufacturer's instructions. The identification of each vaccinated animal was recorded. In addition, calves born subsequently were also vaccinated with a standard dose of the A19 vaccine when they were six months old. No additional booster vaccination was performed as the A19 vaccine has been reported to induce a lifelong antibody response (Anniwaer et al. 2020; Qiao et al. 2019).

Blood sampling and serological tests

Blood samples (5 mL) were collected from the coccygeal vein in the last week of December 2018 (samples collected for diagnosing the cause of abortions). An extra sampling of 33 available heifers vaccinated with a standard dose of A19 was undertaken at one week pre-vaccination and 8, 12, 20 and 24 months post-vaccination. As the cows in this case study had been exposed to a potentially heavily *Brucella*-contaminated environment, they were excluded from the serological follow-up sampling. Each serum sample was tested for antibodies with a commercial Rose Bengal Test (RBT) (IDEXX, USA) and a C-ELISA (Ingezim *Brucella* Compac 2.0, Spain) using the test protocols recommended by the manufacturers. The test results were interpreted in series where a sample was defined as seropositive only if both the RBT and C-ELISA were test-positive. The reported sensitivity and specificity of the RBT are 81.2% and 86.3%, respectively, and for the C-ELISA, 98.0% and 99.9%, respectively (Gall and Nielsen 2005). The sensitivity and specificity of the combined tests interpreted in series were 79.6% and 99.9%, respectively.

Ethical statement

The animal study was reviewed and approved by the institutional ethics committee on animal experimentation of the Laboratory Animal Centre of Huazhong Agricultural University (Protocol No: HZAUCA-2019–006). All animal manipulation in cattle and experiments were strictly performed following the Guidance for the Use and Care of Laboratory Animals, Hubei Province, China. Data were obtained and published with the informed consent of the farm owner. It was considered unethical to include a non-vaccinated group in this study because of the severe impact of brucellosis on cows.

Data collection and analysis

Animal demographic and management data (age, date of calving or abortion, duration of pregnancy, parity, the shed where each animal was located), and animal purchase histories were obtained from the farm owner for all cattle between 2017 and 2020. Data on a history of abortion and calving within each calendar year were collected for the pregnant females. The results of milk testing for brucellosis in January and April 2018, using an individual milk-based indirect ELISA, were sourced from a previous study (Wang et al. 2020c; Robertson et al. 2020). Data on 305-day milk yield for each available lactating animal from 2017 and 2020 were obtained from the Hubei Dairy Herd Improvement (DHI) Centre.

All data were entered into a spreadsheet in Microsoft Excel (Microsoft Excel 2019, Redmond, USA) and then imported into the statistical software R (V. 4.1.2) for further data manipulation and analysis (R Core Team 2021).

An epidemiological curve displaying monthly aborting and calving numbers, animal purchasing histories and emergency vaccination intervention during 2017–2020 was generated to visualise the timeline of the abortion outbreak and management practices in the dairy herd (Fig. 2). The AF of brucellosis seropositivity for abortion was calculated based on the serological test results for December 2018 and the presence of abortion in 2018. The AF was calculated using the following formula:

$$AF = \frac{Incidence_{seropositive} - Incidence_{seronegative}}{Incidence_{seropositive}} \times 100\%$$

where incidence represents the frequency of abortion in each serological group in 2018. The AF is a relative measure of the importance of a risk factor (i.e. *Brucella* seropositivity in this study), and it expresses the proportion of total risk in exposed animals which is due to the risk factor (Dohoo et al. 2009).

Totally 131 animals had at least one abortion or normal calving event in 2018, of which five animals had two records, resulting in a total of 136 records. However, of the 131 cattle, only 84 (64.1%) were available for sampling in December 2018, with the remainder culled mainly due to mastitis (51.7%), low milk yield (20.0%) or abortion (13.3%). Only data from these 84 animals were included in the risk factor analysis and the estimation of AF. To identify the risk factors associated with the presence of abortion at the animal level, univariable and multivariable logistic regression models were developed. Variables with a $p \leq 0.20$ on the univariable analyses were initially offered to a multivariable logistic regression model. The model was generated using a backward stepwise process, and the most appropriate model was identified through assessing the likelihood ratio test and the minimum AIC value (Hosmer and Sturdivant 2013). The model was further assessed with the Hosmer–Lemeshow goodness-of-fit test. A ROC curve was created to display the predictive accuracy of the model using the R package 'pROC' (Robin et al. 2011). OR and their 95% confidence intervals (95% CI) were also calculated to estimate the degree of association between different variables and abortion.

Abortion and calving events in 2019 were not included in the analysis to evaluate the effect of emergency vaccination on reducing the incidence of abortion in subsequent pregnancy because of concerns over the impact of potentially latent infections on the results. RR was computed to determine the degree of association between pre- and post-vaccination on the incidence of abortion. The protection effectiveness of the vaccine was calculated as described by van Straten et al. (2016) using the formula (1− RR) $\times$ 100% based on the incidence of abortion. The AF and RR and their respective 95% CIs were calculated using 'epiR' package (Stevenson et al. 2022). The continuous and categorical variables were presented as median (IQR) and number (percent, %), respectively. The Pearson's Chi-square test and Fisher's exact test were used to compare the difference in the incidence of abortion between test positive and test negative groups, where appropriate. The Shapiro–Wilk test was used to evaluate the normality of the continuous data, indicating that the animal-level 305-day milk yield did not follow a normal distribution ($p < 0.05$) and therefore, the Wilcoxon Rank Sum test and Kruskal–Wallis Rank Sum test were used to compare the statistical difference between groups for these data, depending on the number of groups. If significant, we used the Dunn's test with Holm-Bonferroni correction to determine the difference in the milk yield between individual years. A raincloud plot was created to summarise the distribution, median value and variability of the 305-day milk yield in each group using the R package 'ggplot2' (Wickham 2016).

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Authors' contributions
Y.W.1 and Y.W.2 wrote the main manuscript and analysed the data, Q.P. and Z.X. conducted laboratory tests, Y.C. designed the study, G.W. and X.W. prepared the data, A.G. and I.R. provided funding and supervised this study. All authors reviewed the manuscript. The author(s) read 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

Competing interests
Author Ian D. Robertson was not involved in the journal's review or decisions related to this manuscript. The authors declare no conflicting interests.

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