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RESEARCH ARTICLE

Epidemiological investigation of *Staphylococcus aureus* Infection in Dairy Cattle in Anhui, China

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A B S T R A C T

Staphylococcus aureus is a leading source of foodborne poisoning in the human population also critically severe mastitis in livestock. A total of 671 serum samples were collected and tested through ELISA assays. The results revealed that the total prevalence of *S. aureus* infection in dairy cattle was 29.1% (95% CI: 25.6-32.7). In different farms, the prevalence ranged from 13.9% (95% CI: 8.8-20.5) to 36.6% (30.4-43.2). Similarly, the mean sero-prevalence was 17.0% (13.1-21.4) and 41.6% (36.3-47.2) in male and female animals, respectively. However, at different ages, the mean prevalence of *Staphylococcus aureus* was found to be 14.3% (7.4-24.1) to 38.5% (30.8-46.6). Although in different years, the sero-prevalence was 27.5% (22.2-33.4) in 2019, 19.6% (13.7-26.7) in 2020 and 36.5% (30.6-42.7) in 2021. According to conditional stepwise logistic regression analysis, different farms, gender, age and year were observed as the critical risk factors affecting the prevalence. Our results may contribute to development of prevention and control strategies against this zoonotic pathogen in farm animals in this region.

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INTRODUCTION

The zoonotic *Staphylococcus aureus* (*S. aureus*) is a world-widely distributed pathogen causing serious diseases of skin/tissue infections, sepsis, osteomyelitis and pneumonia in livestock and human beings (Papadopoulosa *et al*., 2019). It is widely known that infectious diseases seriously threaten the health of economic animals, among which *S. aureus* is an important pathogen causing clinical and chronic mastitis in dairy cows, with enormous economic loss to the dairy industry (Aqib *et al*., 2017). The infection of *S. aureus* in cows is verified to have approximately 300 U.S. dollars earned for each cattle (Karahan *et al*, 2019). Due to the abusive use of antibiotics in practice, a resistant organism of *S. aureus* was occasionally found in cattle milk (Aqib *et al*., 2017). Previous research has indicated that *S. aureus* isolates from cattle might endanger the health of persons who work with ruminants regularly (Liu *et al*., 2017). *S. aureus* produces several hemolysins, which enhance its ability to colonize mammalian hosts and then cause different diseases (Elsayed *et al*., 2015). The emerging antibiotics resistant *S. aureus* was also found in hospitals and community environments (Aqib *et al*., 2017). A study found that 13% of *S. aureus* related to skin and tissue infections were people from veterinary practice (Aqib *et al*., 2017). Over 25% of mastitis dairy cases in cattle were caused by *S. aureus* (Petrovski *et al*., 2009). This mastitis agent is mainly spread through milking; the infected animals not only vertically transmit bacteria to their calves, but also spread to people (Papadopoulosa *et al*., 2019).

Anhui province is located in an eastern China with east longitude of 114°54 '--119°37' and north latitude of

29°41'--34°38'. In this warm and subtropical area, since 2016, there have been over annual 790 000 heads of cattle breeding, accounting for nearly 1% of national cattle amounts, and 290 000 tons of milk production, accounting for 1% of whole China's native milk production, respectively (Fig. 1) (National Bureau of Statistics, http://www.stats.gov.cn/). Causative agents like *S. aureus* are critically important in bovine ruminants, previous studies showed that the prevalence of *S. aureus* in dairy cows was 10% in yaks and 21% in cattle in Mongolia (Tsegmed *et al*., 2017), 27.7% in cows in Northern China (Liu *et al*., 2017), 12.4% in yaks in China (Zhang *et al*., 2021). However, as far as we know, there is scanty information regarding *S. aureus* infection in cattle in Anhui province. In this study, an ELISA kit targeting Protein A; 42KDa cell surface protein which is found on the cell walls of 90% of *S. aureus* bacterial strains, was used for detection. Because of its ability to bind the constant regions of antibodies, it can be used to extract immunoglobulins and effective detection.

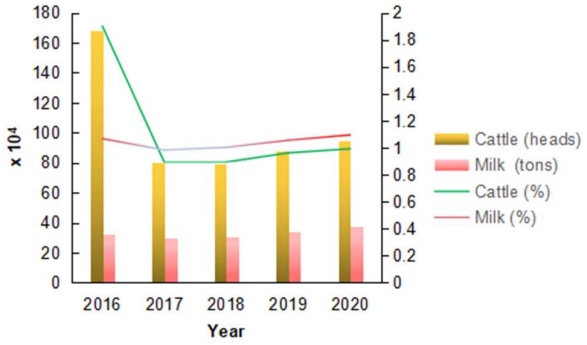

Fig. 1: Cow breeding and milk production during 2016-2020 in Anhui, China.

As *S. aureus* not only limited the development of the cattle industry but became more difficult to prevent and control, the resistant bacteria. Also, *S. aureus* was demonstrated as a main bovine mastitis agent in China. Therefore, it is of great importance to monitor the prevalence of *S. aureus* in dairy farms. The current study herein was carried out to investigate the sero-prevalence of *S. aureus* infection on dairy cows in Anhui, China.

MATERIALS AND METHODS

Ethics statement: All procedures were guided by the instructions and approval of LARC (laboratory animals research center) of Anhui province in P. R. China and the ethics committee of Linyi University, China.

Serum samples: A total of 671 blood samples were obtained in 2019-2021 from 3 dairy cattle farms (A 232, B 288 and C 151) in Anhui, China (Table 1). A sample knowledge of gender and age was collected. All of the samples were kept on dry ice and delivered to the veterinary clinical laboratory at Linyi University, China. Then sera samples were achieved via centrifuge at 2000 × g for 10 min and stored at -20°C for further analysis.

Determination of antibodies against *Staphylococcus aureus* in cattle: All the samples of serum isolated from dairy cattle were examined for antibodies against *S. aureus* by utilizing a commercial enzyme-linked immunosorbent assay (ELISA) kit (Cattle *Staphylococcal* Protein A ELISA Kit, Shanghai yiyan bio-technology Co. Ltd., Shanghai, China) with the sensitivity of (95% CI) 78.6% (60.5–89.8), with a specificity of 98% according to the explanatory memorandum. The detection value was determined according to the optical density (OD) values of OD 450 nm (W1) and 630 nm (W2), S of sample = W1s-W2s, N of negative control = W1n-W2n, where Critical (CUT OFF) = the average value of S/N. The results were interpreted as: positive when the CUT OFF ≥ 2.1. To ensure validity, all samples were examined three times independently.

Statistical analysis: To uncover variables potentially associated with exposure to *S. aureus* infection in dairy cattle, we performed an MLRM (multivariable logistic regression model) analysis. Statistically, levels within factors and interactions were treated as significantly with $P<0.05$. Odds ratios (OR) were shown with 95% confidence intervals (CI). We performed statistical analyses via the IBM SPSS Statistics 22.0 (SPSS Somers, NY, USA).

RESULTS

The total sero-prevalence of *S. aureus* infection in dairy cattle was 29.1% (95 CI: 25.6-32.7) (Table 1). On different farms, the prevalence of *S. aureus* infection in dairy cows ranged from 13.9% (95 CI: 8.8-20.5) to 36.6% (30.4-43.2). In cattle, the sero-prevalence of *S. aureus* infection was 17.0 percent (13.1-21.4) in males and 41.6 percent (36.3-47.2) in females. The frequency of *S. aureus* infection in ruminant cattle varied with age, ranging from 14.3 percent (7.4-24.1) to 38.5 percent (30.8-46.6). Infection with *S. aureus* was found in 27.5 percent of cows (22.2-33.4%) in 2019, 19.6 percent (13.7-26.7%) in 2020, and 36.5 percent (30.6-42.7%) in 2021. Farm, gender, age and year were identified as the most critical

Table 1: The seroprevalence of *Staphylococcus aureus* infection in dairy cattle in Anhui, China

Variable	Category	No. tested	No. positive	% (95% CI)	*P*-value	OR (95% CI)
Farm	C	151	21	13.9 (8.8-20.5)		Reference
	A	232	85	36.6 (30.4-43.2)	< 0.001	3.580 (2.101-6.098)
	B	288	89	30.9 (25.6-36.6)	< 0.001	2.769 (1.639-4.677)
Gender	Male	342	58	17.0 (13.1-21.4)		Reference
	Female	329	137	41.6 (36.3-47.2)	< 0.001	3.494 (2.444-4.995)
Age	1<year≤2	77	11	14.3 (7.4-24.1)		Reference
	0<year≤1	156	60	38.5 (30.8-46.6)	< 0.001	3.750 (1.834-7.667)
	2<year≤4	182	27	14.8 (10.0-20.8)	0.909	1.045 (0.490-2.230)
	4<year	256	97	37.9 (31.9-44.1)	< 0.001	3.660 (1.843-7.272)
Year	2020	158	31	19.6 (13.7-26.7)		Reference
	2019	258	71	27.5 (22.2-33.4)	0.069	1.555 (0.964-2.510)
	2021	255	93	36.5 (30.6-42.7)	< 0.001	2.352 (1.473-3.756)
Total		671	195	29.1% (25.6-32.7)		

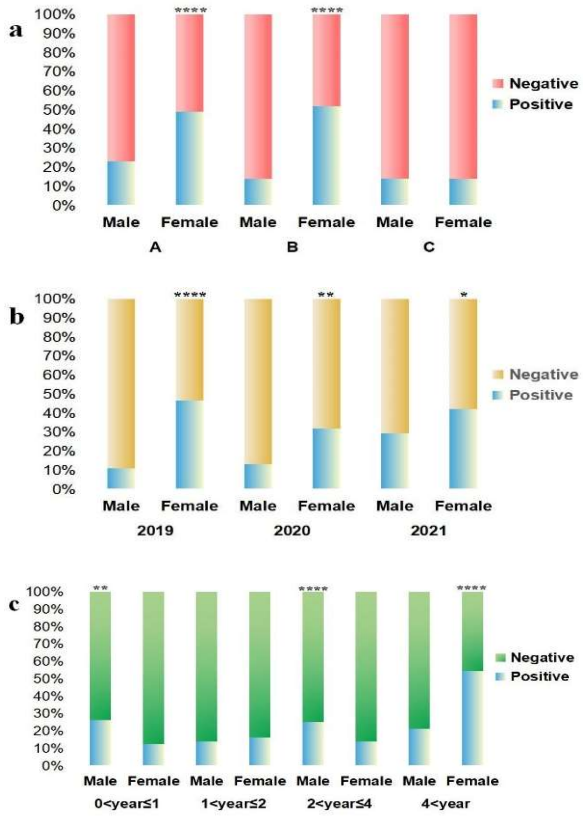

Fig. 2: The seroprevalence of *Staphylococcus aureus* infection in dairy cattle in different genders. a: Farm, b: Age, c: Year. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001.

risk variables using conditional stepwise logistic regression analysis through MLRM. In different farms, ruminant cows in farm A (36.6%) presented a three times (OR=3.580, 95% CI=2.101-6.098, P<0.001) higher risk of infection compared to cattle in farm C (13.9%); however, animals in farm B (30.9%) showed a two-fold increase (OR=2.769, 95% CI=1.639-4.677, P<0.001) in risk of infection while comparing with cows of farm C. Additionally, in different ages, animals in 0<year≤1 (38.5%) and 4<year depicted a threefold increase (OR=3.750, 95% CI=1.834-7.667, P<0.001) and a three times (OR=3.660, 95% CI=1.843-7.272, P<0.001) higher risk of being positive compared to ruminants in 1<year≤2 (14.3%), respectively. While no obvious difference was observed among cattle in 2<year≤4 and 1<year≤2 (p = 0.909 > 0.05). Similarly, while comparing the different years, dairy cattle in 2021 (36.5%) had twice the (OR=2.352, 95% CI=1.473-3.756, P<0.001) risk of being positive for *S. aureus* as compared to cows in 2020 (19.6%). On the contrary, there was no remarkable difference between ruminants in 2019 (27.5%) and 2020 (19.6%) (P=0.069>0.05) was observed. In male and female animals, the prevalence of *S. aureus* infection in female cattle (41.6%) revealed a three-fold (OR=3.494, 95% CI=2.444-4.995, P<0.001) higher risk of infection compared with male dairy cows (17.0%). The sero-prevalence of *S. aureus* infection in female cows in farm A (P<0.0001) and B (P<0.0001) presented significantly higher than the male animals, respectively (Fig 2a). Similarly, the prevalence of *S. aureus* infection in female ruminants in year 2019 (P<0.0001), 2020 (P<0.01) and 2021 (P<0.05) was prominently higher than male cows (Fig 2b). Though, the prevalence of *S. aureus* infection in female cattle in years > 4 (P<0.0001) was consistently higher than in male animals, the prevalence of *S. aureus* infection in male cattle showed significantly higher in 0<year≤1 (P<0.01) and 2<year≤4 (P<0.0001) than female cows (Fig 2c).

DISCUSSION

Milk and milk products have been the most commonly consumed nutritious items for people for a long time, and the demand and the rate of intake of milk products have risen tremendously in the last decade. The consumption of fluid cow milk in India, Europe, the United States, China and Brazil were all over 11 000 000 metric tons in 2020 (https://www.statista.com/statistics/272003/global-annual-consumption-of-milk-by-region/). Also, there is a growing number of people who enjoy taking raw dairy milk and raw-milk processed products (Papadopoulos *et al*., 2019), which greatly contribute to the infection of foodborne pathogens like *S. aureus*. Therefor it is of great importance to perform regular examination the prevalence of *S. aureus* in fresh milk in dairy farms.

A method to be used in epidemiological investigations should be cheap, fast, and easy to apply, but most importantly, repeatable. The protein A gene is a helpful epidemiological marker in investigations of human infections caused by Staph. aureus (Frenay *et al*., 1994). This gene is 2150 bp long and contains three functionally different regions: an FC-binding region, an X region, and a sequence necessary for cell wall attachment at the C terminus (Frenay *et al*., 1994). However, only 93 per cent of Staph. aureus strains isolated from bovine intramammary infections have been shown to contain protein A. Protein A may bind to the Fc component of immunoglobulins from a wide range of species, particularly IgGs. One molecule of protein A has been proven to bind at least two molecules of IgG at the same time. Protein A's IgG-binding domain is made up of three anti-parallel helices, one of which is broken when the protein binds to the Fc region of immunoglobulins. In the present research survey using commercial Cattle Staphylococcal Protein A ELISA Kits, a total of 197 serum samples were detected positive against *S. aureus* (29.1%, 95 CI: 25.6-32.7), which is in line with the study on cattle and buffalos in Pakistan (34%) (Aqib *et al*., 2017). However, contradicting the findings in dairy animals in Greece (47.8%) (Papadopoulosa *et al*., 2019), bulk tank milk in Italy (47.2%) (Cortimiglia *et al*., 2016) and bulk tank milk in Norway (75%) (Jörgensen *et al*., 2015). According to an MLRM assay, factors of the farm, gender, age and year were considered important factors affecting the prevalence of *S. aureus* in ruminants (Table 1). In particular, the prevalence in male and female animals significantly varied in different farms, ages and years, respectively (Fig 2). These findings, including previous studies, revealed that the prevalence of *S. aureus* in different animals was positively associated with geographical location, climate, regions and genders (Aqib *et al*., 2017). The food chain *S. aureus* in raw milk could

introduce this pathogen into calves and the human population (Cortimiglia *et al*., 2016), which could affect the quality and quantity of milk, an early eradication of animals, and increase treatment costs (Sheet *et al*., 2019). Besides being infected through sucking and un-cleaned hands of veterinarians during milking and ticks infestation, there are also ways of transmission from one dairy cattle to another (Aqib *et al*., 2017). Likewise, herdsmen and consumers may potentially get infected via close interaction with infected cattle and ingestion of contaminated food (Papadopoulosa *et al*., 2019). For most people, the infection of *S. aureus* is non-fatal, however, a special group of people i.e. immune-compromised patients, the aged persons, and children are sensitive to this pathogen (Tsegmed *et al*., 2007). This ubiquitous pathogen is well known to seriously threaten animals and public health, however, the treatment methods are limited and compromised due to its strong resistance to antibiotics (Aqib *et al*., 2017).

Conclusions: We reported the seroprevalence of food poisoning bacteria *S. aureus* infection in dairy cows (29.1%) in Anhui, China and found different farms, ages, genders, and years were all factors affecting the prevalence. Our results as a regular monitor survey of the seroprevalence of *S. aureus,* which may contribute to the prevention and control strategies for this zoonotic pathogen in farm animals in this region.

Conflict of interest: The authors state that there are no competing interests.

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Authors contribution: Conceived and designed the experiments: JJL, CLB, ZQH and AFA. Performed the experiments: JJL and JHQ. Analyzed the data: JJL, FA, MUS, HA and CLB. Contributed reagents/materials/ analysis tools: JJL and CLB. Wrote the paper: JJL, CLB and ZQH.

REFERENCES

Aqib AI, M Ijaz, AA Anjum, *et al*., 2017. Antibiotic susceptibilities and prevalence of Methicillin resistant *Staphylococcus aureus* (MRSA) isolated from bovine milk in Pakistan. Acta Tropica 176:168-72.

Cortimiglia C, M Luini, V Bianchini, *et al*., 2016. Prevalence of *Staphylococcus aureus* and of methicillin-resistant *S. aureus* clonal complexes in bulk tank milk from dairy cattle herds in Lombardy Region (Northern Italy). Epidemiol Infect 144: 3046-51.

Elsayed MS, AEM El-Bagoury and MA Dawoud, 2015. Phenotypic and genotypic detection of virulence factors of *Staphylococcus aureus* isolated from clinical and subclinical mastitis in cattle and water buffaloes from different farms of Sadat City in Egypt. Vet World 8:1051-8.

Frenay HM, 1994. Discrimination of epidemic and nonepidemic methicillin-resistant Staphylococcus aureus strains on the basis of protein a gene polymorphism. J Clin Microbiol 32:846-7.

Jörgensen HJ, T Mörk and LM Rörvik, 2005. The occurrence of *Staphylococcus aureus* on a farm with small-scale production of raw milk cheese. J Dairy Sci 88:3810-7.

Karahan M, MN Acik and B Cetinkaya, 2009. Investigation of toxin genes by polymerase chain reaction in *Staphylococcus aureus* strains isolated from bovine mastitis in Turkey. Foodborne Pathog Dis 8:1029-35.

Liu HM, SL Li, L Meng, *et al*., 2017. Prevalence, antimicrobial susceptibility, and molecular characterization of *Staphylococcus aureus* isolated from dairy herds in northern China. J Dairy Sci 100:8796-803.

Papadopoulosa P, AS Angelidisb, T Papadopoulos, *et al*., 2019. *Staphylococcus aureus* and methicillin-resistant *S. aureus* (MRSA) in bulk tank milk, livestock and dairy-farm personnel in north-central and north-eastern Greece: Prevalence, characterization and genetic relatedness. Food Microbiol 84:103249.

Petrovski KR, C Heuer, TJ Parkinson, *et al*., 2009. The incidence and aetiology of clinical bovine mastitis on 14 farms in northland New Zealand. N Z Vet J 57:109–15.

Tsegmed U, G Normanno, M Pringle, *et al*., 2007. Occurrence of Enterotoxic *Staphylococcus aureus* in Raw Milk from Yaks and Cattle in Mongolia. J Food Protect 70:1726-9.

Zhang PF, XY Liu, J Zhang, *et al*., 2021. Prevalence and characterization of *Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus* isolated from retail yak butter in Tibet, China. J Dairy Sci 104:9596-606.