Shang *et al. Animal Diseases* (2024) 4:16
https://doi.org/10.1186/s44149-024-00120-3

Animal Diseases

ORIGINAL ARTICLE

Open Access

Investigation of the epidemiology, pathogenicity and immunogenicity of *Bordetella bronchiseptica* isolated from cats and dogs in China from 2021 to 2023

Qinghua Shang[1,2], Wenhui Gao[1,2], Xiangting Zhang[1,2], Jianqing Zhao[1,2], Ying Wu[1,2], Haoqi Li[1,2], Ming Zhou[2], Zhen F. Fu[1,2], Chengguang Zhang[1,2*] and Ling Zhao[1,2,3*]

Abstract

Bordetella bronchiseptica (*Bb*) is recognized as a leading cause of respiratory diseases in dogs and cats. However, epidemiological data on *Bb* in dogs and cats in China are still limited, and there is no commercially available vaccine. Live vaccines containing *Bb* that are widely used abroad are generally effective but can establish latency and potentially reactivate to cause illness in some immunodeficient vaccinated recipients, raising safety concerns. In this study, 34 canine-derived and two feline-derived *Bb* strains were isolated from 1809 canine and 113 feline nasopharyngeal swab samples collected from eight provinces in China from 2021 to 2023. The PCR results showed that the *percentage of positive Bb* was 22.94% (441/1922), and more than 90% of the *Bb* isolates had four virulence factor-encoding genes (VFGs), namely, *fhaB*, *prn*, *betA* and *dnt*. All the isolated strains displayed a multidrug-resistant phenotype. The virulence of 10 *Bb* strains isolated from dogs with respiratory symptoms was tested in mice, and we found that eight isolates were highly virulent. Furthermore, the eight *Bb* isolates with high virulence were inactivated and intramuscularly injected into mice, and three *Bb* strains (WH1218, WH1203 and WH1224) with the best protective efficacy were selected. Dogs immunized with these three strains exhibited strong protection against challenge with the *Bb* field strain WH1218. Ultimately, the WH1218 strain with the greatest protection in dogs was selected as the vaccine candidate. Dogs and cats that received a vaccine containing 10^9 CFU of the inactivated WH1218 strain showed complete protection against challenge with the *Bb* field strain WH1218. This study revealed that *Bb* is an important pathogen that causes respiratory diseases in domestic dogs and cats in China, and all the isolates exhibited multidrug resistance. The present work contributes to the current understanding of the prevalence, antimicrobial resistance, and virulence genes of *Bb* in domestic dogs and cats. Additionally, our results suggest that the WH1218 strain is a promising candidate safe and efficacious inactivated *Bb* vaccine.

Keywords *Bordetella bronchiseptica*, Epidemiological investigation, Pathogenicity, Immunogenicity, Inactivated vaccine

Introduction

Canine infectious respiratory disease (CIRDC) is a worldwide epidemic syndrome involving multiple viral and bacterial pathogens (Day et al. 2020; Fastrès et al. 2020; Maboni et al. 2019; Matsuu et al. 2020). *Bordetella bronchiseptica (Bb)* is one of the major pathogenic bacteria for CIRDC and can also act as a single pathogen

Handling editor: Fang He.

*Correspondence:
Chengguang Zhang
zhangcg1105@outlook.com
Ling Zhao
zling604@outlook.com; lingzhao@mail.hzau.edu.cn
Full list of author information is available at the end of the article

(Bemis et al. 1977a; Chambers et al. 2019; Maboni et al. 2019). *Bb* is a gram-negative bacillus that colonizes the respiratory tract of mammals, and it is susceptible to nearly all warm-blooded animals (Goodnow 1980; Woolfrey and Moody 1991). This pathogen is the causative agent of kennel cough in dogs and suppurative bronchopneumonia in cats (Speakman et al. 1999). Furthermore, previous studies have demonstrated that *Bb* can infect humans (Goodnow 1980; Woolfrey and Moody 1991), especially immunocompromised patients who are at increased risk of infection (Agarwal et al. 2022; Gupta et al. 2019; Sameed et al. 2019; Tatem et al. 2023). Moreover, coinfections of SARS-CoV-2 and *Bb* have been reported (Faqihi et al. 2020; Mulkoju et al. 2022; Nagarakanti and Bishburg 2021; Papantoniou et al. 2021; Pierce et al. 2022). Like other members of the *Bordetella* genus, *Bb* can produce several important virulence factors, including filamentous hemagglutinin, protein toxin, adenylyl cyclase toxin, skin necrosis toxin, and a type III secretion system and effector proteins involved in its pathogenesis (Kamanova 2020; Linz et al. 2016), and it has been reported that *Bordetella* utilizes a type 3 secretion system to manipulate VIP/VPAC2 signaling to promote colonization and persistence of three classical *Bordetella* species in the lower respiratory tract (First et al. 2023). Porter et al. showed that this pathogen can grow in lake water, which suggests that *Bb* may be a free-living pathogen (Porter et al. 1991; Porter and Wardlaw 1993). It is a great challenge to determine the exact infection rate of *Bb*; it usually takes more than three months to completely eradicate the pathogen after infection (Bemis et al. 1977b), and many infected animals remain asymptomatic (Pecora 1976). Aerosol and direct contact facilitate the transmission of *Bb*, and a previous study reported a transmission rate of 100% by these two means from experimentally infected dogs to fully susceptible dogs within only 1–4 d (McCandlish et al. 1978b; Thompson et al. 1976).

Currently, there is no commercial vaccine against *Bb* in dogs and cats in China. Vaccine strains have been reported to survive in dogs for at least two weeks after immunization with live attenuated vaccines widely used abroad (Shade and Goodnow 1979). In addition, the possibility of virulence reactivation (from avirulent to virulent) appears to be low, but there is still potential for compromised hosts to be clinically significantly infected (Ostle 1989). Furthermore, vaccine-derived *Bb* has been reported to be transmissible from dogs to immunocompromised patients (Doo et al. 2017; Kraai et al. 2023; Yacoub et al. 2014). In veterinary clinics across the U.S., the median canine *Bb* vaccination rate is as high as 68.7%; in cats, it ranges from 1–100% (Malter et al. 2022). Therefore, it is necessary to develop a vaccine with good safety and protective efficacy against the prevalent strain of *Bb* in China. Inactivated vaccines have long been considered safe and have exhibited some effectiveness (Abdoli et al. 2022; Jin et al. 2022; Marouf et al. 2022). The group at the University of Glasgow performed a series of studies in the mid-to-late 1970s and provided proof of the principle that parenteral vaccines could stimulate *Bb* disease-sparing responses (McCandlish et al. 1978a, b). In this study, canine and feline nasopharyngeal swab samples were collected to isolate *Bb* strains, and the drug susceptibility and virulence factor-encoding genes (VFGs) of the *Bb* isolates were evaluated. The virulence of strains isolated from samples with respiratory symptoms was evaluated in mice. Next, the protective efficacy of virulent isolates was compared in mice and dogs, and WH1218, which has the best protection in dogs, was selected. Dogs and cats receiving a vaccine containing 10^9 CFU of the inactivated WH1218 strain showed complete protection. This study indicated that *Bb* is prevalent in dogs and cats in parts of China and that the WH1218 strain is a promising candidate as a safe and efficacious inactivated *Bb* vaccine.

Results

Epidemiological investigation of *Bb* in China from 2021 to 2023

Across different provinces in China, the positivity rates varied between 15.47% and 36.23%, the isolation rates ranged between 0% and 4.48% (Fig. 1A, B), and the overall separation rate was 1.87% (36/1922). Thirty-four strains were isolated from 1809 canine nasopharyngeal swabs, and two strains were isolated from 113 feline nasopharyngeal swabs. The positive rate and isolation rate of dog samples were 23.88% (432/1809) and 1.88% (34/1809), respectively, both of which were greater than those of cat samples (7.96%, 9/113 and 1.77%, 2/113). Furthermore, the percentage of samples isolated from dogs with respiratory symptoms was 6.54% (10/153), which was greater than that from dogs without respiratory symptoms (1.45%, 24/1656) (Fig. 1C). The PCR products of the *Bb*-positive colonies had a specific band at 237 bp on an agarose gel and showed grayish-white, smooth, raised colonies after 28-44 h of incubation on a TSA plate (Fig. 1D). Biochemical experiments revealed that the isolated *Bb* strains were unable to ferment various sugars, such as fructose, glucose, mannitol, maltose, sorbose and lactose. The methyl red (MR), Voges-Proskauer (VP), and indole reactions were all negative, while the oxidase, urease and hydrogen peroxide enzyme reactions were positive (data not shown).

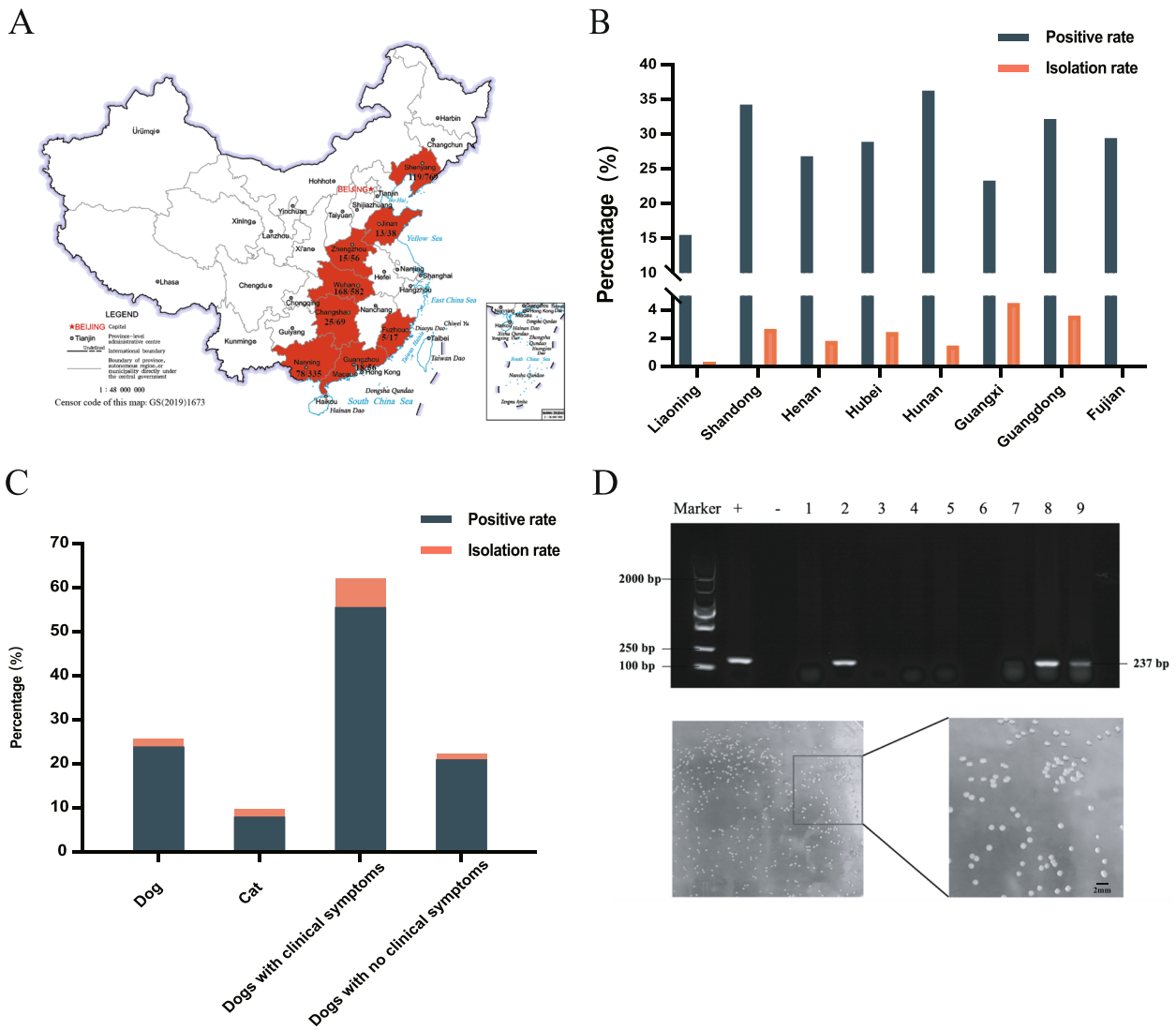

Fig. 1 Isolation of *Bb* (*B. bronchiseptica*) from nasopharyngeal swab samples collected from domestic dogs and cats in various regions of China from 2021 to 2023. **A** The geographic locations of sample collection are represented on the map, with numbers indicating the ratio of *Bb*-positive samples to the total number of samples collected from each province. **B** The percentage of *Bb*-positive nasopharyngeal swabs collected from domestic dogs and cats in select regions of China during the period from 2021 to 2023. **C** Positivity rate and isolation rate of *Bb* from nasopharyngeal swab samples collected from dogs and cats. **D** PCR results of isolates and characterization of colonies on TSA plates

Drug resistance analysis of *Bb* isolates

The antimicrobial susceptibility evaluation results revealed that 100% of the isolated strains ($n=36$) were sensitive to amikacin, gentamicin, kanamycin, doxycycline, florfenicol, enrofloxacin, ciprofloxacin, chloromycetin, and polymyxin B. Over 80% of the isolated strains were sensitive to tetracycline (34 strains, 99.45%) and norfloxacin (32 strains, 88.98%). Approximately 55.56% ($n=20$) of the strains were sensitive to streptomycin, 41.67% ($n=15$) to ceftriaxone, and fewer proportions of the strains were sensitive to rifampicin (27.78%, $n=10$), cefotaxime (13.89%, $n=5$), selectrin (13.89%, $n=5$), and ampicillin (8.33%, $n=3$) (Fig. 2A, Table 1). All the strains ($n=36$) were resistant to fosfomycin. In addition, 100% of the isolated strains ($n=36$) were resistant to at least three antibiotics, 72.22% ($n=26$) to at least four, 50% ($n=18$) to at least five, and 22.22% ($n=8$) to at least six (Fig. 2B). All the feline-derived strains were resistant to the three drugs (Fig. 2C).

The antibiotics tested in this study can be categorized into nine groups: aminoglycosides (AMK, GEN, KAN and SM), broad-spectrum cephalosporins (CRO and CTX), fluoroquinolones (CIP, ENR, NOR and RIF), phenols (CHL and FLO), penicillins (AMP), tetracyclines

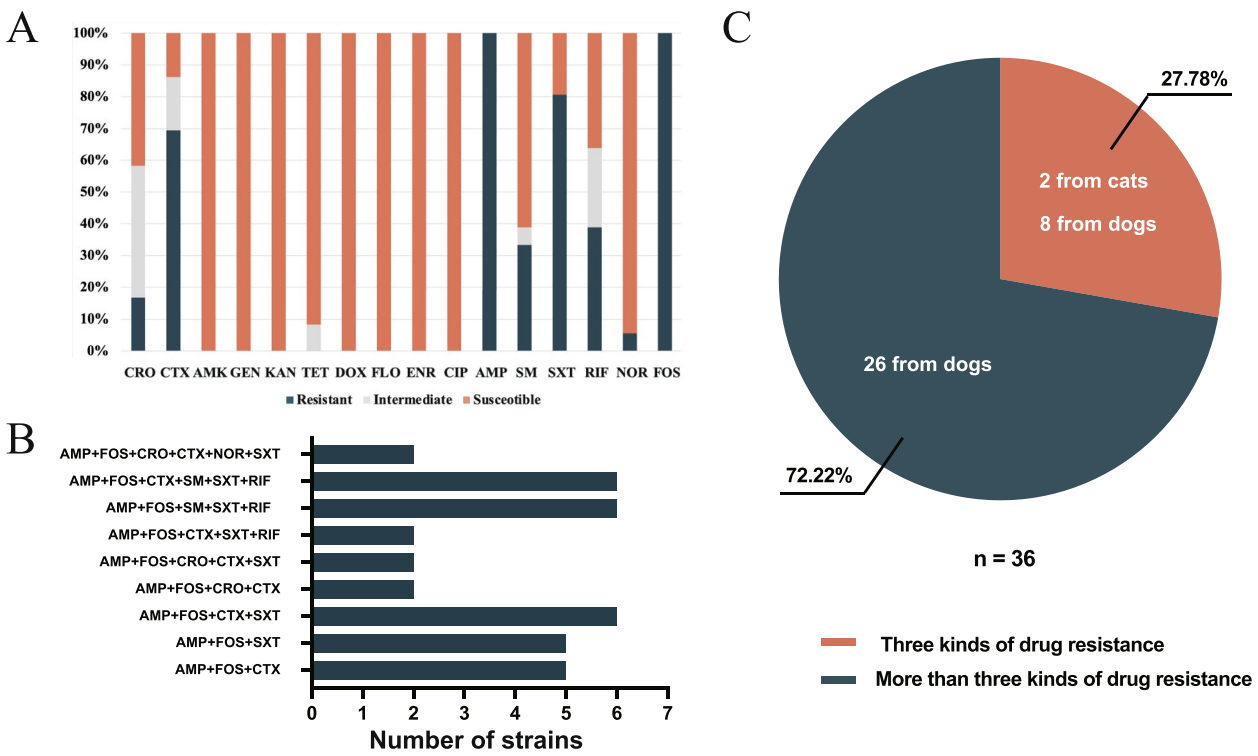

Fig. 2 Drug resistance phenotypes of 36 *Bb* isolates from dogs and cats in parts of China. **A** Percentages of susceptible, intermediate and resistant isolates to the 18 evaluated antibiotics. **B** Distribution of multidrug resistance. Ten *Bb* strains (eight dog-derived and two cat-derived *Bb* strains) exhibited resistance to three antibiotics, and 26 *Bb* strains displayed resistance to more than three antibiotics. **C** Number of isolates with multidrug resistance. The X-axis shows the number of *Bb* strains, and the Y-axis shows multidrug resistance. AMK, amikacin; GEN, gentamicin; KAN, kanamycin; CRO, ceftriaxone; CTX, cefotaxime; DOX, doxycycline; SM, streptomycin; SXT, Selectrin; ENR, enrofloxacin; CIP, ciprofloxacin; CHL, chloramphenicol; FLO, florfenicol; RIF, amoxicillin; AMP, ampicillin; NOR, norfloxacin; FOS, fosfomycin; TET, tetracycline; PMB, polymyxin B

(TET and DOX), polymyxins (PMB), sulfonamide antibiotics (SXT) and polyphosphates (FOS). All *Bordetella bronchiseptica* isolates in this study (100%, $n=36$) were resistant to at least three classes of antibiotics. These 36 *Bb* isolates could be defined as multidrug-resistant (MDR) strains according to international expert recommendations for provisional standard definitions of acquired resistance (Magiorakos et al. 2012). Among these multidrug-resistant strains, 33.33% ($n=12$), 22.22% ($n=8$), 27.78% ($n=10$), and 16.67% ($n=6$) of the isolates were resistant to class III, IV, V and VI drugs, respectively.

Analysis of the virulence factor-encoding genes of *Bb* isolates

The positivity rates of the *fhaB*, *prn*, *cyaA*, *dnt* and *betA* genes were 100% ($n=36$), 100% ($n=36$), 22.22% ($n=8$), 91.67% ($n=33$), and 100% ($n=36$), respectively (Fig. 3A, B). Furthermore, at least three out of five types of VFGs in all 36 strains were detected as positive. A total of 19.44% of the isolated strains ($n=7$) were found to simultaneously contain *fhaB*, *prn*, *cyaA*, *dnt* and *betA* (Fig. 3C). The remaining strains contained the following 3 VFG combinations: "*fhaB*+*prn*+*dnt*+*betA*" (72.22%, $n=26$), "*fhaB*+*prn*+*cyaA*+*betA*" (2.78%, $n=1$), and "*fhaB*+*prn*+*betA*" (5.56%, $n=2$) (Fig. 3C).

Pathogenicity evaluation of *Bb* isolates in mice

Ten strains isolated from dogs with respiratory symptoms were injected intraperitoneally at 10^7 CFU/mouse, and the results showed that eight strains were pathogenic, while two strains were not. The fatality rate of strains WH1218 and WH1201 was 100% (Table 2), and the fatality rate of WH1218 was greater than that of WH1201 in dogs (data not shown). Hence, the strain WH1218 was utilized to conduct subsequent aerosol challenge experiments in mice, dogs and cats.

Immune response and protective efficacy against *Bb* challenge in mice vaccinated with different inactivated wild-type strains

Inactivated vaccines containing eight *Bb* strains with high virulence were injected into mice. Serum samples

Table 1 Drug resistance phenotypes of 36 *Bb* isolates from dogs and cats in parts of China

Antimicrobial	Kirby–Bauer (KB) disk diffusion (mm)				
Type of antibiotic	Median	Range	S (n)	I (n)	R (n)
Ceftriaxone	20	10 - 30	15/36	15/36	6/36
Cefotaxime	10	0 - 26	5/36	6/36	25/36
Amikacin	30	21 - 35	36/36	0/36	0/36
Gentamicin	30	25 - 40	36/36	0/36	0/36
Kanamycin	32	25 - 40	36/36	0/36	0/36
Tetracycline	32	0 - 40	33/36	3/36	0/36
Doxycycline	37	20 - 45	36/36	0/36	0/36
Florfenicol	39	33 - 46	36/36	0/36	0/36
Enrofloxacin	31	26 - 37	36/36	0/36	0/36
Ciprofloxacin	38	29 - 46	36/36	0/36	0/36
Ampicillin	11	0 - 18	0/36	0/36	36/36
Streptomycin	20	0 - 32	22/36	2/36	12/36
Selectrin	0	0 - 37	7/36	0/36	29/36
Rifampicin	17	0 - 25	13/36	9/36	14/36
Norfloxacin	30	12 - 45	34/36	0/36	2/36
Fosfomycin	0	0	0/36	0/36	36/36
Chloromycetin	32	26 - 43	36/36	0/36	0/36
Polymyxin B	27	20 - 34	36/36	0/36	0/36

were collected on days 0, 14, and 28 postimmunization (dpi), and at 28 dpi, the mice were challenged with the wild-type strain WH1218 (Fig. 4A). *Bb*-specific IgG antibodies in the serum of immunized mice were measured by ELISA. No significant differences in *Bb*-specific IgG antibody production were detected among the different inactivated *Bb* strain vaccination groups (Fig. 4B). After challenge with the wild-type strain WH1218, the mice in the PBS group exhibited severe clinical symptoms, such as ocular and nasal discharge, anorexia, depression, intense abdominal respiration, fur dishevelment, and weight loss beginning at 2 days postchallenge (dpc), with a mortality rate of 100% (5/5). Conversely, mice vaccinated with the various inactivated wild strains displayed relatively mild clinical symptoms after challenge, including fur dishevelment and abdominal respiration. Notably, the surviving mice began to recover at 7 dpc. Among the vaccinated groups, mice inoculated with the inactivated vaccine containing the WH1218 strain had the highest survival rate of 80% (4/5). This was followed by the WH1203 and WH1224 strains, both of which exhibited a survival rate of 60% (3/5). The WH1212 and WH1811 strains exhibited a survival rate of 40% (2/5). The WH1816, WH1206, and WH1201 strains had the lowest survival rate of 20% (1/5) (Fig. 4C).

Immune response and protective efficacy against *Bb* challenge in dogs vaccinated with the *Bb* WH1218, WH1203 and WH1224 strains

The inactivated vaccines containing 3 *Bb* strains (WH1203, WH1218 and WH1224) with the best protective efficacy in mice were injected into dogs on days 0 and 21. Serum samples were collected on days 0, 7, 14, 21 28 and 35 after primary vaccination, and at 14 days after the second immunization, the dogs were challenged with the wild-type strain WH1218 (Fig. 5A). *Bb*-specific IgG antibodies in the serum were measured by ELISA. No significant differences in *the levels of Bb*-specific IgG antibodies were detected among the different groups vaccinated with the wild-type strains (Fig. 5B). Upon challenge, the PBS group exhibited severe clinical symptoms, which peaked at 7 dpc and were characterized by ocular and nasal discharge, sneezing, poor appetite, depression, severe abdominal breathing, frequent spontaneous coughing, and retching, with a morbidity rate of 100% (5/5). In contrast, experimental dogs vaccinated with various inactivated wild-type strains showed mild clinical symptoms after challenge, characterized by ocular and nasal discharge, sneezing and episodic coughing. Furthermore, the average clinical scores of the WH1218, WH1203, and WH1224 strain vaccination groups were significantly lower than those of the PBS group (Fig. 5C). Specifically, the WH1218 inactivated vaccine exhibited the highest protection rate of 100% (5/5), which was 80% (4/5) for the WH1203 strain and 60% (3/5) for the WH1224 strain (Fig. 5D). These results showed that the WH1218 strain is a promising *Bb* vaccine candidate.

Immune response and protective efficacy against the WH1218 challenge in dogs and cats inoculated with different doses of the WH1218 strain

Inactivated vaccines containing different doses of the WH1218 strain were injected into dogs on days 0 and 21. Serum samples were collected on days 0, 7, 14, 21, 28 and 35 after primary vaccination, and at 14 days after the second immunization, the dogs were challenged with *Bb* wild-type strain WH1218 (Fig. 5A). The levels of *Bb*-specific IgG antibodies in the sera of dogs and cats were measured using ELISA. In dogs, from 14 to 35 days after the first immunization, IgG antibody levels were significantly greater in the 10^9 CFU-immunized group than in the 10^8 CFU- and 10^7 CFU-immunized groups, and no significant difference in IgG antibody levels was detected between the 10^7 CFU- and 10^8 CFU-immunized groups (Fig. 5E). Dogs in the control group developed severe clinical symptoms after the challenge, including ocular and nasal discharge, sneezing, anorexia, depression, violent abdominal respiration, frequent spontaneous

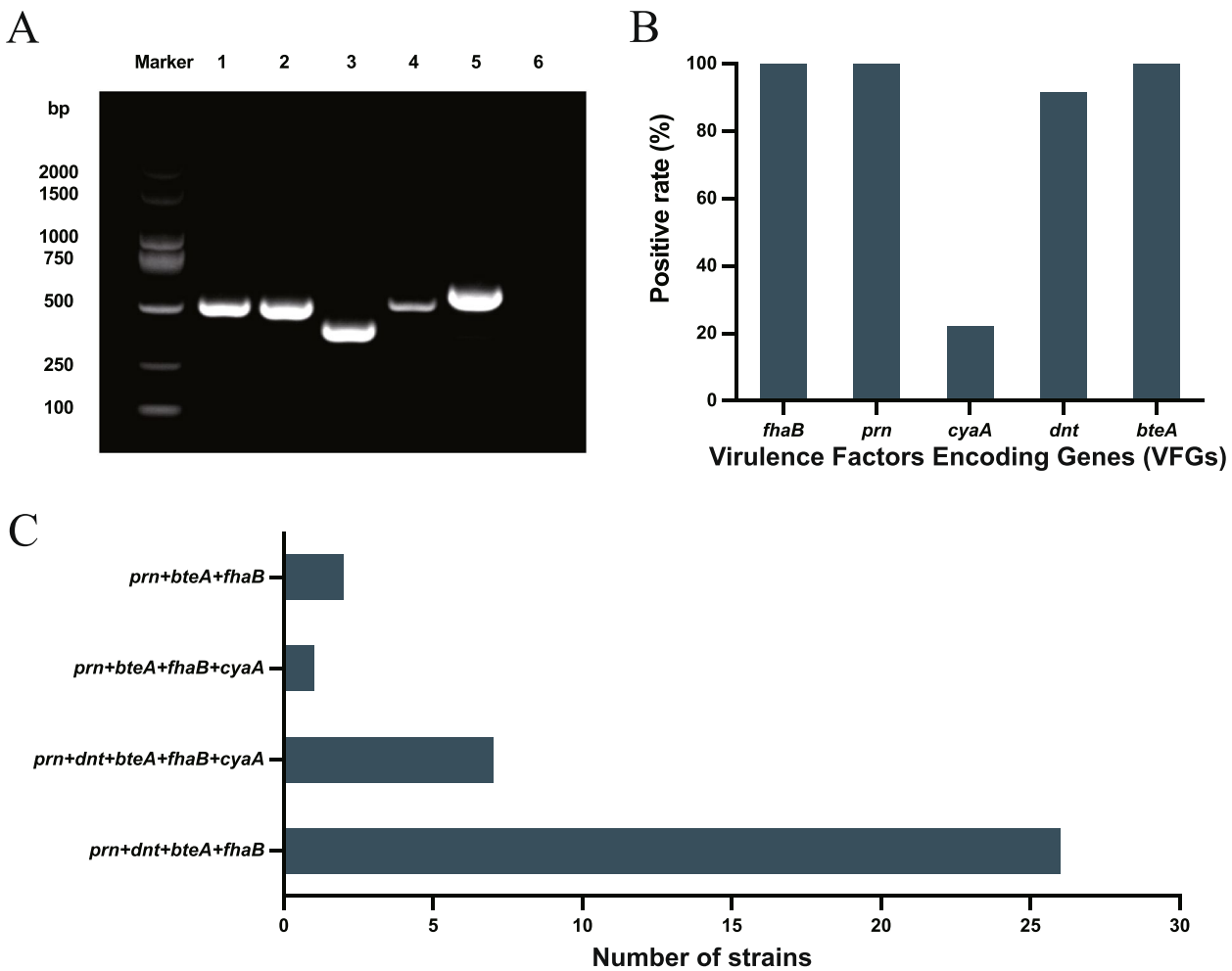

Fig. 3 PCR detection of virulence factor-encoding genes (VFGs) from *Bb* isolates. **A** Agarose gel analysis of the *betA* (band 2, 474 bp), *fhaB* (band 3, 475 bp), *cyaA* (band 1, 377 bp), *dnt* (band 4, 491 bp), and *prn* (band 5, 555 bp) PCR products. **B** Positive rate of 5 VFGs. **C** Number of strains containing different VFGs. X-axis: Number of *Bb* strains; Y-axis: Different VFGs

Table 2 Pathogenicity evaluation of *Bb* isolates in mice

Bacterial strain	Number	Dose (CFU/mouse)	Mortality
WH1201	5	1×10^7	100%
WH1218	5	1×10^7	100%
WH1206	5	1×10^7	80%
WH1224	5	1×10^7	80%
WH1811	5	1×10^7	80%
WH1203	5	1×10^7	60%
WH1212	5	1×10^7	60%
WH1816	5	1×10^7	60%
WH1220	5	1×10^7	0%
WH1808	5	1×10^7	0%

coughing, and retching. The clinical scores peaked at 7 dpc, and the morbidity rate was 100% (5/5). The clinical scores of the immunization groups inoculated with different doses of the WH1218 strain were significantly lower than those of the PBS group (Fig. 5F). The protection rates of the 10^7 CFU-, 10^8 CFU- and 10^9 CFU-immunized groups were 0 (0/5), 60% (3/5) and 100% (5/5), respectively (Fig. 5G). PCR revealed that the nasopharyngeal swabs from the dogs at 7 and 14 dpc were positive for *Bb*. In addition, at 14 dpc, the canine lung *Bb* counts of the 10^9 CFU-immunized group and 10^8 CFU-immunized group were significantly different from those of the controls, while those of the 10^7 CFU-immunized group were not different from those of the controls (Fig. 5H). Pathological examination revealed severe macroscopic

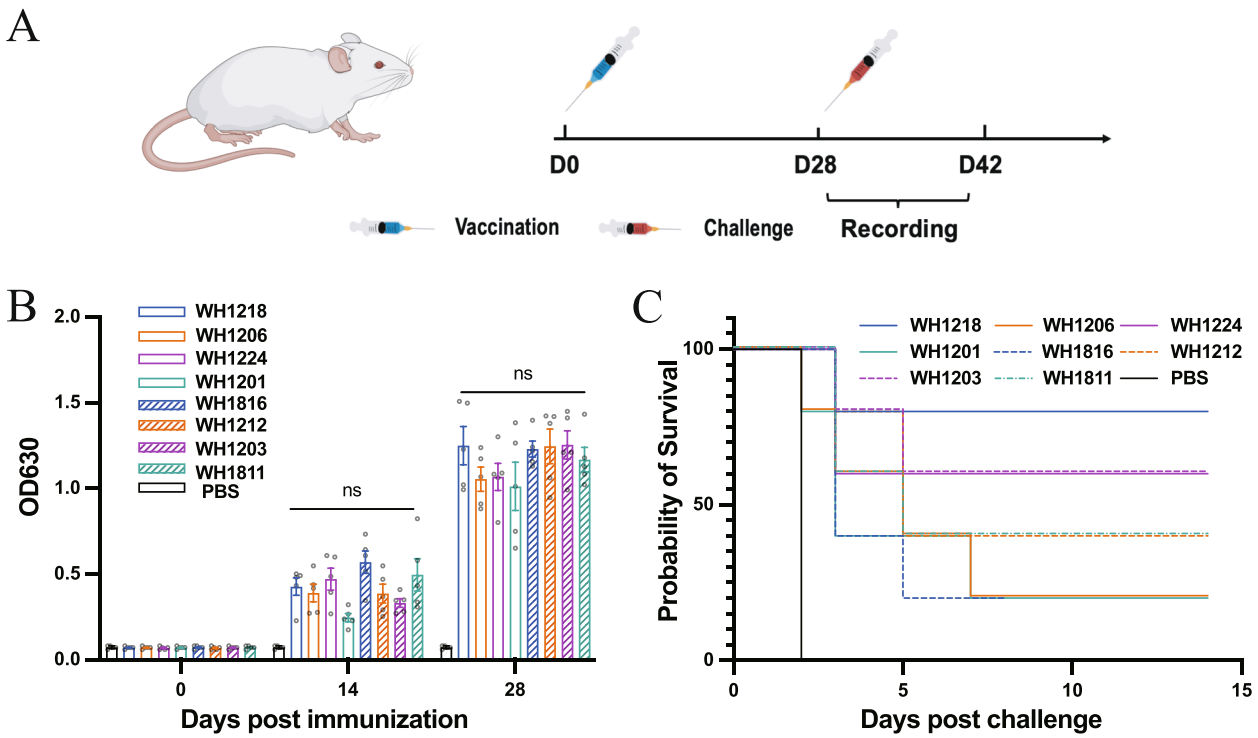

Fig. 4 Immunogenicity evaluation of inactivated *Bb* in mice. **A** Immunization procedures in mice. **B** IgG antibody levels against the WH1218 strain on days 0, 14 and 28 after immunization with eight different inactivated strains. **C** Survival curve of mice after immunization with eight different inactivated *Bb* strains. Ns, not significant; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$, and ****, $p < 0.0001$

pathological lesions in the lungs of the dogs in the PBS group, such as hemorrhage and necrosis; the lungs of the dogs in the low-dose immunization group also showed different degrees of lesions. In addition, the alveolar structure was unclear, with more atrophic collapses and marked thickening of the alveolar wall, as indicated by the black arrows. In contrast, no obvious pathological changes were detected in the 10^9 CFU-immunized group (Fig. 5I).

In cats, the immunization program was the same as that for dogs (Fig. 6A). At 21 to 35 days after the first immunization, the IgG antibody concentration was significantly greater in the 10^9 CFU-immunized group than in the 10^8 CFU- and 10^7 CFU-immunized groups (Fig. 6B). Cats in the control group developed severe clinical symptoms similar to those of dogs after the challenge, mainly spontaneous coughing and retching; the clinical scores peaked at 7 dpc, and the morbidity rate was 100% (5/5). The clinical scores of the immunized groups decreased as the immunization dose increased and were significantly lower than those of the PBS group (Fig. 6C). The protection rates of the 10^7 CFU-, 10^8 CFU- and 10^9 CFU-immunized groups were 40% (2/5), 80% (4/5) and 100% (5/5), respectively (Fig. 6D). Nasopharyngeal swabs from cats at 7 and 14 dpc were positive for *Bb* according to PCR. In addition,

(See figure on next page.)

Fig. 5 Investigation of the immunogenicity of the *Bb* WH1218 strain in dogs. **A** Experimental strategy. **B** The levels of *Bb*-specific IgG antibodies against the *Bb* WH1218 strain in dogs were measured at different time points (days 0, 7, 14, 21, 28, and 35) following the first immunization with three different inactivated strains. **C** Clinical scores of dogs immunized with three different inactivated strains after the WH1218 challenge. **D** Protection curve of dogs immunized with three different inactivated strains after the WH1218 challenge. **E** The levels of *Bb*-specific IgG antibodies against the *Bb* WH1218 strain in dogs were measured at different time points (days 0, 7, 14, 21, 28 and 35) following the first immunization with three different doses of WH1218. **F** Clinical scores of dogs immunized with three different doses of the WH1218 strain measured after the WH1218 challenge. **G** Protection curve of dogs washed after WH1218 challenge and immunized with three different doses of the WH1218 strain. **H** Dogs were immunized with three different doses of WH1218, and the *Bb* load in the lungs was determined 14 days after the WH1218 challenge. **I** Pathological lesions in the lungs of dogs in different immunization dose groups at 14 days after challenge. Pathological lesions are indicated with arrows. Ns, not significant; *, $p < 0.05$; **, $p < 0.01$; ** *, $p < 0.001$ and ****, and $p < 0.0001$

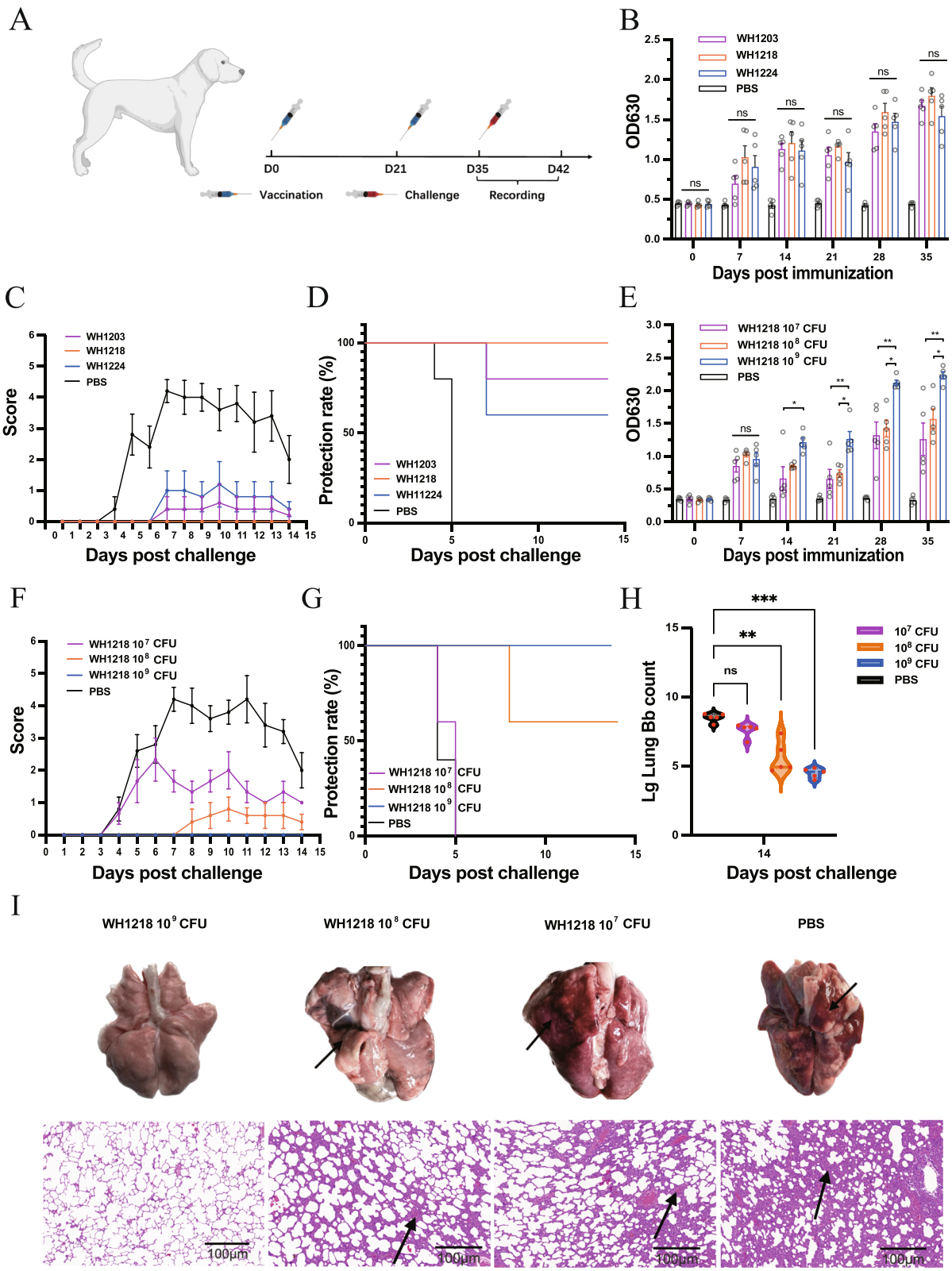

Fig. 5 (See legend on previous page.)

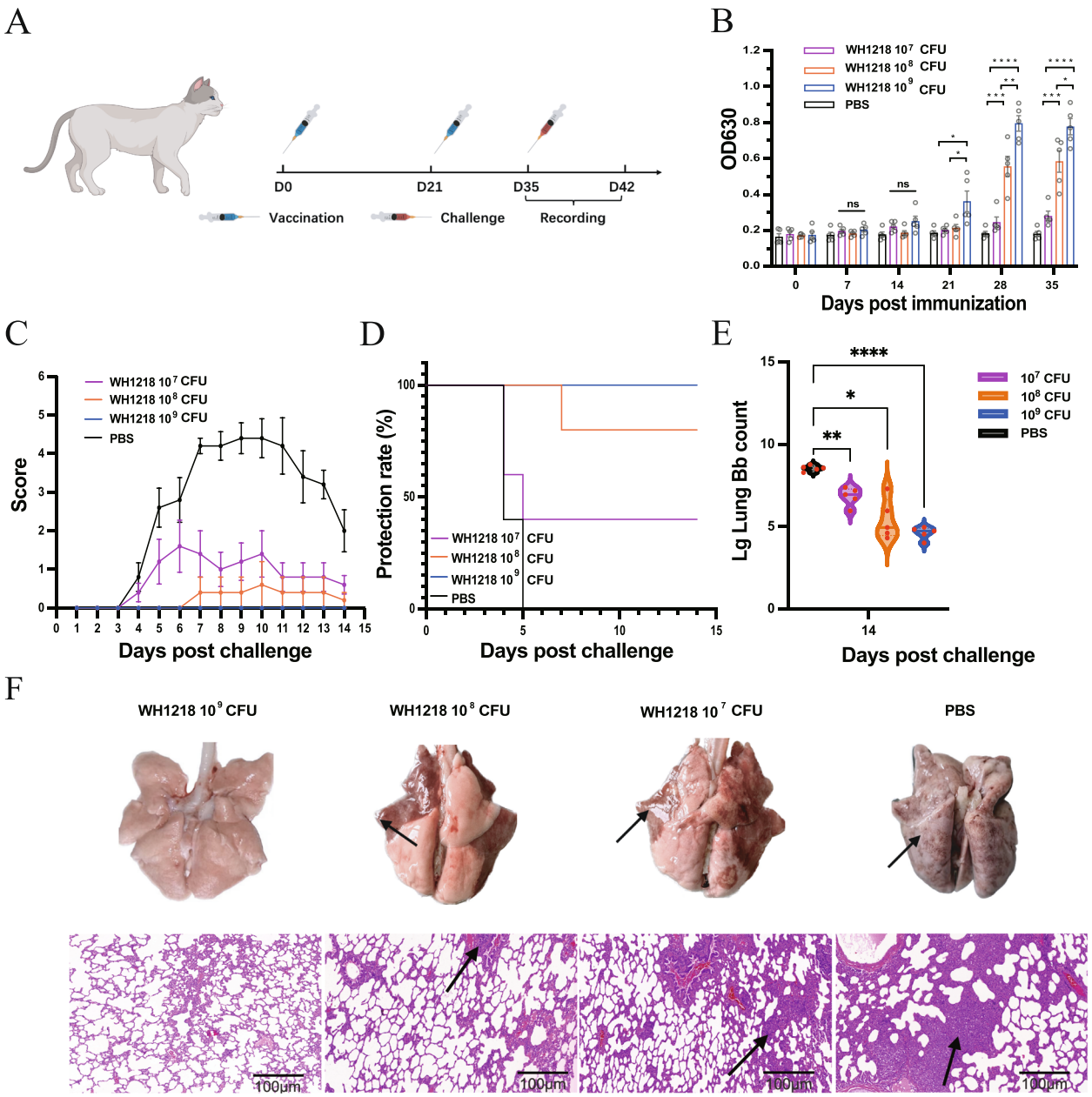

Fig. 6 Investigation of the immunogenicity of the *Bb* WH1218 strain in cats. **A** Experimental strategy. **B** The levels of *Bb*-specific IgG antibodies against the *Bb* WH1218 strain in cats were measured at different time points (days 0, 7, 14, 21, 28 and 35) following the first immunization with three different doses of WH1218. **C** Clinical scores of cats immunized with three different doses of the WH1218 strain measured after the WH1218 challenge. **D** Protection curve of WH1218-challenged cats immunized with three different doses of the WH1218 strain. **E** Cats were immunized with three different doses of WH1218, and the amount of *Bb* in the lungs was determined 14 days after the WH1218 challenge. **F** Pathological lesions in the lungs of cats in different immunization dose groups at 14 days after challenge. Pathological lesions are indicated with arrows. Ns, not significant; *, $p < 0.05$; **, $p < 0.01$; ** *, $p < 0.001$ and ****, and $p < 0.0001$

at 14 dpc, the cat lung *Bb* counts of the 10^9 CFU-immunized group, 10^8 CFU-immunized group, and 10^7 CFU-immunized group were significantly different from those of the controls (Fig. 6E). Pathological examination revealed severe macroscopic pathological lesions, such as hemorrhage and necrosis, in the lungs of the felines in the PBS group; the lungs of the felines in the low-dose immunization group also exhibited different degrees of lesions. In addition, the alveolar structure was unclear, with more atrophic collapses and marked

thickening of the alveolar wall, as indicated by the black arrows. In contrast, kittens in the 10^9 CFU-immunized group showed no obvious pathological changes (Fig. 6F).

Discussion

Between 2021 and 2023, the percentage of *Bb*-positive dogs in eight provinces in China ranged from 15.47% to 36.23%, and the percentage of *Bb*-positive dogs with respiratory diseases also confirmed that *Bb* is one of the main pathogenic bacteria for CIRDC (Bemis et al. 1977a; Maboni et al. 2019). It has been reported in the literature that canine *Bb* infections are common in the UK, USA, Iran, and Japan and dominate the infectious respiratory disease complex of the CIRD, and an Iranian study reported that cats had a positive rate of 39.13% for pharyngeal samples and 15.78% for nasal samples (Matsuu et al. 2020; Singleton et al. 2019; Tabatabaei and Rohani 2022; Yondo et al. 2023). These results demonstrated that *Bb* was prevalent in dogs and cats worldwide. In addition, some of the recent reports of *Bb* infections in China have branched out mainly to economic animals such as pigs and rabbits (Wang et al. 2020; Xu et al. 2023; Zhang et al. 2021). The isolation rate was 1.87% (36/1922) for dogs and cats in China from 2021 to 2023, which is much lower than that reported for pigs with clinical respiratory disease in China from 2018 to 2020 (1.87% vs. 4.25%, $P<0.05$) (Zhang et al. 2021). It could be that we have a smaller percentage of samples with respiratory tracts, or it could be that dogs and cats receive more care as companion animals.

VFGs play a pivotal role in the pathogenesis of bacteria (Sharma et al. 2017). These VFGs facilitate the invasion of *Bb* into its host (Fingermann and Hozbor 2015). Notably, *fhaB, prn* and *bteA* exhibited a 100% detection rate; *dnt,* 91.67%; and *cyaA*, only 22.22%. Of all the *Bb* strains analyzed in this study, only isolates from genotype ST27 were not β-hemolytic (data not shown); these strains lost the hemolytic activity of *CyaA*, which is consistent with previous studies (Buboltz et al. 2008). Since *CyaA* is antigenic (Gueirard and Guiso 1993), *B. bronchiseptica* strains lacking this antigen can evade anti-*CyaA* antibody responses, potentially giving this strain a selective advantage for living in hosts. Another possibility is that strains lacking *CyaA* may not be as lethal and may allow more hosts to survive and transmit the bacteria (Blaser and Kirschner 2007). These studies demonstrated that most isolates lacked *CyaA*. However, more than 90% positivity for *CyaA* has been reported in Chinese pigs and rabbits, possibly because their samples were all from morbid animals (Wang et al. 2020; Zhang et al. 2021), whereas most of the samples in our study were from healthy dogs, resulting in a lower positivity rate of *CyaA*. In a murine model of infection, *CyaA* contributes to pathology, lethality, and the colonization and persistence of *Bb* and *B. pertussis* in the lower respiratory tract (Guiso et al. 1989; Harvill et al. 1999; Khelef et al. 1992; Weingart and Weiss 2000). Surprisingly, we found that cats were more sensitive to the WH1218 strain challenge, since the challenge dose of dogs was tenfold greater than that of cats, despite this strain being isolated from dogs. This result suggests that cats may be more sensitive to *Bb* than dogs and complements the absence of cats from previous reports of *Bb* susceptibility to different species (Bemis 1992).

The administration of antimicrobials is still one of the most effective ways to control *Bb* and other bacteria, but the emergence of drug-resistant bacteria may lead to the failure of the use of antibiotics in the clinic (Lappin et al. 2017). Monitoring the antimicrobial resistance of clinical microbes is essential for epidemiological studies (Wang et al. 2020). In this study, most of the strains were sensitive to tetracycline (94.44%) and norfloxacin (88.89%), which is consistent with previous reports in pigs and rabbits (Wang et al. 2020; Zhang et al. 2021). This result suggested that these two antibiotics could be effective for treating *Bb* infections. However, tetracycline should be avoided as much as possible in young animals, with the potential for adverse effects on bones and teeth (Meyers et al. 2020). In addition, most of the isolates were sensitive to amikacin, gentamicin, kanamycin, doxycycline, florfenicol, enrofloxacin, ciprofloxacin, chloromycetin and polymyxin B, suggesting that these antibiotics might be suitable candidates for treating *Bb* infections when necessary. Cases of bronchial septic bordetellosis treated with nebulized gentamicin have been reported (Clemmons et al. 2021; Morgane Canonne et al. 2020), and the low systemic bioavailability of nebulized gentamicin effectively prevents side effects such as nephrotoxicity and ototoxicity (Al-Amoud et al. 2005). All the isolates were resistant to fosfomycin (100%), followed by ampicillin (91.67%). These findings were consistent with those of previous studies (Kadlec and Schwarz 2018; Wang et al. 2020). Therefore, these drugs are not recommended for use in clinical settings. Our study also revealed that dog-derived strains were generally sensitive to tetracycline and resistant to the compounds neomycin and rifampin, but the opposite results were obtained for cat-derived strains. Doxycycline is regarded as a first-line antibiotic for the treatment of infections caused by *Bb* (Lappin et al. 2017), possibly because of the overuse of doxycycline in the clinical setting. However, more studies on cat-derived isolates are needed to support our conclusion considering the small number of cat-derived *Bb* isolates. Our data showed that most *Bb* isolates in China were multidrug resistant, especially to aminoglycosides, broad-spectrum cephalosporins, and penicillins. This finding is in line

with studies of porcine *Bb* strains in China (Zhang et al. 2021). However, aminoglycoside, broad-spectrum cephalosporin and penicillin antibiotics are commonly used in veterinary medicine for the treatment of Mycobacterium bronchisepticum infections. Therefore, there is a need for continuous clinical monitoring of the prevalence of and trends in these multidrug resistance patterns in isolates (Kadlec and Schwarz 2018; Lappin et al. 2017).

In a series of reports, inactivated parenteral vaccines for *Bb* failed to provide complete protection against *Bb* wild-type strain challenge (Bemis et al. 1977b; McCandlish et al. 1978a, b). In our study, dogs and cats received two subcutaneous immunizations of an inactivated vaccine with 10^9 CFU of WH1218 at three-week intervals, which provided complete protection against *Bb* challenge. This might be due to differences in the immunogenicity and immunizing dose of the *Bb* strain. Our results showed that the levels of *Bb*-specific IgG antibodies in the serum of both cats and dogs inoculated with different doses of the WH1218 strain did not significantly differ after the first vaccination and increased sharply after the second vaccination. In addition, mice that received only a single vaccination could not provide full protection. These results demonstrated that a second or "booster" immunization was necessary for complete protection against *Bb* infection. Several studies have reported an association between both mucosal and systemic *Bb* antibody responses and subsequent disease sparing in *Bb*-infected dogs and other species (Ellis et al. 2001; Goodnow et al. 1979; McCandlish et al. 1978a; Shade and Goodnow 1979; Stephenson et al. 1989). However, we detected serum *Bb*-specific IgA and nasal lavage *Bb*-specific IgA and IgG in dogs and cats, which were not significantly different from those in controls (data not shown). Our results show that reliance on a systemic antibody response is sufficient to prevent infection with wild *Bb*. Th1, Th2 and Th17 responses are reportedly activated in mice after inactivated *Bb* vaccination (Cui et al. 2022). There are also reports that Th1 and Th17 responses contribute to the clearance of bacteria after *Bp* infection in mouse models, which further confirms the feasibility of an all-bacteria inactivated vaccine (Borkner et al. 2021; Dubois et al. 2021; Ross et al. 2013). In our study, *Bb*-specific IgG antibodies were positively correlated with protection rates when dogs were immunized with vaccines containing different concentrations of the WH1218 strain, whereas no correlation was found between *Bb*-specific IgG antibodies and protection rates when dogs were immunized with vaccines containing different *Bb* isolates. We hypothesized that *Bb*-specific IgG antibodies might not be a suitable factor to estimate the protection of different *Bb* strains, and other factors, such as mucosal immunity and Th1 and Th17 responses, should be explored in immunoprotection experiments (Bey et al. 1981; Chamorro et al. 2023; Yount et al. 2019).

Conclusion

This study provides insights into the prevalence, antibiotic resistance, VFGs, virulence and immunization strategies related to *Bb* in dogs and cats in parts of China from 2021 to 2023. The results showed that *Bb* is a crucial pathogen that causes canine respiratory diseases in dogs. Most *Bb* isolates showed multiple drug resistance traits and possessed at least 4 VFGs. These results highlight the need for ongoing monitoring of the prevalence, VFGs and antibiotic resistance of *Bb* in China. Notably, we identified an effective inactivated vaccine candidate *Bb* strain, WH1218, which could provide complete production against *Bb* wild-type strain challenge in dogs and cats.

Methods

Sample collection

From 2021 to 2023, 1809 canine and 113 feline nasopharyngeal swab samples were collected from eight provinces of China, namely, Guangdong, Henan, Hubei, Shandong, Fujian, Guangxi, Hunan and Liaoning (Fig. 1A), for the isolation and identification of *Bb*. Among the canine samples, 153 were supplied by veterinarians or collected from pet markets from dogs with respiratory symptoms, and the other 1656 samples were collected from rescue shelters or kennels from dogs with no respiratory symptoms. In addition, 113 feline samples were collected from field cats or pet cats with no respiratory symptoms via a random sampling method.

Bacterial isolation and identification

PCR was used to identify *Bb* based on the *Fla* gene locus with the primers listed in Table 3 (Hozbor et al. 1999). PCR-positive swab samples were vortexed with TSB medium containing 5% fresh bovine serum. Then, each swab sample was streaked on a tryptic soy agar (TSA; Becton, Dickinson and Company, MD, USA) plate containing 10 μg/mL nicotinamide adenine dinucleotide (NAD; Sigma, St. Louis, MO) and 10% newborn bovine serum. The agar plates were incubated at 37°C for 24–48 h. The plate-grown isolates were then purified and cultured using standard methods for bacterial identification (Jorgensen et al. 2015). On each agar plate, five colonies with morphological characteristics similar to those of *Bb* (small, round, shiny, or rough colonies with a diameter of 0.5 to 1.0 mm) were selected and verified via PCR and biochemical evaluation (Woolfrey and Moody 1991).

Table 3 Primers used in the present study

Primers	Sequences (5′-3′)	Product size (bp)	Tm (°C)	Description	References
Bacterial species identification genes					
*Fla*1	TGGCGCCTGCCCTATC	237	56	*Bb* identification	Hozbor et al. 1999
*Fla*2	AGGCTCCCAAGAGAGAAA				
Virulence factors encoding genes					
FhaB-1	GCGCAGAACATCACCAATG	475	59	Filamentous hemagglutinin encoding gene	Wang et al. 2020
FhaB-2	TGAAATACTCCATGGCGGAC				
Prn-1	GACCTCGCTCAGTCGATC	555	59	Pertactin encoding gene	
Prn-2	GAAGACATTCATGCGGAACAG				
CyaA-1	CTACGAGCAGTTCGAGTTTC	377	59	Adenylate cyclase-hemolysin toxin encoding gene	
CyaA-2	TATTCATGTCGCCGTCGTA				
Dnt-1	TGATCCTGCAGTGGTTGATC	491	59	Dermonecrotic toxin encoding gene	
Dnt-2	ATCGGCATACGCCAGATC				
BteA-1	TGTTGAGCAACAACGTCAATC	474	59	Bordetella type-III secretion system effector A encoding gene	
BteA-2	TATGCAGGTCTTCGAGGTTC				

Antimicrobial susceptibility evaluation

The disc diffusion method was used to evaluate the antimicrobial susceptibility of the *Bb* isolates (CLSI 2022). Specifically, overnight-cultured colonies of *Bb* from TSA plates were purified and suspended in sterile 0.9% normal saline to obtain a 0.5 McFarland standard. The resulting suspension was coated onto Mueller–Hinton (MH) agar (Sigma-Aldrich, 102 St. Louis, MO) using sterile swabs, after which the discs impregnated with specific antibiotics (Hangzhou Microbial Reagent, Hangzhou, China) were placed on agar plates. After five min of drying, the agar plates were incubated overnight at 37°C. The antibiotics used in this study included amikacin (AMK; 30 μg), kanamycin (KAN; 30 μg), doxycycline (DOX; 30 μg), streptomycin (SM; 10 μg), selectrin (SXT; 250 μg), rifampicin (RIF; 5 μg), norfloxacin (NOR; 10 μg), fosfomycin (FOS; 200 μg), ciprofloxacin (CIP; 5 μg), gentamicin (GEN; 10 μg), ceftriaxone (CRO; 30 μg), cefotaxime (CTX; 30 μg), enrofloxacin (ENR; 10 μg), chloramphenicol (CHL; 30 μg), florfenicol (FLO; 30 μg), ampicillin (AMP; 10 μg), tetracycline (TET; 30 μg), and polymyxin B (PMB; 300 IU). After incubation, the bacterial inhibition zone diameter was measured, and the results were interpreted according to the Clinical and Laboratory Standards Institute document (CLSI 2022). Owing to the limited availability of clinical breakpoints specific to *Bb* (Kadlec and Schwarz 2018), we employed breakpoints of Enterobacteriaceae in the CLSI document M100 to interpret our results. In addition, we utilized *Escherichia coli* ATCC®*25922 for quality control.

Detection of virulence factor-encoding genes (VFGs)

The presence of five well-characterized VFGs of *Bb* isolates, including the filamentous hemagglutinin-encoding gene *fhaB,* the pertactin-encoding gene *prn*, the adenylate cyclase-hemolysin toxin-encoding gene *cyaA*, the dermonecrotic toxin-encoding gene *dnt*, and the *Bordetella* type-III secretion system effector A-encoding gene *bteA*, was examined by PCR with the primers listed in Table 3. The 20 μL PCR system contained 2 μL of bacterial DNA, 1 μL of each forward and reverse primer, 10 μL of 2 × Taq Master Mix (Dye Plus), 2 μL of DMSO, and 4 μL of ultrapure water. PCR procedures were as follows: an initial phase at 94°C for 5 min, followed by 35 cycles of 94°C for 30 s, 59°C for 30 s, and 72°C for 30 s, with a final extension at 72°C for 5 min. *Bb* strain HH0809 (Ai et al. 2019), preserved in the laboratory of Huazhong Agricultural University, was used as a positive control, and sterile ultrapure water was used as a negative control. Finally, the PCR products were analyzed by electrophoresis on a 1% agarose gel.

Virulence and protective efficacy of *Bb* isolates in mice

To determine the virulence of the isolated *Bb* strains, 55 4-week-old female BALB/c mice were divided into 11 groups with 5 mice per group, and Groups 1–10 were challenged intraperitoneally with one of 10 *Bb* strains isolated from the dogs with respiratory symptoms (0.2 mL/mouse, 10^7 CFU/mouse). The same volume of normal saline (0.2 mL/mouse) was injected into Group 11 as a control. After challenge, the clinical symptoms of the mice were observed for 14 d, and

the mice were euthanized when the weight decreased to 75% of their original body weight. At 14 d postchallenge (dpc), all the mice were euthanized.

To determine the protective efficacy of *Bb* strains in mice, 8 *Bb* strains with high virulence were adjusted to 10^9 CFU/mL, inactivated by beta-propiolactone (1:1000) and mixed with aluminum glue adjuvant at a final concentration of 0.5 mg/mL to obtain vaccines. Four-week-old 90 BALB/c mice were divided into nine groups with 10 mice per group. Groups 1-8 were injected intramuscularly with the inactivated vaccines, and Group 9 was injected with the same amount of PBS as a negative control (Fig. 4A). On 0, 14, and 28 dpi, serum samples were collected from the tail vein of the mice to track their *Bb*-specific IgG antibodies. At 28 dpi, the mice were challenged with *Bb* wild-type strain WH1218 (method 2.8). After challenge, the clinical symptoms of the mice were observed for 14 days, and the mice were euthanized when their body weight decreased to 75%.

Immune responses and protective efficacy after immunization with WH1203, WH1218 and WH1224 in dogs

To evaluate the immune responses and protective efficacy of *Bb* isolates in dogs, three isolates (WH1203, WH1218 and WH1224) with the best protective efficacy in mice were adjusted to 10^9 CFU/mL, inactivated by beta-propiolactone (1:1000), and mixed with a water adjuvant to obtain an inactivated vaccine. Twenty healthy 6- to 10-week-old dogs free of antigens and antibodies to *Bb* were randomly allocated to one of four groups ($n=5$ dogs/group). Groups 1-3 were injected subcutaneously with 1 mL (10^9 CFU/mL) of the inactivated vaccine containing the WH1203 strain, the WH1218 strain or WH1224 on days 0 and 21, and group 4 was injected with the same amount of PBS as a control (Fig. 5A). Serum samples were collected on days 0, 7, 14, 21, 28 and 35 after primary vaccination to track their *Bb*-specific IgG antibodies. At 14 d after the second immunization, all the canines were challenged with the *Bb* wild-type strain WH1218. After challenge, clinical scores (according to Table 4) (Ellis et al. 2016) were recorded daily for 14 days, and the dogs were euthanized when their scores exceeded 12 points. At 14 dpc, all dogs were euthanized.

Immune responses and protective efficacy after immunization with different doses of the WH1218 strain in dogs and cats

To further evaluate the immune responses and protective efficacy of the selected strain WH1218 in dogs and cats, the WH1218 strain was inactivated by beta-propiolactone (1:1000) and mixed with a water adjuvant to obtain an inactivated vaccine containing the WH1218 strain at final concentrations of 10^7 CFU/mL, 10^8 CFU/mL, or 10^9 CFU/mL. Twenty healthy 6- to 10-week-old dogs or cats free of antigens and antibodies to *Bb* were randomly allocated to one of four groups ($n=5$ dogs each). The dogs and cats in groups 1-3 were injected subcutaneously with 1 mL of the inactivated vaccine containing 10^7 CFU/mL, 10^8 CFU/mL or 10^9 CFU/mL of the WH1218 strain on days 0 and 21, and group 4 was injected with the same amount of PBS as a control (Fig. 5A). Serum samples were collected on days 0, 7, 14, 21, 28 and 35 after primary vaccination to track their *Bb*-specific IgG antibodies. At 14 days after the second immunization, all the dogs or cats were challenged with the *Bb* wild-type strain WH1218. After the challenge, clinical scores (according to Table 4) (Ellis et al. 2016) were recorded daily for 14 days, and dogs or cats were euthanized when their scores exceeded 12 points. At 14 dpc, all dogs or cats were euthanized, and lung samples were collected for analysis of pathological lesions and *Bb* counts.

Mouse, dog and cat challenge experiments

The highly virulent *Bb* WH1218 strain was cultured on selective Bordet Gengou (BG) agar plates following previously described procedures (Ellis et al. 2001). The relationship between colony forming units (CFUs) and turbidity during the culture process was predetermined, and the turbidity of the culture suspension was measured to determine the amount of live WH1218 strain in the culture suspension before challenge. Ten mice were exposed to 12 mL of an atomized culture suspension containing approximately 1.2×10^{13} CFU/mL (1×10^6 CFU per mouse) of the WH1218 strain for 45 min in a confined space (35 cm in length, 20 cm in width, 20 cm in height). At one time, 5 dogs or cats were exposed to 8 ml of atomized culture suspension containing approximately 8×10^{10} CFU for dogs (1×10^{10} per dog) or 8×10^9 for cats (1×10^9 per cat) for 30 min in a confined space (60 cm in length, 50 cm in width, and 50 cm in height).

Measurement of *Bb*-specific IgG and IgA by ELISA

Bb-specific IgG and IgA antibodies were measured by ELISA as previously described (Boot et al. 1993). Briefly, the culture suspension of the WH1218 strain was centrifuged, and the collected bacteria were washed with PBS 3 times. The bacteria were subsequently sonicated and resuspended in physiological saline and stored at -70°C until use. Ninety-six-well flat-bottomed microtiter plates were coated with 7.5 μg of antigen per well dissolved in carbonate buffer and incubated overnight at 4°C. After removing the antigen coating, the plate was washed with double-distilled water containing 0.05% Tween 20 and then incubated with 1% bovine serum albumin (BSA) at 37°C for 2 h. Serum samples were diluted in PBS at a

Table 4 Clinical scoring rubric (Ellis et al. 2016)

Clinical sign	Score	Description
Nasal discharge	0	Absent: includes normal, moist nose
	1	Mild: serous (clear, watery) discharge
	2	Moderate: evidence of mucopurulent discharge
	3	Severe: bloody discharge, or a combination of mucopurulent and bloody discharge
Ocular discharge	0	Absent
	1	Mild: serous discharge
	2	Moderate: evidence of mucopurulent discharge
	3	Severe: bloody discharge, or a combination of mucopurulent and bloody discharge
Cough	0	Absent
	1	Mild: one cough episode
	2	Moderate: spontaneous and frequent coughing; two or more coughing episodes
	3	Severe: spontaneous coughing with frequent retching; animal had persistent and prolonged cough
Sneezing	0	Absent
	1	Mild: animal sneezed once or twice
	2	Moderate: animal sneezed repeatedly
	3	Severe: animal presented paroxysmal sneeze
Depression	0	Absent
	2	Moderate: animal is able to rise and move, but inactive other than to eat or drink
	3	Severe: animal is recumbent, unable to rise, and refuses food and/or drink
Retching	0	Absent
	1	Mild: animal retches or vomits once briefly or occasionally
	2	Severe: animal retches or vomits multiple times for a prolonged period
Respiration	0	Normal respiration
	2	Moderate: small clicking, bubbling, or ratting sounds in the lung (rales)
	3	Severe: difficult or labored breathing; shortness of breath (dyspnea)

1:50 ratio and added to the plate at 50 µL per well. The plate was incubated at 37°C for 1 h, washed, and incubated with horseradish peroxidase (HRP)-labeled rabbit anti-cat IgG (1:10000), rabbit anti-dog IgG (1:10000), goat anti-mouse IgG (1:10000), and goat anti-mouse IgA (1:10000) at 37°C for 60 min. The results were read at 630 nm. Sera from species that were neither vaccinated nor challenged with *Bordetella* were used as negative controls, and those from species that were vaccinated or challenged with *Bordetella* were used as positive controls. Blank wells contained a PBS solution with 0.05% Tween 20 and 0.2% gelatin.

Clinical scoring and morbidity criteria for dogs and cats

Dogs and cats were clinically scored according to a previously reported scale involving the assessment of nasal discharge, eye droppings, coughing, sneezing, depression, regurgitation, and respiration, with the severity of each symptom ranging from 0 to 3 points (Table 4) (Ellis et al. 2016). The morbidity criteria were when dogs or cats coughed or their clinical scores were greater than 3 for two consecutive days.

Statistical analysis

Statistical analysis was conducted using one-way ANOVA or Student's t test in GraphPad Prism V. 8.3.0. The data are expressed as the mean ± standard deviation (SD). The levels of significance were set as follows: $P < 0.05$ (*) denoting a significant difference, $P < 0.01$ (**) indicating a highly significant difference, and $P < 0.001$ (***) representing an extremely significant difference.

Abbreviations

Bb	*Bordetella bronchiseptica*
VFGs	Virulence factor encoding genes
CIRDC	Canine infectious respiratory disease
CFU	Colony forming unit
MR	The methyl red
VP	Voges-Proskauer
dpi	Days postimmunization

dpc	Days postchallenge
CLSI	Clinical and Laboratory Standards Institute
fhaB	Filamentous hemagglutinin-encoding gene
prn	Pertactin-encoding gene
cyaA	Adenylate cyclase-hemolysin toxin-encoding gene
dnt	Dermonecrotic toxin-encoding gene
bteA	The *Bordetella* type-III secretion system effector A-encoding gene

Acknowledgements
Not applicable.

Authors' contributions
QS, LZ, and CZ delineated the study conception and design. LZ and CZ supervised the study. QS, WG, XZ, HL, YW, and JZ collected the bacterial isolates, performed laboratory tests and analyzed the data, and QS, WG and XZ participated in the animal experiments and analyzed the data. QS, CZ and JZ wrote the manuscript and approved the final version for publication. CZ, LZ, MZ, ZF, and HC participated in the manuscript discussion and revision. All authors have read and approved the final version of the manuscript.

Funding
This study was supported by the Guangdong Major Project of Basic and Applied Basic Research (2020B0301030007).

Availability of data and materials
The data that support the findings of this study are openly available in this manuscript and are available from the corresponding author upon request.

Declarations

Ethics approval and consent to participate
All mouse studies were conducted at the Laboratory Animal Center of Huazhong Agricultural University, and the mice were fed in specific pathogen-free (SPF) environments. During the *Bb* challenge study, the mice with a body weight decrease of more than 25%, severe paralysis, or inability to feed were euthanized by CO_2. The experimental protocol was reviewed and approved by the Scientific Ethics Committee of Huazhong Agricultural University with the approval number HZAUMO-2020–0096. The dogs and cats used in this study were fed in isolation environments as recommended by the Regulations for the Administration of Affairs Concerning Experimental Animals of the Ministry of Science and Technology of China. The immunization and challenge of *Bb* experiments were performed using protocols that were approved by the Scientific Ethics Committee of Huazhong Agricultural University (permit numbers: HZAUCA-2020-002 and HZAUDO-2020–003).

Consent for publication
Our research paper agreed to be published in Animal Diseases.

Competing interests
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this study. Author Ling Zhao was not involved in the journal's review or decisions related to this manuscript.

Author details
[1]National Key Laboratory of Agricultural Microbiology, Huazhong Agricultural University, Wuhan 430070, China. [2]Key Laboratory of Preventive Veterinary Medicine of Hubei Province, College of Veterinary Medicine, Huazhong Agricultural University, Wuhan 430070, China. [3]Hubei Hongshan Laboratory, Wuhan 430070, China.

Received: 30 January 2024 Accepted: 31 March 2024
Published online: 17 May 2024

References

Abdoli, A., R. Aalizadeh, H. Aminianfar, Z. Kianmehr, A. Teimoori, E. Azimi, N. Emamipour, M. Eghtedardoost, V. Siavashi, H. Jamshidi, M. et al. 2022. Safety and potency of BIV1-CovIran inactivated vaccine candidate for SARS-CoV-2: a preclinical study. *Reviews in Medical Virology* 32:e2305. https://doi.org/10.1002/rmv.2305.

Agarwal, L., H. Singh, C. Jani, P. Banankhah, M. Abdalla, J.S. Kurman, and R.A. Franco. 2022. A wolf in sheep's clothing: fogs confer an unrecognized risk for their immunocompromised master. *Respiratory Medicine Case Reports* 38: 101672. https://doi.org/10.1016/j.rmcr.2022.101672.

Ai, W., Z. Peng, F. Wang, Y. Zhang, S. Xie, W. Liang, L. Hua, X. Wang, H. Chen, and B. Wu. 2019. A Marker-Free *Bordetella bronchiseptica* aroA/bscN double deleted mutant confers protection against lethal challenge. *Vaccines* (Basel) 7.https://doi.org/10.3390/vaccines7040176.

Al-Amoud, A.I., B.J. Clark, K.A. Assi, and H. Chrystyn. 2005. Determination of the bioavailability of gentamicin to the lungs following inhalation from two jet nebulizers. *British Journal of Clinical Pharmacology* 59: 542–545. https://doi.org/10.1111/j.1365-2125.2005.02360.x.

Bemis, D.A. 1992. Bordetella and Mycoplasma respiratory infections in dogs and cats. *The Veterinary Clinics of North America. Small Animal Practice* 22: 1173–1186. https://doi.org/10.1016/s0195-5616(92)50308-4.

Bemis, D.A., L.E. Carmichael, and M.J. Appel. 1977a. Naturally occurring respiratory disease in a kennel caused by *Bordetella bronchiseptica*. *The Cornell Veterinarian* 67:282–293.

Bemis, D.A., H.A. Greisen, and M.J. Appel. 1977b. Pathogenesis of canine bordetellosis. *Journal of Infectious Diseases* 135: 753–762. https://doi.org/10.1093/infdis/135.5.753.

Bey, R.F., F.J. Shade, R.A. Goodnow, and R.C. Johnson. 1981. Intranasal vaccination of dogs with liver avirulent *Bordetella bronchiseptica*: correlation of serum agglutination titer and the formation of secretory IgA with protection against experimentally induced infectious tracheobronchitis. *American Journal of Veterinary Research* 42:1130–1132.

Blaser, M.J., and D. Kirschner. 2007. The equilibria that allow bacterial persistence in human hosts. *Nature* 449: 843–849. https://doi.org/10.1038/nature06198.

Boot, R., R.H. Bakker, H. Thuis, and J.L. Veenema. 1993. An enzyme-linked immunosorbent assay (ELISA) for monitoring guineapigs and rabbits for *Bordetella bronchiseptica* antibodies. *Laboratory Animals* 27: 342–349. https://doi.org/10.1258/002367793780745624.

Borkner, L., L.M. Curham, M.M. Wilk, B. Moran, and K.H.G. Mills. 2021. IL-17 mediates protective immunity against nasal infection with *Bordetella pertussis* by mobilizing neutrophils, especially Siglec-F(+) neutrophils. *Mucosal Immunology* 14: 1183–1202. https://doi.org/10.1038/s41385-021-00407-5.

Buboltz, A.M., T.L. Nicholson, M.R. Parette, S.E. Hester, J. Parkhill, and E.T. Harvill. 2008. Replacement of adenylate cyclase toxin in a lineage of *Bordetella bronchiseptica*. *Journal of Bacteriology* 190: 5502–5511. https://doi.org/10.1128/jb.00226-08.

Chambers, J.K., I. Matsumoto, T. Shibahara, M. Haritani, H. Nakayama, and K. Uchida. 2019. An outbreak of Fatal *Bordetella bronchiseptica* Bronchopneumonia in Puppies. *Journal of Comparative Pathology* 167: 41–45. https://doi.org/10.1016/j.jcpa.2018.12.002.

Chamorro, B.M., K. Luca, G. Swaminathan, N. Rochereau, J. Majorel, H. Poulet, B. Chanut, L. Piney, E. Mundt, and S. Paul. 2023. Mucosal vaccination with live attenuated *Bordetella bronchiseptica* protects against challenge in wistar rats. *Vaccines* (Basel) 11.https://doi.org/10.3390/vaccines11050982.

Clemmons, E.A., D. Chavez, L. Condel, J.W. Dutton 3rd., S. Price, and R. Lanford. 2021. Comparison of oral, nebulized and combination antibiotic treatment of *Bordetella bronchiseptica* in baboons (Papio spp.). *Journal of Veterinary Pharmacology and Therapeutics* 44: 836–841. https://doi.org/10.1111/jvp.12975.

CLSI. Performance standards for antimicrobial susceptibility testing. 32th ed. CLSI standard M100. Clinical and Laboratory Standards Institute; 2022.

Cui, X., X. Xu, P. Huang, G. Bao, and Y. Liu. 2022. Safety and efficacy of the *Bordetella bronchiseptica* vaccine combined with a vegetable oil adjuvant and multi-omics analysis of Its potential role in the protective response of rabbits. *Pharmaceutics* 14.https://doi.org/10.3390/pharmaceutics14071434.

Day, M.J., S. Carey, C. Clercx, B. Kohn, F. Marsillo, E. Thiry, L. Freyburger, B. Schulz, and D.J. Walker. 2020. EAetiology of canine infectious respiratory disease complex and prevalence of its pathogens in Europe. *Journal of Comparative Pathology* 176: 86–108. https://doi.org/10.1016/j.jcpa.2020.02.005.

Doo, K., R. Pillai, A. Kazeros, and R. Smith. 2017. A case of presumed infection with *Bordetella Bronchiseptica*. In: *An Immunocompromised Human Subject. American journal of respiratory and critical care medicine* 195.

Dubois, V., J. Chatagnon, A. Thiriard, H. Bauderlique-Le Roy, A.S. Debrie, L. Coutte, and C. Locht. 2021. Suppression of mucosal Th17 memory responses by acellular pertussis vaccines enhances nasal

Bordetella pertussis carriage. *NPJ Vaccines* 6: 6. https://doi.org/10.1038/s41541-020-00270-8.

Ellis, J.A., D.M. Haines, K.H. West, J.H. Burr, A. Dayton, H.G. Townsend, E.W. Kanara, C. Konoby, A. Crichlow, K. Martin, and G. Headrick. 2001. Effect of vaccination on experimental infection with *Bordetella bronchiseptica* in dogs. *Journal of the American Veterinary Medical Association* 218: 367–375. https://doi.org/10.2460/javma.2001.218.367.

Ellis, J.A., S.P. Gow, C.L. Waldner, S. Shields, S. Wappel, A. Bowers, S. Lacoste, Z. Xu, and E. Ball. 2016. Comparative efficacy of intranasal and oral vaccines against *Bordetella bronchiseptica* in dogs. *The Veterinary Journal* 212: 71–77. https://doi.org/10.1016/j.tvjl.2016.04.004.

Faqihi, F., A. Alharthy, P. Pirompanich, A. Noor, A. Shahzad, N. Nasim, A. Balhamar, Z.A. Memish, and D. Karakitsos. 2020. CoCo-infection of SARS-CoV-2 and *Bordetella bronchiseptica* in a young man with idiopathic nonnon-cystic bronchiectasis and vitamin D (3) deficiency. *Respiratory Medicine Case Reports* 31: 101203. https://doi.org/10.1016/j.rmcr.2020.101203.

Fastrès, A., M.A. Canonne, B. Taminiau, F. Billen, M.M. Garigliany, G. Daube, and C. Clercx. 2020. Analysis of the lung microbiota in dogs with *Bordetella bronchiseptica* infection and correlation with culture and quantitative polymerase chain reaction. *Veterinary Research* 51: 46. https://doi.org/10.1186/s13567-020-00769-x.

Fingermann, M., and D. Hozbor. 2015. Acid tolerance response of *Bordetella bronchiseptica* in avirulent phase. *Microbiological Research* 181: 52–60. https://doi.org/10.1016/j.micres.2015.09.001.

First, N.J., J. Pedreira-Lopez, M.R.F. San-Silvestre, K.M. Parrish, X.H. Lu, and M.C. Gestal. 2023. Bordetella spp. utilize the type 3 secretion system to manipulate the VIP/VPAC2 signaling and promote colonization and persistence of the three classical Bordetella in the lower respiratory tract. *Frontiers in cellular and infection microbiology* 13: 1111502. https://doi.org/10.3389/fcimb.2023.1111502.

Goodnow, R.A. 1980. Biology of *Bordetella bronchiseptica*. *Microbiological Reviews* 44: 722–738. https://doi.org/10.1128/mr.44.4.722-738.1980.

Goodnow, R.A., F.J. Shade, and W.P. Switzer. 1979. Efficacy of *Bordetella bronchiseptica* bacterin in controlling enzootic atrophic rhinitis in swine. *American Journal of Veterinary Research* 40: 58–60.

Gueirard, P., and N. Guiso. 1993. Virulence of *Bordetella bronchiseptica*: role of adenylate cyclase-hemolysin. *Infection and Immunity* 61: 4072–4078. https://doi.org/10.1128/iai.61.10.4072-4078.1993.

Guiso, N., M. Rocancourt, M. Szatanik, and J.M. Alonso. 1989. Bordetella adenylate cyclase is a virulence associated factor and an immunoprotective antigen. *Microbial Pathogenesis* 7: 373–380. https://doi.org/10.1016/0882-4010(89)90040-5.

Gupta, S., P. Goyal, and J. Mattana. 2019. *Bordetella bronchiseptica* pneumonia a thread in the diagnosis of human immunodeficiency virus infection. *Idcases* 15: e00509. https://doi.org/10.1016/j.idcr.2019.e00509.

Harvill, E.T., P.A. Cotter, M.H. Yuk, and J.F. Miller. 1999. Probing the function of *Bordetella bronchiseptica* adenylate cyclase toxin by manipulating host immunity. *Infection and Immunity* 67: 1493–1500. https://doi.org/10.1128/iai.67.3.1493-1500.1999.

Hozbor, D., F. Fouque, and N. Guiso. 1999. Detection of Bordetella bronchisepticaBordetella bronchiseptica by the polymerase chain reaction. *Research in Microbiology* 150: 333–341. https://doi.org/10.1016/s0923-2508(99)80059-x.

Jin, L., Z. Li, X. Zhang, J. Li, and F. Zhu. 2022. CoronaVac: A review of efficacy, safety, and immunogenicity of the inactivated vaccine against SARS-CoV-2. *Human Vaccines & Immunotherapeutics* 18: 2096970. https://doi.org/10.1080/21645515.2022.2096970.

Jorgensen, J.H.P.M., K.C. Carroll, G. Funke, M.L. Landry, S.S. Richter, et al. 2015. *Manual of Clinical Microbiology*. Eleventh. Washington, DC: ASM Press. https://doi.org/10.1128/9781555817381.

Kadlec, K., and S. Schwarz. 2018. Antimicrobial resistance in *Bordetella bronchiseptica*. Microbiol Spectr 6. https://doi.org/10.1128/microbiolspec.ARBA-0024–2017.

Kamanova, J. 2020. Bordetella type III secretion injectosome and effector proteins. *Frontiers in Cellular and Infection Microbiology* 10: 466. https://doi.org/10.3389/fcimb.2020.00466.

Khelef, N., H. Sakamoto, and N. Guiso. 1992. Both adenylate cyclase and hemolytic activities are required by *Bordetella pertussis* to initiate infection. *Microbial Pathogenesis* 12: 227–235. https://doi.org/10.1016/0882-4010(92)90057-u.

Kraai, I., M. Knoester, E. Bathoorn, M. Bakker, and M. Nijland. 2023. Zoonotic transmission of vaccine-derived *Bordetella bronchiseptica*. *Open Forum Infect Dis* 10: ofad421. https://doi.org/10.1093/ofid/ofad421.

Lappin, M.R., J. Blondeau, D. Boothe, E.B. Breitschwerdt, L. Guardabassi, D.H. Lloyd, M.G. Papich, S.C. Rankin, J.E. Sykes, J. Turnidge, and J.S. Weese. 2017. Antimicrobial use guidelines for treatment of respiratory tract disease in dogs and cats: antimicrobial guidelines working group of the international society for companion animal infectious diseases. *Journal of Veterinary Internal Medicine* 31: 279–294. https://doi.org/10.1111/jvim.14627.

Linz, B., Y.V. Ivanov, A. Preston, L. Brinkac, J. Parkhill, M. Kim, S.R. Harris, L.L. Goodfield, N.K. Fry, A.R. Gorringe, T.L. et al. 2016. Acquisition and loss of virulence-associated factors during genome evolution and speciation in three clades of Bordetella species. *BMC Genomics* 17: 767. https://doi.org/10.1186/s12864-016-3112-5.

Maboni, G., M. Seguel, A. Lorton, R. Berghaus, and S. Sanchez. 2019. Canine infectious respiratory disease: new insights into the etiology and epidemiology of associated pathogens. *PLoS One* 14: e0215817. https://doi.org/10.1371/journal.pone.0215817.

Magiorakos, A.P., A. Srinivasan, R.B. Carey, Y. Carmeli, M.E. Falagas, C.G. Giske, S. Harbarth, J.F. Hindler, G. Kahlmeter, B. Olsson-Liljequist, et al. 2012. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clinical Microbiology & Infection* 18: 268–281. https://doi.org/10.1111/j.1469-0691.2011.03570.x.

Malter, K.B., M.E. Tugel, M. Gil-Rodriguez, G. Guardia, S.W. Jackson, W.G. Ryan, and G.E. Moore. 2022. Variability in nonnon-core vaccination rates of dogs and cats in veterinary clinics across the United States. *Vaccine* 40: 1001–1009. https://doi.org/10.1016/j.vaccine.2022.01.003.

Marouf, S., H.M. Ibrahim, M.S. El-Naggar, A.A. Swelum, A.H. Alqhtani, M.T. El-Saadony, K.A. El-Tarabily, and H.M. Salem. 2022. Inactivated pentavalent vaccine against mycoplasmosis and salmonellosis for chickens. *Poultry Science* 101: 102139. https://doi.org/10.1016/j.psj.2022.102139.

Matsuu, A., M. Yabuki, E. Aoki, and M. Iwahana. 2020. Molecular detection of canine respiratory pathogens between 2017 and 2018 in Japan. *Journal of Veterinary Medical Science* 82: 690–694. https://doi.org/10.1292/jvms.20-0017.

McCandlish, I.A., H. Thompson, and N.G. Wright. 1978a. Vaccination against canine bordetellosis using an aluminum hydroxide adjuvant vaccine. *Research in Veterinary Science* 25: 51–57.

McCandlish, I.A., H. Thompson, and N.G. Wright. 1978b. Vaccination against canine bordetellosis: protection from contact challenge. *The Veterinary Record* 102: 479–483. https://doi.org/10.1136/vr.102.22.479.

Meyers, R.S., J. Thackray, K.L. Matson, C. McPherson, L. Lubsch, R.C. Hellinga, and D.S. Hoff. 2020. Key potentially inappropriate drugs in pediatrics: the KIDs List. *Journal of Pediatric Pharmacology and Therapeutics* 25: 175–191. https://doi.org/10.5863/1551-6776-25.3.175.

Morgane Canonne, A., E. Roels, M. Menard, L. Desquilbet, F. Billen, and C. Clercx. 2020. Clinical response to 2 protocols of aerosolized gentamicin in 46 dogs with *Bordetella bronchiseptica* infection (2012–2018). *Journal of Veterinary Internal Medicine* 34: 2078–2085. https://doi.org/10.1111/jvim.15843.

Mulkoju, R.C., V. Rajuri, S. Leo, and R.R. Kolan. 2022. A tale of three in symbiosis: TB-COVID-19-Bordetella coinfection. *Int J Mycobacteriol* 11: 463–465. https://doi.org/10.4103/ijmy.ijmy_166_22.

Nagarakanti, S., and E. Bishburg. 2021. Coinfection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and *Bordetella bronchiseptica* pneumonia in a renal transplant patient. *Cureus* 13: e13113. https://doi.org/10.7759/cureus.13113.

Ostle, G.N. 1989. Cough associated with canine vaccination? *The Veterinary Record* 125: 446. https://doi.org/10.1136/vr.125.17.446-a.

Papantoniou, S., A. Tsakiris, T. Ladopoulos, G. Kranidiotis, and C. Tamvakos. 2021. A case of *Bordetella bronchiseptica* bacteremia in a patient with COVID-19: brief report. *Cureus* 13: e15976. https://doi.org/10.7759/cureus.15976.

Pecora, D.V. 1976. Bacteriologic cultural examination of the lower respiratory tract of laboratory dogs. *American Journal of Veterinary Research* 37: 1511–1513.

Pierce, M., W. Slipke, and M. Biagi. 2022. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and *Bordetella bronchiseptica* co-infection in a stem cell transplant patient. *Antibiotics* (Basel) 11.https://doi.org/10.3390/antibiotics11091200.

Porter, J.F., and A.C. Wardlaw. 1993. Long-term survival of *Bordetella bronchiseptica* in lakewater and in buffered saline without added nutrients. *FEMS Microbiology Letters* 110: 33–36. https://doi.org/10.1111/j.1574-6968.1993.tb06291.x.

Porter, J.F., R. Parton, and A.C. Wardlaw. 1991. Growth and survival of *Bordetella bronchiseptica* in natural waters and in buffered saline without added nutrients. *Applied and Environment Microbiology* 57: 1202–1206. https://doi.org/10.1128/aem.57.4.1202-1206.1991.

Ross, P.J., C.E. Sutton, S. Higgins, A.C. Allen, K. Walsh, A. Misiak, E.C. Lavelle, R.M. McLoughlin, and K.H. Mills. 2013. Relative contribution of Th1 and Th17 cells in adaptive immunity to *Bordetella pertussis*: towardds the rational design of an improved acellular pertussis vaccine. *PLoS Pathogens* 9: e1003264. https://doi.org/10.1371/journal.ppat.1003264.

Sameed, M., Sullivan, S., Marciniak, E.T., Deepak, J., 2019. Chronic cough and cystic lung disease caused by *Bordetella bronchiseptica* in a patient with AIDS. BMJ Case Rep 12.https://doi.org/10.1136/bcr-2018-228741.

Shade, F.J., and R.A. Goodnow. 1979. Intranasal immunization of dogs against *Bordetella bronchiseptica*-induced tracheobronchitis (kennel cough) with modified live-*Bordetella bronchiseptica* vaccine. *American Journal of Veterinary Research* 40: 1241–1243.

Sharma, A.K., N. Dhasmana, N. Dubey, N. Kumar, A. Gangwal, M. Gupta, and Y. Singh. 2017. Bacterial virulence factors: secreted for survival. *Indian J Microbiol* 57: 1–10. https://doi.org/10.1007/s12088-016-0625-1.

Singleton, D.A., J. Stavisky, C. Jewell, S. Smyth, B. Brant, F. Sánchez-Vizcaíno, S. Dawson, G.L. Pinchbeck, P.J.M. Noble, and A.D. Radford. 2019. Small animal disease surveillance 2019: respiratory disease, antibiotic prescription and canine infectious respiratory disease complex. *The Veterinary Record* 184: 640–645. https://doi.org/10.1136/vr.l3128.

Speakman, A.J., S. Dawson, S.H. Binns, C.J. Gaskell, C.A. Hart, and R.M. Gaskell. 1999. *Bordetella bronchiseptica* infection in the cat. *Journal of Small Animal Practice* 40: 252–256. https://doi.org/10.1111/j.1748-5827.1999.tb03074.x.

Stephenson, E.H., C.J. Trahan, J.W. Ezzell, W.C. Mitchell, T.G. Abshire, D.D. Oland, and G.O. Nelson. 1989. Efficacy of a commercial bacterin in protecting strain 13 guineapigs against *Bordetella bronchiseptica* pneumonia. *Laboratory Animals* 23: 261–269. https://doi.org/10.1258/002367789780810581.

Tabatabaei, M., and H.R. Rohani. 2022. Identification of *Bordetella bronchiseptica* in the throat and nose of dogs and cats by PCR. *Mol Biol Res Commun* 11: 127–131. https://doi.org/10.22099/mbrc.2022.43873.1755.

Tatem, L.L., T. Veale, C. Richardson, and T. Luckhardt. 2023. Canine acquired pneumonia caused by *Bordetella bronchiseptica*. *Idcases* 34: e01922. https://doi.org/10.1016/j.idcr.2023.e01922.

Thompson, H., I.A. McCandlish, and N.G. Wright. 1976. Experimental respiratory disease in dogs due to *Bordetella bronchiseptica*. *Research in Veterinary Science* 20: 16–23.

Wang, J., S. Sun, Y. Chen, D. Chen, L. Sang, and X. Xie. 2020. Characterization of *Bordetella bronchiseptica* isolated from rabbits in Fujian. *China. Epidemiol Infect* 148: e237. https://doi.org/10.1017/s0950268820001879.

Weingart, C.L., and A.A. Weiss. 2000. *Bordetella pertussis* virulence factors affect phagocytosis by human neutrophils. *Infection and Immunity* 68: 1735–1739. https://doi.org/10.1128/iai.68.3.1735-1739.2000.

Woolfrey, B.F., and J.A. Moody. 1991. Human infections associated with *Bordetella bronchiseptica*. *Clinical Microbiology Reviews* 4: 243–255. https://doi.org/10.1128/cmr.4.3.243.

Xu, X., J. Li, P. Huang, X. Cui, X. Li, J. Sun, Y. Huang, Q. Ji, Q. Wei, G. Bao, and Y. Liu. 2023. Isolation, identification and drug resistance rates of bacteria from pigs in Zhejiang and Surrounding areas during 2019-2021. *Vet Sci* 10.https://doi.org/10.3390/vetsci10080502.

Yacoub, A.T., M. Katayama, J. Tran, R. Zadikany, M. Kandula, and J. Greene. 2014. *Bordetella bronchiseptica* in the immunosuppressed population - a case series and review. *Mediterr J Hematol Infect Dis* 6: e2014031. https://doi.org/10.4084/mjhid.2014.031.

Yondo, A., A.A. Kalantari, I. Fernandez-Marrero, A. McKinney, H.K. Naikare, and B.T. Velayudhan. 2023. Predominance of canine parainfluenza virus and mycoplasma in canine infectious respiratory disease complex in dogs. *Pathogens* 12.https://doi.org/10.3390/pathogens12111356.

Yount, K.S., J. Jennings-Gee, K. Caution, A.R. Fullen, K.N. Corps, S. Quataert, R. Deora, and P. Dubey. 2019. Bordetella colonization factor A (BcfA) elicits protective immunity against *Bordetella bronchiseptica* in the absence of an additional adjuvant. *Infect Immun* 87.https://doi.org/10.1128/iai.00506-19.

Zhang, Y., H. Yang, L. Guo, M. Zhao, F. Wang, W. Song, L. Hua, L. Wang, W. Liang, X. Tang, Z. Peng, and B. Wu. 2021. Isolation, antimicrobial resistance phenotypes, and virulence genes of *Bordetella bronchiseptica* from pigs in China, 2018–2020. *Front Vet Sci* 8: 672716. https://doi.org/10.3389/fvets.2021.672716.

Publisher's Note