The Journal of Veterinary Medical Science

FULL PAPER

Laboratory Animal Science

Pathogenicity of *Bordetella bronchiseptica* isolated from apparently healthy rabbits in guinea pig, rat, and mouse

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ABSTRACT. *Bordetella bronchiseptica* (*B. bronchiseptica*) is associated with respiratory tract infections in laboratory animals. In our laboratory animal facility, *B. bronchiseptica* was isolated from 21 of 27 apparently healthy rabbits obtained from a breeding farm contaminated with *B. bronchiseptica*. Restriction fragment length polymorphism (RFLP) analysis showed that the flagellin genotype of isolates from the laboratory animal facility and breeding farm was type A, which is seen relatively frequently in rabbits in Europe. To examine its pathogenicity, guinea pigs, rats, and mice were inoculated intranasally with a representative strain isolated in the laboratory animal facility. Following inoculation of 10^7 colony forming unit (cfu), severe inflammation was observed in the lungs of guinea pig and mice, although the inflammation was less severe in rats. The strain was recovered from the trachea and lungs of these species after inoculation with lower dose such as 10^3 or 10^4 cfu. These results suggest that the isolated strain causes respiratory tract infection in guinea pigs, rats, and mice, and that its pathogenicity higher in mice than in rats. This study extends our knowledge of interpreting the microbiologic status of laboratory animals, which will contribute to the development of reliable and reproducible animal experiments.

KEYWORDS: *Bordetella bronchiseptica*, infection, inflammation, mice, pathogenicity

J. Vet. Med. Sci.
84(4): 574–581, 2022
doi: 10.1292/jvms.21-0494

Received: 12 September 2021
Accepted: 29 January 2022
Advanced Epub:
11 February 2022

Bordetella bronchiseptica (*B. bronchiseptica*) is a broad-host-range gram-negative and motile bacterium. It is associated with respiratory disease in numerous mammals and has been isolated from dog, guinea pig, swine, rabbit, and rat [4]. *B. bronchiseptica* infection is clinically significant in guinea pigs and rabbits among laboratory animals, whereas natural infection in rats and mice is of minor importance.

In guinea pigs, morbidity and mortality due to *B. bronchiseptica* are most common in young individuals. *B. bronchiseptica* was detected in 20 of 45 Hartley guinea-pig colonies (17 institutional and 28 breeding colonies) in Japan in 1986 [12]. Most infected guinea pigs do not show obvious symptoms, however, 20% develop bronchopneumonia [10], which decreases the animal's growth rate.

Although rabbits are susceptible to *B. bronchiseptica*, most do not show signs of infection; thus, the pathogenicity of *B. bronchiseptica* in rabbits is uncertain, potentially contributing to upper respiratory tract infections collectively described as "snuffles" under co-infection with *Pasteurella multocida*. It seems that *B. bronchiseptica* has been noted to prefer the cilia of the respiratory epithelium in rabbits and can impair mucociliary clearance, resulting in allowance for the entry of other pathogens.

The sensitivity of rat and mouse to *B. bronchiseptica* is currently poorly understood, although animals with innate immune system defects may be more susceptible to clinical disease caused by *B. bronchiseptica* [9]. Routine microbial monitoring is not recommended for mice in Japan because of the rarity of natural infection [1].

We previously isolated *B. bronchiseptica* from apparently healthy rabbits from a breeding farm contaminated with *B.*

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bronchiseptica [7]. In this study, we performed experimental infections using guinea pigs, rats, and mice to examine the pathogenicity of the isolated strain in these species.

MATERIALS AND METHODS

Animals

Japanese White rabbits (one per cage) were reared in *a self-cleaning* metal cage system (Auto Scraper Unit, CL-1340-KS, CLEA Japan, Inc., Tokyo, Japan) in our laboratory animal facility. The rabbits were obtained from two breeding farms. Seventeen 7–9-month-old males (rearing period: 6–10 weeks), one 8-month-old female (rearing period: 10 weeks), two 24-month-old males (rearing period: 16 months), and seven 30-month-old males (rearing period: 24 months), for a total of 27 rabbits, were obtained from the farm at which *B. bronchiseptica* was detected. Twelve 4-month-old males (rearing period: 3 weeks), and ten 5-month-old males (rearing period: 3 months), for a total of 22 rabbits, were also obtained from a farm at which *B. bronchiseptica* was not detected.

Four-week-old female ddY mice, ICR mice, Wistar rats, and Hartley guinea pigs were purchased from Japan SLC, Inc. (Hamamatsu, Japan). These animals were free of respiratory infections caused by the following microbes: *Pasteurella pneumotropica*, *Mycoplasma pulmonis*, Sendai virus and *B. bronchiseptica*. Infectious and histopathological analyses also confirmed that *B. bronchiseptica* had not colonized the respiratory organs of these animals. The animals were maintained in plastic cages (2–4 mice per cage, 2 rats per cage, and 1 guinea pig per cage).

They were kept in rooms maintained at 23 ± 3°C and 55 ± 15% relative humidity under a 12:12-hr light:dark cycle. Rabbits and guinea pigs were fed commercial chow once a day (LRC4 for rabbits and GOC4 for guinea pigs; ORIENTAL YEAST Co., Ltd., Tokyo, Japan) and rats and mice had free access to chow (CRF-1LID10 for mice and rats; ORIENTAL YEAST Co., Ltd.). All animals had free access to water throughout the study.

Animal experiments were carried out humanely in accordance with the Regulations for Animal Experiments of Kyushu University. All protocols and procedures were approved by the Institutional Animal Experiment Committees of Kyushu University (approval number: A27-319, A29-051, and A30-192).

Restriction fragment length polymorphism (RFLP) analysis for flagella genotyping

B. bronchiseptica was isolated from rabbits in our facility and a representative strain designated as KCBR10 was analyzed for flagella genotyping [7]. Swab samples were obtained from the nasal cavities of all 49 rabbits under anesthetization with sevoflurane (Mylan Inc., Canonsburg, PA, USA) and incubated on deoxycholate hydrogen sulphide lactose (DHL) agar medium (Eiken Chemical Co., Ltd., Tokyo, Japan) for 72 hr at room temperature. Pink colonies were selected for a *B. bronchiseptica*-specific polymerase chain reaction (PCR) assay. A bacterial suspension diluted in distilled water (20 µl) was boiled for 15 min and centrifuged at 6,000 rpm for 1 min. The supernatant was used as a DNA template. An upstream sequence (237 bp) of the flagellin structural gene was used as the target DNA region [6]. The primer pair Fla2 (5′-AGGCTCCCAAGAGAGAAAGGCTT-3′) and Fla4 (5′-TGGCGCCTGCCCTATC-3′) was used to amplify the *flaA* gene specific for *B. bronchiseptica*. DNA amplification was performed using the Gene Atlas 482 thermal cycler (ASTEC Co., Ltd., Fukuoka, Japan) in a final volume of 20 µl. The reaction mixture contained 0.5 µl of template, 0.1 µl of 5 U/µl ExTaq (Takara Bio Inc., Kusatsu, Japan), 2 µl of 10×Taq buffer, 1.6 µl of 2.5 mM dNTP, 1 µl of 10 pmol/µl of each primer, and 13.8 µl of distilled water. The PCR reaction conditions were as follows: 35 cycles of denaturation at 98°C for 10 sec, annealing at 50°C for 30 sec, and extension at 72°C for 1 min, 20 sec. Electrophoresis was performed on 0.8% agarose gels following standard procedures.

RFLP analysis of the *flaA* gene of KCBR10 was conducted as previously described [2]. The region encompassing nucleotides 2–1166 of the *flaA* gene was amplified using the primer pair BflaF (5′-TGGCTGCAGTCATCAATACC-3′) and BFlaR (5′-AGCGACAGGACGTTTTGC-3′) [2]. Amplification was performed in an 80 µl reaction mixture containing 1 µl of template, 0.4 µl of 5U/µl ExTaq (Takara Bio Inc.), 8 µl of 10×Taq buffer, 6.4 µl of 2.5 mM dNTP, 4 µl of 10 pmol/µl of each primer, and 56.2 µl of distilled water. The PCR reaction conditions were as described above. Electrophoresis was performed as described above. The band on the agarose gel was removed and purified using the Zymoclean Gel DNA Recovery Kit (Zymo Research Corp., Irvine, CA, U.S.A) according to the manufacturer's instructions. Purified DNA was digested with *Bgl*I (Takara Bio Inc.) for 17 hr at 37°C. Electrophoresis was performed on 2.0% agarose gels using standard procedures. The RFLP of the *flaA* gene was determined as previously described [8].

Antiserum preparation for immunohistochemical staining

Lipopolysaccharide (LPS) and acetone-treated cells of *B. bronchiseptica* strain KCBR10 were used for preparation of antisera. LPS was extracted by hot phenol-water method as described previously with some modifications [14]. In order to eliminate contaminating nucleic acids, treatment with DNase and RNase was performed before ethanol precipitation. LPS was recovered from aqueous phase by ethanol precipitation. The bacterial cells were treated with cold acetone and dried. We immunized one mouse (n=1) and one rat (n=1) four times in total as follows: 5-week-old female ICR mouse and Wistar rat were injected subcutaneously with 50–200 µl of emulsion two times, separated by a 3-week interval. The emulsion consisted of antigen solution containing 0.5–2 mg of LPS and Freund's complete adjuvant or TiterMax Gold (TiterMax USA, Inc., Norcross, GA, USA). Three weeks later, the mouse and rat were boosted with 150 or 300 µl of emulsion containing 7.5 or 15 mg of acetone-treated bacteria in the same manner as the third injection. Three weeks later, a final injection consisting of 250 or 500 µl of solution containing 12

or 23 mg of acetone-treated bacteria without adjuvant was given intraperitoneally to the mouse or rat. Whole blood was collected from the heart of each animal following euthanasia using sevoflurane (Mylan Inc.) at 2 weeks after the final injection; antisera were verified using antibody titers and agglutination tests on slides, using standard procedures.

Experimental infection

B. bronchiseptica KCBR10 incubated for 48 hr at 37°C on trypticase soy agar (NISSUI PHARMACEUTICAL Co., Ltd., Tokyo, Japan) with 5% horse blood were suspended in saline solution and adjusted to the appropriate density. Experimental infections with *B. bronchiseptica* strain KCBR10 were carried out in the BSL-2 facility at the Center of Biomedical Research, Research Center for Human Disease Modeling, Graduate School of Medical Sciences, Kyushu University. For intranasal infections Hartley guinea pigs, Wistar rats and ddY mice (5 weeks of age) were anesthetized by sevoflurane (Mylan Inc.) and then intranasally inoculated with the bacterial suspension (day 0). Guinea pigs were inoculated intranasally with 1.88×10^2(n=1), 1.88×10^4(n=1), 1.88×10^5(n=1) or 1.88×10^7 colony forming unit (cfu) (n=1) of strain KCBR10 in 40 µl saline solution and used without inoculation as control (n=1). Rats were inoculated intranasally with 6.36×10^3(n=2), 6.36×10^5(n=2), or 6.36×10^7 cfu (n=2) of strain KCBR10 in 40 µl saline solution and used without inoculation as control (n=1). Mice were inoculated intranasally with 5.40×10^3(n=3), 5.40×10^5(n=3), 2.76×10^7(n=4) or 5.40×10^7 cfu (n=4) of strain KCBR10 in 20 µl saline solution and used without inoculation as control (n=6).

The survival and body weights of infected animals were recorded for 14 or 15 days. Percent body weight was calculated based on the body weight on day 0, as body weight (%)=100 × (body weight [g]/body weight [g] on day 0). Data are presented as the mean ± standard error of the mean (SEM). Statistical significance was performed using GraphPad Prism software (version 9; GraphPad Software Inc., San Diego, CA, USA). Data were analyzed using analysis of variance (ANOVA) followed by the Bonferroni correction *post hoc* test for multiple comparisons between groups when appropriate. A P-value <0.05 was considered significant.

At the end of infectious experiments, surviving animals were euthanized using sevoflurane (Mylan Inc.) and swab samples were obtained from external naris and nasal cavity. To determine bacterial colonization in lungs and trachea, animals were sacrificed using sevoflurane (Mylan Inc.) and collected lungs and swab samples from trachea. Swab samples were suspended using voltex in 1 ml saline solution and lung samples were homogenized in 10 times weight of saline solution using a disposable homogenizer (BioMasherII, Nippi. Inc., Tokyo, Japan). The swab suspension and lung homogenate were incubated on trypticase soy agar (NISSUI PHARMACEUTICAL Co., Ltd.) with 5% horse blood for 48 hr at 37°C for counting the bacterial number.

Histopathological analysis

Lung tissues collected from euthanized animals were fixed with 4% paraformaldehyde phosphate buffer solution (Wako Pure Chemical Industries, Ltd., Tokyo, Japan) and embedded in paraffin wax. Sections (2–4 µm thick) were cut and stained with haematoxylin and eosin (HE). Immunohistochemical staining was performed by polymer method using Histofine Simple Stain MAX-PO (Rat) (NICHIREI BIOSCIENCE INC, Tokyo, Japan) or Histofine Simple Stain MAX-PO (M) (NICHIREI BIOSCIENCE INC). Endogenous peroxidase activity was blocked by 3% hydrogen peroxide in methanol. Subsequently, sections were incubated with protein block serum-free reagent (DAKO, Carpinteria, CA, USA) for blocking non-specific reactions. Then they were incubated with the antisera for 16 hr at 4°C, as primary immunoreaction. The antisera from rat (1:1,000) were used for sections of guinea pig and mouse, and the antisera from mouse (1:1,000) were used for sections of rat. Then they were treated with Histofine Simple Stain MAX-PO (Rat) for tissues of guinea pig and mouse, and with Histofine Simple Stain MAX-PO (M) for rat tissues for 30 min at room temperature. For immunohistochemical detection, 3,3′-diaminobenzidine tetrahydrochloride solution (ImmPACT DAB Peroxidase Substrate; Vector Laboratories, Burlingame, CA, USA) was used.

RESULTS

Pathogenicity of B. bronchiseptica from rabbits reared in the laboratory animal facility

B. bronchiseptica was isolated from Japanese White rabbits reared in our laboratory animal facility and characterized as reported previously [7]. Briefly, B. *bronchiseptica* was detected by PCR assay in 21 of 27 apparently healthy rabbits from the breeding farm contaminated with *B. bronchiseptica*, but not in 22 rabbits from a second, non-contaminated, farm. *B. bronchiseptica* strain GTC28 (type strain; isolated from dog) and strain Fukuoka2014 (isolated from swine) were provided by Department of Microbiology, Gifu University School of Medicine, through the National Bioresource Project (NBRP) of the MEXT, Japan and Fukuoka Chuo Livestock Hygiene Service Center, respectively. Strain Hita5 was isolated from a guinea pig at the contaminated breeding farm. Using a biochemical identification system for non-fastidious, non-enteric gram-negative rods (API20NE; bioMérieux Japan Ltd., Tokyo, Japan), five strains (KCBR2, KCBR10, KCBR41, KCBR44, and KCBR48) isolated from 5 of 21 rabbits showed similar properties to the Hita5 and Fukuoka2014 strains, although the type strain GTC28 exhibited differences in terms of reduction of nitrates, malic acid assimilation, and phenylacetic acid assimilation. In the RFLP analysis for flagella genotyping, these five strains showed similar patterns to type strain GTC28 and strain Hita5. Figure 1 shows the RFLP patterns of strain KCBR10, which indicate RFLP type A flagellin. This type is frequently isolated from rabbits in Europe [8]. To examine the pathogenicity of the isolated *B. bronchiseptica*, one of the five strains was chosen for experimental infection and designated strain KCBR10.

Hartley guinea pigs were infected intranasally. Because the typical inflammatory regions extend progressively to the right and left lung lobes in guinea pigs infected with *B. bronchiseptica* at 15 days post-infection [11], we euthanized guinea pigs at 14 or

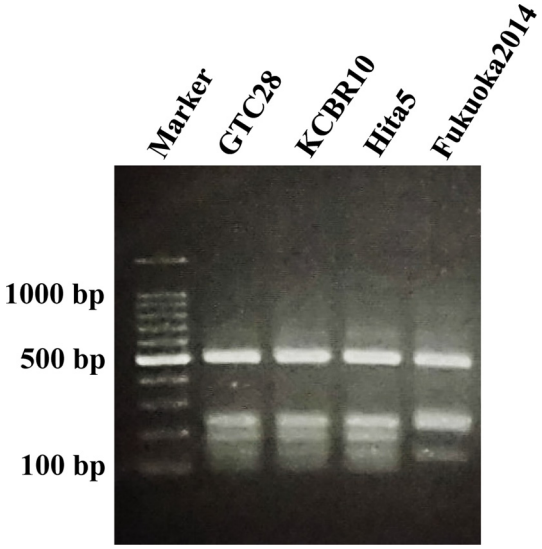


Fig. 1. Restriction flagment length polymorphism (RFLP) patterns of strain KCBR10 *flaA* gene by digestion with *Bgl* I.

15 days after infection to examine pathogenicity. Ruffled fur, cough, discharge of nasal mucus, which are typical symptoms in infected guinea pigs, were not observed. However, the rate of body weight gain showed lower increase in all infected guinea pigs compared with control (Fig. 2A), although there was no correlation with infectious dose. Bacteria were recovered from the nasal cavity, trachea, and lung irrespective of inoculum, except for animals inoculated with 1.88×10^2 cfu (Fig. 2B).

In Wistar rats, the rate of body weight increase showed similar patterns in all infected animals (Fig. 2C), and no symptoms were observed. Bacteria were recovered from the nasal cavity, trachea, and lung (Fig. 2D), albeit in smaller quantities compared to the other animals.

In ddY mice, the rate of increase in body weight was significantly difference among groups ($F4,64=8.63$, $P<0.01$) (Fig. 2E). The difference in body weight gain reached significance after Bonferroni correction (control mice vs. inoculated mice with 5.40×10^7 cfu, $P<0.01$). Additionally, following inoculation with 5.40×10^7 cfu, infected mice showed a mean body weight loss of more than 10% on day 2 after infection. One infected mouse showed severe weight loss and died at 10 days post-infection. Five of 11 mice inoculated with $>5.40 \times 10^5$ cfu showed inactivity at 1–8 days post-infection. Bacteria were recovered from all sites of the examined respiratory tract, even at the lowest dose (Fig. 2F). Therefore, *B. bronchiseptica* strain KCBR10, isolated in our facility, causes respiratory disease in ddY mice.

Histopathological analysis

At 14 days post-infection, peribronchial and alveolar inflammation, consisting of infiltrations and accumulations of neutrophils, lymphocytes, and macrophages with interstitial thickening were observed in the lungs of guinea pigs inoculated with 1.88×10^7 cfu (Fig. 3A and 3B) and milder inflammation with slight interstitial thickening was observed in those inoculated with 1.88×10^4 (data not shown) and 1.88×10^5 cfu (data not shown), but not in those inoculated with 1.88×10^2 cfu (data not shown) and control (Fig. 3C and 3D). Milder inflammation was observed in the lungs of Wistar rats inoculated with 6.36×10^7 cfu, (Fig. 3E and 3F) whereas the infiltration was not detected in control (Fig. 3G and 3H). Severe inflammatory cell infiltration was observed in the lungs of ddY mice inoculated with 5.40×10^7 cfu (Fig. 3I and 3J) and milder inflammation with slight interstitial thickening was observed in the lungs of mice inoculated with 5.40×10^3 (data not shown) and 5.40×10^5 cfu (data not shown), but not detected in the control (Fig. 3K and 3L).

Furthermore, immunohistochemistry with anti-*B. bronchiseptica* antisera revealed some aggregates of coccobacilli on the alveolar epithelial surface of infected guinea pig (Fig. 4A), rats (Fig. 4B), and mice (Fig. 4C) whereas these aggregates were not detected in controls (data not shown).

DISCUSSION

Approximately 80% of rabbits (21/27) from the contaminated breeding farm and reared in our facility were infected with *B. bronchiseptica* despite different introduction dates, ages, and feeding periods. In contrast, none of rabbits obtained from the non-contaminated breeding farm and reared in our facility were infected. Five representative strains (KCBR2, KCBR10, KCBR41, KCBR44, and KCBR48) isolated from rabbits and strain Hita5 isolated from guinea pigs at the contaminated breeding farm were designated type A by RFLP analysis. Therefore, it is likely that rabbits were infected at the breeding farm prior to procurement for our facility.

The flagellin type of Hita5 was consistent with that which is frequently isolated from rabbits in Europe, and different from that carried by guinea pigs (flagellin genotype C) [8]. Although no data are available to determine the exact infection route of the flagellin genotype A *B. bronchiseptica* strain, it may have originated from the contaminated farm, with rabbits as the initial hosts, followed by expansion to the guinea pig colony.

Following introduction into our facility, all 49 rabbits were reared in a single room containing two racks, one containing 27 rabbits from the contaminated breeding farm and the other containing 22 rabbits from the non-contaminated breeding farm. The racks faced each other, and were separated by a distance of 1.8 m. All rabbits from the non-contaminated breeding farm remained uninfected despite 3 weeks or 3 months of rearing in our facility in the same room as the infected rabbits, i.e., no horizontal infection occurred during this study. Thus, *B. bronchiseptica* type A strain KCBR10, isolated in this study, appears to be difficult to transmit cage-to-cage, with low infectivity among rabbits.

The pathogenicity of *B. bronchiseptica* strain KCBR10 was examined by experimental infection of guinea pigs, rats, and mice. Intranasal inoculation with KCBR10 caused severe inflammation in the lungs of Hartley guinea pigs without typical symptoms including ruffled fur, cough, and nasal mucus discharge. Although the rate of body weight gain was lower in infected guinea pigs than

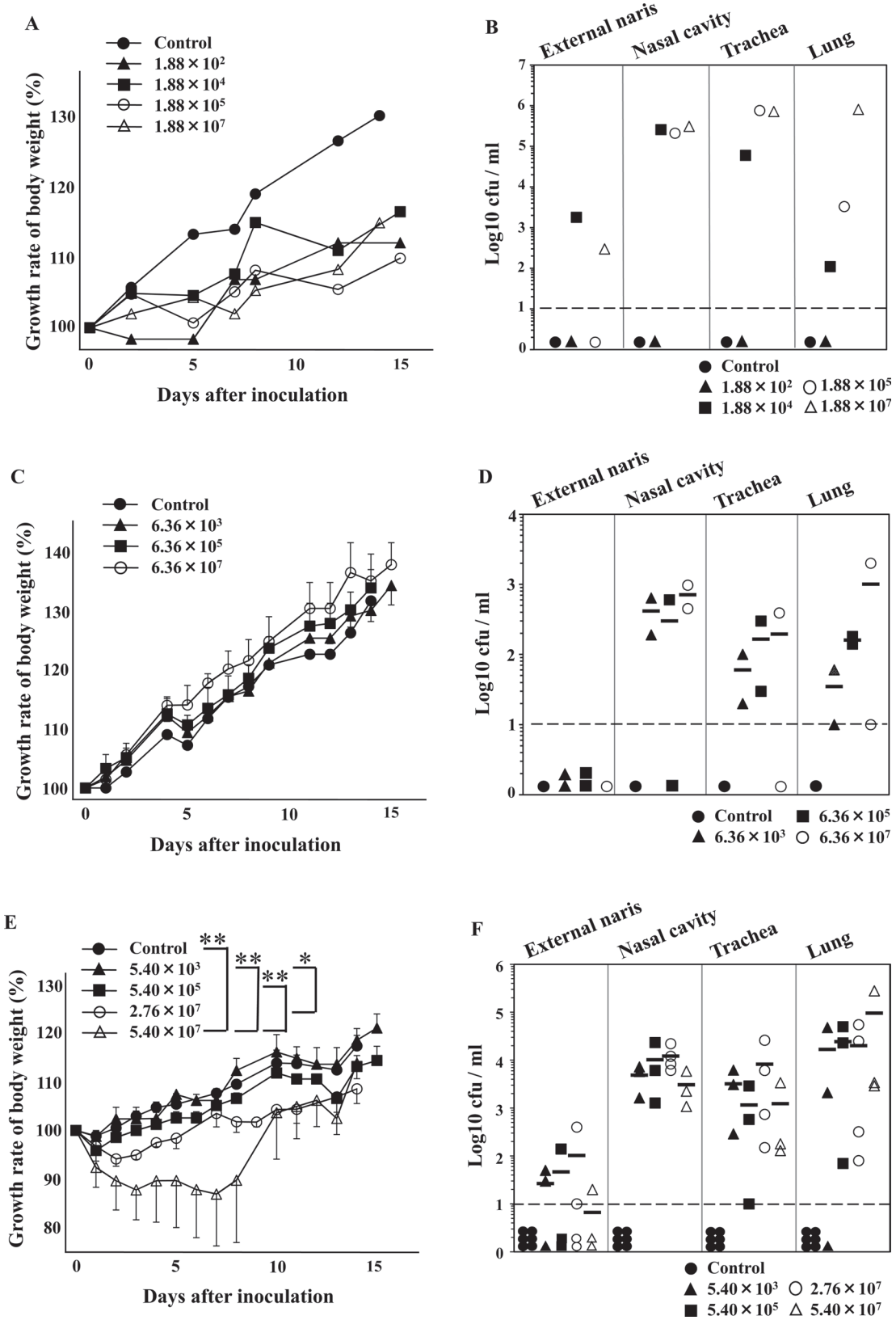
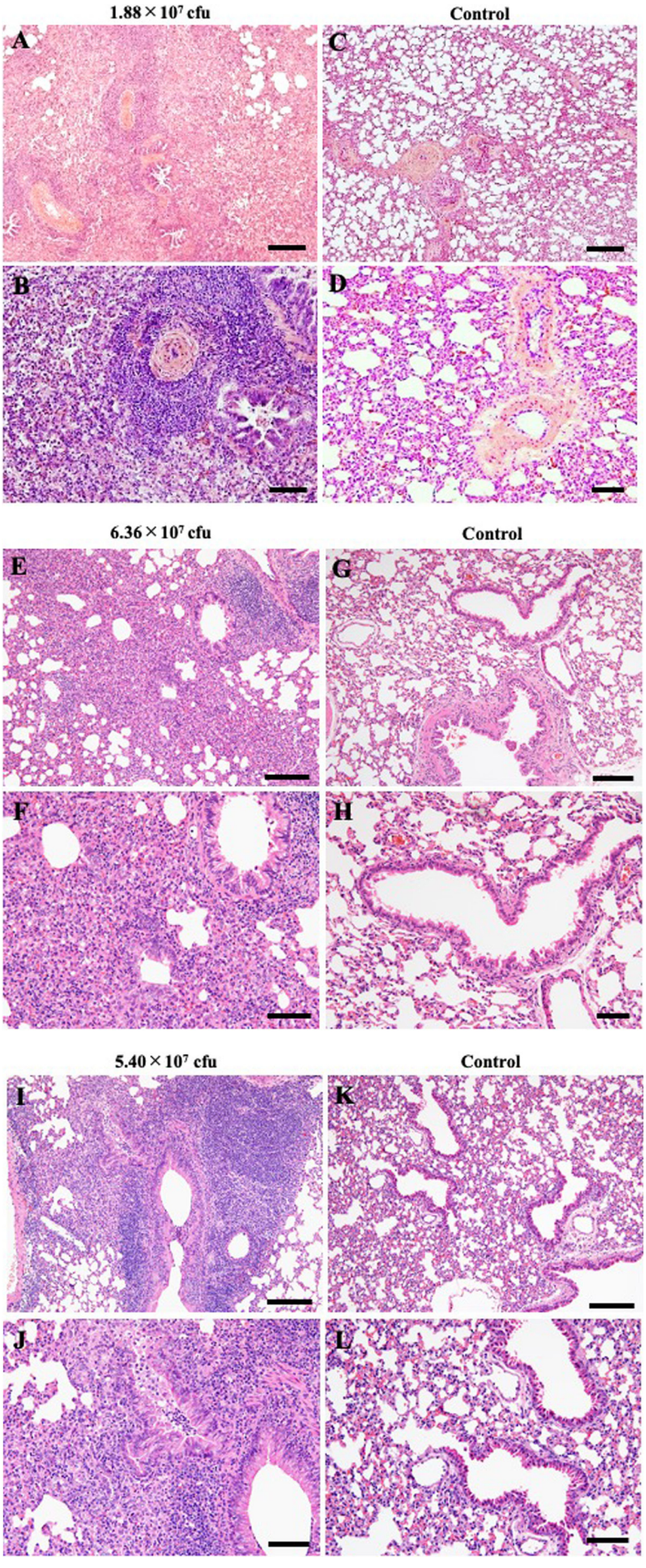


Fig. 2. Susceptibility of guinea pig, rats and mice to strain KCBR10. Hartley guinea pig (**A** and **B**), Wistar rats (**C** and **D**), and ddY mice (**E** and **F**) were inoculated with strain KCBR10 intranasally as described in Materials and Methods. Growth rate of body weight (A, C, E) and appearance of the bacteria in respiratory tracts (B, D, F) were shown. Each symbols represents the value of the detected bacteria in an individual animal, and the bar represents the mean for the group (B, D, F). Dotted line means detection limit of 10 colony forming unit (cfu) (B, D, F). Data are presented as means ± SEM. **P*<0.05, ***P*<0.01.



in the control, it was not correlated with infectious dose. No correlation was detected between the rate of body weight gain and either the number of detected bacteria in the respiratory tract or the severity of lung inflammation. Although bacteria were not recovered from the nasal cavity, trachea, or lung in animals at 15 days post-inoculation with 1.88×10^2 cfu, body weight gain differed between animals inoculated with 1.88×10^2 cfu and controls. These results suggest that infection may have been established in guinea pigs inoculated with 1.88×10^2 cfu in the early phase, inducing a decrease in body weight gain as a main symptom. By contrast, there was no effect on the body weight gain of Wistar rats, although milder inflammation was observed in the lung. Surprisingly, it was noteworthy that symptoms and histopathological findings such as inflammation were observed in ddY mice. Almost half of mice inoculated with $>5.40 \times 10^5$ cfu became inactive. The rate of body weight loss increased with increasing inoculum size (mice inoculated with 5.40×10^3 cfu vs. mice inoculated with 2.76×10^7 cfu, $P<0.05$; mice inoculated with 5.40×10^3 cfu vs. mice inoculated with 5.40×10^7 cfu, $P<0.01$, mice inoculated with 5.40×10^5 cfu vs. mice inoculated with 5.40×10^7 cfu, $P<0.01$, Bonferroni correction). The highest dose caused severe weight loss at several days post-infection and lung inflammation. To our knowledge, this is the first report to investigate *B. bronchiseptica* infectivity and pathogenicity in guinea pigs, rats, and mice inoculated with the same strain isolated from rabbits. Therefore, we conclude that Hartley guinea pigs and ddY mice are more susceptible to KCBR10 than Wistar rats. Importantly, these results indicate that *B. bronchiseptica* strain KCBR10 causes serious respiratory disease in mice.

Under low-dose inoculation, strain RB50 (unclassified flagellin genotype) isolated from New Zealand White rabbits colonized the nasal cavity and trachea of Wistar rats at 26 days post-inoculation with 5×10^3 cfu [5]. In addition, strain RB50 colonized the nasal cavity, trachea, and right lung of BALB/c mice at 14 days post-inoculation with 7.5×10^4 cfu [13]. Strain KCBR10 also colonized the nasal cavity, trachea, and lungs of Hartley guinea pigs, Wistar rats, and ddY mice at 15 days post-inoculation with 1.88×10^4, 6.36×10^3, and 5.40×10^3 cfu, respectively. Therefore, RB50 and KCBR10 appear to colonize easily via inhalation in these rodents.

Fig. 3. Representative photomicrographs of hematoxylin and eosin staining of respiratory organs at 14 days after infection. Lung sections of inoculation with 1.88×10^7 (**A**, **B**) and control (**C**, **D**) of Hartley guinea pig, 6.36×10^7 (**E**, **F**) and control (**G**, **H**) of Wistar rats, and inoculated with 5.40×10^7 colony forming unit (cfu) (**I**, **J**) and control (**K**, **L**) of ddY mice were shown. Scale bar=250 µm (A, C), 200 µm (E, G, I, K), and 100 µm (B, D, F, H, J, L).

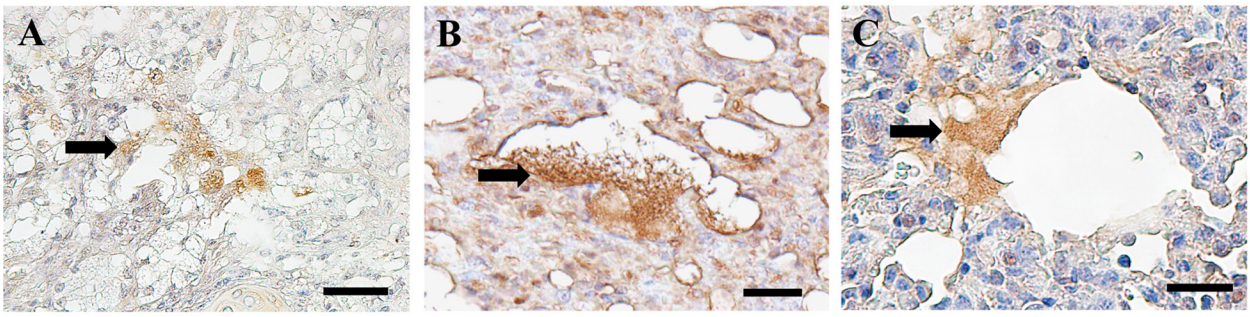


Fig. 4. Representative photomicrographs of immunohistochemistry of lung alveolus from Hartley guinea pig, Wistar rats, and ddY mice inoculated with 1.88×10^7, 6.36×10^7, and 5.40×10^7 colony forming unit (cfu), respectively. Black arrows show *Bordetella bronchiseptica* stained by 3,3′-diaminobenzidine tetrahydrochloride solution using mouse or rat anti-*B. bronchiseptica* antisera on guinea pig (**A**), rat (**B**), and mouse (**C**) sections. Scale bar=50 μm (A) and 25 μm (B, C).

Intranasal inoculation with 5.2×10^6 cfu of strain HH0809 (unclassified flagellin genotype) isolated from pigs resulted in the death of approximately 90% of BALB/c mice after 2–7 days [15]. In strain RB50, 10^6 cfu of bacteria were detected in the lungs of BALB/c mice infected with 7.5×10^4 cfu at 7 days post-inoculation [13] and 10^3 cfu of bacteria were detected in the trachea of Wistar rats infected with 5×10^3 cfu at 15 days post-inoculation [5]. Inflammatory cell recruitment continued, filling alveoli in large lesions of the lung of BALB/c mice at 7 days post-inoculation with 5×10^5 cfu of RB50 [5]. In contrast, only 10^4 cfu of KCBR10 were detected in the lungs of ddY mice infected with 2.76 or 5.40×10^7 cfu at 15 days post-inoculation and only 10^1 cfu were detected in the tracheae of Wistar rats infected with 6.36×10^3 cfu at 15 days post-inoculation. The lungs of ddY mice showed milder inflammation at 15 days post-inoculation with 5.40×10^3 or 5.40×10^5 cfu of KCBR10. Although we cannot compare these results directly because there are differences in days post-inoculation and genetic background, and flagellin genotypes are uncertain, our results suggest that the virulence of strain KCBR10 may be lower than that of strains HH0809 and RB50, which were used in these previous studies. To investigate the symptomatic state of these three rodent species in further detail, we will examine aspects of disease caused by strain KCBR10 in a future study, including visible symptoms, bacterial colonization/localization rates, and lung histopathology at more time points after inoculation.

There are few reports of natural infection with *B. bronchiseptica* in mice or rats. It is unclear whether mice and rats are natural hosts of *B. bronchiseptica.* The low prevalence of *B. bronchiseptica* in laboratory mice and rats may reflect recent hygiene management, barrier system, and health-monitoring practices. Although more information is required to determine the risk of natural infection, our results suggest that mice can become natural hosts of the strain derived from Japanese White rabbits. Although *B. bronchiseptica* is a common pathogen in the respiratory tract of wild and domestic animal species, it is rarely seen in *humans [3]*. However, the role of non-murine hosts, particularly humans, as sources of the organism in animal facilities needs to be investigated. This study extends our knowledge of interpreting the microbiologic status of laboratory animals, which will contribute to the development of reliable and reproducible animal experiments.

CONFLICT OF INTEREST. The authors declare no conflict of interest.

ACKNOWLEDGMENTS. We thank K. Ichikawa, G. Nagura, K. Akahoshi and K. Nakai for technical assistance and Y. Nomiyama for kindly providing *B. bronchiseptica* Fukuoka2014 strain.

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