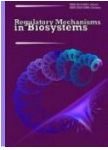

Regulatory Mechanisms in Biosystems

ISSN 2519-8521 (Print)
ISSN 2520-2588 (Online)
Regul. Mech. Biosyst.

Relationship between the Bvg$^+$ phenotype of clinical isolates of *Bordetella bronchiseptica* and their immunogenic potential

D. V. Hadzevych*, A. P. Paliy*, O. V. Hadzevych*, O. V. Pavlichenko**, L. V. Kovalenko*, Y. K. Dunaev*, I. O. Gerilovych*

*Institute of Experimental and Clinical Veterinary Medicine, Kharkiv, Ukraine
**State Biotechnological University, Kharkiv, Ukraine

Article info

Received 16.01.2026
Received in revised form 15.02.2026
Accepted 04.03.2026

Institute of Experimental and Clinical Veterinary Medicine, Hryhoriia Skovorody st., 83, Kharkiv, 61023, Ukraine.
Tel.: +38-066-225-34-34.
E-mail: paliy.dok@gmail.com

State Biotechnological University, Alchevskikh st., 44, Kharkiv, 61002, Ukraine.
Tel.: +38-050-760-62-84. E-mail: pavlichenkoelena777@gmail.com

Hadzevych, D. V., Paliy, A. P., Hadzevych, O. V., Pavlichenko, O. V., Kovalenko, L. V., Dunaev, Y. K., & Gerilovych, I. O. (2026). Relationship between the Bvg$^+$ phenotype of clinical isolates of Bordetella bronchiseptica and their immunogenic potential. Regulatory Mechanisms in Biosystems, 17(2), e26045. doi:10.15421/0226045

Bordetella bronchiseptica is an important etiological agent of respiratory infections in companion and livestock animals and is characterized by pronounced phenotypic plasticity regulated by the two-component BvgAS system. The stability of the Bvg$^+$ phase determines the level of expression of adhesive and toxigenic virulence factors; however, its applied significance for the selection of production strains for inactivated vaccines remains insufficiently studied. The aim of the study was to determine the relationship between the stability of the Bvg$^+$ phenotype of clinical isolates of *B. bronchiseptica*, their adhesion activity, dermonecrotic activity, ability to accumulate biomass, and induction of a specific humoral response. A comprehensive phenotypic analysis of 25 clinical isolates was performed using the hemagglutination reaction, an adhesion assay with Vero cells, evaluation of dermonecrotic activity *in vivo*, and determination of biomass accumulation. According to the results, five isolates were classified as demonstrating a stable Bvg$^+$ phenotype. The immunogenic potential was assessed by the level of specific IgG after immunization with inactivated bacterial preparations (1×10^8 CFU/mL before inactivation). Isolates with a stable Bvg$^+$ phenotype were characterized by higher adhesion activity (2.6 ± 0.4 vs 1.4 ± 0.5 adhesion scores), dermonecrotic activity (8.2 ± 1.1 vs 3.9 ± 0.8 mm), and biomass accumulation (8.7 ± 0.3 vs 7.8 ± 0.5 log CFU/mL). The level of specific IgG in the Bvg$^+$ group was significantly higher (0.88 ± 0.12 vs 0.54 ± 0.11 OD_{450}). A moderate positive correlation between dermonecrotic activity and IgG level was established ($r = 0.61$). ROC analysis demonstrated good predictive ability of phenotypic markers (AUC = 0.84; 95% CI 0.70–0.98). The obtained results indicate that the stability of the Bvg$^+$ phenotype is associated with increased expression of virulence factors, a greater capacity for biomass accumulation, and a significantly higher induction of the humoral immune response. Phenotypic screening of Bvg$^+$ phase stability may be used as a tool for selecting promising production strains for the development of inactivated bacterial preparations.

Keywords: *Bordetella bronchiseptica*; BvgAS regulation; biomass accumulation; heterogeneity of phenotypic characteristics; adhesion activity to Vero cell line; immunogenic potential of clinical isolates.

Introduction

Diseases of productive and companion animals are a widespread problem worldwide. Infectious diseases of bacterial etiology cause significant economic losses to the livestock industry, and control of their occurrence and spread is one of the main tasks of veterinary specialists (Rahman et al., 2020; Zavgorodnii et al., 2021; Skowron et al., 2023). The complexity of diagnosis and treatment of these diseases is associated with the adaptive variability of pathogens to environmental conditions and the development of resistance to antimicrobial agents (Paliy, 2018; Zhang & Cheng, 2022; Paliy et al., 2024).

Bordetella bronchiseptica is a Gram-negative aerobic coccobacillary microorganism that belongs to the classical representatives of the genus *Bordetella* and plays a leading role in the development of respiratory infections in dogs, cats, pigs, rabbits, and other animal species. In canine pathology, this pathogen is a component of the canine infectious respiratory disease complex (CIRDC), which is characterized by polyetiology, high contagiousness, and considerable variability in clinical course (Day et al., 2020; Kaul et al., 2025). In addition to classical respiratory manifestations, *B. bronchiseptica* is capable of causing systemic infections under immunodeficiency conditions and can also persist in extra-host ecological niches, indicating its pronounced adaptive plasticity (Ma et al., 2022; Badhai & Das, 2023).

Modern molecular and genetic studies demonstrate considerable genetic diversity among clinical isolates of *B. bronchiseptica*, including variability of virulence genes, the presence of mobile genetic elements, and different profiles of antimicrobial resistance (Zhang et al., 2021; Nicholson & Shore, 2024). Chen et al. (2020) showed that the integration of temperate bacteriophages may alter the level of bacterial pathogenicity, whereas Petrovic Fabijan et al. (2021) emphasized the complex interaction between *Bordetella* and bacteriophages and the potential influence of these interactions on the expression of virulence factors. In addition, the study by Yi et al. (2024) demonstrated a relationship between biofilm formation and the development of antimicrobial resistance, highlighting the complexity of the phenotypic organization of *B. bronchiseptica* populations.

The central mechanism regulating the virulence of classical *Bordetella* species is the two-component BvgAS system, which provides phase modulation of the expression of a wide range of genes. The structural features of the sensor kinase BvgS and the mechanisms of signal transduction were described in detail by Dupré et al. (2021). In addition, Luu et al. (2021) demonstrated the role of serine-, threonine-, and tyrosine-phosphorylation in the coordination of *Bordetella* virulence proteins. The global nature of this regulation was confirmed in the study by Nicholson et al. (2024), which showed the involvement of BvgR, RisA, and RisS in the control of transcription, intracellular c-di-GMP levels, motility, and biofilm formation. At the same time, Vondrova et al. (2026) demonstrated the complex architecture of the c-di-GMP signaling network in classical *Bordetella*, which is integrated with Bvg-dependent regulation. The possibility of pharmacological modulation of the BvgAS system was confirmed in the work of Ota et al. (2025), further emphasizing its central position in the regulatory network.

In the Bvg$^+$ phase, the expression of key adhesive determinants is activated, particularly filamentous hemagglutinin (FHA) and pertactin. Johnson et al. (2021) demonstrated the regulated processing of

FhaB required for effective adhesion. Ma et al. (2021) confirmed the role of pertactin in transmission and colonization, while Nash et al. (2024) showed the interaction of FHA with adenylate cyclase toxin on the bacterial surface. At the same time, clinical observations by Rodriguez & Berliner (2023) indicate the influence of variability of virulence determinants on the course of infectious outbreaks.

Dermonecrotic toxin is another Bvg-regulated factor reflecting the active virulent state of the bacterium. Jang et al. (2024) demonstrated the structural and immunological characteristics of the toxin in clinical isolates, whereas Gutierrez et al. (2024) confirmed the role of BvgR in maintaining the virulent phenotype. Belhart et al. (2023) showed that diguanylate cyclases can influence secretory systems and immune responses, integrating environmental signals with virulence programs.

Biofilm formation and the ability to accumulate biomass are considered important factors of persistence (Kolchyk et al., 2022). Mugni et al. (2025) demonstrated the role of albumin and calcium in the regulation of biofilm formation through signaling molecules, whereas Price & Skaar (2025) described metal-induced mechanisms of biofilm structure stabilization. Yasbolaghi Sharahi et al. (2025) systematized the mechanisms of biofilm dispersion and their therapeutic potential. At the same time, Ma et al. (2022) demonstrated the ability of *Bordetella* to interact with amoebae, indicating evolutionarily developed regulatory flexibility and the potential stability of Bvg-dependent phenotypic programs.

From the perspective of vaccinology, the preservation of the full spectrum of Bvg-regulated antigens is critically important. Gestal et al. (2022) demonstrated the formation of a long-lasting Th17-mediated response under conditions of expression of the complete virulence profile. Jang et al. (2025) confirmed the effectiveness of vaccines based on outer membrane proteins. Lee & Joo (2023) described the immunomodulatory properties of the lipopolysaccharide of *B. bronchiseptica*, whereas Pérez-Ortega et al. (2021, 2023) demonstrated the possibility of reducing its endotoxin activity through lipid A engineering without loss of immunogenicity. Miguelena Chamorro et al. (2023) and Munoz Navarrete et al. (2025) emphasize the need to revise the antigenic composition of vaccines considering the regulatory plasticity of *Bordetella*.

Despite the considerable number of molecular and genomic studies, the applied significance of Bvg^+ phase stability for the selection of production strains remains insufficiently studied. Most studies focus on pathogenesis or antimicrobial resistance (Zhang et al., 2021; Nicholson & Shore, 2024), whereas the integral relationship between phenotypic stability, expression of virulence factors, and the ability to induce a humoral immune response requires systematic analysis. In particular, it remains insufficiently clarified whether the stability of the Bvg^+ phenotype of clinical isolates of *B. bronchiseptica* is associated with their immunogenic properties and whether phenotypic analysis can be used as a practical criterion for selecting promising production strains.

In this regard, the aim of the present study was to comprehensively assess the relationship between the stability of the Bvg^+ phenotype of clinical isolates of *B. bronchiseptica*, their adhesion activity, dermonecrotic activity, ability to accumulate biomass, and induction of a specific humoral immune response, with subsequent substantiation of the feasibility of using phenotypic screening as a tool for selecting promising production strains for the development of inactivated bacterial preparations.

Materials and methods

All experimental studies involving laboratory animals were conducted in accordance with the general principles of bioethics. All procedures involving experimental animals were approved by the Bioethics Commission of the National Scientific Center "Institute of Experimental and Clinical Veterinary Medicine" (Kharkiv, Ukraine). Particular attention was given to procedures that could potentially cause discomfort or pain to the animals, including intradermal administration of culture supernatants and immunization with inactivated bacterial preparations. All manipulations were performed under conditions aimed at minimizing stress and suffering of the animals. The study was conducted in accordance with the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (Simmonds, 2017).

The study included 25 clinical isolates of *B. bronchiseptica* obtained from dogs and cats with clinical signs of respiratory syndrome during 2023–2025. The microorganism cultures are stored in the collection of the National Scientific Center "Institute of Experimental and Clinical Veterinary Medicine" (Kharkiv) (Table 1).

Table 1
Clinical isolates of *B. bronchiseptica* used in the study

Isolate ID	Host species	Year of isolation	Sample source
Bb-01	dog	2023	nasal swab
Bb-02	dog	2023	nasal swab
Bb-03	cat	2023	tracheal swab
Bb-04	dog	2023	nasal swab
Bb-05	cat	2023	nasal swab
Bb-06	dog	2024	nasal swab
Bb-07	dog	2024	tracheal swab
Bb-08	cat	2024	nasal swab
Bb-09	dog	2024	nasal swab
Bb-10	cat	2024	nasal swab
Bb-11	dog	2024	tracheal swab
Bb-12	dog	2024	nasal swab
Bb-13	cat	2024	nasal swab
Bb-14	dog	2024	nasal swab
Bb-15	dog	2024	tracheal swab
Bb-16	cat	2025	nasal swab
Bb-17	dog	2025	nasal swab
Bb-18	dog	2025	tracheal swab
Bb-19	cat	2025	nasal swab
Bb-20	dog	2025	nasal swab
Bb-21	dog	2025	nasal swab
Bb-22	cat	2025	tracheal swab
Bb-23	dog	2025	nasal swab
Bb-24	cat	2025	nasal swab
Bb-25	dog	2025	tracheal swab

The species identity of the isolates was confirmed based on morphological, cultural, and biochemical characteristics in accordance with generally accepted diagnostic criteria for the identification of *Bordetella* spp. (Zhang et al., 2021; Nicholson & Shore, 2024).

The isolates were cultivated in liquid Stainer-Scholte medium (Stainer & Scholte, 1971) at a temperature of 35.5 ± 0.5 °C for 24 h under aerobic submerged cultivation on an orbital shaker (220 rpm). Biomass accumulation was determined using the method of serial tenfold dilutions followed by plating onto solid nutrient medium and counting colony-forming units (CFU). The results were expressed as log CFU/mL in accordance with standard microbiological methods for the cultivation of *Bordetella* spp. (Johnson et al., 2021; Ma et al., 2021).

Hemagglutination was performed using a 1.0% suspension of sheep erythrocytes. Hemagglutination was used as a screening test to assess the expression of surface adhesins of *B. bronchiseptica* associated with the Bvg^+ phenotype (Johnson et al., 2021; Nash et al., 2024). The reaction is based on the ability of bacterial cells to bind erythrocytes and induce their aggregation, which is mediated by adhesive proteins, particularly filamentous hemagglutinin. The bacterial suspension was mixed with erythrocytes and incubated at room temperature. The presence of visible agglutination was interpreted as a positive result.

Adhesion activity was determined in an interaction assay with Vero cell line cells. After incubation and removal of non-adherent bacteria, the degree of attachment was evaluated using a semi-quantitative scale from 0 to 3 scores according to previously described methodological approaches (Johnson et al., 2021; Nash et al., 2024). The assessment was performed based on the number of bacterial cells adhering to the surface of Vero cells:

0 – no adhesion;
1 – weak adhesion (1–10 bacterial cells per Vero cell);
2 – moderate adhesion (10–50 bacterial cells per Vero cell);
3 – intensive adhesion (>50 bacterial cells per Vero cell).

Phenotypic tests (hemagglutination, determination of adhesion activity, and biomass accumulation) were performed in three independent replicates.

Dermonecrotic activity was evaluated using culture supernatants of 24-hour cultures of *B. bronchiseptica* isolates obtained after cultivation in liquid Stainer-Scholte medium followed by centrifugation to remove bacterial cells. Before collecting the supernatant, the concentration of the bacterial suspension was standardized to 1×10^8 CFU/mL. Twenty-five experimental groups of outbred laboratory mice were formed (n = 250), with 10 animals in each group. Each group received the culture supernatant of the corresponding isolate. Additionally, one control group (n = 10) was formed, whose animals received sterile physiological saline. The supernatant was administered intradermally in a volume of 0.1 mL after preliminary dilution 1:4 in sterile physiological saline. The diameter of the necrotic lesion (mm) was measured 24 hours after administration according to previously described methods for evaluating dermonecrotic activity of *Bordetella* (Dupré et al., 2021; Jang et al., 2024). For each isolate, the assessment was performed in a group of 10 animals, and the results were expressed as the mean diameter of the necrotic lesion.

The isolates were classified as demonstrating a stable Bvg^+ phenotype based on a combination of phenotypic characteristics: positive hemagglutination reaction; high adhesion activity (≥ 2 scores); pronounced dermonecrotic activity (≥ 6 mm diameter of necrotic lesion); and high biomass accumulation (≥ 8.5 log CFU/mL). These selection criteria were based on functional indicators of the expression of Bvg-regulated virulence factors and are consistent with experimental models for evaluating adhesive and toxigenic properties of *Bordetella* described in previous studies (Johnson et al., 2021; Jang et al., 2024; Nicholson et al., 2024).

To assess immunogenicity, an inactivated bacterial suspension of *B. bronchiseptica* isolates standardized to a concentration of 1×10^8 CFU/mL prior to inactivation was used. Inactivation was carried out using a 0.3% formalin solution for 24 hours at 37.0 ± 0.5 °C followed by sterility control of the preparation.

For immunization, 25 experimental groups of outbred laboratory mice were formed (n = 250), with 10 animals in each group. Each group received an inactivated bacterial preparation obtained from the corresponding clinical isolate of *B. bronchiseptica*, standardized to a concentration of 1×10^8 CFU/mL before inactivation. Additionally, one control group (n = 10) was formed, whose animals received sterile physiological saline. The inactivated bacterial preparation was administered subcutaneously at a dose of 0.5 mL twice with an interval of 14 days. The level of specific IgG antibodies was determined using enzyme-linked immunosorbent assay (ELISA). The results were evaluated based on optical density values measured at a wavelength of 450 nm (OD_{450}) according to previously described approaches for assessing the humoral immune response in infections caused by *B. bronchiseptica* (Gestal et al., 2022; Jang et al., 2025).

Before performing parametric analysis, the normality of data distribution was assessed using the Shapiro-Wilk test. Variables with normal distribution were analyzed using one-way analysis of variance (one-way ANOVA). For semi-quantitative indicators (adhesion activity), the non-parametric Mann-Whitney test was used. Correlation analysis was performed by determining the Pearson correlation coefficient (r). ROC analysis was applied to evaluate the predictive ability of phenotypic indicators for identifying isolates with a stable Bvg+ phenotype with determination of the area under the ROC curve (AUC) and the 95% confidence interval. Statistical significance was considered established at $P < 0.05$.

Results

The studied clinical isolates of *B. bronchiseptica* demonstrated different growth intensities in liquid medium. The values of biomass accumulation ranged from 7.39 to 9.05 log CFU/mL, indicating heterogeneity of the growth characteristics of the studied *Bordetella* population. Individual values of biomass accumulation for *B. bronchiseptica* isolates were determined (Table 2), and a comparative analysis of the obtained data was also performed (Table 3).

Table 2
Biomass accumulation of clinical isolates
of *B. bronchiseptica* (mean ± SD, n = 3)

Isolate	Biomass, log CFU/mL
Bb-01	8.72 ± 0.18
Bb-02	7.86 ± 0.21
Bb-03	8.91 ± 0.17
Bb-04	7.63 ± 0.19
Bb-05	9.05 ± 0.23
Bb-06	8.18 ± 0.20
Bb-07	8.83 ± 0.16
Bb-08	7.52 ± 0.22
Bb-09	8.41 ± 0.25
Bb-10	7.74 ± 0.19
Bb-11	8.02 ± 0.17
Bb-12	8.55 ± 0.21
Bb-13	7.81 ± 0.18
Bb-14	8.27 ± 0.23
Bb-15	8.47 ± 0.24
Bb-16	7.39 ± 0.20
Bb-17	8.09 ± 0.19
Bb-18	7.61 ± 0.18
Bb-19	8.88 ± 0.17
Bb-20	7.68 ± 0.21
Bb-21	8.16 ± 0.22
Bb-22	7.54 ± 0.20
Bb-23	8.34 ± 0.23
Bb-24	7.92 ± 0.18
Bb-25	8.65 ± 0.19

The mean value of biomass accumulation in the Bvg^+ group was 8.9 ± 0.3 log CFU/mL, whereas in the other isolates the values were lower and mainly ranged from 7.39 to 8.88 log CFU/mL. The highest biomass accumulation values were observed in isolates Bb-01, Bb-03, Bb-05, Bb-07, and Bb-19. It should be noted that some isolates (Bb-12 and Bb-25) demonstrated a relatively high level of biomass accumulation (> 8.5 log CFU/mL); however, they did not meet the other phenotypic criteria of the Bvg^+ phase and therefore were not classified in this group.

Table 3
Comparative analysis of biomass accumulation
in *B. bronchiseptica* isolates (mean ± SD)

Group	Biomass, log CFU/mL
Bvg^+ isolates (n = 5)	8.9 ± 0.3
Other isolates (n = 20)	7.8 ± 0.5*

Note: $^*P < 0.05$ (one-way ANOVA).

The results of hemagglutination testing showed that a positive hemagglutination reaction was observed in isolates Bb-01, Bb-03, Bb-05, Bb-07, Bb-12, Bb-19, Bb-23, and Bb-25, whereas the other studied isolates (Bb-02, Bb-04, Bb-06, Bb-08, Bb-09, Bb-10, Bb-11, Bb-13, Bb-14, Bb-15, Bb-16, Bb-17, Bb-18, Bb-20, Bb-21, Bb-22, and Bb-24) did not exhibit hemagglutinating activity. The obtained results indicate that hemagglutinating activity was characteristic only for a portion of the studied *B. bronchiseptica* isolates. A positive hemagglutination reaction was observed in 9 (36%) of the 25 studied isolates, which may indicate variability in the expression of surface adhesive factors in clinical strains.

The results of determining the adhesive activity of clinical isolates of *B. bronchiseptica* in the interaction assay with Vero cell line cells demonstrated pronounced variability of this parameter. High adhesive activity (3 scores) was observed in isolates Bb-01, Bb-03, Bb-05, Bb-07, and Bb-19. Moderate adhesion (2 scores) was observed in isolates Bb-06, Bb-12, Bb-15, Bb-21, Bb-23, and Bb-25. The other studied isolates (Bb-02, Bb-04, Bb-08, Bb-09, Bb-10, Bb-11, Bb-13, Bb-14, Bb-16, Bb-17, Bb-18, Bb-20, Bb-22, and Bb-24) were characterized by low adhesive activity (1 score). No isolates with complete absence of adhesion (0 scores) were detected in this study. The distribution of isolates according to the level of adhesive activity showed that low adhesion (1 score) was observed in 14 isolates (56%), moderate adhesion (2 scores) in 6 isolates (24%), whereas high adhesive activity (3 scores) was detected in 5 isolates (20%) (Table 4).

Table 4
Distribution of clinical isolates
of *B. bronchiseptica* according to adhesion activity (n = 3)

Adhesion score	Number of isolates	%
1 (low)	14	56
2 (moderate)	6	24
3 (high)	5	20

The analysis of the obtained results showed variability in adhesive activity among the studied clinical isolates of *B. bronchiseptica*. A portion of the isolates (44%) was characterized by a high capacity for attachment to Vero cells (2–3 scores), whereas the majority of isolates (56%) demonstrated low adhesive activity (1 score). The highest adhesion values were observed in isolates Bb-01, Bb-03, Bb-05, Bb-07, and Bb-19. A comparative analysis of the mean values of adhesive activity between groups of clinical isolates of *B. bronchiseptica* was performed (Table 5).

Table 5
Comparative analysis of adhesion activity
of *B. bronchiseptica* isolates (score, mean ± SD, n = 3)

Group	Adhesion activity
Bvg^+ isolates (n =5)	2.6 ± 0.4
Other isolates (n = 20)	1.4 ± 0.5*

Note: see Table 3.

The obtained results indicate that clinical isolates of *B. bronchiseptica* with a stable Bvg^+ phenotype are characterized by significantly higher adhesive activity compared with other isolates. This may be associated with active expression of Bvg-regulated adhesive factors, particularly filamentous hemagglutinin and pertactin, which ensure effective attachment of bacteria to epithelial cells.

It was established that the studied clinical isolates of *B. bronchiseptica* exhibit different levels of dermonecrotic activity (Table 6). The results presented in Table 6 indicate that clinical isolates of *B. bronchiseptica* are characterized by varying levels of dermonecrotic activity. The largest diameter of necrotic lesions was observed after administration of supernatants from isolates Bb-01, Bb-03, Bb-05, Bb-07, and Bb-19, indicating their increased dermonecrotic activity. The mean dermonecrotic activity in the group of isolates with a stable Bvg^+ phenotype was 8.2 ± 1.1 mm, whereas in the other isolates this value was 3.9 ± 0.8 mm (Table 7).

To assess the immunogenic potential of clinical isolates of *B. bronchiseptica*, five isolates characterized by the most pronounced phenotypic properties were selected, as well as two isolates with lower indicators for comparison (Table 8). The analysis of the obtained data showed that isolates with a stable Bvg^+ phenotype were characterized by a higher level of induction of specific antibodies compared with other isolates. The mean optical density value in the Bvg^+ group was 0.88 ± 0.12 OD_{450}, whereas in the other isolates this value did not exceed 0.54 ± 0.11 OD_{450} (Table 9).

Correlation analysis revealed positive relationships between the main phenotypic characteristics of clinical isolates of *B. bronchiseptica*. The strongest correlation was observed between dermonecrotic activity and the level of specific IgG ($r = 0.61$; $P < 0.01$), indicating a possible association between the toxigenic properties of the isolates and their immunogenic potential. Moderate positive correlations were also observed between adhesive activity and dermonecrotic activity ($r = 0.58$; $P < 0.05$), as well as between adhesion and IgG level ($r = 0.54$; $P < 0.05$). The relationship between biomass accumulation and the other indicators was less pronounced (r = 0.44–0.49), but was also positive in nature (Table 10, Fig. 1).

To evaluate the predictive ability of phenotypic markers for identifying isolates with a stable Bvg^+ phenotype, ROC analysis was performed. The obtained results demonstrated sufficiently high diagnostic accuracy of the studied indicators. The area under the ROC curve was 0.84 (95% CI 0.70–0.98; P = 0.01), indicating good predictive performance of the phenotypic characteristics (Fig. 2).

The generalization of the obtained results indicates that clinical isolates of *B. bronchiseptica* are characterized by pronounced phenotypic variability in the main virulence indicators. Five isolates (Bb-01, Bb-03, Bb-05, Bb-07, and Bb-19) demonstrated increased values of adhesive activity, dermonecrotic activity, and biomass accumulation capacity. These isolates exhibited a combination of characteristics typical of the active Bvg^+ phenotype. Immunization with inactivated bacterial preparations obtained from these isolates was accompanied by a higher level of induction of specific IgG antibodies compared with other isolates. The performed correlation analysis confirmed the presence of a positive relationship between dermonecrotic activity and the level of specific antibodies.

Table 6
Dermonecrotic activity of clinical isolates
of *B. bronchiseptica* (mean ± SD, n = 10)

Isolate	Diameter of necrosis, mm
Bb-01	8.4 ± 0.42
Bb-02	3.2 ± 0.31
Bb-03	8.1 ± 0.38
Bb-04	2.9 ± 0.27
Bb-05	9.0 ± 0.45
Bb-06	4.1 ± 0.34
Bb-07	7.8 ± 0.36
Bb-08	3.5 ± 0.29
Bb-09	3.7 ± 0.33
Bb-10	3.1 ± 0.28
Bb-11	3.4 ± 0.30
Bb-12	4.5 ± 0.37
Bb-13	3.6 ± 0.26
Bb-14	3.3 ± 0.24
Bb-15	4.2 ± 0.32
Bb-16	2.7 ± 0.23
Bb-17	3.8 ± 0.31
Bb-18	3.0 ± 0.27
Bb-19	8.6 ± 0.40
Bb-20	3.1 ± 0.29
Bb-21	4.4 ± 0.35
Bb-22	3.2 ± 0.28
Bb-23	4.6 ± 0.33
Bb-24	3.5 ± 0.26
Bb-25	4.0 ± 0.34

Table 7
Comparative analysis of dermonecrotic activity
of *B. bronchiseptica* isolates (mean ± SD, n = 3)

Group	Dermonecrotic activity, mm
Bvg^+ isolates (n = 5)	8.2 ± 1.1
Other isolates (n = 20)	3.9 ± 0.8**

Note: **$P < 0.01$ (one-way ANOVA).

Table 8
Immunogenic activity of selected clinical isolates
of *B. bronchiseptica* (mean ± SD, n = 10)

Isolate	Phenotype	IgG, OD_{450}
Bb-01	Bvg^+	0.89 ± 0.08
Bb-03	Bvg^+	0.92 ± 0.10
Bb-05	Bvg^+	0.90 ± 0.09
Bb-07	Bvg^+	0.87 ± 0.07
Bb-19	Bvg^+	0.88 ± 0.08
Bb-04	Bvg^- / non-Bvg phenotype	0.52 ± 0.06
Bb-11	Bvg^- / non-Bvg phenotype	0.49 ± 0.05

Table 9
Comparative analysis of IgG antibody levels
induced by *B. bronchiseptica* isolates (mean ± SD, n = 3)

Group	IgG level, OD_{450}
Bvg^+ isolates (n = 5)	0.88 ± 0.12
Other isolates (n = 20)	0.54 ± 0.11*

Note: see Table 3.

Discussion

The obtained results demonstrate a statistically significant relationship between the stability of the Bvg^+ phenotype of clinical isolates

of *B. bronchiseptica* and their adhesive activity, dermonecrotic activity, ability to accumulate biomass, and induction of a specific humoral immune response. Significant differences between the groups were observed ($P < 0.05$), and large effect sizes (Cohen's $d > 0.8$) confirm the biological significance of phenotypic stratification of the isolates and indicate the systemic nature of regulatory differences. Similar phenotypic heterogeneity of clinical strains has been described by Wang et al. (2020), Zhang et al. (2021), and Kameyama et al. (2022), which is consistent with the concept of adaptive plasticity of *Bordetella*. In addition, Badhai & Das (2023), based on genomic analysis, demonstrated the diversity of virulence determinants and adaptive traits of *B. bronchiseptica* outside the host organism, which confirms the high ecological and phenotypic plasticity of this species.

Table 10
Correlation analysis of phenotypic characteristics
of *B. bronchiseptica* isolates (Pearson correlation coefficients, n = 7)

Parameter	Adhesion	Dermonecrosis	Biomass	IgG
Adhesion (score)	1.000	0.580*	0.460	0.540*
Dermonecrosis, mm	0.580*	1.000	0.490	0.610**
Biomass, log CFU/mL	0.460	0.490	1.000	0.440
IgG, OD_{450}	0.540*	0.610**	0.440	1.000

Note: * – $P < 0.05$; ** – $P < 0.01$.

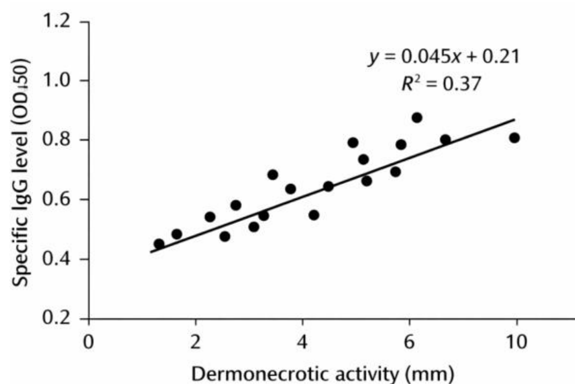

Fig. 1. Correlation between dermonecrotic activity and specific IgG levels (Pearson's $r = 0.61$, $R^2 = 0.37$, $y = 0.045x + 0.21$, $P = 0.01$)

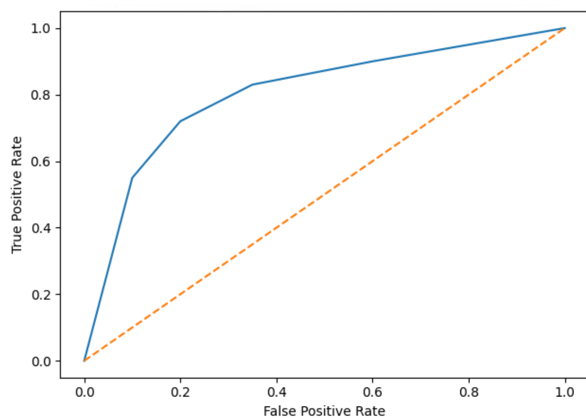

Fig. 2. Receiver operating characteristic (ROC) curve evaluating the predictive performance of phenotypic markers for identification of isolates with stable Bvg^+ phenotype (AUC = 0.84; 95% CI 0.70–0.98)

Within the framework of the study, a primary phenotypic analysis of 25 clinical isolates was performed with a comprehensive evaluation of Bvg-regulated virulence factors. Five isolates were classified as demonstrating a stable Bvg^+ phenotype based on a combination of characteristics (positive hemagglutination reaction, high adhesion to Vero cells, increased dermonecrotic activity, and intensive biomass accumulation). Such an integrated approach minimizes the risk of erroneous phenotypic interpretation and is consistent with the recommendations for comprehensive characterization of isolates (Chen et al., 2020; Tabatabaei & Rohani, 2022; Cole et al., 2024). In the context of polyetiological respiratory infections in dogs, the necessity for accurate differentiation of *B. bronchiseptica* from other pathogens of the canine infectious respiratory disease complex (CIRDC) has also been emphasized by Kaul et al. (2025), which further justifies the use of standardized phenotypic and molecular identification algorithms.

The BvgAS system is the central global regulator of virulence in classical *Bordetella* species. The structural mechanisms underlying activation of the sensor kinase BvgS were described in detail by Dupré et al. (2021). Luu et al. (2021) demonstrated the importance of post-translational phosphorylation in the coordination of *Bordetella* virulence proteins. Nicholson et al. (2024) showed the involvement of BvgR, RisA, and RisS in the global control of transcription, intracellular c-di-GMP levels, and biofilm formation. Ota et al. (2025) confirmed the possibility of pharmacological modulation of the BvgAS system, further emphasizing its central role in the regulatory network. Thus, the stability of the Bvg^+ phase reflects systemic activation of both virulence and metabolic programs.

The adhesive activity of isolates with a stable Bvg^+ phenotype was significantly higher (2.6 ± 0.4 vs 1.4 ± 0.5; $P = 0.018$). This is consistent with the Bvg-dependent expression of filamentous hemagglutinin and pertactin. Johnson et al. (2021) demonstrated the role of FhaB processing, whereas Nash et al. (2024) described the interaction between FHA and adenylate cyclase toxin. Ma et al. (2021) confirmed the importance of pertactin in transmission and colonization. In addition, Rodriguez & Berliner (2023) emphasized the clinical consequences of variability in adhesive determinants, while Petrovic Fabijan et al. (2021) highlighted the possible influence of mobile genetic elements on virulence.

The dermonecrotic activity of supernatants from Bvg^+ isolates was significantly higher (8.2 ± 1.1 vs 3.9 ± 0.8 mm; $P = 0.031$). Regulation of dermonecrotic toxin through the BvgAS system has been confirmed by Dupré et al. (2021) and Gutierrez et al. (2024). Jang et al. (2024) demonstrated structural and immunological differences of dermonecrotic toxin, whereas Belhart et al. (2023) reported the influence of signaling molecules on virulence systems. Reduced activity observed in some isolates may reflect a partial transition to the Bvg^- or intermediate phase.

Higher biomass accumulation in Bvg^+ isolates (8.7 ± 0.3 vs 7.8 ± 0.5 log CFU/mL; $P = 0.021$) indicates the involvement of the BvgAS system in physiological adaptation. Mugni et al. (2025) and Vondrova et al. (2026) described the regulation of biofilm formation through c-di-GMP signaling. Perslow et al. (2025) demonstrated the role of BvgS in morphological adaptations. Yi et al. (2024) and Tang et al. (2026) associated biofilm formation with antimicrobial resistance. Price & Skaar (2025) and Yasbolaghi Sharahi et al. (2025) emphasized the role of stress-related mechanisms in the stabilization of bacterial populations. In addition, interactions of *B. bronchiseptica* with protozoan organisms and its ability to survive in extra-host niches, described by Ma et al. (2022), indicate evolutionarily developed regulatory flexibility that may indirectly influence the stability of Bvg-dependent phenotypic programs.

A key finding of the present study was the significantly higher level of specific IgG following immunization with preparations derived from Bvg^+ isolates (0.88 ± 0.12 vs 0.54 ± 0.11; $P = 0.015$). Gestal et al. (2022) demonstrated the formation of a Th17-mediated immune response when the full virulence profile is preserved. Jang et al. (2025) confirmed the effectiveness of vaccines based on outer membrane proteins (OMPs). Lee & Joo (2023) described the immunomodulatory properties of lipopolysaccharide (LPS), whereas Pérez-Ortega et al. (2021, 2023) demonstrated the possibility of reducing its endotoxicity while maintaining immunogenicity. Miguelena Chamorro et al. (2023) and Muñoz Navarrete et al. (2025) emphasize the need to revise the antigenic composition of vaccines taking into account the regulatory plasticity of *Bordetella* species.

The established correlation between dermonecrotic activity and IgG levels ($r = 0.61$; $P = 0.01$) indicates coordinated expression of Bvg-regulated factors. Considering the global regulatory function of the BvgAS system (Luu et al., 2021; Nicholson et al., 2024), it can be assumed that the active Bvg^+ phase ensures coordinated synthesis of several immunodominant proteins, forming an integrated immunoge-

nic effect. Such consistency of phenotypic indicators is biologically expected, since most of the studied virulence factors of *B. bronchiseptica* are under the control of the global regulatory BvgAS system, which coordinates the expression of adhesive, toxigenic, and metabolic bacterial programs. ROC analysis (AUC = 0.84; 95% CI 0.70–0.98; P = 0.01) confirmed the good predictive ability of the phenotypic markers. In the context of genetic diversity, mobile genetic elements, and antimicrobial resistance (Nicholson et al., 2024; Yi et al., 2024; Tang et al., 2026), the implementation of standardized phenotypic screening may have practical value for optimizing the selection of production strains.

Thus, the stability of the Bvg^+ phenotype of clinical isolates of *B. bronchiseptica* is associated with increased expression of adhesive and toxigenic factors, a greater capacity for biomass accumulation, and a significantly higher induction of the humoral immune response. The obtained data substantiate the feasibility of including phenotypic screening in the algorithm for selecting promising production strains for the development of inactivated bacterial preparations.

Despite the statistically significant results obtained, the study has several limitations. First, the sample of clinical isolates was relatively small (n = 25), which may limit the generalization of the results to a broader population of *B. bronchiseptica* from different geographical regions and production conditions. Second, the phenotypic assessment of Bvg^+ phase stability was based on functional tests without direct molecular analysis of gene expression within the Bvg regulatory cascade, which does not allow a complete characterization of the transcriptional profile of the isolates. In addition, immunogenicity was evaluated based on the level of specific IgG in a laboratory model without analysis of the cellular immune response and without conducting an infection challenge study, which does not allow a definitive assessment of the protective efficacy of the obtained preparations. Further studies should therefore focus on expanding the isolate sample size, incorporating molecular-genetic methods to verify Bvg^+ status, and evaluating protective efficacy in target animal species.

Conclusion

It was established that the stability of the Bvg^+ phenotype of clinical isolates of *Bordetella bronchiseptica* is associated with complex activation of Bvg-regulated virulence determinants, manifested by increased adhesive activity, more pronounced dermonecrotic activity, and more intensive biomass accumulation.

Isolates with a stable Bvg^+ phenotype were characterized by a significantly higher level of induction of the specific humoral immune response following immunization with inactivated preparations, indicating their increased immunogenic potential and functional integration of Bvg-dependent factors.

The identified correlation between dermonecrotic activity and IgG levels confirms the coordinated expression of virulence and immunodominant components within the Bvg+ phase, while the results of ROC analysis demonstrate sufficient predictive accuracy of the phenotypic markers.

The obtained data substantiate the feasibility of using phenotypic screening of Bvg^+ phase stability as a practical tool for selecting promising production strains for the development of inactivated bacterial preparations and expand the understanding of the applied significance of BvgAS regulation in vaccinology.

The authors declare they have no conflict of interest.

References

Badhai, J., & Das, S. K. (2023). Genomic evidence and virulence properties decipher the extra-host origin of *Bordetella bronchiseptica*. Journal of Applied Microbiology, 134(9), lxad200.

Belhart, K., Sisti, F., Gestal, M. C., & Fernández, J. (2023). *Bordetella bronchiseptica* diguanylate cyclase *BdcB* inhibits the type three secretion system and impacts the immune response. Scientific Reports, 13(1), 7157.

Chen, Y., Yang, L., Yang, D., Song, J., Wang, C., Sun, E., Gu, C., Chen, H., Tong, Y., Tao, P., & Wu, B. (2020). Specific integration of temperate phage decreases the pathogenicity of host bacteria. Frontiers in Cellular and Infection Microbiology, 10, 14.

Cole, M., Simon, A. K., Faulkner, A., Skoff, T., Tondella, M. L., Montero, C., Nye, M. B., & Williams, M. (2024). Comparison of *Bordetella* species identification among differing rt-PCR assays in the United States. Microbiology Spectrum, 12(8), e0078324.

Day, M. J., Carey, S., Clercx, C., Kohn, B., MarsilIo, F., Thiry, E., Freyburger, L., Schulz, B., & Walker, D. J. (2020). Aetiology of canine infectious respiratory disease complex and prevalence of its pathogens in Europe. Journal of Comparative Pathology, 176, 86–108.

Dupré, E., Clantin, B., Yuan, Y., Lecher, S., Lesne, E., Antoine, R., Villeret, V., & Jacob-Dubuisson, F. (2021). Structural insight into the role of the PAS domain for signal transduction in sensor-kinase *BvgS*. Journal of Bacteriology, 203(9), e00614-20.

Gestal, M. C., Howard, L. K., Dewan, K. K., & Harvill, E. T. (2022). Bbvac: A live vaccine candidate that provides long-lasting anamnestic and Th17-mediated immunity against the three classical *Bordetella* spp. mSphere, 7(1), e0089221.

Gutierrez, M. D. L. P., Damron, F. H., Sisti, F., & Fernández, J. (2024). BvgR is important for virulence-related phenotypes in *Bordetella bronchiseptica*. Microbiology Spectrum, 12(11), e0079424.

Jang, J. Y., Oh, M. W., Na, C., Im, Y. B., Shim, S., Moon, H. J., & Yoo, H. S. (2024). Comparative structural and immunological analysis of outer membrane proteins and dermonecrotic toxin in *Bordetella bronchiseptica* canine isolate. Veterinary Immunology and Immunopathology, 272, 110756.

Jang, J. Y., Park, B. J., Song, H., Rha, S., Moon, H. J., & Yoo, H. S. (2025). Safety and efficacy of a *Bordetella bronchiseptica* outer membrane proteins (OMPs) subunit vaccine in a murine model. Journal of Veterinary Science, 26(3), e39.

Johnson, R. M., Nash, Z. M., Dedloff, M. R., Shook, J. C., & Cotter, P. A. (2021). DegP initiates regulated processing of filamentous hemagglutinin in *Bordetella bronchiseptica*. mBio, 12(3), e0146521.

Kameyama, H., Fujimoto, Y., Tomioka, Y., Yamamoto, S., Suyama, H., Inoue, H., Takahashi, E., & Ono, E. (2022). Pathogenicity of *Bordetella bronchiseptica* isolated from apparently healthy rabbits in guinea pig, rat, and mouse. Journal of Veterinary Medical Science, 84(4), 574–581.

Kaul, R., Bhagwan, J., Batra, K., Kumar, P., & Agnihotri, D. (2025). Development of multiplex PCR assay for detection of canine infectious respiratory disease complex (CIRDC) pathogens in dogs. Frontiers in Cellular and Infection Microbiology, 15, 1661499.

Kolchyk, O., Illarionova, T., Buzun, A., Paliy, A., & Palii, A. (2022). Influence of probiotic microorganisms on microbial biofilms in feeds. Scientific Horizons, 25(1), 41–50.

Lee, Y. J., & Joo, H. G. (2023). Immunostimulatory activity and safety evaluation of *Bordetella bronchiseptica*-derived lipopolysaccharide, a new vaccine adjuvant candidate. Immunobiology, 228(5), 152709.

Luu, L. D. W., Zhong, L., Kaur, S., Raftery, M. J., & Lan, R. (2021). Comparative phosphoproteomics of classical Bordetellae elucidates the potential role of serine, threonine and tyrosine phosphorylation in *Bordetella biology* and virulence. Frontiers in Cellular and Infection Microbiology, 11, 660280.

Ma, L., Dewan, K. K., Taylor-Mulneix, D. L., Wagner, S. M., Linz, B., Rivera, I., Su, Y., Caulfield, A. D., Blas-Machado, U., & Harvill, E. T. (2021). Pertactin contributes to shedding and transmission of *Bordetella bronchiseptica*. PLoS Pathogens, 17(8), e1009735.

Ma, L., Linz, B., Caulfield, A. D., Dewan, K. K., Rivera, I., & Harvill, E. T. (2022). Natural history and ecology of interactions between *Bordetella* species and amoeba. Frontiers in Cellular and Infection Microbiology, 12, 798317.

Miguelena Chamorro, B., De Luca, K., Swaminathan, G., Longet, S., Mundt, E., & Paul, S. (2023). *Bordetella bronchiseptica* and *Bordetella pertussis*: Similarities and differences in infection, immuno-modulation, and vaccine considerations. Clinical Microbiology Reviews, 36(3), e0016422.

Mugni, S. L., Ambrosis, N., O'Toole, G. A., Sisti, F., & Fernández, J. (2025). Interplay of virulence factors and signaling molecules: Albumin and calcium-mediated biofilm regulation in *Bordetella bronchiseptica*. Journal of Bacteriology, 207(4), e0044524.

Munoz Navarrete, K., Edwards, K. M., Mills, K. H. G., Kamanová, J., Rodriguez, M. E., Gorringe, A., Preston, A., Kampmann, B., Gestal, M. C., Harvill, E. T., Dubey, P., Diavatopoulos, D. A., Mattoo, S., Scanlon, K. M., Locht, C., & Sebo, P. (2025). Highlights of the 14th International *Bordetella* Symposium. mSphere, 10(6), e0018925.

Nash, Z. M., Inatsuka, C. S., Cotter, P. A., & Johnson, R. M. (2024). *Bordetella* filamentous hemagglutinin and adenylate cyclase toxin interactions on the bacterial surface are consistent with FhaB-mediated delivery of ACT to phagocytic cells. mBio, 15(5), e0063224.

Nicholson, T. L., & Shore, S. M. (2024). Comparative analysis of antimicrobial resistance and genetic diversity of *Bordetella bronchiseptica* isolates ob-

tained from swine within the United States. Frontiers in Microbiology, 15, 1501373.

Nicholson, T. L., Waack, U., Fleming, D. S., Chen, Q., Miller, L. C., Merkel, T. J., & Stibitz, S. (2024). The contribution of BvgR, RisA, and RisS to global gene regulation, intracellular cyclic-di-GMP levels, motility, and biofilm formation in *Bordetella bronchiseptica*. Frontiers in Microbiology, 15, 1305097.

Ota, N., Nishida, T., Standley, D. M., Sherif, A. A., Iwano, S., Nugraha, D. K., Ueno, T., & Horiguchi, Y. (2025). Lonidamine, a novel modulator for the BvgAS system of *Bordetella* species. Microbiology and Immunology, 69(3), 133–147.

Paliy, A. P. (2018). Dyferentsiyna chutlyvist mikobakteriy do khlornykh dezinfektantiv [Differential sensitivity of mycobacterium to chlorine disinfectants]. Mikrobiolohichnyi Zhurnal, 80(2), 104–116 (in Ukrainian).

Paliy, A., Pavlichenko, O., Berezovskyi, A., Fotin, A., Kisil, D., & Panasenko, O. (2024). Bactericidal properties of inorganic acids against mycobacteria. Veterinarska Stanica, 55(4), 375–386.

Pérez-Ortega, J., van Harten, R. M., Haagsman, H. P., & Tommassen, J. (2023). Physiological consequences of inactivation of *lgmB* and *lpxL1*, two genes involved in lipid – A synthesis in *Bordetella bronchiseptica*. Research in Microbiology, 174(5), 104049.

Pérez-Ortega, J., van Harten, R. M., van Boxtel, R., Plisnier, M., Louckx, M., Ingels, D., Haagsman, H. P., & Tommassen, J. (2021). Reduction of endotoxicity in *Bordetella bronchiseptica* by lipid A engineering: Characterization of *lpxL1* and *pagP* mutants. Virulence, 12(1), 1452–1468.

Perslow, N. G., Meadows-Graves, S. J., & Luallen, R. J. (2025). The virulence regulator *bvgS* controls nutrient-induced filamentation in *Bordetella avium*. Journal of Bacteriology, 207(9), e0003025.

Petrovic Fabijan, A., Aleksic Sabo, V., Gavric, D., Doffkay, Z., Rakhely, G., & Knezevic, P. (2021). Are *Bordetella bronchiseptica* siphoviruses (genus *Vojvodinavirus*) appropriate for phage therapy – bacterial allies or foes? Viruses, 13(9), 1732.

Price, S. L., & Skaar, E. P. (2025). Forging biofilms: Metal-induced microbial responses in biofilm formation. Journal of Bacteriology, 207(11), e0024725.

Rahman, M. T., Sobur, M. A., Islam, M. S., Ievy, S., Hossain, M. J., El Zowalaty, M. E., Rahman, A. T., & Ashour, H. M. (2020). Zoonotic diseases: Etiology, impact, and control. Microorganisms, 8(9), 1405.

Rodriguez, L., & Berliner, E. A. (2023). Outbreak management of multidrug-resistant *Bordetella bronchiseptica* in 16 shelter-housed cats. Journal of Feline Medicine and Surgery, 25(2), 1098612X231153051.

Simmonds, R. C. (2017). Chapter 4. Bioethics and animal use in programs of research, teaching, and testing. In: Weichbrod, R. H., Thompson, G. A. H., & Norton, J. N. (Eds.). Management of animal care and use programs in research, education, and testing. 2nd edition. CRC Press, Taylor & Francis, Boca Raton. Pp. 1–28.

Skowron, K., Grudlewska-Buda, K., & Khamesipour, F. (2023). Zoonoses and emerging pathogens. BMC Microbiology, 23(1), 232.

Stainer, D. W., & Scholte, M. J. (1971). A simple chemically defined medium for the production of phase I *Bordetella pertussis*. Journal of General Microbiology, 63(2), 211–220.

Tabatabaei, M., & Rohani, H. R. (2022). Identification of *Bordetella bronchiseptica* in the throat and nose of dogs and cats by PCR. Molecular Biology Research Communications, 11(3), 127–131.

Tang, B., Hu, X., Song, Y., Liu, X., Zhao, G., & Yue, M. (2026). Acquired antimicrobial resistance genes in *Bordetella* species: A global genomic analysis. Journal of Antimicrobial Chemotherapy, 81(1), dkaf418.

Vondrova, D., Mugni, S. L., Blumenstein, J., Kasiztky, C., Sisti, F., Fernández, J., & Kamanova, J. (2026). Architecture and regulatory functions of c-di-GMP signaling in classical *Bordetella* species. FEMS Microbiology Reviews, 50, fuaf065.

Wang, J., Sun, S., Chen, Y., Chen, D., Sang, L., & Xie, X. (2020). Characterisation of *Bordetella bronchiseptica* isolated from rabbits in Fujian, China. Epidemiology and Infection, 148, e237.

Yasbolaghi Sharahi, J., Fayyazi, A., Kodori, M., Hosseinpour, M., Hashemi, A., Mahmoudi, M., & Rafiei, F. (2025). Dispersal mechanisms in biofilm control: Characteristics, induction, impacts, and therapeutic potential. Current Microbiology, 83, 3.

Yi, L., Fan, H., Yuan, S., Li, R., Wang, H., Quan, Y., Zhang, H., Wang, Y., & Wang, Y. (2024). Antimicrobial resistance and biofilm formation of *Bordetella bronchiseptica* in Central China, with evidence of a rare heteroresistance strain to gentamicin. Animals, 14(9), 1301.

Zavgorodnii, A. I., Pozmogova, S. A., Kalashnyk, M. V., Paliy, A. P., Plyuta, L. V., & Palii, A. P. (2021). Etiological factors in triggering non-specific allergic reactions to tuberculin in cattle. Regulatory Mechanisms in Biosystems, 12(2), 228–233.

Zhang, F., & Cheng, W. (2022). The mechanism of bacterial resistance and potential bacteriostatic strategies. Antibiotics, 11(9), 1215.

Zhang, Y., Yang, H., Guo, L., Zhao, M., Wang, F., Song, W., Hua, L., Wang, L., Liang, W., Tang, X., Peng, Z., & Wu, B. (2021). Isolation, antimicrobial resistance phenotypes, and virulence genes of *Bordetella bronchiseptica* from pigs in China, 2018–2020. Frontiers in Veterinary Science, 8, 672716.