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STUDY OF THE BIOCHEMICAL PROPERTIES OF *BORDETELLA BRONCHISEPTICA* CLINICAL ISOLATES

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Summary. During the first half of 2025, we isolated five isolates of *Bordetella bronchiseptica* from dogs with bordetellosis. These isolates exhibited low enzymatic activity, which is characteristic of *B. bronchiseptica*. Specifically, they did not produce indole, liquefy gelatin, ferment sugars, or produce the enzyme tyrosinase. However, they produced the enzyme urease and exhibited catalase and oxidase activity. Additionally, they grew on Simmons citrate agar and reduced nitrates to nitrites. Notably, they did not produce hydrogen sulfide on trisaccharide agar with the addition of iron. Our improved method for identifying and differentiating *B. bronchiseptica* isolates enabled us to significantly simplify and accelerate the isolation of pure *Bordetella* cultures by 2–3 days. Identification of clinical isolates of *B. bronchiseptica* took 3–4 days

Keywords: Bordetellosis, dogs, identifying, differentiating, pure culture

Introduction. There are many modern methods for detecting, characterizing, and identifying microorganisms. Some methods rely on phenotypic biochemical characteristics, while others use genotypic identification. The biochemical characteristics of bacteria offer numerous features that facilitate the classification and identification of microorganisms (Korniienko et al., 2008; Gerlach et al., 2001; Gueirard et al., 1995; Harvill et al., 2000).

The preferred and generally accepted approach for determining the genus and species of a bacterium is to analyze the nutritional and metabolic capabilities of a bacterial isolate. This approach allows for the quick and accurate identification of microorganisms without the need for costly equipment and materials (Korniienko et al., 2008; Henderson and Nataro, 2001; Ellis, 2015; Hadzevych, 2024).

Studying enzymes in bacteria is also of great practical importance. Determining microbial enzyme activity allows one to differentiate between bacterial species and identify the nature of a pathogen. Additionally, microbial enzyme activity determines the pathogenesis and clinical presentation of infectious diseases.

Studying biochemical activity in bacterial isolates is crucial for microbiological laboratories and clinical practice because the results guide treatment choices and help prevent and control disease spread among animals and humans. For example, cases of salmonellosis require rapid and precise identification of the pathogen to trace the infection source and enable healthcare providers to prescribe the right treatment quickly. Accurately identifying the genus and species of the pathogen during an outbreak is also vital so that an appropriate response can be implemented promptly to control the epizootic (epidemic) (Gueirard et al., 1995; Harvill et al., 2000; Kameyama et al., 2022; Milanko, Kholodylo and Dushkyn, 1995).

There are currently many reports about the difficulty of diagnosing bordetellosis in animals. The etiological agent of the disease is the bacterium *B. bronchiseptica*. This Gram-negative bacterium colonizes the respiratory tract of mammals. Bordetellosis is a highly contagious infectious disease in animals that often progresses chronically and asymptomatically, which greatly complicates diagnosis, prevention, and treatment (Korniienko et al., 2008; Harvill et al., 2000; Henderson and Nataro, 2001; Hadzevych, 2024). Laboratory diagnosis of bordetellosis is also complicated by the fact that the disease is often uncontrollable and spreads rapidly through asymptomatic carrier animals. Therefore, it is urgent to search for and develop test systems suitable for the early detection and identification of the pathogen and its phase states. The main conditions for successfully eliminating outbreaks of bordetellosis in animals are the early isolation of the pathogen and determination of its properties and antibiotic sensitivity; timely initial treatment; and the comprehensive, systematic implementation of remedial measures, including general and specific veterinary and sanitary measures (Korniienko et al., 2008; Milanko, Kholodylo and Dushkyn, 1995; Mylanko and Dushkin, 1996; Milanko, Gerilovich and Dushkin, 1995).

According to the literature, the bacteriological method remains the main tool for diagnosing bordetellosis in animals. This method includes isolating a pure culture of the bacterium and determining its biochemical activity. At the same time, many scientists claim that the study takes 6–9 days (MHU, 2005). The duration of the study is associated with the pathogen's slow growth, the untimely and incomplete examination of animals with prolonged coughs, contamination of the test material by other microorganisms, the use of antibacterial drugs before the study's start, violations of the rules for collecting and transporting material, and unsuccessful or imperfect formulations of diagnostically selective media. In particular, there is an insufficient selection of selective and nutrient components (Harvill et al., 2000; MHU, 2007; Gadzevich, 2024; Moore, Rendall and Millar, 2021a, 2021b; Inatsuka, Julio and Cotter, 2005; Jacob-Dubuisson et al., 2000; Chambers

et al., 2019; Schulz et al., 2014; Taha-Abdelaziz et al., 2016; Miguelena Chamorro et al.; 2023, Kadlec and Schwarz, 2018; Clements, McGrath and McAllister, 2018).

Thus, diagnosing bordetellosis and studying the properties of *B. bronchiseptica* require careful research and study. Resolving these issues will enable the integration of modern tools into veterinary practices, enhance diagnostic methodologies, and provide valuable insights into effective outbreak prevention strategies. Our study aimed to investigate the biochemical properties of clinical isolates of *B. bronchiseptica* that we collected from sick dogs in 2025. We **aimed** to determine the terms for studying the biochemical activity of *Bordetella*, identify weaknesses in studying bacterial enzymes, and find solutions to eliminate them. Based on the results, we planned further work to improve the diagnosis of bordetellosis in animals.

Materials and methods. Clinical isolates of *B. bronchiseptica* were isolated from dogs with or suspected of having bordetellosis in accordance with the methodological guidelines for the microbiological diagnosis of whooping cough and paracough. These guidelines were approved by Order No. 169 of the Ministry of Health of Ukraine on April 15, 2005, in our modification (Hadzevych, 2024; MHU, 2005; Woods et al., 2019; Bemis, Shek and Clifford, 2003). The process of isolating *B. bronchiseptica* clinical isolates from nasopharyngeal secretions consisted of two stages. The first stage included selecting and culturing the biological material (nasopharyngeal secretions) on a nutrient medium. Based on positive results from previous studies, we used a CCA (casein-charcoal agar) medium supplemented with 5% sheep blood and the selective component cephalexin (4 mg/100 ml), adding the antibiotic to the medium. Samples of nasopharyngeal secretions for *Bordetella* isolation were collected using a sterile, disposable probe swab from the mucous membranes of the tonsillar and peripharyngeal areas. In parallel, the biological material was inoculated onto other nutrient media (MPA, MPB, blood agar, Endo, MacConkey, Sabouraud, etc.) to isolate and identify other associated microorganisms (Hadzevych, 2024; MHU, 2005). Inoculations were performed to obtain separate colonies (MHU, 2005). The inoculations were placed in a thermostat at a temperature of 35.5 ± 0.5°C for 48 h. The growth of microorganisms on the CCA medium was assessed daily. The morphology of the colonies was examined using an MBS-9 binocular magnifying microscope with a long focal length (Korniienko et al., 2008; Harvill et al., 2000; Henderson and Nataro, 2001; MHU, 2005; Bemis, 1992).

Samples from sick animals were collected early in the disease process, no later than three weeks after the onset of symptoms, because the viability of the pathogen drops significantly later on. It was crucial to ensure that the animals were not treated with antibiotics before the biological material was collected. To obtain objective results for isolating clinical isolates of *B. bronchiseptica*, eight samples of nasopharyngeal secretions were collected from each dog's tonsillar and peripharyngeal mucous membranes using a sterile, disposable probe-swab for each sample. Samples of nasopharyngeal secretions from the same sick dogs were collected twice in a row during repeated examinations at 24-hour intervals. The media and individual nutrient components were produced by HiMedia Laboratories Pvt. Ltd. (India) and Farmaktiv LLC (Ukraine) (Korniienko et al., 2008; Gerlach et al., 2001; MHU, 2005, 2007).

The second stage was to obtain a pure culture of the *B. bronchiseptica* clinical isolate. To this end, cultures on nutrient media were examined. Based on the appearance of the colonies (size, color, and shape), certain rapid tests, and light microscopy after staining with aniline dyes, the type of microorganisms and their significance in a particular case were determined (Ellis, 2015; Hadzevych, 2024; MHU, 2005, 2007). Selected colonies were screened on nutrient media to accumulate a pure culture of microorganisms. If a sufficient number of colonies of only one species grew, identification and determination of antibiotic sensitivity were carried out without the accumulation stage.

The identification of the isolated microorganisms, including the determination of their biochemical, serological, and antigenic properties, was performed using a fixed set of substrates, diagnostic selective media, and sera. These substrates included carbohydrates, amino acids, polyhydric alcohols, and other complex compounds. The results were used to determine the genus and species of the microorganism (Ellis, 2015; Hadzevych, 2024; Kameyama et al., 2022; MHU, 2005). To study the biochemical properties of the microorganisms, we used selective and diagnostic selective media, as well as individual components, which were manufactured by HiMedia Laboratories Pvt. Ltd. (India) and Farmaktiv LLC (Ukraine). These included Giss medium with sugars and polyhydric alcohols, acetate agar, nitrate agar to determine nitrate reduction to nitrite, a medium to determine gelatinase, Piz medium, and Simmons citrate agar. The reference strains used were *B. bronchiseptica* B-C2, *B. bronchiseptica* No. K16, and *B. bronchiseptica* No. K17. These strains were stored in the Microorganism Strain Museum of the National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine' (Kharkiv, Ukraine).

To determine the catalytic activity of clinical isolates of *Bordetella bronchiseptica*, a microbial sample was placed on a microscope slide with a loop, and a drop of 3% hydrogen peroxide solution was immediately added to it. The release of oxygen bubbles indicated the presence of catalase in the microbes.

To detect urease activity, the Zaks method was used, which is based on the ability of microorganisms with the urease enzyme to break down urea into ammonia. This causes a change in the pH of the medium, which is detected by a change in the color of the indicator. After incubation, the results were recorded. If there was no change in color, the tubes were left at room temperature,

and the results were recorded the next day. If the microorganism has the urease enzyme, the urea breaks down into ammonia, which leads to alkalization of the medium and a color change from yellow to crimson (Hadzevych, 2024; Mylanko and Dushkin, 1996).

The ability to use citrate as the sole carbon source was determined using Simmons medium. The test culture was inoculated onto sloping Simmons medium and incubated at 36 ± 1°C for one day. *B. bronchiseptica*, a citrate-utilizing bacterium, grows well, alkalizes the medium, and causes it to turn blue. To determine pigment formation, the culture was inoculated onto simple nutrient agar containing 0.1% tyrosine and incubated at 36 ± 1°C for 24 h. When tyrosine is broken down, the medium turns yellow-brown.

Results and discussion: Based on bacteriological studies of nasopharyngeal secretion samples collected in the first half of 2025, five clinical isolates of *B. bronchiseptica* were isolated from five dogs (n = 5) with bordetellosis. The disease progressed acutely and classically in all dogs. The animals exhibited decreased or lost appetite, lethargy, and mucopurulent (Fig. 1) or purulent (Fig. 2) nasal discharge. They also displayed conjunctivitis (Fig. 3), enlarged submandibular lymph nodes, and an increased body temperature of 40–41°C. The dogs experienced catarrhal and subsequently catarrhal-purulent inflammation of the upper respiratory tract, as well as shortness of breath, frequent sneezing, and a painful, suffocating cough. The cough intensified when the dogs stood up or moved, changing from wet to dry with severe attacks of suffocation and vomiting. As pathogenic microflora accumulated, pulmonary emphysema, pneumonia, exhaustion, and death were observed (Fig. 4).

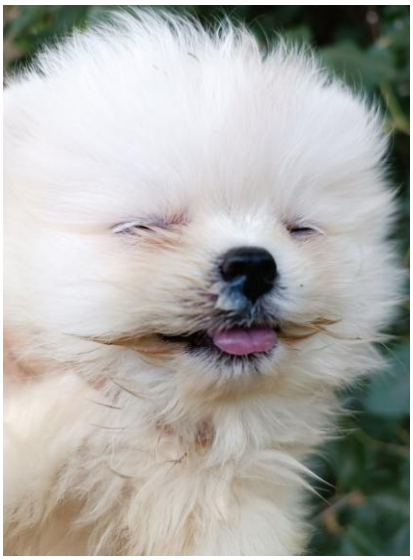

Figure 1. Conjunctivitis and mucopurulent exudate from the nasal passages in a puppy with bordetellosis.

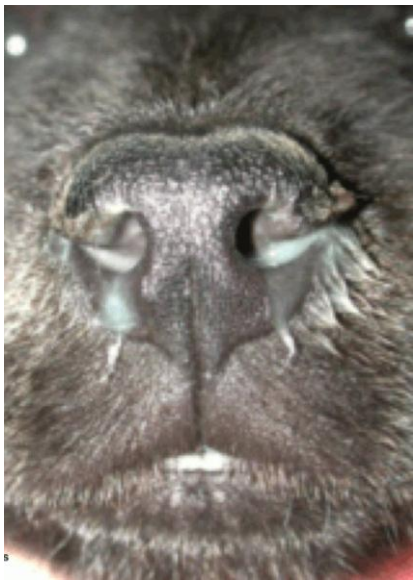

Figure 2. Purulent exudate from the nasal passages in a puppy with bordetellosis.

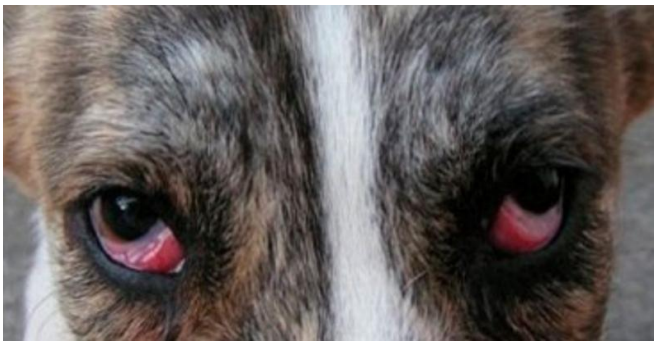

Figure 3. Conjunctivitis in a puppy with bordetellosis.

Figure 4. Signs of exhaustion in a dead animal with pale and dull visible mucous membranes and dry, inelastic skin.

Similar clinical pictures of acute *Bordetella* infection in dogs have been observed by Chambers et al. (2019) and Miguelena Chamorro et al. (2023) among other researchers. However, some reports indicate that bordetellosis in dogs is more often manifested by sneezing and nasal discharge and other scientists also indicate that, in most cases, bordetellosis is mild and resolves within two to three weeks (Miguelena Chamorro et al., 2023; Kadlec and Schwarz, 2018). However, complications can develop, which can lead to pneumonia and death. In our opinion, risk factors for developing complications include an impaired immune system and chronic respiratory diseases, such as bronchitis, ciliary dyskinesia, and tracheal collapse. This view is consistent with studies by Clements, McGrath and McAllister (2018), Woods et al. (2019), and other scientists. These studies also indicate that the nature and intensity of the infection depend solely on the pathogen's aggressive properties, the animal's immune status, and the presence of accompanying microflora.

Based on our study of the phenotypic characteristics of the *B. bronchiseptica* clinical isolates selected, we established that all five isolates formed colonies on a dense CCA medium within 24–48 h of cultivation at 35.5 ± 0.5°C during primary isolation. Subsequent recultivations on CCA medium reduced the time required for colony formation to 18–24 h. Similar growth dynamics and colony formation times for *B. bronchiseptica* were observed by Schulz et al. (2014) and other scientists. They noted that, due to *Bordetella*'s adaptation to artificial cultivation conditions, the growth rate of microorganisms and colony formation accelerated. The *Bordetella* isolates grew on dense media in the form of polymorphic colonies that varied greatly in color, shape, size, and consistency.

We more often recorded the formation of colonies of three types of subcultures of isolated clinical isolates of *B. bronchiseptica*:

— convex, moist, smooth, and shiny with even edges. They were gray in color, sometimes with a blue, pearl, metallic, yellowish, or whitish tint. They were 0.5–2 mm in diameter. These colonies had a soft, oily consistency and could be easily removed with a loop;

— flat colonies or those with a raised (or conversely, depressed) center were oily and gray with a blue, yellowish, or whitish tint;

— in the form of a diffuse, oily, gray mass (diffuse growth) with different shades and often without clear boundaries.

Morphologically, three phases of colony development were distinguished. Phase I corresponded to the S-shape, colonies were round, convex, moist, microorganisms were monomorphic and small. Recently isolated and young cultures were predominantly in Phase I of colony development, as determined by morphological characteristics (Fig. 5). Phases II (SR-form) and III (R-form) were transitional, with changes in bacterial morphology. In the second phase, rods of various sizes were predominantly found, while in the third phase, coccoid formations of various sizes were found. Colonies in phases II and III were slightly larger in size, with a diameter of 1–2 mm. The colonies were flat, slimy, with a tendency to merge, and had predominantly uneven contours and different segments (Figs. 6 and 7).

As shown in Fig. 6, growth of *B. bronchiseptica* culture on blood agar was observed after 24 h of incubation at a temperature of 35.5 ± 0.5°C in the form of a diffuse oily gray mass (confluent growth) with different shades and uneven edges. Fig. 7 shows an increase in the size of the growth zone of *B. bronchiseptica* culture on blood agar after 48 h of incubation due to diffuse growth of *Bordetella* on the surface of the medium. Fig. 8 shows the growth of *B. bronchiseptica* culture in the form of a continuous film on the surface of semi-liquid (0.4%) nutrient agar after 48 h of incubation at a temperature of 35.5 ± 0.5°C.

Based on our analysis of the literature, we found that Miguelena Chamorro et al. (2023), Kadlec and Schwarz (2018) and other researchers distinguish three phases of *B. bronchiseptica* colony development based on morphology: I — the virulent phase, which corresponds to the S-form; II — the weakly virulent phase (SR-form); III — the avirulent phase (R-form). Foreign researchers have determined that phase I, the virulent phase, is cultivated on Bordet-Gengou agar during primary isolation. The resulting colonies are round, convex, and moist. Prolonged cultivation results in antigenic modulation and transformation of the microorganism into phases II and III. Phase II is weakly virulent, and phase III is avirulent. The colonies are flat and mucous in morphology with a tendency to merge. They have predominantly uneven contours and different shades. This phenomenon is reversible, and scientists have shown that a return to phase I is possible even after 15 passages. This conclusion is fully consistent with the results of our research. Our research shows that cultures isolated after 18–24 h of incubation at a temperature of 35.5 ± 0.5°C more frequently formed small, smooth, shiny, translucent colonies with even edges and a convex surface. These colonies ranged from 0.5 to 2.0 mm in diameter and turned grayish-white after 48–72 h. The diversity of *B. bronchiseptica* colonies makes it harder to distinguish them from foreign microflora, which extends the time needed to isolate a pure *Bordetella* culture. To shorten this process when suspicious colonies are present on the culture medium, we transferred a pure *Bordetella* culture onto Petri dishes with CCA medium. The medium's surface was divided into several sectors, and each colony was transferred to a different sector. The cultures were then incubated at a temperature of 35.5 ± 0.5°C for 24–48 h. Since extraneous microflora can grow when sample material is cultured on nutrient media, we transferred the maximum number of colonies, on average, at least five.

Based on the results of our analysis of literature data and our own research on the phenotypic characteristics of *B. bronchiseptica* clinical isolates, we can conclude that the typical bacterial cell morphology, color characteristics,

and ability to grow on specific nutrient media — taking into account biochemical substrate degradation — as well as phenotypic colony heterogeneity, are unique morphological, tinctorial, biochemical, and cultural properties of *Bordetella*. These properties can be used to assert the presence of the pathogen with a high degree of probability and as an additional tool for its bacteriological detection.

To speed up the isolation of pure *B. bronchiseptica* cultures, we improved the method of identifying and isolating pure *Bordetella* cultures by setting up separate rapid biochemical tests for preliminary identification. The method consisted of the following steps:

1. Obtaining a sufficient quantity of typical colonies on CCA medium by successively plating individual colonies on sectors.

2. Preparing smears and determining the morphological and tinctorial properties of the culture by Gram staining. *B. bronchiseptica* bacteria are Gram-negative, monomorphic, small, ovoid rods (coccobacilli). They do not form spores and are motile and evenly distributed in the smear or arranged in single groups.

3. Performance of the Zaks test to determine the presence of the urease enzyme. If the microorganism produces urease, the urea will break down into ammonia. This leads to the alkalization of the medium, changing its color from yellow to crimson. *B. bronchiseptica* bacteria produce urease (Fig. 9).

4. Determining the catalytic activity of suspicious colonies. *B. bronchiseptica* is catalase-positive (Fig. 10).

5. Determination of the ability to utilize citrate on Simmons medium. *B. bronchiseptica* is a citrate-utilizing microorganism. They grow well, acidify the medium, and cause it to turn blue (Fig. 11).

Based on the study of the pure culture's morphology, the results of the urease, citrate utilization, tyrosinase, and catalase activity tests (Table 1), we made a preliminary conclusion that the microorganism belongs to the *B. bronchiseptica* species. If no colonies suspicious for *Bordetella* bacteria were detected on the culture medium within five days, the study was terminated, and a final negative result was issued.

As shown in Table 1, all five *B. bronchiseptica* clinical isolates that we obtained from dogs with bordetellosis in 2025 had biochemical properties consistent with those of *B. bronchiseptica*, as determined by rapid biochemical testing. To confirm the isolates belonged to the *B. bronchiseptica* species, we performed PCR diagnostics and studied the enzymatic activity of the clinical isolates in more detail. The *B. bronchiseptica* isolates we obtained from dogs exhibited low enzymatic activity, which is characteristic of this species (Table 2): they did not produce indole, liquefy gelatin, or ferment sugars; they exhibited oxidase activity; they reduced nitrates to nitrites; they grew well on MPA, MacConkey agar, and SS agar; and they did not produce hydrogen sulfide on trisaccharide agar with iron. The low biochemical activity of clinical isolates of *B. bronchiseptica* has also been reported by Clements, McGrath and McAllister (2018), Bemis (1992) and other researchers. They note that *B. bronchiseptica* differs from other *Bordetella* species in its ability to reduce nitrates to nitrites. *Bordetella* utilizes certain organic compounds (succinate, citrate, pyruvate, acetate, fumarate, lactate, oxaloacetate, α-ketoglutarate, or amino acids — proline, glutamate, glutamine, and tyrosine) as its sole source of energy (electrons and protons). They suggest that the tricarboxylic acid cycle (Krebs cycle) in the metabolism of *B. bronchiseptica* is nonfunctional. There is speculation that it may be inactive due to the acquisition of additional biochemical pathways for utilizing alternative nutrient sources, rather than due to a lack of enzymes. When studying the biological properties of isolates, scientists found that *Bordetella* bacteria can be cultivated on conventional and selective media. We obtained similar results when studying the cultural properties of isolates. However, there are many reports that *B. bronchiseptica* bacteria are demanding in terms of cultivation conditions and require special nutrient components, especially during primary isolation.

Our improved method for indicating and differentiating clinical isolates of *B. bronchiseptica* has allowed us to significantly simplify and speed up the process of isolating a pure *Bordetella* culture by 2–3 days. From the time of collecting nasopharyngeal swabs from animals and inoculating the sample on CCA to obtaining a pure culture, it now takes only 24–48 h. We allocate 24 h for morphological and tinctorial assessment of *B. bronchiseptica*, rapid testing, and preliminary species identification, and an additional 24 h for the final confirmation of isolates belonging to *B. bronchiseptica* based on enzymatic activity assessment. Overall, the identification process for *Bordetella* now takes 3–4 days.

The literature and our research results indicate that there is a need to improve bacteriological diagnostic methods for bordetellosis, especially for rapid isolation, typing, and differentiation of the pathogen. Developing high-quality selective media and enhancing biochemical tests play a crucial role in this effort. When designing differential and diagnostic media, it is essential to consider which microorganisms — either primary symbionts or associates of the pathogen — should be separated. Specifically, it is necessary to identify which morphologically and phenotypically similar bacterial genera need to be differentiated from clinical isolates of *B. bronchiseptica* first. We believe that establishing and implementing clear criteria for differentiation, including biochemical markers, would be beneficial. Chambers et al. (2019), Miguelena Chamorro et al. (2023), and other researchers report that the primary associated microorganisms in the upper respiratory tract of animals, which need to be differentiated from *B. bronchiseptica*, include *Pseudomonas* spp., *Corynebacterium* spp., *Proteus* spp., *Haemophilus influenzae*, *H. haemolyticus*, *H. parainfluenzae, H. parahaemolyticus, M. catarrhalis, N. meningitidis, N. flavescens, Yersinia pseudotuberculosis, Morganella coli*, and closely related species such as *Bordetella parapertussis* and *Bordetella pertussis*.

Figure 5. Convex, moist, and smooth 0.5–2 mm colonies of the recently isolated *B. bronchiseptica* clinical culture on blood agar after 24 h of incubation.

Figure 6. Growth of *B. bronchiseptica* on blood agar after 24 h of incubation.

Figure 7. Growth of *B. bronchiseptica* on blood agar after 48 h of incubation.

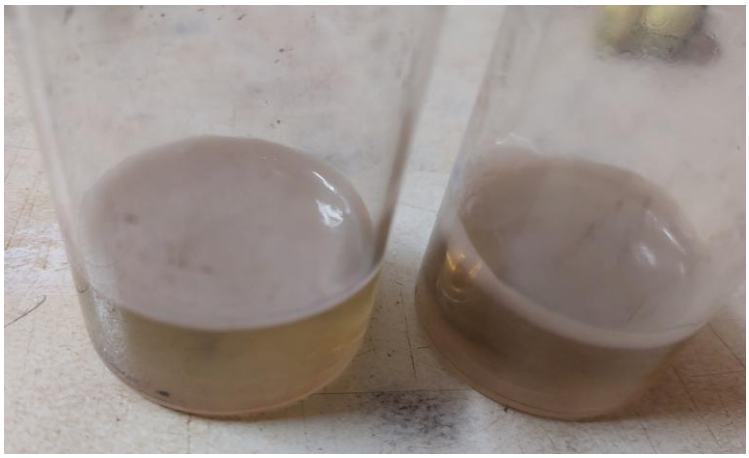

Figure 8. Growth of *B. bronchiseptica* on semi-liquid (0.4%) nutrient agar after 48 h of incubation in the form of a continuous film.

Figure 9. Zaks' tests for determining the urease enzyme. *B. bronchiseptica* has the urease enzyme; we observed the alkalization of the medium and a change in its color from yellow to crimson due to the breakdown of urea into ammonia.

Figure 10. Determination of catalytic activity in clinical isolates of *B. bronchiseptica*. The release of oxygen bubbles indicated the presence of catalase in the microbes.

Figure 11. Determination of the ability to utilize citrate as the sole source of carbon. Citrate-utilizing bacteria *B. bronchiseptica* grew, acidified the Simmons medium, and caused it to turn blue.

Table 1 — Results of biochemical rapid tests for preliminary differential identification of clinical isolates of *B. bronchiseptica*

Designation of microorganisms	Tyrosidase activity	Presence of the urease enzyme	Catalytic activity	Growth on Simmons citrate agar
B. bronchiseptica strain B C2	–	+	+	+
B. bronchiseptica strain K16	–	+	+	+
Isolate 25/1	–	+	+	+
Isolate 25/2	–	+	+	+
Isolate 25/3	–	+	+	+
Isolate 25/4	–	+	+	+
Isolate 25/5	–	+	+	+

Table 2 — Results of biochemical tests for final differential identification of clinical isolates of *B. bronchiseptica*

Designation of microorganisms	Ability to ferment sugars	Ability to liquefy gelatin	Ability to produce indole	Mobility	Oxidase activity	Ability to reduce nitrites to nitrites	Ability to produce hydrogen sulfide on trisaccharide agar with iron
B. bronchiseptica strain B C2	–	–	–	+	+	+	–
B. bronchiseptica strain K16	–	–	–	+	+	+	–
Isolate 25/1	–	–	–	+	+	+	–
Isolate 25/2	–	–	–	+	+	+	–
Isolate 25/3	–	–	–	+	+	+	–
Isolate 25/4	–	–	–	+	+	+	–
Isolate 25/5	–	–	–	+	+	+	–

Conclusions. 1. During the first half of 2025, we isolated five clinical isolates of *B. bronchiseptica* from dogs with bordetellosis. These isolates exhibited low enzymatic activity, which is characteristic of *B. bronchiseptica*. Specifically, they did not produce indole, liquefy gelatin, ferment sugars, or produce the enzyme tyrosinase. However, they did produce the enzyme urease and exhibited catalase and oxidase activity. Additionally, they grew on Simmons citrate agar and reduced nitrates to nitrites. Notably, they did not produce hydrogen sulfide on trisaccharide agar with iron.

2. The characteristics of typical bacterial cell morphology, coloration, and the ability to grow on specific nutrient media, considering the biochemical degradation of various substrates, as well as phenotypic colony heterogeneity, constitute a set of unique morphological, tinctorial, biochemical, and cultural properties of *Bordetella*. These properties can be used to preliminarily identify the microorganism and serve as an additional tool for the bacteriological detection of the pathogen.

3. Our improved method for identifying and differentiating clinical isolates of *B. bronchiseptica* significantly reduces the time required to isolate a pure culture of *Bordetella* by 2–3 days. Identifying clinical isolates of *B. bronchiseptica* took 3–4 days.

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