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Prevalence and Etiological Significance of *Bordetella bronchiseptica* from Swine with Respiratory Disease in Northeast India

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Abstract

Atrophic rhinitis is a polymicrobial disease with *Bordetella bronchiseptica* being the primary bacterial agents involved and control measures are essential for the prevention and management of atrophic rhinitis in pig populations. Therefore, a systematic study was carried out to characterize the phenotypic, toxigenic and biochemical properties of of *B. bronchiseptica*. All 47 *B. bronchiseptica* isolates recovered from local and cross-breed pigs having symptoms of atrophic rhinitis, chronic respiratory disease and severe pneumonia were screened for Congo-red binding, phase modulation and Hemolytic activity. Isolates were examined for *dnt*, *bvg*AS, *achly*A, *fha*B, *fha*C, *ftm*2, fimS and *tcf*A, of which all isolates were positive for virulent genes based on amplicon sizes of 600bp, 599bp,745bp, 320bp, 1767bp, 301bp, 454bp and 598 bp. In order to examine the pathogenicity, rabbits were inoculated intradermally with *B. bronchiseptica* strain. Further isolates with strong dermonecrotic lesion in rabbit were selected for cell culture assay. Following inoculation, severe inflammation and lesions were observed in the lungs and trachea. Likewise, cytopathic changes were also observed in Vero cells with enlarged cells and cytolysis. This study confirmed recovered *B. bronchiseptica* as the cause of atrophic rhinitis in pigs from Meghalaya. The findings from this study would be employed in the development of new protocols to mitigate the occurrence of outbreak caused by *B. bronchiseptica.*

Keywords *Bordetella bronchiseptica*, Dermonecrotic toxin (DNT), Atrophic rhinitis, Pig, PCR

Introduction

Pig farming is a vital component of the agricultural economy in Northeast India, contributing significantly to rural livelihoods and socio-economic development (Talukdar et al., 2019). According to the Livestock Census, the Northeast region accounts for 38.42% of the total pig population in India. However, the sustainability of the sector is challenged by several endemic and emerging diseases, some of which possess zoonotic potential. Among the major infectious diseases affecting swine, Atrophic Rhinitis (AR) remains a significant concern for pig production systems. AR is a polymicrobial respiratory disease primarily associated with *Pasteurella multocida* and *Bordetella bronchiseptica* and is characterized by inflammation and progressive degeneration of the nasal mucosa and turbinate bones, resulting in distortion and shortening of the snout (Chanter et al., 1989). Although *B. bronchiseptica* is enzootic in many pig populations worldwide, reports from India are limited, with only two documented outbreaks from Meghalaya (Shome et al., 2006; Kumar et al., 2014).

Infection with Bordetella bronchiseptica induces a mild and reversible form of atrophic rhinitis (AR) in swine. In contrast, infection with toxigenic strains of Pasteurella multocida in combination with *B. bronchiseptica* results in a severe and progressive form of the disease (Magyar et al., 1988). *B. bronchiseptica* facilitates the colonization of the nasal mucosa by *P. multocida*, promoting its establishment and subsequent development of atrophic rhinitis. Furthermore, the immunomodulatory effects of *B. bronchiseptica* enhance the colonization and persistence of itself and other pathogens in the nasal passages, thereby exacerbating AR progression. Disease severity within a herd is largely determined by the presence of key virulence factors in *B. bronchiseptica* strains, including hemagglutinin, adenylate cyclase-hemolysin toxin, and dermonecrotic toxin (DNT). These factors, primarily responsible for nasal turbinate degeneration, are positively regulated by the bvg locus. Therefore, targeted control measures against B. bronchiseptica are essential for the effective prevention and management of atrophic rhinitis in pig populations. Since pig rearing is an important sector in North-Eastern states of India, this study would provide insights for the development of effective diagnostics test kit for effective health monitoring of program and disease management.

Materials and methods

Collection, Isolation and Identification of *B. bronchiseptica*

A total of 50 clinical samples were collected aseptically from pigs exhibiting mild rhinitis, chronic respiratory disease, and severe pneumonia across various geographical locations in Meghalaya. Samples were obtained from the lungs, liver, heart, lymph nodes, and deep nasal swabs based on observed respiratory infection symptoms. The specimens were inoculated onto Bordet Gengou Agar (Hi-Media, India) supplemented with *Bordetell*a selective supplement (Cephalexin, 20 mg/ml) and blood agar containing 10% sheep blood. Isolates were identified as *B. bronchiseptica* based on bacterial motility, Leifson's staining, cultural characteristics, and biochemical tests including catalase, cytochrome oxidase, indole, hydrogen sulphide production, nitrate reduction, citrate utilization, and triple sugar iron tests.

Ultrastructure study of *B. bronchiseptica* by Scanning Electron microscopy (SEM)

B. bronchiseptica was cultured on Bordet–Gengou agar at 37°C for 48 h. Agar blocks (2–3 mm) containing colonies were excised and immersed in Karnovsky's fixative for 4 h. Specimens were washed three times in 0.1 M sodium cacodylate buffer for 15 min at 4°C. Post-fixation used 1% osmium tetroxide in 0.1 M sodium cacodylate buffer at 4°C, followed by three washes in buffer. Dehydration through graded acetone (30–100%) at 4°C. Samples were immersed in tetramethylsilane for two changes of 5–10 min and air-dried for 15 min. Dried samples were mounted, sputter-coated with gold for 10 min, and examined scanning electron microscopy (JEOL/JSM-6360, Japan).

Detection of hemolytic activity of *B. bronchiseptica*

The hemolytic activity of *B. bronchiseptica* isolates was assessed using sheep, goat, cattle, and chicken erythrocytes on nutrient agar supplemented with 5% blood. Hemolysis was determined by the presence and extent of clear zones surrounding bacterial colonies after incubation at 37°C for 48 h.

Congo red binding test

In this study, all *B. bronchiseptica* isolates were evaluated for Congo red dye binding using the standard method by (Xue et al., 2025). The test was performed on trypticase soya agar containing 0.03% Congo red dye. Cultures were spot-inoculated, and intense brick-red colonies observed after 24–48 h incubation at 37°C were considered positive.

Phase Modulation study of *B. bronchiseptica*

Phase modulation studies were conducted on four randomly selected B. bronchiseptica isolates using physical and chemical modulators. Differentiation between Bvg+ and Bvg− phases was

assessed based on cultural characteristics, including colony morphology, colony type, and hemolytic activity, to identify phase-specific phenotypic variations.

Detection of Virulence gene by PCR

Isolates harboring the virulence genes *dnt, bvgAS, achlyA, fhaB, fhaC, ftm2, fimS,* and *tcfA* were identified by PCR using primers listed in Table 1. Amplification conditions included initial denaturation at 94°C for 7 min, followed by cycles of denaturation (94°C, 30 s), annealing (54°C, 30 s), and extension (72°C, 30 s), with a final extension at 72°C for 7 min. Products were analyzed by electrophoresis and visualized using a Gel Documentation System (Thermofisher Scientific, USA).

In-vitro adhesion and colonization of *B. bronchiseptica*

The isolates of B. bronchiseptica (Bb1 and Bb11) were cultured in Brain Heart Infusion (BHI) broth at 37°C for 20 h. Following centrifugation at 4000 × g for 10 min, the pellets were washed with 1× PBS, resuspended to 1×10^8 cells/mL, and stored at 4°C.

Three-month-old New Zealand White rabbits were procured from ICAR for the NEH Region, Meghalaya, and confirmed negative for *B. bronchiseptica* by culturing nasal swabs on Bordet–Gengou agar (BGA) with or without cephalexin supplementation, exploiting the intrinsic cephalexin resistance of *Bordetella* spp. Rabbits were placed in dorsal recumbency and inoculated intranasally with 100 µl of bacterial suspension per nostril. To prevent cross-contamination, animals were housed with an empty cage separating each experimental unit. Rabbits were monitored daily for clinical signs, discomfort, and respiratory distress. On day 14 post-inoculation, animals were euthanized intravenously using pentobarbital (1 ml of 6 g/ml stock solution), and the trachea, larynx, and lung lobes were aseptically collected, fixed in Karnovsky's solution, and processed for scanning electron microscopy.

Purification of dermonecrotic toxin (DNT) by fast protein liquid chromatography (FPLC)

B. bronchiseptica isolate Bb9(a) was cultured in two test tubes containing 10 mL of brain heart infusion (BHI) broth (Oxoid, UK) and incubated at 37°C for 18 h in a rotary shaker at 200 rpm. The inoculum was subsequently transferred into two separate conical flasks containing 490 mL BHI broth and incubated under identical conditions. Following incubation, the cultures were centrifuged at 5500 × g for 10 min at 4°C. The harvested cells were washed twice with autoclaved Milli-Q water and resuspended to a concentration of 100 mg wet weight/mL. The suspension was

sonicated at 100% amplitude for 10 min using 60-s bursts, centrifuged at 13000 × g for 30 min, membrane-filtered (0.22 µm), and stored at 4°C for FPLC analysis.

Immunogenicity of purified DNT

The purified DNT was characterized by Western blotting using rabbit hyperimmune serum raised against DNT, following the method by Towbin et al. (1979). Purified DNT was separated on a precast SDS-PAGE gel (Page Duramide® 4–20% Tris-glycine gel, Cambrex, USA) and electrotransferred onto a nitrocellulose membrane (NCM). The membrane was dried at 37°C for 15 min, blocked with 1% bovine serum albumin fraction-V in PBS for 1 h, and washed thrice with PBST. Subsequently, the membrane was incubated with anti-DNT rabbit serum (1:10^3 dilution) for 1 h at 37°C. After washing, it was incubated with HRP-conjugated anti-rabbit IgG (1:5×10^4 dilution) for 1 h. The blot was developed using FAST-DAB substrate containing hydrogen peroxide and rinsed with distilled water to terminate the reaction after complete color developmen

Dermonecrotic (DNT) assay

To evaluate the dermonecrotic activity of B. bronchiseptica, dermonecrotic toxin (DNT) was purified from sonic extracts of the isolates following a previously described method (Jang et al., 2024). Toxin homogeneity was confirmed by SDS-PAGE. New Zealand White rabbits (6–8 months old) obtained from ICAR for NEH Region, Meghalaya, were intradermally inoculated with 100 µl DNT, while PBS served as the control. Eleven inoculations per rabbit were administered, and lesion development was monitored on the ventral surface 48–72 h post-inoculation.

Cytotoxicity assay of *B. bronchiseptica* on Vero cell line

Vero cells, obtained from the Institute of Animal Health and Veterinary Biologicals, Hebbal, Bangalore, were used for cytotoxicity assessment. Cells were seeded in 96-well microtitre plates at a density of 4×10^4 cells/ml and incubated at 37°C in 5% CO_2 for 72–96 h. Following incubation, cells were maintained in 2% fetal bovine serum (FBS; pH 7.4) supplemented with antibiotics. Sterilized crude cell extract (20 µl) was added to the first row, followed by serial two-fold dilutions. Control wells contained confluent Vero cell monolayers with 2% FBS. After 72 h incubation, cytotoxic effects were evaluated microscopically based on cell rounding.

Results

Identification of *Bordetella bronchiseptica* isolates

The B. bronchiseptica isolates were identified as Gram-negative, motile, small rod-shaped bacteria capable of growth on MacConkey agar and positive for citrate, oxidase, catalase, nitrate, and urease

tests, with no reaction in the butt of a triple sugar iron agar slant. A total of 47 isolates were recovered, predominantly from nasal swabs, followed by lungs, liver, trachea, and one lymph node sample **(Fig. 1).** Co-isolation of B. bronchiseptica and P. multocida was observed in samples H_2R_1, NS-10a (b), NS-10b (S), 5T/a, and NS3/a, whereas remaining samples yielded only B. bronchiseptica. Scanning electron microscopy revealed scattered, clustered, single, and paired bacterial arrangements within colonies **(Fig. 2).**

Detection of hemolytic activity

B. bronchiseptica isolates exhibited strong hemolysis on sheep blood agar, weak hemolysis on goat, cattle, and rabbit blood agar, and no hemolysis on chicken erythrocytes. Hemolytic activity varied among strains, with some producing distinct, broad β-hemolytic zones after 24 h at 37°C, while others showed initially hazy hemolysis that intensified by 48 h. Hemolytic zones became more pronounced following storage at 4°C for 48–72 h.

Congo red binding test of *B. bronchiseptica*

In the present study, all 47 *B. bronchiseptica* isolates were tested for Congo red binding ability All the isolates produced intense brick red-coloured colonies after 24 to 48 hours of incubation at 37ºC (**Fig. 3**).

Phase modulation study

The *B. bronchiseptica* isolates examined were readily differentiated into two major phase variants (PVs) based on colonial morphology across the culture media evaluated. Among the incubation temperatures tested (37°C, 30°C, and room temperature [24°C]), room temperature (24°C) most effectively distinguished the characteristic Bvg phase phenotypes. Consequently, variant classification was performed exclusively following incubation at 24°C. The non-modulated (Bvg+) phase exhibited domed, smooth-surfaced, hemolytic colonies (Dom^+ Scs^+ Hly^+), whereas the modulated (Bvg−) phase produced flat, smooth-surfaced, non-hemolytic colonies (Dom^- Scs^+ Hly^-). All isolates developed characteristic colonial morphology within 48 h, with colonies on Bordet–Gengou agar appearing larger than those on other media.

The phase variants of both Bvg+ and Bvg phases were analyzed by SDS-PAGE using 5–15% gradient polyacrylamide gels. Distinct polypeptide profiles were observed among the phase variants of B. bronchiseptica isolates (Fig. 4). Non-modulated (Bvg+) isolates exhibited 20–21 polypeptide bands, whereas modulated (Bvg) isolates displayed only 10–12 bands (Fig. 4). Variations in band intensity were also evident. Notably, two prominent polypeptide bands within

the 20–25 kDa range were consistently detected in all modulated isolates, irrespective of culture media.

Virulence Profiling of *B. bronchiseptica*

It was observed that all isolates of *B. bronchiseptica* showed positive results for *dnt*, *bvg*AS, *achly*A, *fha*B, *fha*C, *ftm*2, *fim*S and *tcf*A. These virulent genes were amplified in PCR and amplicon sizes were 600 bp, 599 bp,745bp, 320bp,1767bp, 301bp, 454bp, and 598 bp for *dnt*, *bvg*AS, *achly*A, *fha*B, *fha*C, *ftm*2, *fim*S and *tcf*A., respectively.

Adhesion and colonization of *B. bronchiseptica*

Postmortem examination of 3-month-old rabbits experimentally infected with B. bronchiseptica revealed severe tracheal and pulmonary lesions. The trachea exhibited extensive mucus accumulation and necrosis, while the lungs showed widespread necrotic lesions and multifocal areas distributed across the pulmonary surface **(Fig. 5).** No evidence of B. bronchiseptica infection was detected in control rabbits. Re-isolation of B. bronchiseptica from infected tracheal and lung tissues confirmed its primary etiological role. Scanning electron microscopy demonstrated rupture of the tracheal epithelial membrane, ciliary disintegration and detachment, bacterial adherence to cilia, marked epithelial damage, loss of over 90% ciliary density, reduced epithelial height, and severe necrosis.

The ultrastructural examination of lungs by SEM demonstrated pathological alterations following intranasal inoculation of live B. bronchiseptica, although lesion severity varied among strains **(Fig. 6).** Compared with normal lungs, which exhibited uniformly organized septa, alveolar cells, and edibular spaces, infected lungs showed marked pathological changes. These included increased erythrocyte accumulation, deposition of cellular debris, deformation of alveolar cells, and bacterial colonization of the lung surface. Extensive alveolar damage, heavy cellular debris deposition, and complete destruction of edibular spaces and connecting septa were also observed **(Fig. 6).**

Western blot analysis of the purified DNT

The immunogenicity of the purified DNT preparation was also detected by western blot analysis with the DNT antiserum raised in rabbit. A single band with approximately 160 kDa (**Fig.7**) was detected in the Western blot.

Dermonecrotic activity in rabbit

The dermonecrotic assay was performed using 20 *B. bronchiseptica* isolates in two healthy 8-month-old New Zealand White rabbits. Among the isolates, six (30%) induced severe necrotic lesions, six (30%) produced moderate lesions, and six (30%) caused mild lesions. Previous studies suggest that dermonecrotic toxin (DNT) contributes to nasal turbinate atrophy and pneumonia in infected pigs. The present findings confirm that DNT production is essential for lesion development, as severe necrotic lesions occurred only in rabbits exposed to DNT-producing isolates, while controls remained lesion-free. Two isolates failed to induce clinical signs.

Cytotoxicity assay of *B. bronchiseptica* on Vero cell line

The cytopathic effect of DNT in Vero cells was evident after 72 h of incubation. Consistent with previous studies, the Vero cell culture assay proved to be a sensitive and effective method for assessing toxin-induced cytopathic changes. Toxin-treated cells exhibited rapid morphological alterations within 24 h, including cell rounding and cytoplasmic retraction, as observed under an inverted microscope **(Fig. 8).** The cytotoxic response was characterized by the formation of dense cell clusters surrounded by enlarged cells, leading to uneven monolayer distribution. Progressive disruption of the monolayer culminated in its detachment from the substrate. The severity of cytopathic effects increased markedly from 24 to 72 h **(Fig. 8).** Additionally, numerous polynucleated, predominantly binucleated, cells were observed, exceeding 50% by day 3.

Discussion

The occurrence of infectious atrophic rhinitis and bronchopneumonia continues to pose a significant challenge to the swine industry in Northeast India. Swine atrophic rhinitis is frequently associated with reduced growth performance and poor feed conversion efficiency, leading to substantial economic losses (De Jong, 1992). Although atrophic rhinitis is recognized as a polymicrobial disease involving *Pasteurella multo*cida and *Bordetella bronchiseptica*, the independent role of *B. bronchiseptica* has received limited attention. In the present investigation, 47 *B. bronchiseptica* isolates were recovered from cases of atrophic rhinitis and bronchopneumonia across different outbreaks. This finding indicates a prominent role of *B. bronchiseptica* in these disease conditions, corroborating the observations of Cowart et al. (1989), who reported its recovery from most outbreak cases, with only infrequent involvement of P. multocida. Similarly, Zhao et al. (2011) documented a high prevalence of *B. bronchiseptica* infection in pigs with respiratory disease. Furthermore, the organism's ability to survive in

nutrient-poor soil and water environments may facilitate persistence and transmission (Porter et al., 1991).

The adherence of pathogenic microorganisms to the mucosal surface is recognized as a critical initial step in colonization and infection (Hughes, 2018). In the present study, scanning electron microscopy demonstrated extensive colonization of *B. bronchiseptica* on the cilia of the ciliated respiratory epithelium, accompanied by necrosis and marked damage to ciliary structures. Colonization of pneumocytes in the lungs following intranasal inoculation with live *B. bronchiseptica* cells was also observed. These findings establish the pathogenicity of *B. bronchiseptica* and its efficient ability to colonize the ciliary apparatus of the respiratory tract. Matsuyama and Takino (1980) similarly reported, using scanning electron microscopy of rabbit tracheal mucosa, the entanglement of numerous *B. bronchiseptica* cells among tracheal epithelial cilia. Furthermore, Sekiya et al. (1989) demonstrated progressive tracheal epithelial damage in mice after intranasal inoculation, characterized by rupture of ciliary membranes, ciliary constriction and breakage, adherence of organisms to ciliary apexes, and colonization within interciliary spaces.

Phase variation in *B. bronchiseptica* is associated with the loss of multiple virulence factors (Ezzell et al., 1981; Peppler and Schrumpf, 1984). In this study, the Bvg-modulated phase was evident at 24°C and on BGA supplemented with 20 mM $MgSO_4$. Although biochemical characteristics remained unchanged, hemolysis and colony morphology differed distinctly.

In the present study, all major virulence-associated genes, namely dnt, bvgAS, achlyA, fhaB, fhaC, ftm2, fimS, and tcfA, were detected in the isolates by polymerase chain reaction. PCR analysis confirmed the presence of the bvgAS locus, a key transcriptional regulator of Bordetella virulence factors, in all 47 isolates. The 600 bp bvgAS amplicon is also known to activate genes encoding additional virulence determinants (Melvin et al. 2014). Detection of fhaB, fhaC, ftm2, and fimS indicated the presence of major attachment factors, while the tcfA gene suggested tracheal colonization potential. Furthermore, primer pairs targeting bvgAS, fhaB, fhaC, dnt, achlyA, fim2, fim3, and tcfA exhibited high specificity, with no cross-reactivity observed against Pasteurella multocida, Staphylococcus aureus, or Streptococcus sp.

Conclusion

In the present study, Bordetella bronchiseptica was identified as the primary etiological agent associated with atrophic rhinitis in pigs. PCR analysis revealed the presence of several important virulence-associated genes, including dnt, bvgAS, achlyA, fhaB, fhaC, ftm2, fimS, and tcfA. The study further confirmed the critical role of dermonecrotic toxin (DNT) in lesion development, as severe necrotic lesions were observed only in rabbits infected with DNT-producing strains. These findings may facilitate the development of effective control strategies against B. bronchiseptica outbreaks. Further investigations are required to assess the genetic diversity of Bordetella strains infecting pigs and support targeted disease control measures.

Author contributions

Conceptualization: Y.M., S.S., P.H., A.K., D.S., M.P.S. Analysis: Y.M., S.S., P.H., Methodology: Y.M., S.S., P.H., A.K., D.S., M.P.S. Software: A.K., Writing, Y.M., S.S., P.H., Writing - original draft preparation: Y.M., S.S., P.H., Writing - review and editing: Y.M., S.S., P.H., A.K., D.S., M.P.S. Supervision: M.P.S. All authors have read and agreed to the published version of the manuscript.

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Data Availability The dataset used during this study is available from the author upon reasonable request.

Declarations

Ethical approval The experimental protocols applied in this study were approved by Institutional Animal Ethics Committee, Assam down town University with approval number AdtU/IAEC/2022/005

Conflict of interest The authors declare no conflict of interest.

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List of Tables and Figures

Table captions

Table 1 Primers used for *dnt*, *bvg*AS, *achly*A ,*fha*B, *fha*C, *ftm*2, *fim*S and *tcf*A genes.

Figure captions

Fig. 1 Number of isolates observed in various sample categories

Fig. 2 Scanning electron micrograph (SEM) of *B. bronchiseptica* cells grown on Bordet Gengou agar at an acceleration voltage of 20 kv; a: cells are scattered; b: cells are clumped together; c: cells are paired together; d: single cells; at different magnifications; Bar ranged from 1-5 µm

Fig. 3 Congo red binding test of *B. bronchiseptica* isolates showing positive brick red-colored colonies whereas pale-colored colonies showing a negative reaction

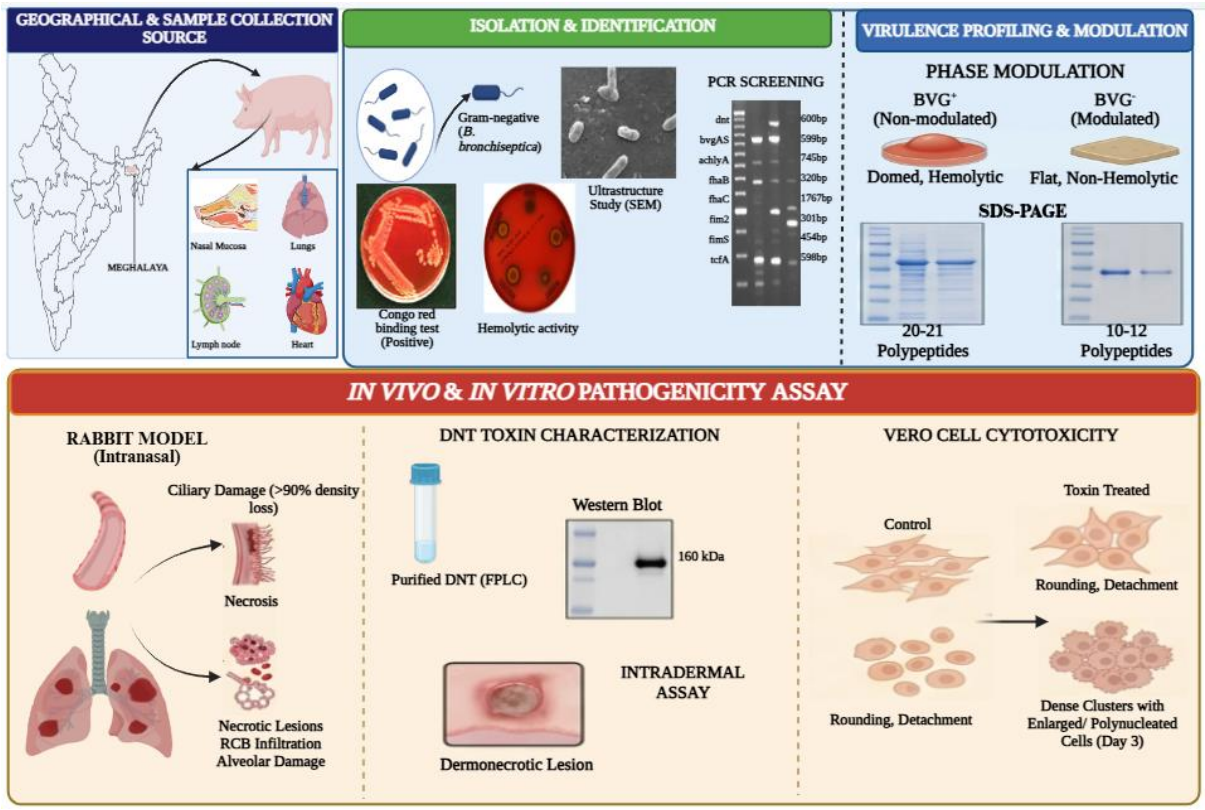
Fig. 4 SDS-PAGE protein profile of non-modulated and modulated B. bronchiseptica isolates under different culture conditions and media supplements (C, BGA, BrA, SBA, BGA+$MgSO_4$) with molecular weight marker (Promega, USA)

Fig. 5 Pulmonary Lesions on Post-Mortem (14 Days Post-Inoculation of B. bronchiseptica in Rabbit): Necrotic Foci and Surface Lesions (arrows)

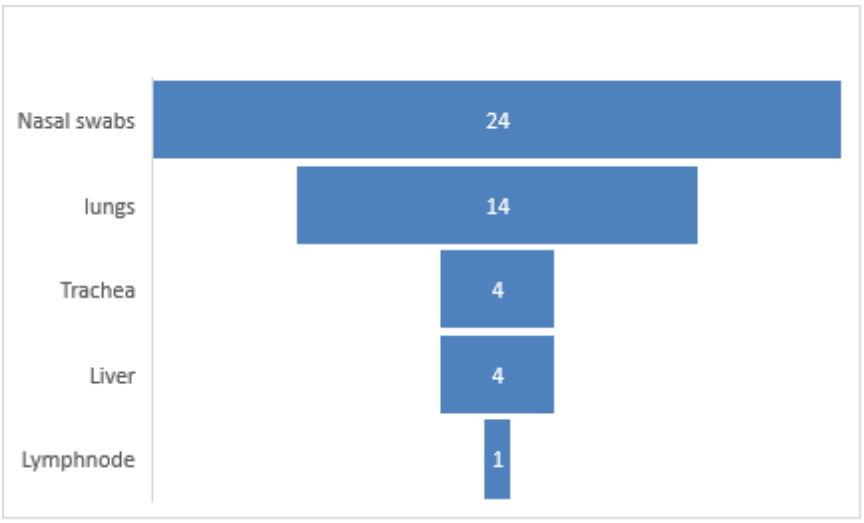
Fig. 6 Scanning Electron Micrograph of Rabbit Lung (14 Days Post-Inoculation with B. bronchiseptica): Control vs Experimental Changes in Alveolar Architecture, Wall Thickening, and Intra-Alveolar Cellular Infiltration (arrow heads)

Fig.7 Detection of DNT Toxin Immunogenicity by Western Blot Using Rabbit Hyperimmune Serum (Lane A: Purified DNT; M: Protein Molecular Weight Marker, Promega, USA)

Fig. 8 Cytopathic Effect of B. bronchiseptica Crude Toxin on Vero Cells at 24, 48, and 72 h (Giemsa Stain, 20× Inverted Microscopy)



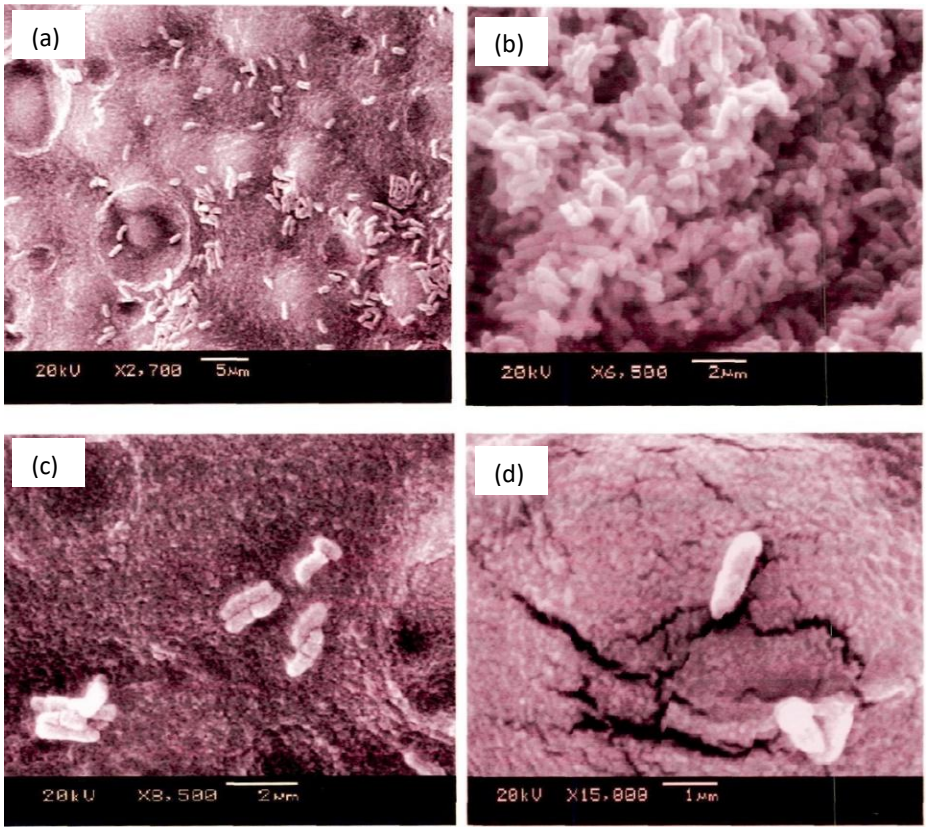
Pictorial Diagram



403

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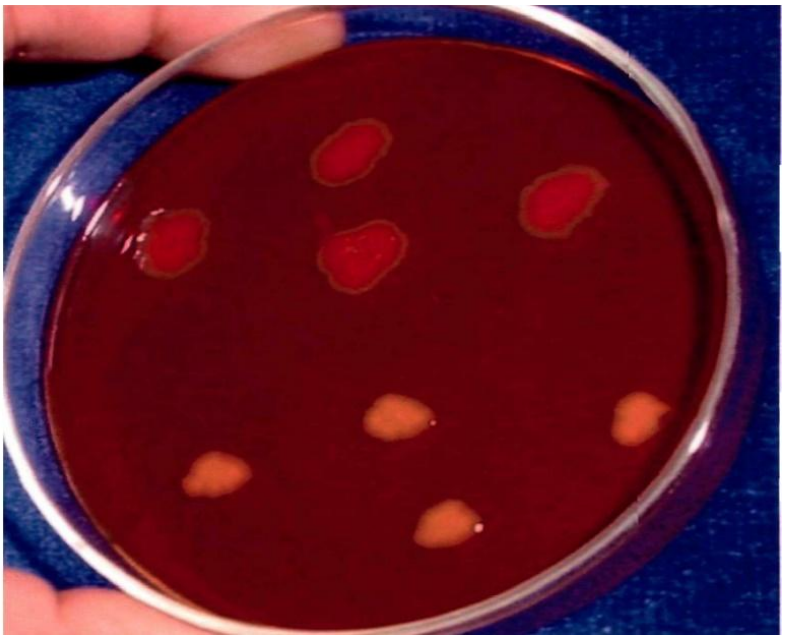


Fig. 3 Congo red binding test of *B. bronchiseptica* isolates showing positive brick red-colored colonies whereas pale-colored colonies showing a negative reaction

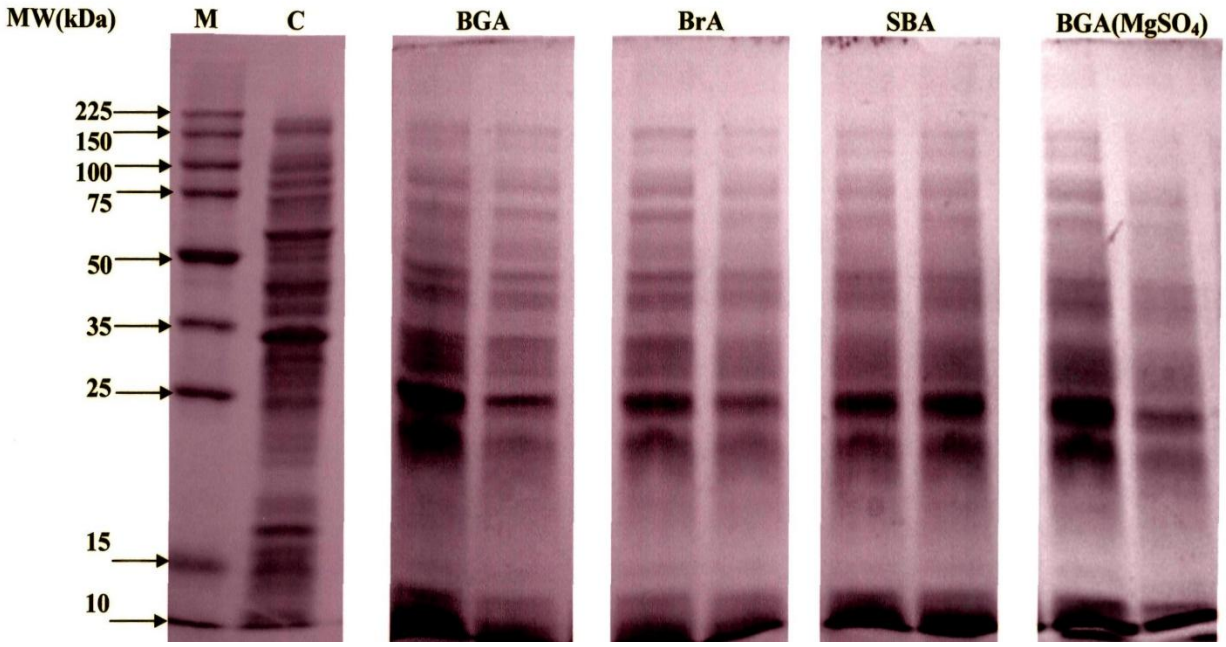


Fig. 4 SDS-PAGE protein profile of non-modulated and modulated B. bronchiseptica isolates under different culture conditions and media supplements (C, BGA, BrA, SBA, BGA+$MgSO_4$) with molecular weight marker (Promega, USA)

Fig. 5

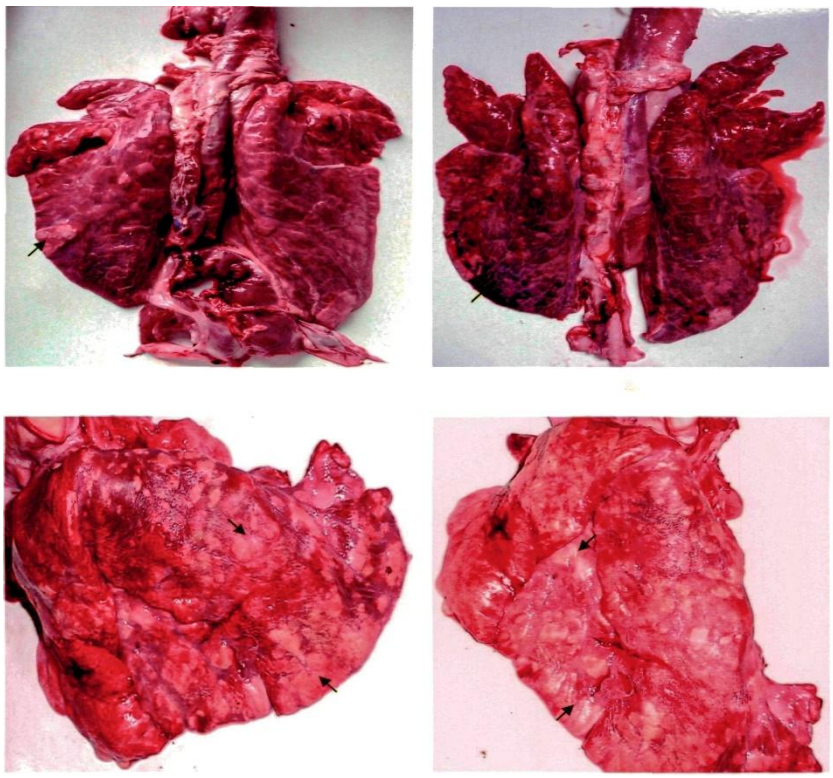
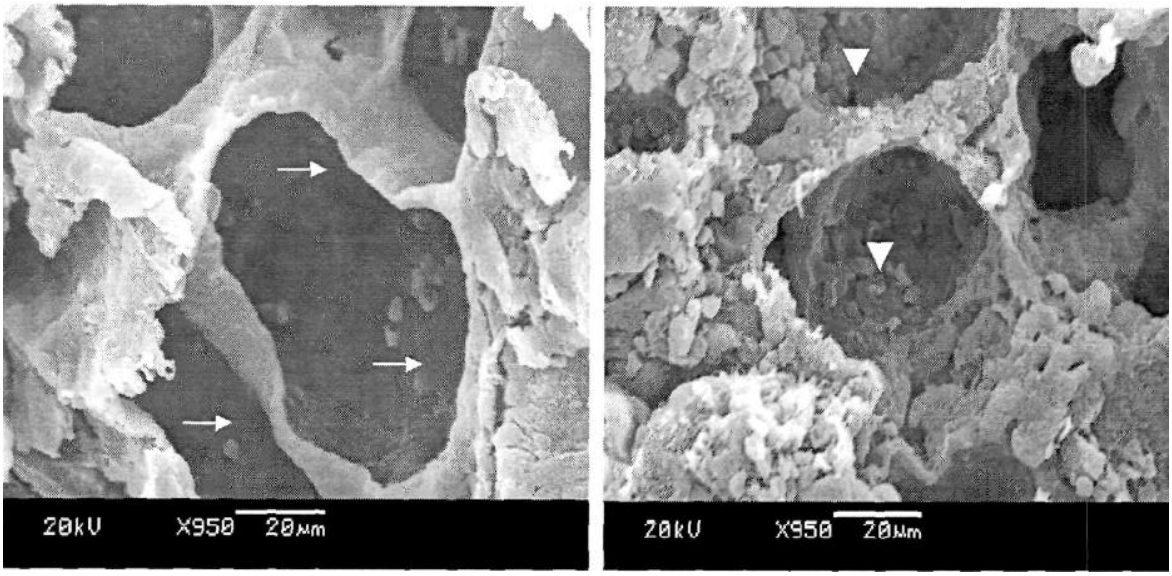


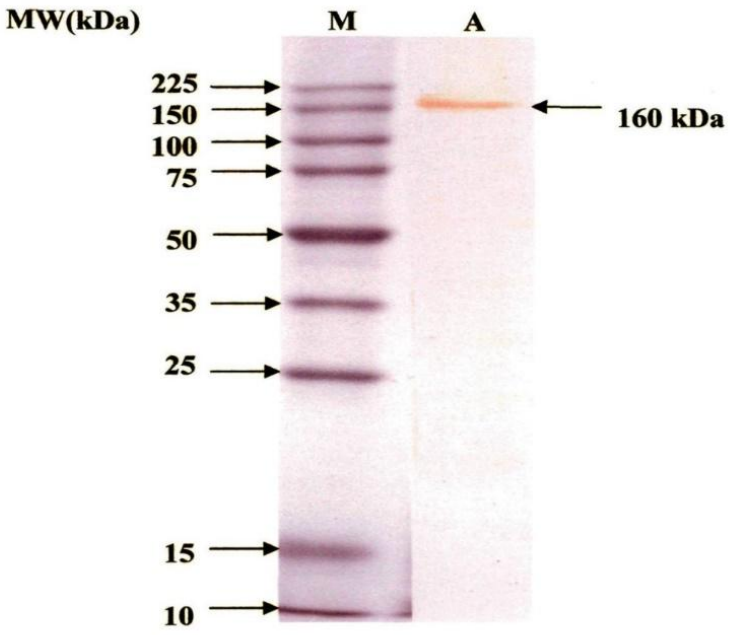
Fig. 5 Pulmonary Lesions on Post-Mortem (14 Days Post-Inoculation of B. bronchiseptica in Rabbit): Necrotic Foci and Surface Lesions (arrows)



428

429 **Fig. 6** Scanning Electron Micrograph of Rabbit Lung (14 Days Post-Inoculation with B. bronchiseptica): Control vs
430 Experimental Changes in Alveolar Architecture, Wall Thickening, and Intra-Alveolar Cellular Infiltration (arrow
431 heads)

432



433

434 **Fig.7** Detection of DNT Toxin Immunogenicity by Western Blot Using Rabbit Hyperimmune Serum (Lane A: Purified
435 DNT; M: Protein Molecular Weight Marker, Promega, USA)

436

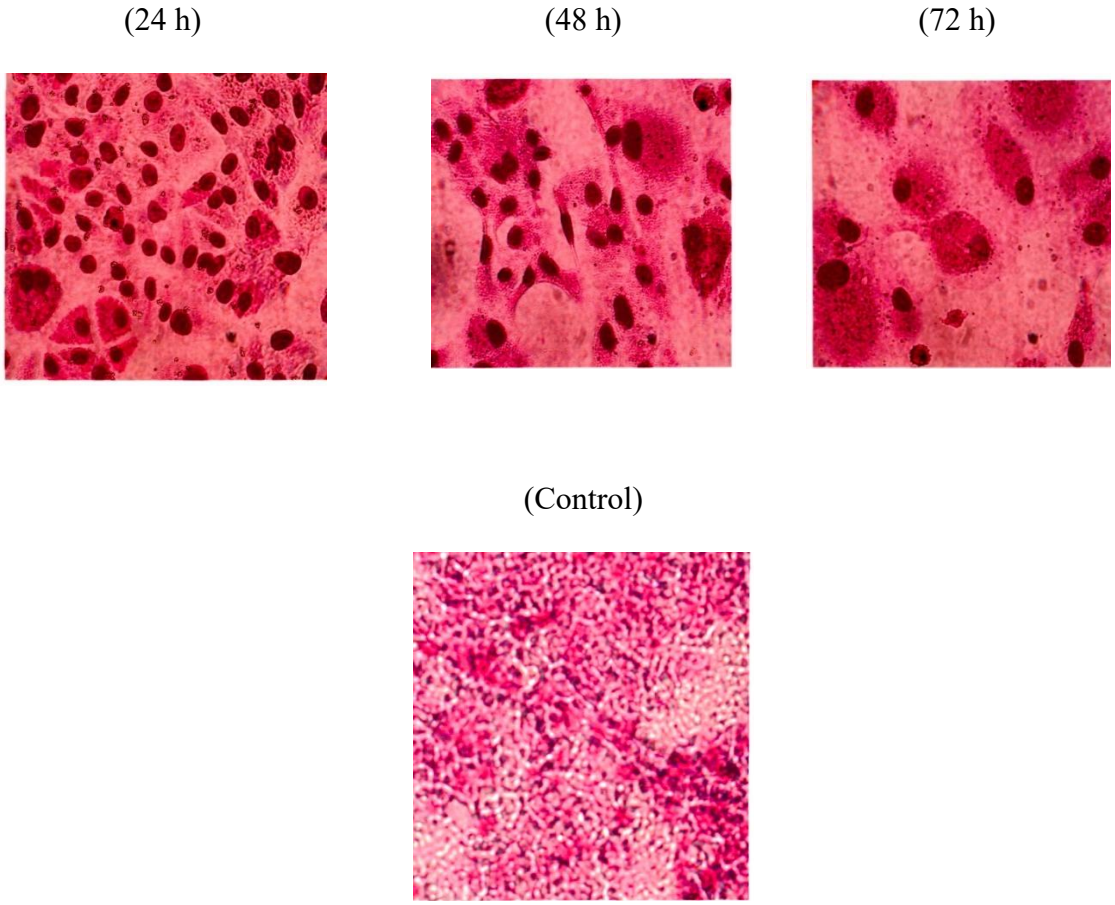


Fig. 8 Cytopathic Effect of B. bronchiseptica Crude Toxin on Vero Cells at 24, 48, and 72 h (Giemsa Stain, 20× Inverted Microscopy)

Supplementary Files

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