Indian Journal of Poultry Science (2024) 59(2): 161–170, Research Article
https://doi.org./10.56093/ijps.v59i2.165168

Study of molecular variations in field isolates of *Ornithobacterium rhinotracheale* from respiratory infections of poultry in Rayalaseema regions of Andhra Pradesh

P. SWETHASRI, T. NAGENDRA REDDY*, S. VIJAYALAKSHMI AND A. ANAND KUMAR

Department of Veterinary Microbiology, College of Veterinary Science,
Sri Venkateswara Veterinary University, Tirupati-517502, India

(Received on December 18, 2023; accepted for publication on July 20, 2024)

ABSTRACT

Swethasri, P., Reddy, T.N., Vijayalakshmi, S. and Kumar, A.A. 2024. Study of molecular variations in field isolates of Ornithobacterium rhinotracheale *from respiratory infections of poultry in Rayalaseema regions of Andhra Pradesh. Indian Journal of Poultry Science, 59(2): 161-170.*

The respiratory diseases are the most frequent causes of death and morbidity in chicken, which leads to significant financial losses to poultry farmers and industry. In the present study was aimed to isolate, identify and study of molecular variations in one of the common respiratory bacteria, *Ornithobacterium rhinotracheale(ORT),* is difficult bacterium to grow in lab and is the emerging pathogen. A total of 107 oral swabs ,107 tracheal swabs, 107 nasal swabs, 18 tracheal tissues, 22 exudates from infra orbital sinuses, 6 larynx and 24 lung tissue samples were collected from 18 suspected poultry farms, located in Rayalaseema region of Andhra Pradesh and these samples were cultured and then molecular detection was done by targeting 16S *r*RNA gene. Out of 18 farms screened 14(77.77%) farms were found positive. Among the 14 positive farms, 52 oral swabs, 58 nasal swabs, 56 tracheal swabs, 6 infra orbital sinus exudates, 4 tracheal tissues were positive with positivity of 48.6, 54.2, 52.3, 27.2, 22.2 % respectively and all 24 lung tissues and 6 larynx tissues were found to be negative. The *ORT* was isolated from the clinical samples by streaking on selective blood agar with gentamicin. The organism was confirmed phenotypically by cultural, biochemical tests and genotypically by targeting 16S *r*RNA gene. The gene products of representative samples were sent to sequencing. The obtained nucleotide sequences were verified by National Center for Biotechnology Information- Basic Local Alignment Search Tool (NCBI-BLAST) showed homology of 97.96- 98.37%. The clinical ORT isolates showed 11 nucleotide substitution at G6C, C7G, T22, T27C, A30T, A32T, G33C, A36G, G95A, T352C and A728T. OP919604 isolate showed 4 nucleotide substitution at G3T, T4G, G6A and G33A. OP919610 isolate showed single nucleotide substitution at G33A. OP919613 showed 8 nucleotide substituitons at G74T, G80A, A81G, T140C, A149G, A199T, C507T and A723C. Phylogenetic tree reveals, three present isolates (OP919513, OP919604, OP919610) segregated into close group with KY809801 ESV-301 strain of Mexico and the sequences OP919513 and OP919604 clustered into a distinct clade. The isolate OP919613 segregated in distinct clade with HF548215 B293-2B strain of United Kingdom (UK)

Keywords: *Ornithobacterium rhinotracheale(ORT),* Blood agar, 16S *r*RNA gene, Sequencing, Phylogenetic analysis

INTRODUCTION

Ornithobacteriosis, commonly known as *Ornithobacterium rhinotracheale(ORT)* infection, is a respiratory distress illness of avian species leading to economic losses in poultry due to mortality, condemnation, decreased egg production, and impaired growth. It is a gram-negative rod, non sporeformer and non-motile bacterium, comes under family *Flavobacteriaceae*; phylum *Bacteroidetes* and also noted as *Pasteurella*-like, Kingella-like, Taxon28, the present name *O.rhinotracheale* was suggested in 1994 (Van Empel and Hafez, 1999; Chin *et al*., 2008) is responsible for respiratory tract infection. There are few reports in India indicating that *ORT* has been implicated as a main or secondary agent in the respiratory illness complex. Clinical signs includes depression, reduced appetite, weight gain losses, increased mortality rates, gasping, severe dyspnoea, mucous discharge, presence of nasal exudates, wet eyes, swelling of infraorbital sinus (Zorman – Rojs *et al*. 2000; Rahimi and Banani 2007; Hablolvarid *et al*. 2013; Umali *et al*. 2017; Hassan *et al*. 2020). Usually, the symptoms are also accompanied by a decline in egg production, a reduction in egg size, and a poorquality egg shell. The clinical signs are similar to other pathogens like *Mycoplasma* and *Avibacterium* and mostly combination of these infections are common.The confirmatory diagnosis is essential to implement control steps. The most of the times the disease causing isolates were shows different mortality and morbidity rates, so the present study is aimed to study the molecular variations in field isolates of *Ornithobacterium rhinotracheale(ORT)* from respiratory infections of poultry in Rayalaseema regions of Andhra Pradesh.

MATERIALS AND METHODS

Samples

Samples were collected from birds that were showing respiratory symptoms and were pooled farm wise and organ wise separately. A total of 107 oral swabs ,107 tracheal swabs, 107 nasal swabs, 18 tracheal tissues, 22 exudates from infra orbital sinuses, 6 larynx and 24 lung tissue samples were collected from 18 suspected

*Corresponding author Email: tnreddy32@gmail.com

poultry farms located in Rayalaseema region of Andhra Pradesh.

Isolation

The collected samples were inoculated on to the 10% blood agar medium with gentamicin (10 µg/ml) and incubated at 37°C for 48 hr in candle jar. Following incubation, colony shape and growth parameters were examined. Colonies that were taken straight from the appropriate culture plates were used to make smears for Gram staining. Conventional biochemical methods were used to confirm the presence of bacteria Chin *et al*. (2008); Murthy *et al*. (2008); Zahra *et al.* (2013); Mayahi *et al*. (2016) and Asadi *et al.* (2022).

Method used for DNA extraction

Genomic DNA was extracted from *ORT* pure cultures using boiling method (Thopireddy *et al*., 2022). Active culture (1mL) was taken in Eppendorf tube, by centrifugation at 10000 rpm for one min was done for pelleting of bacterial cells and the supernatant was discarded. The pellet was washed with 1mL of PBS and again centrifuged at 10000 rpm for 1min and the supernatant solution was removed. The pellet was resuspended in 0.5ml of distilled water by gentle mixing and boiled at 100ºC for 10 min. This was followed by rapid chilling at -20ºC for 6 min. The suspension was centrifuged at 10000 rpm for 1 min and the resultant supernatant containing DNA was transferred to a new centrifuge tube and stored at -20p C for further procedures.

PCR assay for molecular detection of ORT by targeting 16SrRNA gene

PCR for detection of 16S*r*RNA gene of *Ornithobacterium rhinotracheale* was designed by Van Empel and Hafez,1999. Details of the primers sequence and PCR conditions are listed in Table 1 & 2

RESULTS AND DISCUSSION

Indian poultry industry has progressed during the last few decades from backyard poultry to a organized commercial industry. The organized commercial poultry sector is contributing to 70 per cent output and the remaining 30 per cent comes from unorganized sector. At the same time the poultry industry is facing several problems like decreased production, mortality and disease incidence and leads to economic losses to the poultry industry. The production rates in poultry industry is difficulty because of changes in pathogens due to mutations and antimicrobial resistance. The respiratory diseases are the major concern due to involvement of multiple organisms leading to trouble in implementation of control strategies. Among the poultry diseases, the mixed respiratory infections (Roussan *et al*. 2008) due to *Ornithobacterium rhinotracheale, Mycoplasma, Avibacterium and E.coli* are the common bacterial pathogens and causing huge economic losses to the industry. In Andhra Prades maintaining different age birds in poultry farms and stressful conditions are suitable for growth of respiratory pathogens. The confirmatory diagnosis of each pathogen, its differentiation from the others is essential to treat, control and for prevention of respiratory diseases. Hence the present study is aimed to isolate one of the important respiratory bacterial pathogens of poultry with reference to *Ornithobacterium rhinotracheale* and to study molecular variations in clinical *ORT* isolates. The birds affected with different respiratory diseases showed signs of dullness, loss of appetite, nasal discharge, sneezing, gasping, coughing, open mouth breathing, respiratory rales, dyspnoea, drooped wings, ruffled feathers, oedema of the face and eyelids, conjunctivitis with purulent exudate, soiled vent and watery greenish and whitish diarrhoea, some birds were showed paralysis(Fig.1,2,3). Drop in egg production and egg quality was also observed from the flocks that had respiratory infections similar findings were noticed Ley *et al.* (2003); Roussan *et al*. (2008); Murthy *et al*. (2008); Hassan *et al.* (2020); Gowthaman *et al*. (2017); Chaudhary *et al*. (2018); Kaore *et al*. (2018) and Thopireddy *et al*. (2022). The clinical signs given some hint, the birds were suspected for *O.rhinotracheale, Mycoplasma and E.coli* infections. The clinical signs are almost similar in different respiratory diseases of chicken and it is difficult to diagnose based on clinical signs alone. Kaore *et al*. (2018) stated that all the respiratory diseases produce almost similar clinical signs and lesions which often make the diagnosis challenging. The isolation and conformation of these infections needed to reduce losses Patel *et al*. (2018) and Ellakany *et al*. (2019). The *ORT* has been neglected in poultry farms, mainly due to the lack of appropriate early diagnostic method and consequent treatment failure (Barbosa *et al*., 2019).

Table 1: Primer details

Organism	Gene	Primers	Product size (bp)
ORT	16S *r*RNA	F-GAGAATTAATTTACGGATTAAG	784 (Van Empel
		R-TTCGCTTGGTCTCCGAGAT	and Hafez,1999)

Table 2: PCR conditions

Primers	Cycling conditions				
	Initial denaturation	Denaturation	Annealing	Extension	Final extension
ORT	94°C 5 min	94°C 30 sec	52°C 1 min	72°C 1 min 30 sec	72°C 7 min
	Repeated for 44 cycles				

Several scientists described initial isolation, typical morphology of organism, biochemical investigations of *ORT* (Mayahi *et al.,* 2016; Ellakany *et al*., 2019; Al-Hasan *et al.*, 2022) but only limited studies on molecular diagnosis as have been described. In focused more on molecular diagnosis of *ORT*. The clinical signs noticed in the *O. rhinotracheale* infected birds in the present study includes weakness, gasping, dyspnoea and mucous discharge (Fig.1,2,3). Similar signs were also reported by several researchers (Van Empel and Hafez 1999; Rahimi and Banani 2007; Umali *et al.,* 2017; Hassan *et al.,* 2020). Observed presence of mucous exudate in trachea in the present study in accordance with the findings of with Hauck *et al*. (2015) and Umali *et al*. (2017).

All the suspected samples were initially tested for the presence of *O. rhinotracheale* by isolation and identification of bacteria were done on the basis of cultural, morphological and biochemical characteristics. A total of 40 (37.3%) from oral swabs, 50 (46.7%) from tracheal swabs and 45 (42%) from nasal swabs, 4 (18.1%) from exudates of infraorbital sinuses, samples from tracheal tissues, Lung tissues and larynx tissue are negative by isolation these results were in accordance to Murthy *et al*. (2008) in their study reported 34.6% positive and higher compared to Nayak *et al*. (2017) in his study found only 0.56 % positive. In this study, 46.7% prevalence of *ORT* infections was observed might be attributed to heavy rains, stressful and suitable environment, due to increased ammonia levels, improper ventilation, high temperature and humidity, and concentration birds and similar causes were reported by other workers (Espinosa *et al*.,2011; Ellakany *et al*., 2018).

In this study, *ORT* isolated from tracheal swabs, oral swabs, infraorbital sinus exudates and nasal swabs but not from tracheal tissues, larynx and lungs, it might be due to the organisms mostly present in the discharges and localised on surface mucus membranes of respiratory tract, in accordance with Espinosa *et al*. (2011) where they isolated only from the infraorbital sinus exudates from four layer hens but not from trachea and lung tissues.

ORT requires specialised growth conditions with long incubation for cultivation. Thus, attempts of isolation of *ORT*, often unsuccessful and plates were mostly overgrown by other bacteria during incubation periods (Travers *et al*., 1996; Amal, 2002). To avoid difficulties in isolation, in this study, the successful isolation of *ORT* might be due to addition of gentamicin. Since it has been shown that most of *ORT* isolates are resistant to gentamicin (Back *et al*., 1997).In the present study, the small, non-haemolytic, grey to greyish white, opaque, convex with entire edge, circular and with butyrous odour colonies were observed after 48hrs of incubation at 37p C under anaerobic conditions on 5-10% sheep blood agar

Fig. 1: Bird showing dyspnoea

Fig. 2: Infected bird showing sticky nasal discharges, facial edema

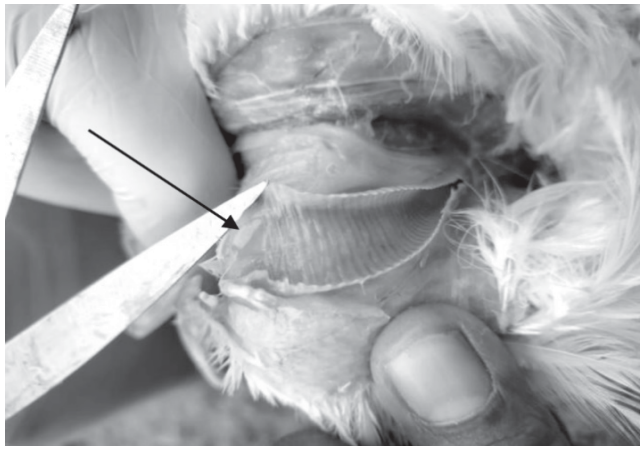


Fig. 3: Mucous exudate in trachea

with gentamicin (Fig. 4, 5). Similar findings were also described by several scientists (Murthy *et al*., 2008; Sivaseelan *et al*., 2014; Mayahi *et al*., 2016; Hassan *et al.,* 2020; Mohamed *et al*.,2022; Asadi *et al.*,2022). The *ORT* also isolated on BHI agar in the present study and not on MacConkey agar, these characters were described by previous studies (Hafez *et al*., 1993; Roepke *et al*., 1998; Post *et al*., 1999; Amal, 2002; Asadpour *et al*., 2008; Mayahi *et al*., 2016). Concerning cellular morphology of *ORT*, it appeared as Gram negative,

pleomorphic rods bacteria as described by several workers (Van Empel and Hafez 1999; Amal,2002; Bisshop,2003; Shahata *et al*.,2006 and Espinosa *et al*., 2011). The *ORT* pathogen mostly known to cause respiratory tract infections with reference to Mayahi *et al*. (2016) and Hassan *et al.* (2020). In present study pleomorphism observed more when smears prepared from BHI broth culture than agar plate colonies are in line with reports of Murthy *et al.* (2008) All the *ORT* isolates in this study were further confirmed by biochemical tests and were showed catalase negative and oxidase positive(Fig.6,7) agree with EI-Gohary and Awaad (1998), Chin and Charlton (2008) and Ellakany *et al.* (2018). The result of the *ORT* isolates were positive for Arginine dihydrolase, Urease test and Voges-Praskauer and negative for L-lysine decarboxylase, Ornithine decarboxylase and H2S(Fig.8,9,10) and our findings agrees with Chin *et al*. (2008), Murthy *et al*. (2008), Zahra *et al.* (2013), Mayahi *et al.* (2016) and Asadi *et al*. (2022).The isolation of *ORT* is time consuming and required special incubation conditions to overcome the difficulties the molecular based PCR tests are alternative for the confirmatory, accurate and rapid diagnosis of *ORT* directly from the field samples (Doosti *et al*. 2011). Many investigators (Hung and Alvarado 2001; Ozbey *et al*., 2004 and Hassanzadeh *et al*., 2010) have applied the PCR method for verification of *ORT* strains, as it is a sensitive, quick, and specific approach.

In the present study, all the suspected samples were initially tested for the presence of *ORT* by targeting the *16S rRNA* gene. The Primers(Table.1) used in the study were genus specific primers specified by Van Empel and Hafez (1999). A 44 cycle PCR reaction with annealing temperature of 52°C were found optimum(Table.2) for amplification of 784 bp product of *16S rRNA* gene in positive DNA sample(Fig.11) and the similar PCR conditions were also used for detection of *O. rhinotracheale* by Asadpour *et al*. (2008) and Hassan *et al.* (2020).

All the 18 farms were screened by PCR (Fig.12) for the presence of *ORT* and found 14 farms were positive, with percent positivity of 77.77% but the severity of its effects depends on the strain and other predisposing factors such as viral infections, improper ventilation, age of the birds at infection. Several research workers also reported incidence of 8.57% (Mayahi *et al*., 2016) ,100 % (Umali *et al*., 2017) and 28.57 % (Kursa *et al.*, 2022) in suspected poultry respiratory infections. Among the 14 positive farms, 52, 58, 56 and 6 oral, nasal swabs, tracheal swabs and infraorbital sinus exudates were positive with positivity of 48.6, 54.2, 52.3 and 27.2 % respectively (Graph.1). A total of 4 tracheal tissues (22.2 %) were positive for *O.rhinotracheale*. Several researchers were reported the positivity of *ORT* 1.03 %

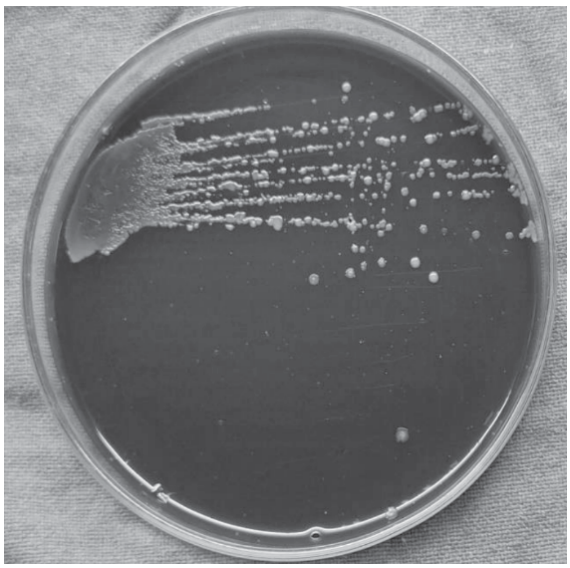


Fig. 4: *ORT* on Blood agar plate showing non- hemolytic, greyish white colonies

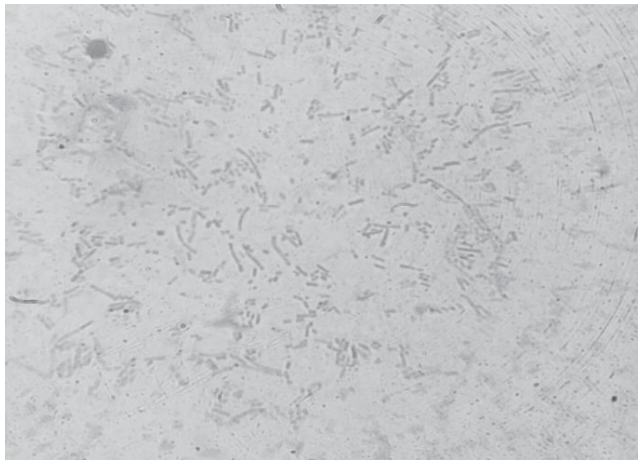


Fig. 5: On Gram staining-*ORT* showing gram negative, pleomorphic, rod shaped

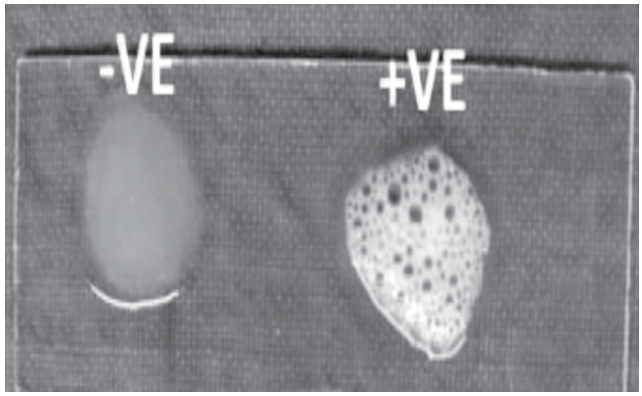


Fig. 6: *ORT* showing catalase test -ve on left side

(Asadpour. *et al*. 2008), 21.00 % (Roussan *et al*. 2011), 8.57 % (Mayahi *et al*. 2016) from tracheal swabs, 1% from tracheal tissues by Hassanzadeh *et al*. (2010) and 5 % from infraorbital sinus exudates by Espinosa *et al*. (2011). More prevalence recorded in this study (77.77%) might be due to heavy rains and climate cause stress and

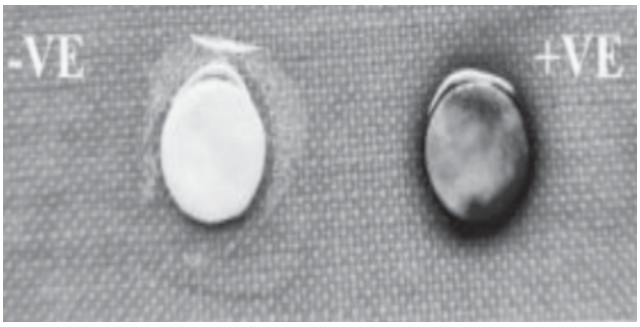


Fig. 7: *ORT* showing Oxidase test +ve on right side

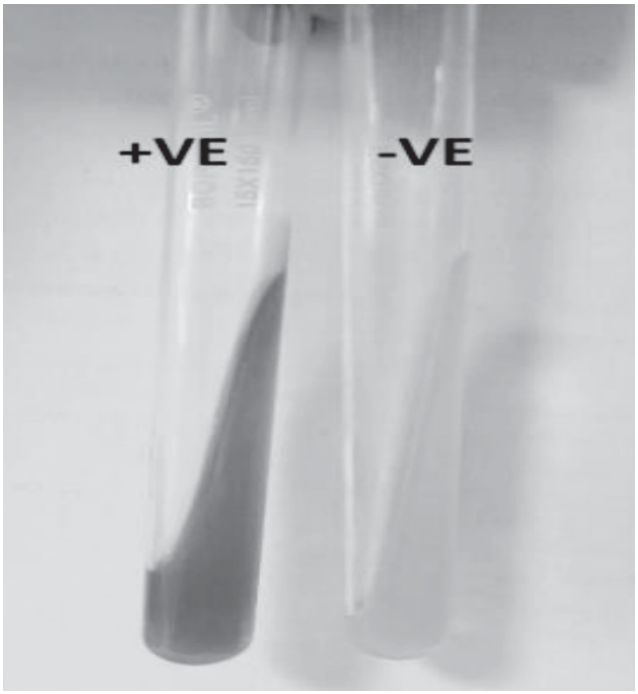


Fig. 8: *ORT* showing Urease test – Positive

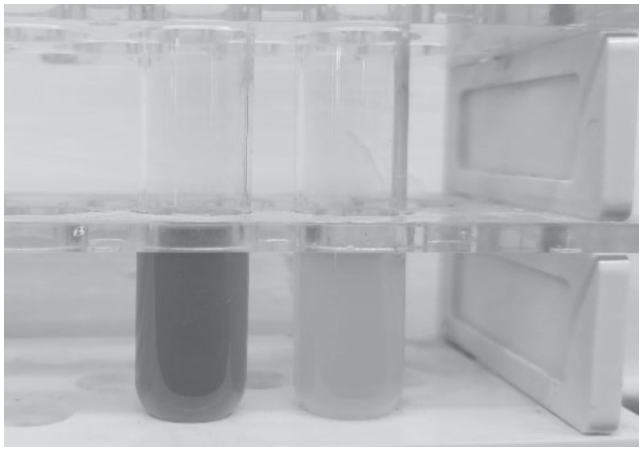


Fig. 9: *ORT* showing Arginine dihydrolyase test positive

more suitable conditions for pathogens.

In the present study, the positive PCR *16S r RNA* gene products from four representative farms located at Tirupati, Kadapa, Annamayya and Chittoor region of Rayalaseema samples of DNA were sequenced and found

Fig. 10: *ORT* showing IMViC test (- - + -)

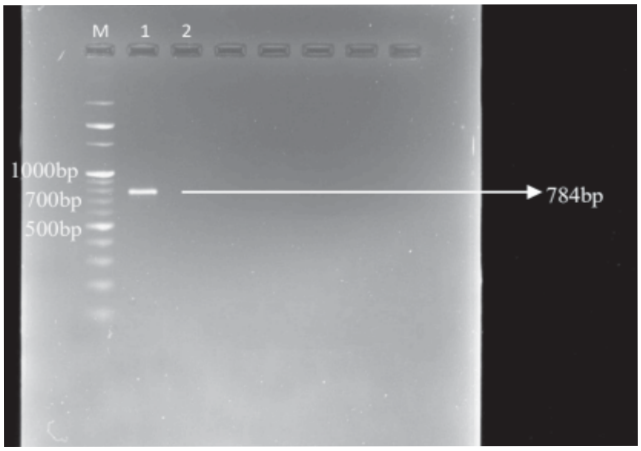


Fig. 11: Standardization of PCR for detection of *O.rhinotracheale* targeting *16S rRNA* gene
Lane M: Ladder (100bp)
Lane 1: Positive control
Lane 2: Negative control

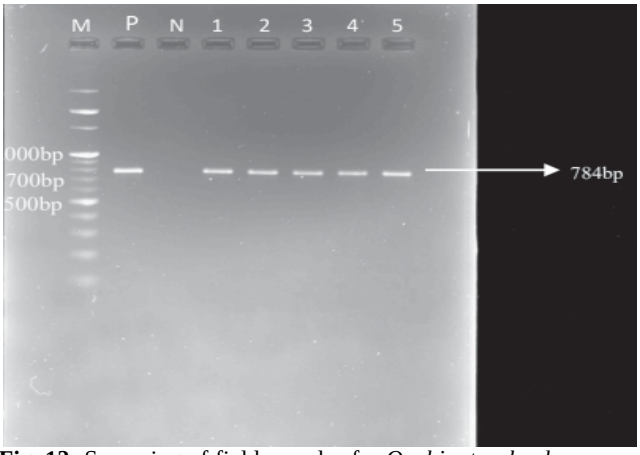
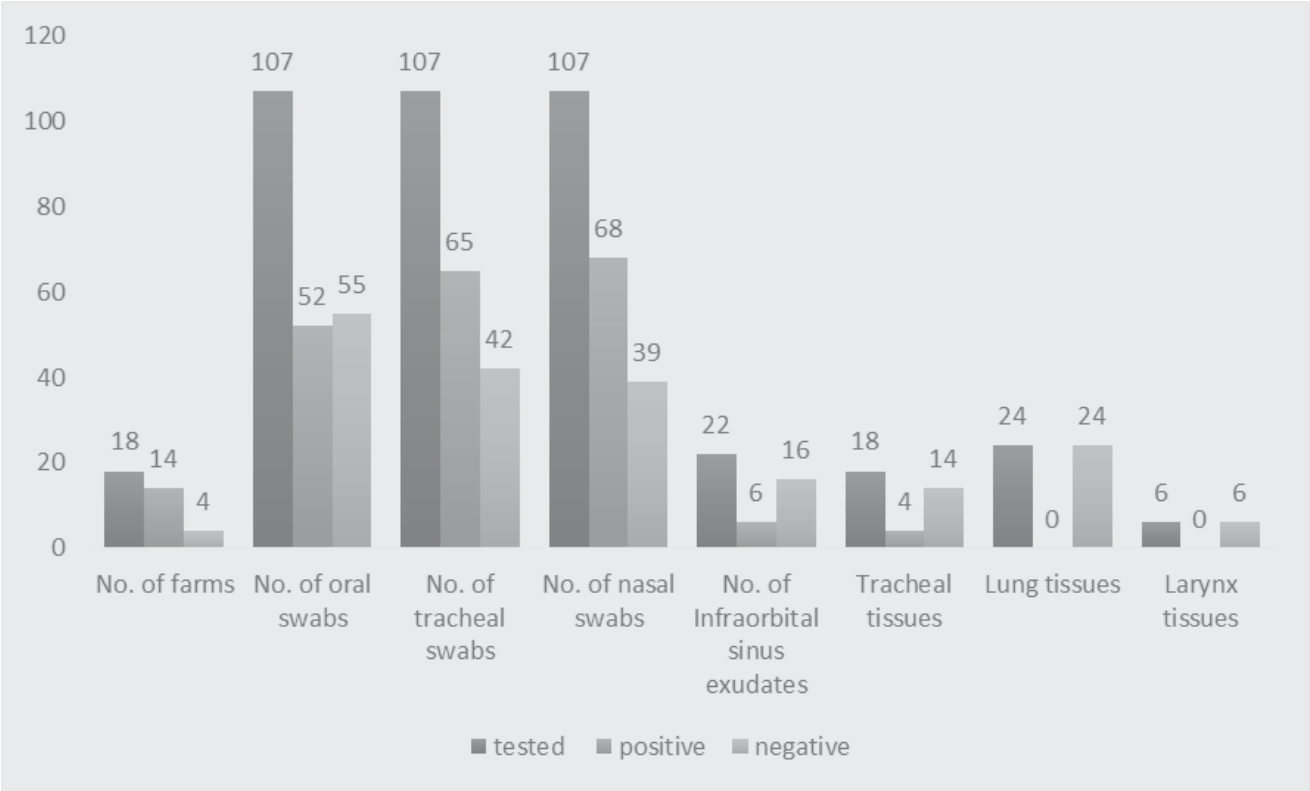
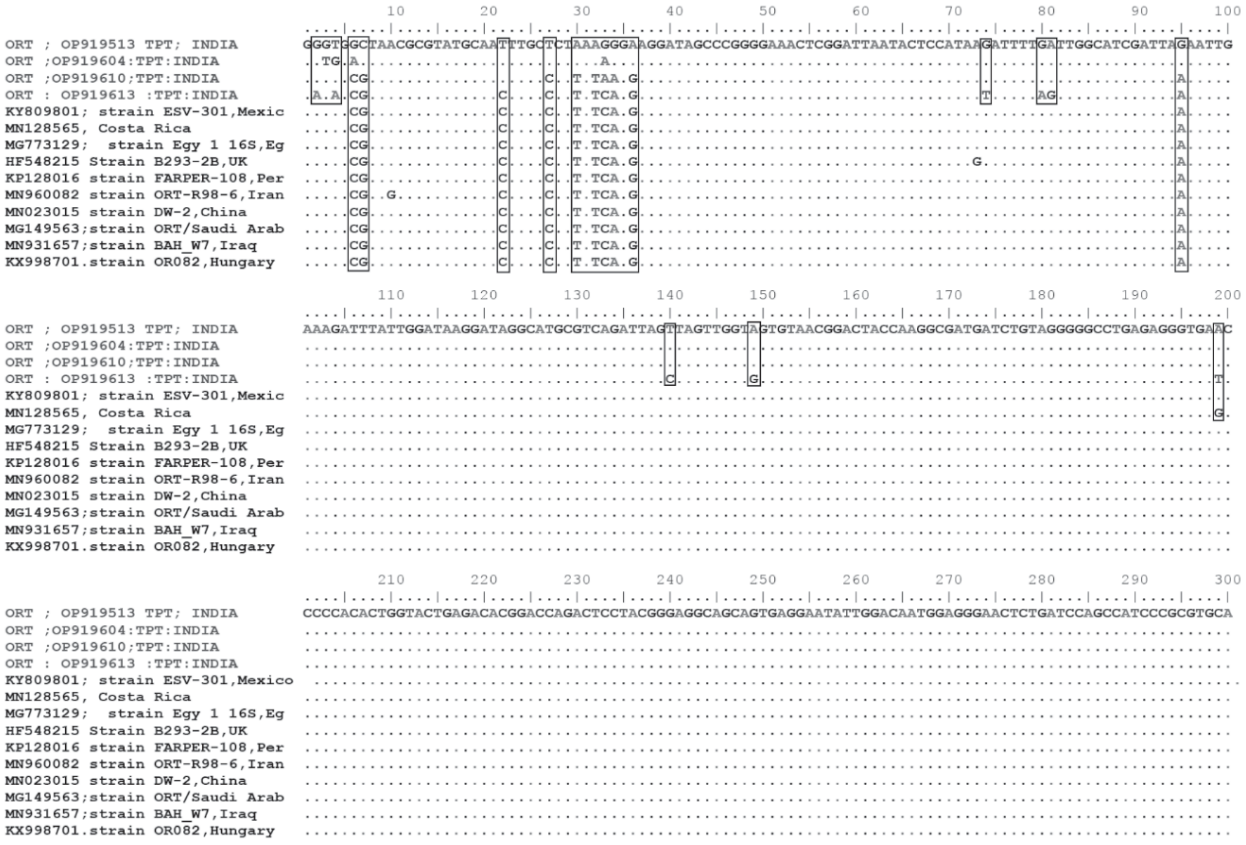


Fig. 12: Screening of field samples for *O. rhinotracheale*
Lane M: Ladder (100bp)
Lane P: Positive control
Lane N: Negative control
Lane 1,2,3,4&5: Field samples positive for *16S rRNA* genus *O.rhinotracheale*



Graph 1 Sreening of *ORT* by PCR



97.96- 98.37 % homology with *O. rhinotracheale* strains of NCBI. These nucleotide sequences were then submitted to Gen Bank and accession numbers were allotted for sequences i.e, OP919513, OP919604, OP919610 and OP919613. Our four strain sequences were published in NCBI and noticed that our publications are the first as a local Indian strain.

The multiple sequence nucleotide analysis of partial *16S rRNA* gene of the present *O. rhinotracheale* isolates revealed nucleotide substitutions. The *ORT* isolates

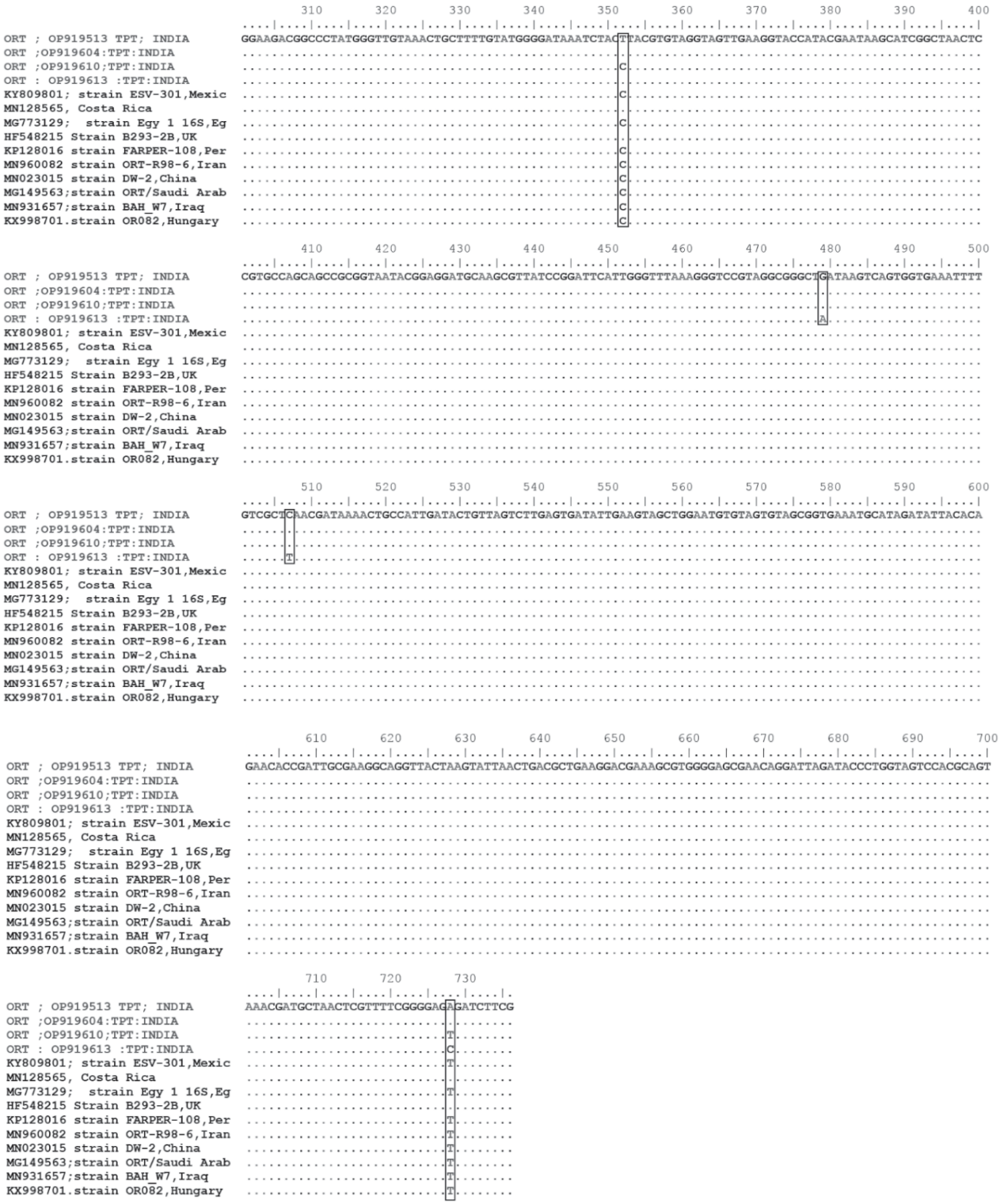


Fig. 13: Multiple sequence alignment of *Ornithobacterium rhinotracheale* field isolates with published NCBI GenBank sequences (Bio Edit 7.2.5, Hall, 1999)

showed 11 nucleotide substitution at G6C, C7G, T22, T27C, A30T, A32T, G33C, A36G, G95A, T352C and A728T. The OP919604 *ORT* isolate showed 4 nucleotide substitution at G3T, T4G, G6A and G33A whereas the OP919610 *ORT* isolate showed single nucleotide substitution at G33A and OP919613 showed 8 nucleotide substitutions at G74T, G80A, A81G, T140C, A149G, A199T, C507T, A723C(Fig.13). Similarly, Canal *et al*. (2005) observed no variations in poultry isolates and one base pair variations among 4 isolates of turkey Minnesota in 1995.

The multiple alignment of amino acid sequences

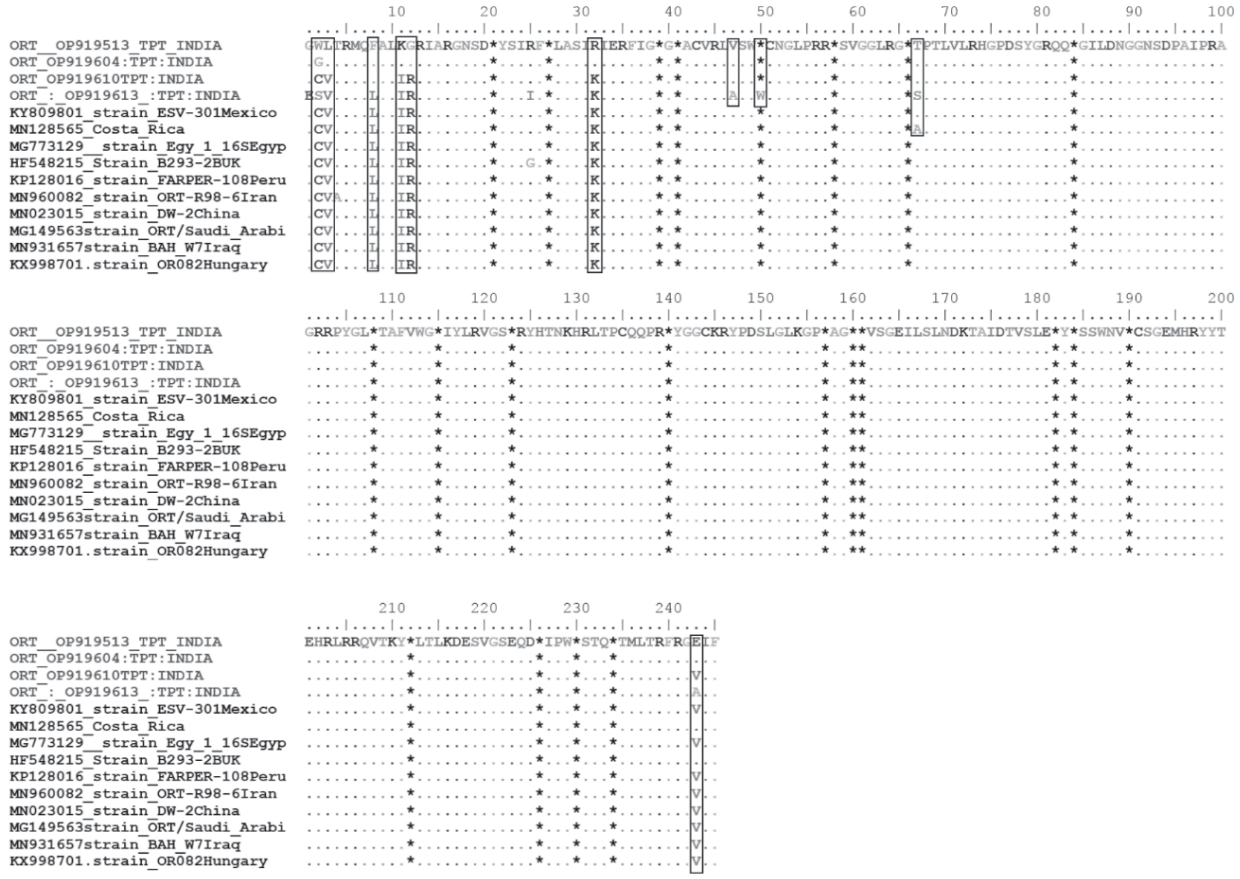


Fig. 14: Comparison of *Ornithobacterium rhinotracheale* amino acid sequences with Published GenBank sequences

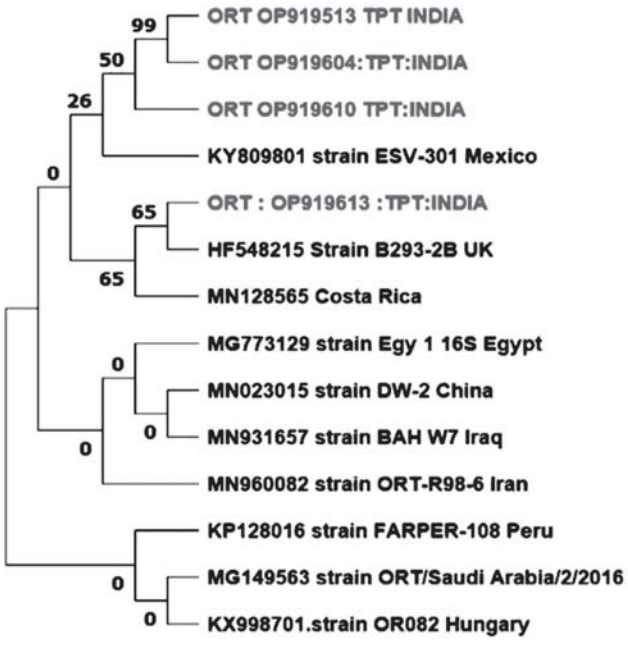


Fig. 15: Phylogenetic analysis of *Ornithobacterium rhinotracheale* sequences with other reference sequences available in NCBI database

The phylogenetic tree was constructed using the MEGA version 11 by Maximum Parsimony method with 1000 bootstrap replicates using Kimura 2-parameter model (Tamura *et al*. 2021).

of partial *16S rRNA* gene revealed that amino acid changes and insertion were observed at different positions between 1st to 32rd and 43rd. The isolates showed 6 changes at W2C, F8L, K11I, G12R, R32K and E243V i.e, change of amino acids at 2nd position tryptophan to cysteine, 8th position phenylalanine to leucine, 11th position lysine to isoleucine, 12th position glycine to arginine, 32nd position arginine to lysine and 243rd position glutamic acid to valine, OP919604 showed single change at W2G *i.e*, at 2nd tryptophan to glycine OP919610 showed single change at W2C *i.e*, at 2nd tryptophan to cysteine and OP919613 showed 7 amino acid changes at G1E, W2S, R25I, V47A, T67S and E243A *i.e*, at 1st position glycine to glutamic acid, 2nd position tryptophan to serine, 25th position arginine to isoleucine, 47th position valine to alanine, 67th position threonine to serine and 243rd position glutamic acid to alanine insertion at -50W(Fig.14). The variations in the sequences might be due to mutations from generation to another generation (Thopireddy *et al*., 2023).

The phylogenetic tree was constructed with the present *O. rhinotracheale* isolate OP919513, OP919604, OP919610, OP919613 and selected published sequences of partial *16S rRNA* gene. Three isolates of present study (OP919513, OP919604, OP919610) segregated into close group with KY809801 ESV-301 strain of Mexico and the sequences OP919513 and OP919604 clustered

into a distinct clade (Fig.15). The sequence OP919613 segregated in distinct clade with HF548215 B293-2B strain of United Kingdom (UK). Similarly De oca-jimenez *et al*. (2018) observed 99.1-100 % homology in his 8 Mexican ORT isolates from poultry and also noticed 10SNPs.

CONCLUSION

This study concluded that ornithobacterial infections are more common in poultry causing economic losses along with other pathogens like *Mycoplasma*, *Avibacterium* and *E.coli* .Isolation and molecular detection methods are confirmatory and implementation of the control strategies is essential. This study also helps to know the variations in field isolates due to mutations.

REFERENCES

Asadi, N., Bozorgmehri-Fard, M.H., Seifi, S., Khoshbakht, R. and Sheikhi, N. 2022. Isolation, characterization, and genotyping of *Ornithobacterium rhinotracheale* isolated from broiler and broiler breeder flocks in Mazandaran province. Northern Iran. *Iranian Journal of Veterinary Research*, **23**(1):32.

Al-Hasan, B.A., Alhatami, A.O., Abdulwahab, H.M., Bustani, G.S., Hameed, M.A. and Jawad, A.H. 2022. First report of Avian metapneumovirus type B in Iraqi broiler flocks with swollen head syndrome. *Veterinary World*, **15**(1): 16.

Asadpour, Y., Rezaei, M. and Rahimabadi, E. 2008. Infection of broiler chickens to *Ornithobacterium rhinotracheale* In Urmia city. *Iranian Veterinary Journal*, ***12***(2): 5-10.

Barbosa, E.V., Cardoso, C.V., Silva, R.D.C.F., Cerqueira, A.D.M.F., Liberal, M.H.T. and Castro, H.C. 2019. *Ornithobacterium rhinotracheale*: An update review about an emerging poultry pathogen. *Veterinary sciences*, ***7***(1): 3.

Chaudhary, S.K., Rokade, J.J., Aderao, G.N., Singh, A., Gopi, M., Mishra, A. and Raje, K. 2018. Saponin in poultry and monogastric animals: A review. *Int. J. Curr. Microbiol. Appl. Sci*, ***7***(7): 3218-3225.

Chin, R.P. and Charlton, B.R. 2008. Ornithobacteriosis In: A laboratory manual for the isolation, identification, and characterization of avian pathogens, Fifth Ed. *American Association of Avian Pathologists*, Madison, Wisconsin, United States, pp. 75-76.

De oca-jimenez, R.M., Vega-Sanchez, V., Morales-Erasto, V., Salgado-Miranda, C., Blackall, P.J. and Soriano-Vargas, E. 2018. Phylogenetic relationship of *Ornithobacterium rhinotracheale* strains. *Journal of Veterinary Medical Science*, **17**:0474.

El-Gohary, A. 1998. *Ornithobacterium rhinotracheale* (ORT) associated with hatching problems in chicken and turkey eggs. *Veterinary Medical Journal-Giza*, **46**:183-191.

Ellakany, H.F., Abd-Elhamid, H.S., Ibrahim, M.S., Mostafa, N.S., Elbestawy, A.R. and Gado, A.R. 2019. Isolation, serotyping, pathogenicity and antibiotic sensitivity testing of *Escherichia coli* from broiler chickens in Egypt. *Alexandria Journal for Veterinary Sciences*, **61**(2).

Ellakany, H., F., Elbestawy, A., R., Abd-Elhamid, H., S., Gado, A., R., Nassar, A., A., Abdel-Latif, M., A. and Alowaimer, A., N. 2019. Effect of experimental *Ornithobacterium rhinotracheale* infection along with live infectious bronchitis vaccination in broiler chickens. *Poultry Science*, ***98***(1):105-111.

Espinosa, I., Colas, M., Vichi, J., Báez, M. and Martínez, S. 2011. Isolation and identification of *Ornithobacteriun rhinotracheale* from laying hens in farms of la Habana Province. *Revista de Salud Animal*, **33**(1): 38-38.

Gowthaman, V., Singh, S.D., Dhama, K., Srinivasan, P., Saravanan, S., Murthy, T.R.G.K. and Ramakrishnan, M.A. 2017. Molecular survey of respiratory and immunosuppressive pathogens associated with low pathogenic avian influenza H9N2 subtype and virulent Newcastle disease viruses in commercial chicken flocks. *The Journal of Poultry Science*, ***54***(2): 179-184.

Hablolvarid, M.H., Abdoshah, M. and Bijanzad, P. 2013. Evaluation of clinical signs, gross lesions and antibody response in experimental of indvidual and co-infection of H9N2 avian influenza and *Ornithobacterium rhinotracheale* in SPF chickens. *European Journal of Experimental Biology*, **3**(1): 503-507.

Hassan, A., Medhat, M.A., Shahata, M. and Bekheet, A. 2020. Phenotypic and molecular characterization of *Ornithobacterium rhinotracheale* isolates in broiler chickens. *Journal of Advanced Veterinary Research*, **10**(4):193-199.

Hassanzadeh, M., Karrimi, V., Fallah, N., Ashrafi, I. 2010. Molecular characterization of *Ornithobacterium rhinotracheale* isolated from broiler chicken flocks in Iran. *Turkish Journal of Veterinary and Animal Sciences*, **34**: 373-378.

Hauck, R., Chin, R.P. and Shivaprasad, H.L. 2015. Retrospective Study on the Isolation of *Ornithobacterium rhinotracheale* from Chickens and Turkeys in Central California: 294 cases (2000–12). *Avian Diseases*, **59**(1):130-137.

Kaore, M., Singh, K.P., Palanivelu, M., Kumar, A.M., Reddy, M.R. and Kurkure, N.V. 2018. Patho-epidemiology of respiratory disease complex pathogens (RDPs) in commercial chicken. *Indian J. Vet. Pathol.*, **42**(4) : 231-238.

Kursa, O., Tomczyk, G., Adamska, K., Chrzanowska, J. and Sawicka-Durkalec, A. 2022. The microbial community of the respiratory tract of commercial chickens and turkeys. *Microorganisms*, **10**(5): 987.

Mayahi, M., Gharibi, D., Ghadimipour, R. and Talazadeh, F. 2016 .Isolation, identification and antimicrobial sensitivity of *Ornithobacterium rhinotracheale* in broilers chicken flocks of Khuzestan, Iran. *In Veterinary Research Forum*, **7**(4): 341.

Mohamed, E.S., Hamouda, A.M. and El Enbaawy, M.I. 2022. Current status of multidrug resistance of *Ornithobacterium rhinotracheale* from avian host. *International Journal of Veterinary Science*, **11**(4): 539-543.

Murthy, T.R.G.K., Dorairajan, N., Balasubramaniam, G.A., Dinakaran, A.M. and Saravanabava, K. 2008. Pathogenic bacteria related to respiratory diseases in poultry with reference to *Ornithobacterium rhinotracheale* isolated in India. *Veterinary Archives*, **78**: 131–140

Nayak, A., Bordoloi, S., Soni, R., Sharma, V. and Jogi, J. 2017. Incidence of Ornithobacterium rhinotracheale from poultry in and around Jabalpur. *Immunology and Immunopathology*, **19**: 26-30.

Patel, J.G., Patel, B.J., Patel, S.S., Raval, S.H., Parmar, R.S., Joshi, D.V. and Patel, B.K. 2018. Metagenomic of clinically diseased and healthy broiler affected with respiratory disease complex. *Data in brief*, **19**: 82.

Rahimi, M. and Banani, M. 2007. Isolation of *Ornithobacterium rhinotracheale* from the chickens of a broiler farm in Kermanshah province, west of Iran. *Iranian Journal of Veterinary Research* **8**(4): 355-359.

Roussan, D.A., Haddad, R. and Khawaldeh, G. 2008. Molecular survey of avian respiratory pathogens in commercial broiler chicken flocks with respiratory diseases in Jordan. *Poultry Science*, **87**(3): 444-448.

Roussan, D.A., Al-Rifai, R.H. and Khawaldeh, G.Y. 2011. *Ornithobacterium rhinotracheale* and *Mycoplasma synoviae* in broiler chickens in Jordan. *Rev Sci Tech*, **30**(3): 931-937.

Tamura, K., Stecher, G. and Kumar, S. 2021. MEGA 11: Molecular Evolutionary Genetics Analysis Version 11. Molecular Biology and Evolution https://doi.org/10.1093/molbev/msab120.

Thopireddy, N.R., Bollini, S. and Nagaram,V.K. 2022. Molecular detection and differentiation of *Mycoplasma gallisepticum* field isolates of poultry in Andhra Pradesh. *Indian journal of poultry Science*, **57**(02):181-186.

Thopireddy, N.R., Bollini, S. and Nagaram, V.K. 2023. Comparative study of molecular variations in *Mycoplasma synoviae* field isolates from common respiratory infections of poultry in Andhra Pradesh. *Indian journal of poultry science*, **58**(02):159-163.

Umali, D.V., Shirota, K., Sasai, K. and Katoh, H. 2017. Characterization of *Ornithobacterium rhinotracheale* from commercial layer chickens in eastern Japan. *Poultry Science*, ***97***(1):24-29.

Van Empel, P.C.M. and Hafez, H.M. 1999 *Ornithobacterium rhinotracheale*: a review. *Avian Pathology*, **28**: 217-227.

Van Empel, P., van den Bosch, H., Loeffen, P. and Storm, P. 1997. Identification and serotyping of *Ornithobacterium rhinotracheale*. *Journal of Clinical Microbiology*, **35**(2): 418-421.

Zahra, M., Ferreri, M., Alkasir, R., Yin, J., Han, B. and Su, J. 2013. Isolation and characterization of small-colony variants of *Ornithobacterium rhinotracheale*. *Journal of Clinical Microbiology*, **51**(10): 3228-3236.

Zorman-Rojs, O., Zdovc, I., Bencina, D. and Mrzel, I. 2000. Infection of turkeys with *Ornithobacterium rhinotracheale* and *Mycoplasma synoviae*. *Avian Diseases*, **44**: 1017-1022.