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Occurrence of *Ornithobacterium rhinotracheale* in Polish turkey flocks

Olimpia Kursa✉, Grzegorz Tomczyk, Anna Sawicka-Durkalec

Department of Poultry Diseases, National Veterinary Research Institute, 24-100 Puławy, Poland
olimpia.kursa@piwet.pulawy.pl

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Abstract

Introduction: *Ornithobacterium rhinotracheale* (ORT) causes significant economic losses to the poultry industry around the world. The bacterium often affects poultry as part of multiple infections causing very serious clinical signs that are usually not limited only to the respiratory system. This study's main objective was the retrospective detection and identification of ORT in turkey flocks. **Material and Methods:** ORT identification was performed in 6,225 samples taken from 133 different flocks between 2015 and 2020. Molecular methods were used, specifically real-time PCR and traditional PCR. We focused on partial 16S rRNA gene sequences of isolates, which were compared with sequences obtained from GenBank. The reaction products were analysed phylogenetically. Molecular methods indicating secondary infections was carried out, and the bacterial composition of the upper respiratory tract was 16S metasequenced for selected flocks to identify any other pathogens. **Results:** The presence of ORT was detected in 30.83% of samples by real-time PCR and 28.57% by PCR. Phylogenetic analysis of the PCR products from the turkeys samples showed that their sequences resolved into two main genetic groups. Tests for the occurrence of secondary infections showed the presence of *Mycoplasma gallisepticum* and *M. synoviae* in some samples but the total absence of *Bordetella avium*. The upper respiratory tract in turkeys was dominated by two major phyla Firmicutes and Proteobacteria. At the genus level, the genera *Ornithobacterium, Mycoplasma, Gallibacterium, Avibacterium*, and *Escherichia-Shigella* were found which may include pathogenic bacteria that can cause clinical symptoms. **Conclusion:** The results of the analysis of multiple infection carried out in flocks with respiratory signs are probably associated with outbreaks of ornithobacteriosis in turkey flocks in Poland.

Keywords: turkey, *Ornithobacterium rhinotracheale*, phylogenetic analysis, 16S rRNA.

Introduction

Infections of poultry with *Ornithobacterium rhinotracheale* (ORT) cause respiratory disease, arthritis, decreased egg production and egg quality, and also increased mortality (40). This Gram-negative bacterium was first described in South Africa in 1991 as an aetiological agent of a poultry respiratory disease with accompanying increased mortality and poor performance parameters (39, 40). The first ORT strains were isolated in Hungary, Germany and the Netherlands (16, 40, 43). In the 1990s, ORT spread across the world and was isolated in Israel, Belgium, France, the UK and the USA (6, 7, 24, 44). Transmission of ORT can be horizontal *via* direct contact and this pathogen can be a primary or secondary aetiological agent depending on the strain virulence and the immune status of the host (43). Factors increasing the severity of ORT infections are poor management, high stocking density, high levels of ammonia and inadequate ventilation, and the occurrence of other respiratory coinfections such as *Escherichia coli*, *Mycoplasma gallisepticum*, *Mycoplasma synoviae*, *Bordetella avium* or *Chlamydophila psittaci* (1, 8, 10, 25, 36, 42). Viral respiratory pathogens such as avian metapneumovirus, infectious bronchitis virus, Newcastle disease and avian influenza virus also predispose infected birds to display more extensive clinical signs (25, 29, 43). Ornithobacteriosis causes respiratory tract lesions in chickens, turkeys, geese and ducks and in a wide range of wild birds such as pigeons, pheasants, quail, gulls, partridges, rooks and falcons (12, 37, 40). In recent years, outbreaks of infection with this bacteria with serious economic consequences have been reported all over the world, including Germany, Hungary, the Netherlands, Iran, Algeria, Brazil, New Zealand, the USA, Japan and Peru (3, 5, 6, 11, 14, 27, 32, 35, 38).

Serological studies have identified the presence of at least 18 different serotypes and these have the designations A–R. Serotype A has been the most frequently isolated strain in chickens so far, whereas

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serotypes A, B, D and E have been isolated in turkeys. Some serotypes such as F, K and M are isolated in both chickens and turkeys as serotype A is, but sporadically (41). However, serotyping is complicated by cross-reactivity between strains and inconsistent results (35). Differentiation of strains based on phenotypic methods is also not completely satisfactory because attempts at such differentiation have often given inconclusive results or suggested atypical strains (5). The use of molecular techniques has overcome the problematic heterogeneity of the results offered by other methods and contributed to a better understanding of the phylogenetic relationships of ORT. The great variety of species and subspecies of the genus *Ornithobacterium* validates the partial sequencing of the 16S rRNA gene as a means of comparing isolates from poultry from different countries as well as from different avian hosts (1, 3).

The occurrence of ornithobacteriosis in turkey flocks is of high significance to the poultry industry. Monoinfections with ORT are more aggressive in turkeys than in hens and therefore represent a very important problem in broiler turkey flocks, which does not spare Polish turkeys farmers. The prevalence of ORT in turkeys (41%) is much higher than in chicken broilers (6.9%) (15). This difference is particularly salient for the Polish poultry industry, considering that Poland is one of the largest producers of turkey meat in the EU. The lesions that are observed when turkeys are infected with ORT are often flattened tracheal mucosa, with reddish or haemorrhagic spots and accumulation of mucus. Blood may be excreted through the oral cavity from haemorrhagic lesions in the lungs (3).

To our knowledge, this is the first molecular characterisation of ORT sequences isolated from Polish turkey flocks. So far, only a serological analysis of ORT has been published and it dates back to 2000 (47). Mixed infections with different pathogens or two types of *Ornithobacterium* may influence the clinical course of the disease. Epidemiologically, it is highly advantageous to know if ORT multiple infections are present, and therefore, to determine such we used 16S rRNA sequencing. To partially address the paucity of data concerning ORT in Polish turkey flocks, this study investigated bacterial community of upper respiratory tract of turkeys with clinical signs.

Material and Methods

Sampling procedures. During the period 2015–2020, tracheal swab or tracheal tissue samples were collected from 133 turkey flocks all over Poland. The number of samples used in this study is presented in Table 1. Trachea tissues were aseptically obtained from birds sent for diagnostic purposes. Swab samples were brought to the Department of Poultry Diseases at the National Veterinary Research Institute in Poland as part of a routine diagnostic test and monitoring programme. In some flocks, clinical signs had been observed in the respiratory tract.

DNA extraction. Genomic DNA was extracted from trachea swab samples using a QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's recommendations. Tissue samples were pooled separately into Eppendorf tubes containing Tris-EDTA buffer and processed for DNA extraction. The samples were frozen at −20°C until further analysis. DNA was extracted from the tissue using Maxwell RSC Tissue DNA kits (Promega, Madison, WI, USA) according to the manufacturer's protocol and its quantity and quality were determined using the NanoDrop 1000 spectrometry system (Thermo Scientific, Waltham, MA, USA). The negative control was the Tris-EDTA used for sample preparation.

Real-time PCR. For the detection of the 16S rRNA gene of ORT, a real-time PCR was performed as described by Abdelwhab *et al.* (2) with minor modifications. The reaction was carried out in a total volume of 25 µL using a 12,5 µL of QuantiFast Probe PCR Kit (Qiagen), 1.3 µL of each 10 µM primer, 0.5 µL of probe, 7.4 µL of distilled water, and 2 µL of DNA in an ABI 7500 thermal cycler (Applied Biosystems, Foster City, CA, USA) under the following conditions: 95°C for 3 min and 40 cycles of 95°C for 3 s. The fluorescence data were collected during an annealing and extension step at 60°C for 32 s.

Traditional PCR and sequence analysis. The PCR was conducted according to van Empel and Hafez (40) using previously described specific ORT primers which amplify a partial 784 bp region of the 16S rRNA. The PCR assays were performed on positive samples obtained in the real-time PCR. The reaction mixture contained Taq PCR Master Mix (Eurx, Gdańsk, Poland) in a volume of 12.5 µL, 1.5 µL of each 10 µM primer and 7.5 µL of distilled water with the addition of 2 µL of DNA to give a total reaction volume of 25 µL. The PCR procedure included an initial incubation at 95°C for 1 min, 35 cycles of 95°C for 40 s, annealing at 50°C for 40 s, and extension at 72°C for 40 s, with a final extension at 72°C for 2 min. The PCR amplicons were separated by electrophoresis on a 2% agarose E-gel plate (Invitrogen, Carlsbad, CA, USA) containing ethidium bromide and visualised by ultraviolet transillumination. The identification of ORT was confirmed by sequencing the amplified fragments. Selected PCR products were sent for sequencing by the Sanger method to a commercial service (Genomed, Warsaw, Poland). Closely related sequences of ORT were downloaded from GenBank. Multiple sequence alignments were established and phylogenetic trees were constructed using ClustalW in MEGA 7 software and the neighbour-joining tree inference method, with evolutionary distances computed using the maximum likelihood method with 1000 bootstrap replicates (21).

Nucleotide sequence accession numbers. The nucleotide sequences from this study have been submitted to GenBank and assigned accession numbers MW298686–MW298723.

Table 1. Number of samples used in this study

Year	Number of flocks	Samples (flocks)		Number of positive flocks (%) in real-time PCR	Number of positive flocks (%) in PCR	Flocks with signs
		Swabs	Tissue			
2015	67	66	1	11 (16.42)	10	3
2016	17	10	7	6 (35.29)	5	5
2017	14	14	-	0	0	0
2018	10	8	2	6 (60)	6	2
2019	20	17	3	14 (70)	13	3
2020	5	3	2	4 (80)	4	0
Total	133	118	15	41 (30.83)		13

Statistical analysis. Chi-squared analysis was used to determine the statistical significance of differences between the PCR and real-time PCR tests' results, and P values of <0.05 were considered statistically significant. Statistical analyses were performed using the Social Science Statistics program (www.socscistatistics.com).

Presence of other avian pathogens. Turkeys positive for ORT from field outbreaks were tested for *Mycoplasma gallisepticum* (MG) targeting the mgc2, *Mycoplasma synoviae* (MS) targeting the 16S–23S rRNA region in a real-time PCR according to Raviv and Kleven (30), *Mycoplasma meleagridis* (MM) targeting 16S rRNA in a PCR, and *Bordetella avium* (BA) also targeting 16S rRNA in a PCR. Swab samples from up to five birds were pooled. Additionally, to confirm multiple infections, we characterised total bacterial communities from 13 selected positive samples using PCR amplification with universal primers for the V3–V4 hypervariable regions of the 16S rRNA gene, and used the purified product for MiSeq library preparation and sequencing as previously described using MiSeq (Illumina, San Diego, CA, USA) (20). The 16S rRNA gene taxonomy was assigned using Krona charts that allow comparison between bacterial communities based on detailed phylogenetic composition and identification of differential abundances of operational taxonomic units (OTUs) and other phylogenetic classifications (28). The Krona charts were generated using the quantitative insights into microbial ecology krona_qiime.py package from Qiime2 (4) (https://github.com/lokeshbio/AmpliSeq/blob/master/Qiime2_pipeline_IT_EMP.md#krona-plots). The sequences were clustered into OTUs using dada2 allowing regions of sequences below quality score 15 to be removed. The chimeric sequences were filtered and the reads were corrected. A trained Silva 132 99% OTU (full-length) classifier was used to assign taxonomy to sequences (31). DNA samples for the 16S gene sequencing were selected from the flocks where clinical symptoms such as respiratory problems were present, the presence of ORT was confirmed, and DNA quality allowed for further analysis.

Results

Real-time PCR for ORT detection. The percentage of positive samples was 30.83%, and the cycle threshold (Ct) range was from 19.45 to 36.9 (mean Ct 30.75) where amplifications below 37 cycles were considered positive for ORT. All real-time PCR-positive samples were also tested by traditional PCR. Positive samples were detected in the Warmińsko-Mazurskie, Wielkopolskie, Lubelskie, Kujawsko-Pomorskie, Lubuskie and Śląskie provinces (Fig. 1).

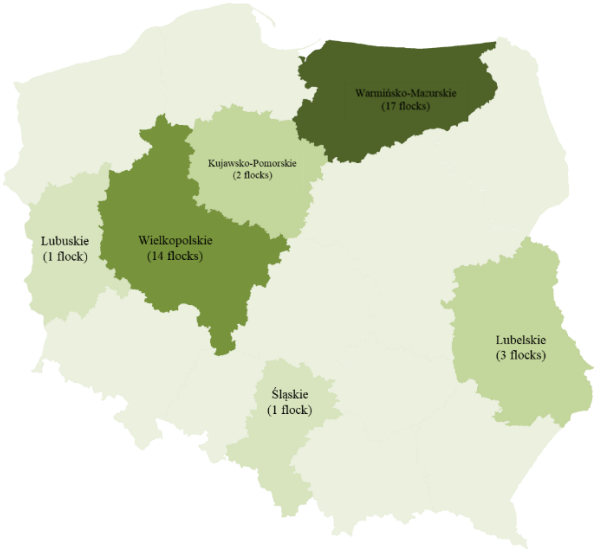
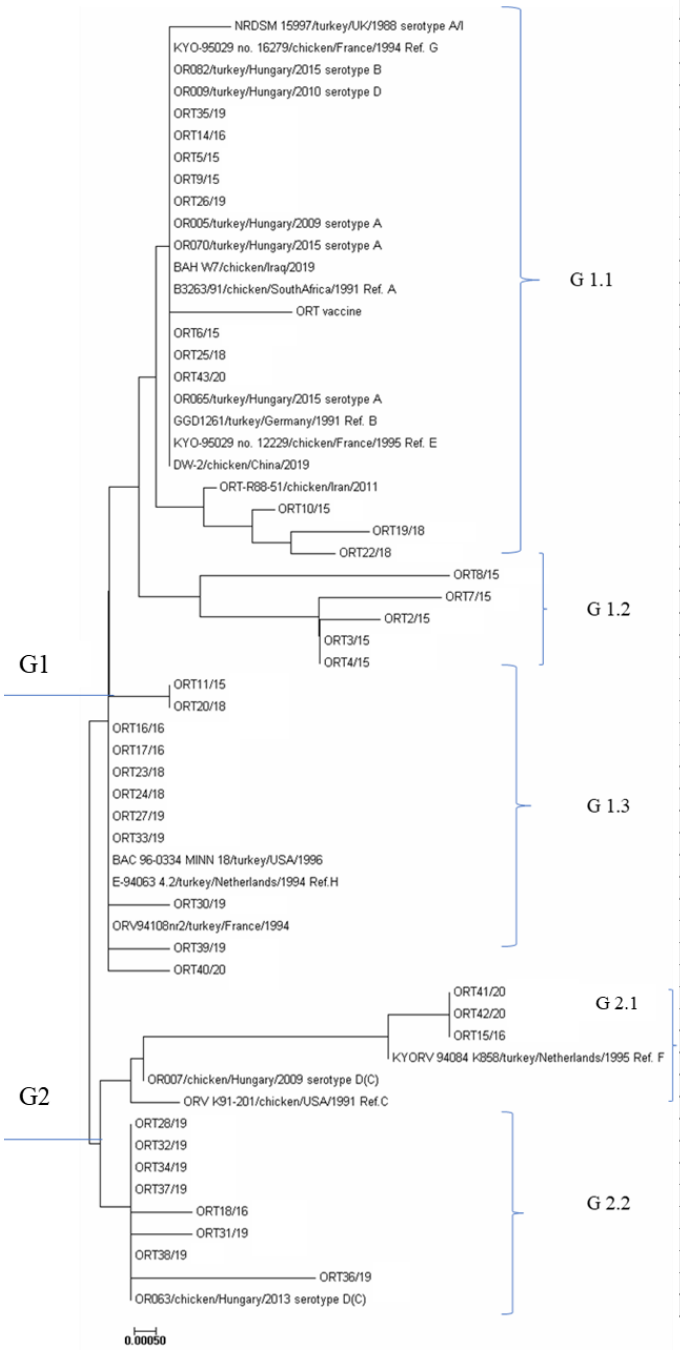


Fig. 1. Map of Poland showing the number of positive cases (n = 38) of ORT per province in turkey flocks between 2015 and 2020

Traditional PCR for ORT detection and sequence analysis. The 16S rRNA gene of ORT was successfully amplified by PCR reactions in 28.57% of turkey flocks. There were no significant differences between the traditional PCR and real-time PCR results (P>0.05).We performed a phylogenetic analysis of 38 selected sequences and obtained scores for the 16S rRNA partial gene with the NCBI BLAST tool showing between 98% and 100% identity with ORT. The phylogenetic tree is shown in Fig. 2, and arranges the ORT sequences in two main genetic groups by nucleotides. One of the most frequent allocations was to genetic group G1, which was divided into three subgroups. The first, the G1.1 subgroup, contained 11 sequence isolates from tissue and swab samples which were closely related to ORT detected in chickens from France, Iraq, South Africa, China and Iran (accession nos. KY809792, MN931657, KX998702, KX998704, MN023015 and JF810493) and turkeys from Hungary and Germany (accession nos. KX998701, KX998672, KX998668, KX998697, KX998692 and KX998705).



Strain designation	Coutry	Host	Year of isolation	Acession number
NRDSM 15997	UK	turkey	1988	WR102940
KYO-95029 16279	France	chicken	1994	KY809792
OR082	Hungary	turkey	2015	KX998701
OR009	Hungary	turkey	2010	KX998672
ORT35/19	Poland	turkey	2019	MW298715
ORT14/16	Poland	turkey	2016	MW298696
ORT5/15	Poland	turkey	2015	MW298689
ORT9/15	Poland	turkey	2015	MW298693
ORT26/19	Poland	turkey	2019	MW298707
OR005	Hungary	turkey	2009	KX998668
OR070	Hungary	turkey	2015	KX998697
BAH W7	Iraq	chicken	2019	MN931657
B3263/91	South Africa	chicken	1991	KX998702
ORT vaccine	Poland	turkey	2020	MW298715
ORT6/15	Poland	turkey	2015	MW298690
ORT25/18	Poland	turkey	2018	MW298706
ORT43/20	Poland	turkey	2020	MW298723
OR065	Hungary	turkey	2015	KX998692
GGD1261	Germany	turkey	1991	KX998705
KYO-95029 12229	France	chicken	1995	KX998704
DW-2	China	chicken	2019	MN023015
ORT-R88-51	Iran	chicken	2011	JF810493
ORT10/15	Poland	turkey	2015	MW298694
ORT19/18	Poland	turkey	2018	MW298701
ORT22/18	Poland	turkey	2018	MW298703
ORT8/15	Poland	turkey	2015	MW298692
ORT7/15	Poland	turkey	2015	MW298691
ORT2/15	Poland	turkey	2015	MW298686
ORT3/15	Poland	turkey	2015	MW298687
ORT4/15	Poland	turkey	2015	MW298688
ORT11/15	Poland	turkey	2015	MW298695
ORT20/18	Poland	turkey	2018	MW298702
ORT16/16	Poland	turkey	2016	MW298698
ORT17/16	Poland	turkey	2016	MW298699
ORT23/18	Poland	turkey	2018	MW298704
ORT24/18	Poland	turkey	2018	MW298705
ORT27/19	Poland	turkey	2019	MW298708
ORT33/19	Poland	turkey	2019	MW298713
BAC 96-0334MIN18	USA	turkey	1996	KY809794
E-940634.2	Netherlands	turkey	1994	KY809793
ORT30/19	Poland	turkey	2019	MW298710
ORV94108nr2	France	turkey	1994	KX998706
ORT39/19	Poland	turkey	2019	MW298719
ORT40/20	Poland	turkey	2020	MW298720
ORT41/20	Poland	turkey	2020	MW298721
ORT42/20	Poland	turkey	2020	MW298722
ORT15/16	Poland	turkey	2016	MW298697
KYORV 94084K858	Netherlands	turkey	1995	KY809791
OR007	Hungary	chicken	2009	KX998670
ORVK91-201	USA	chicken	1991	KY809788
ORT28/19	Poland	turkey	2019	MW298709
ORT32/19	Poland	turkey	2019	MW298712
ORT34/19	Poland	turkey	2019	MW298714
ORT37/19	Poland	turkey	2019	MW298717
ORT18/16	Poland	turkey	2016	MW298700
ORT31/19	Poland	turkey	2019	MW298711
ORT38/19	Poland	turkey	2019	MW298718
ORT36/19	Poland	turkey	2019	MW298716
OR063	Hungary	chicken	2013	KX998690

Fig. 2. Phylogenetic tree showing the relationship of *O. rhinotracheale* sequences from GenBank (n = 20), sequences used in this study (n = 38) and a sequence of the commercial *Ornithobacterium rhinotracheale* (ORT) vaccine strain in the Ornitin product from Abic Polska, based on the neighbour-joining analysis of their 16S rRNA gene sequences

One vaccine strain sequence had high similarity to sequences in this group. In the second, the G1.2 subgroup, there were only five Polish sequences, which had been isolated from swab samples in 2015. Lastly, in the third, the G1.3 subgroup, there were eleven amplicons found in swab and tissue samples from turkeys which were closely related to ORT detected in turkeys from the Netherlands, France and the USA (accession nos. KY809793, KX998706 and KY809794).

Group 2 (G2), the second main branch, included two subgroups. In the first subgroup, G2.1, there were three sequences isolated from swab samples that were similar to a Dutch sequence from turkeys (accession no. KY809791) and also to Hungarian and US American sequences from chickens (accession nos. KX998670 and KY809788). Eight Polish sequences were classified to the second and last subgroup, G2.2. They were closely related to a sequence isolated from chickens from Hungary (accession no. KX998690).

Detection of other pathogens. The presence of MG DNA was demonstrated in tracheal swabs collected from 13 turkey farms positive for ORT and the presence of MS DNA was confirmed in eight flocks. No genetic material of MM and BA was found in any of the

investigated flocks. Secondary infections in ORT-positive samples were detected in ORT2-9/15, in which MS was found, and in ORT27/19, ORT28/19 and ORT30-39/19, in which MG was identified.

In this study to identify bacterial multiple infections, 13 samples from flocks with typical respiratory clinical signs were sequenced on MiSeq (Illumina) using the 16S rRNA V3–V4 region. In every sample, ORT taxa were detected in relative abundances which differed widely. Firmicutes (54.92%), Proteobacteria (34.22%), Bacteroidetes (7.63%), Actinobacteria (1.7%) and Tenericutes (1.13%) were the most abundant phyla. Others were also identified in low abundance (less than 0.01%), and these included Verrucomicrobia, Acidobacteria, Dependentiae and Patescibacteria. In six flocks bacteria from an unclassified phylum were also detected in low abundance (Fig. 3).

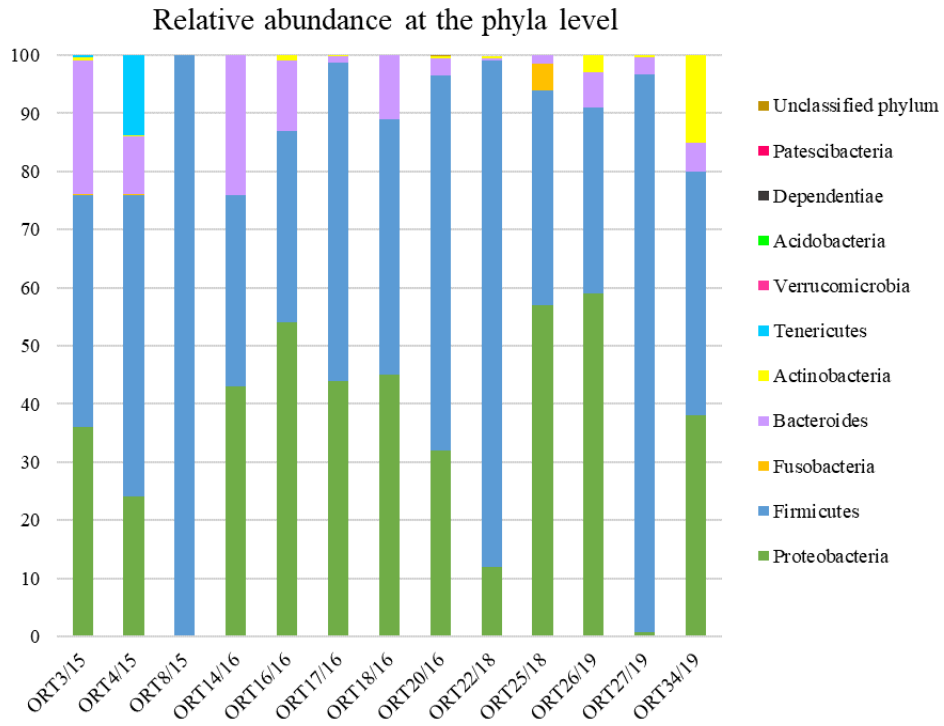


Fig. 3. Relative abundance of all organisational taxonomic units at the phylum level

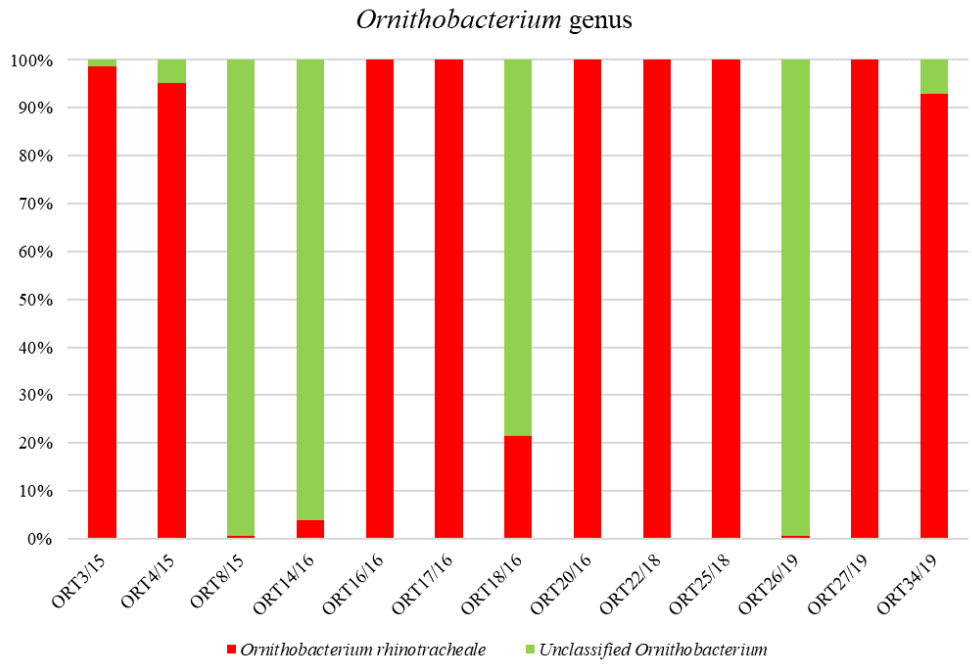


Fig. 4. Relative abundance in the *Ornithobacterium* genus

The abundance data at the family level show the presence of 87 taxa. The *Enterobacteriaceae* family (18.62% ± 20.27) was the most common in the *Proteobacteria* phylum. In descending order of frequency, the other families were *Pasteurellaceae* (6.48% ± 12.77), *Moraxellaceae* (5.26% ± 15.36), *Neisseriaceae* (1.57% ± 3.16), *Xanthomonadaceae* (1.23% ± 3.97) and *Pseudomonadaceae* (0.94% ± 2.91). This phylum also included bacteria that were not classified below the Betaproteobacteria (ORT27/19) or Gammaproteobacteria classes (ORT17/16 and ORT4/15). In the *Firmicutes* phylum, the most common family was *Enterococcaceae* (31.91% ± 33.19), followed by *Streptococcaceae* (8.01% ± 14.83), *Carnobacteriaceae* (6.84% ± 21.71) and *Lactobacillaceae* (4.19% ± 5.13). In this phylum, unclassified Lactobacillales (0.46% ± 1.15) were observed (ORT22/18, ORT3/15 and ORT4/15). The most common family in the *Bacteroides* phylum was *Weeksellaceae* (7.35% ± 7.78) and uncultured *Chitinophagaceae* were also identified (ORT3/15). However, in the *Actinobacteria* phylum the *Micrococcaceae* family was detected in relative abundance (1.52% ± 4.25) and uncultured *Actinobacterium* was observed (ORT16/16).

The Ornithobacterium genus was represented by *Ornithobacterium rhinotracheale* in all bacterial communities and all 13 samples. Additionally, unclassified Ornithobacterium were also identified in 7 samples (Fig. 4).

The *Mycoplasma* genus was found in five samples (ORT3/15, ORT4/15, ORT20/16, ORT26/16 and ORT27/19). In some samples *Mycoplasma* were present only in an average abundance of less than 0.001%.

Discussion

Infections with ORT can occur in a variety of bird species, but in turkeys they pose a greater problem. In this study, the presence of ORT was not associated with a health problem in most of the tested flocks, which confirms that infection when it is the primary pathogen occurs subclinically in the host.

Analysis of the tracheal swabs and tissue revealed that 30.83% of the flocks were positive in real-time PCR (Fig. 1). Phylogenetic analysis of the 16S rRNA gene showed that 38 Polish isolates from samples collected between 2015 and 2020 were similar to sequences registered in GenBank. The sequences were 98–100% homologous, which is similar to the findings of other studies (26, 35). All the compared strains clustered into two main groups. Most strains were assigned to G1 (n = 27) (Fig. 2). Our results are similar to those obtained in Hungary and Mexico (26, 35). The isolates were assigned to G1 together with sequences from turkeys isolated in the 1990s from Germany in 1991, the USA in 1996, and the Netherlands and France in 1994. In G1 there were also sequences isolated from chickens from South Africa in 1991 and France in 1994 and 1995. This group was also the place for a sequence of the Ornitin vaccine strain used for flock vaccination on turkey farms and containing a mix of the three ORT serotypes A, B and C. This group also contains sequences isolated in Hungary in 2009, 2010 and 2015, Iran in 2011, China in 2019 and Iraq in 2019. The second group (G2) contains Polish sequences similar to sequences isolated from chickens from the USA in 1991 and Hungary in 2009 and 2013. There was also one sequence isolated from turkeys from the Netherlands in 1995. Analysis of the 16S rRNA sequences did not differentiate the isolates obtained from poultry into separate groups for those from chickens and those from turkeys. Group G2 was smaller and sequences numbering only 11 were assigned to this group. In this study, there was no clear relationship between the year of isolation and the group.

Many bacterial pathogens present in the avian host may not give clinical signs of the associated disease when the pathogens are monoinfections, but the occurrence of secondary infections may enhance their virulence and prompt the manifestation of clinical symptoms (10, 15, 18). Most clinical cases of coinfection do not have a clear explanation of the mechanisms in which microorganisms interact during the development of infection. A host's response to infection with one pathogen can determine its response to infection with a second pathogen, such that the course of the disease may be more virulent or be subclinical (9, 19, 40). Various combinations of several infectious bacterial and viral pathogens may be responsible for respiratory disease in poultry, and often this disease has just such a multifactorial aetiology. The most common respiratory tract coinfections with ORT in turkeys are *E. coli*, *Gallibacterium anatis*, *Bordetella avium*, *Pasteurella multocida*, *Proteus* spp. and *Staphylococcus* spp. (3, 15). Various coinfections of ORT with other bacterial and viral pathogens have also been reported (27, 31, 42). However, the pathogenicity of a microorganism in a coinfection depends on its type and strain. Simultaneous infections of the respiratory tract with ORT and MG, the most important a etiological agents, have significant economic impact on worldwide poultry production; the association of ORT infection with chronic respiratory disease (18) being just one example of this problem. It is well known that the additional presence of pathogenic mycoplasmas such as MG and MS can induce the appearance of clinical symptoms (22, 34). In this study, 20 flocks of turkeys were positive for *Mycoplasma* spp. The presence of *Mycoplasma gallisepticum* and *M. synoviae* was found in samples collected from ORT natural outbreaks. The outbreak flocks had clinical symptoms from the respiratory tract such as airsacculitis and neurological signs. Similar symptoms were observed in birds infected experimentally with ORT and MG (33).

Many pathogens associated with respiratory infections in poultry have been detected by sequencing

the 16S rRNA gene (17, 23, 46). However, the respiratory tract of turkeys may contain bacteria the role of which is unclear or completely unknown, such as those which were not classified below the Betaproteobacteria class – a class which includes more than 400 species (45). Other unelucidated turkey respiratory tract bacteria were those identified only to the class level as Gammaproteobacteria, which includes groups of bacteria of high importance in medicine, ecology and science, such as the *Salmonella*, *Yersinia*, and *Pseudomonas* genera and *E. coli* species. Further study is needed to understand the influence of unidentified bacteria on the development of respiratory diseases in turkeys and other birds.

In samples in which the 16S rRNA gene was sequenced, the presence of diverse communities of members of the *Ornithobacterium* genus was detected. The taxa of *Ornithobacterium rhinotracheale* and unclassified *Ornithobacterium* were identified in samples from seven flocks, and in four of them the genus content was dominated by unclassified *Ornithobacterium* species (Fig. 4). The presence of multiple *Ornithobacterium* species at the same time can impact the virulence of the infection at supraspecific level in exposed hosts (40). Future work will be aimed at identifying *Ornithobacterium* species and amassing information about multiple ORT infections. The respiratory tract is one of the main routes of entry into the bird for a variety of microorganisms, and understanding the diversity of coinfections during ORT infection will help to improve treatment and prevention of infections with these pathogens. The use of metasequencing methods to study the bacterial diversity of samples represents an important step in advancing the knowledge of turkey respiratory coinfections.

Conflict of Interests Statement: The authors declare that there is no conflict of interests regarding the publication of this article.

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