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Original Article

Diagnosis of Gallibacterium Anatis in Layers: First Report in Turkey

■Author(s)

Yaman S[I] https://orcid.org/0000-0002-9998-3806
Sahan Yapicier O[II] https://orcid.org/0000-0003-3579-9425

[I] Burdur Mehmet Akif Ersoy University, The Health Sciences Institute, Department of Microbiology, 15030, Burdur, Turkey.
[II] BurdurMehmet Akif Ersoy University, Faculty of Veterinary Medicine, Department of Microbiology, 15030, Burdur, Turkey.

■Mail Address

Corresponding author e-mail address
Ozlem ŞAHAN YAPICIER, DVM, PhD
Faculty of Veterinary Medicine, Burdur Mehmet Akif Ersoy University, Department of Microbiology, 15030, Burdur, Turkey.
Phone: +90 248 2132064
Email: ozlemsahan@mehmetakif.edu.tr

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Gallibacterium anatis, isolation and identification, layers, PCR.

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ABSTRACT

Gallibacterium anatis, a member of the Pasteurellaceae family, leads to decrease in egg-production, animal welfare and increase in mortality. This study-aimed to diagnose *G. Anatis*, which caused economic losses in laying hens by using conventional and molecular techniques. In this study, *G. anatis* was examined from a total of 200 dead chicken tissues (heart, liver, lung, spleen and trachea) in laying hen farms that observed a decrease in egg production with respiratory system infection. Conventional methods based on colony morphology, sugar fermentation tests and hemolytic properties and molecular conformation using 16S rRNA-23S rRNA specific primers were performed to identify *G. anatis*. *G. anatis* was isolated in 20 (10%) of the examined samples and isolates were confirmed by conventional PCR. A total of 11 (2.2%) positivity was obtained as isolates were the result of PCR performed on tissues and organs directly. As a result, the presence of *G. anatis* was detected for the first time in Turkey by this study. It was thought that *G. anatis* may have a role in egg production losses due to respiratory tract infection in poultry and this situation may be a guide for poultry clinicians and microbiologists.

INTRODUCTION

Major health problems of the poultry industry have certain effects on egg production. Especially, infectious diseases which may drop in egg production and egg quality by affecting the reproductive system directly and the health status of poultry indirectly (Clauer, 2009).

Gallibacterium anatis (G. anatis) has been known to be a part of the normal microflora of the lower genital and upper respiratory tract (Bojesen *et al.*, 2004; Jones *et al.*, 2013; Lawal *et al.*, 2018; Persson & Bojesen 2015; Rzewuska *et al.*, 2007). Paudel *et al.*, 2013. In recent years, decreased egg production associated with oophoritis, follicule degeneration, salpingitis, respiratory system disorders and increased mortality in commercial layers has accelatered interest in *G. anatis* infections (Alispahic *et al.*, 2011; Bisgaard *et al.*, 2009; Bager *et al.*, 2013; Bojesen *et al.*, 2003; Bojesen, 2003; Sing, 2016; Chaveza *et al.*, 2017; Johnson *et al.*, 2013; Paudel *et al.*, 2014). The epidemiology and bacteria-host interactions of *Gallibacterium* spp. are little understood due to a lack of published literature and previous uncertainty with regard to the identification of bacteria representing this genus (Bisgaard, 1993).

The aim of this study was to investigate the *Gallibacterium anatis* from commercial layers that suffered respiratory tract disease and decrease in egg production as well as determine a convenient microbiological and molecular diagnostic technique.

MATERIALS AND METHODS

G. anatis strains

G. anatis F149T (non-hemolytic strain, ATCC 43329) and 12656-12 strain (hemolytic strain) were obtained from Prof. Anders Miki Bojesen (Department of Biology, Department of Veterinary Diseases, Copenhagen University) and used in this study.

Sampling

G. anatis was examined from in a total of 200 dead hens tissue samples (heart, liver, lung, spleen and trachea) were collected from 31 commercial layer houses from three different cities (Afyonkarahisar, Kütahya, Gaziantep) during the period from August 2017 to January 2018 in Turkey. The number of samples collected are summarized in Table 1.

Table 1 – Layer houses where samples were collected

Flocks	Breed	Age(week)	Number of sampled animal
1	Lohmann white	24	5
2	Lohmann white	76	3
3	Lohmann white	64-66	2
4	Lohmann white	64-66	3
5	Lohmann white	16	3
6	Supernick	18	19
7	Lohmann white	60	17
8	Lohmann white	65	4
9	Lohmann white	55	4
10	Lohmann white	85	12
11	Lohmann white	12	16
12	Lohmann white	52	2
13	Lohmann white	60	6
14	Lohmann white	85	11
15	Lohmann white	60	6
16	Lohmann white	48-50	6
17	Lohmann white	36	4
18	Lohmann white	70	5
19	Atak-S	32	2
20	Lohmann white	68	4
21	Supernick	75	14
22	Supernick	19	5
23	Lohmann white	65	9
24	Lohmann white	75	7
25	Lohmann white	35	5
26	Lohmann white	66	4
27	Lohmann white	75	3
28	Lohmann white	57	4
29	Lohmann white	30	6
30	Nick chick white	16	5
31	Lohmann white	36	4
Total			200

A total of 10 to 45.000 flock sized, 12-85 week-old laying hens were housed in 60 x 60 cm cages (n:5-8 birds in each cage) in a 40x10m farm building. The litter of the poultry houses was of good quality, although ventilation by mechanical fans or windows was poor in some of these. Water and feed were provided *ad libitum*. Average body weight of birds was 1500-1600 g.

All of the examined dead birds included in the study had recent histories of respiratory disease and reproductive problems with a cumulative mortality rate during the week of sampling which ranged from0.4-0.7%.

Isolation and identification: Tissue samples were inoculated to 5% sheep blood (Oxoid, USA) and Mac-Conkey agar (Oxoid, USA). The plates were incubated at 37°C for 18-24 hours aerobically. Beta haemolytic, circular, smooth, shiny and greyish suspect colonies were stained by Gram staining and biochemical tests were performed to identify the Gram negative rods (Bager *et al.*, 2013; Bojesen & Shivaprasad, 2006). *Gallibacterium* isolates were suspensed in seven hundred microlitres were mixed with 300 µl sterile glycerol 50% and stored at -80 oC until further use (Bojesen *et al.*, 2003).

Molecular identification: DNA extraction from *G. anatis* isolates and tissues (heart, lung, trachea, spleen) was performed according to the instructions of the GeneJET Genomic DNA Purification Kit (Thermo Scientific, USA) and the QIAamp DNA Stool Kit (Qiagen, Hilden, Germany). DNAs were stored for use as template DNA at -20°C until amplification.

A primer pair specific for 16S-23S rRNA genes [1133F(5'-TATTCTTTGTTACCARCGG-3') and 114R (5'-GGTTTCCCCATTCGG-3')] of *G. anatis* were selected. PCR was performed with the default settings of the thermocycler (Nyx Technik, A6-00150, USA) and the PCR assay was carried out in a 25 µl reaction solution containing 3 µl MgCl (25 mM), 0.5 µl dNTP (10 mM), 10 pmols of primers and 0.2 µl Taq polymerase (5U/µl). The following cycling conditions were used: 3 min at 94°C, followed by 30 cycles of 1 min at 94°C (denaturation) and 1 min at 54°C (primer annealing), 1 min at 72°C (extension), and 7 min at 72°C (final extension).

The amplification products (790 bp and 1080 bp for the *G. anatis*) were examined by the separation of PCR products during electrophoresis on 1.5 % agarose gel stained with safe dye (Jena Bioscience, Germany).

RESULTS

Isolation and Identification

In the present study, 20(10%) *Gallibacterium* spp. were isolated from tissue and organ specimens (trachea, heart, liver, lungs and spleen) from 8 out

of 31 flocks. *Gallibacterium* spp. was isolated from lung (5%), heart (0.5%), liver (1%), and from trachea (3.5%) as shown in Table 2.

Table 2 – Isolation and identification results from the tissue samples.

Positive flocks	Lung	Spleen	Heart	Liver	Trachea
6	6(%3)	-	1(%0.5)	1(%0.5)	1(%0.5)
7	1(%0.33)	-	-	-	1(%0.5)
10	-	-	-	-	2(%1)
11	-	-	-	-	1(%0.5)
14	-	-	-	1(%0.5)	1(%0.5)
23	2(%1)	-	-	-	-
24	1(%0.5)	-	-	-	-
31	-	-	-	-	1(%0.5)
Total	10(%5)	-	1(%0.5)	2(%1)	7(%3.5)

According to the tissue samples collected from the different provinces, 25.8% was isolated from Afyonkarahisar, while no *Gallibacterium* spp. was isolated from Gaziantep or Kütahya.

Beta haemolytic *Gallibacterium* spp. isolates (Figure 1), all tested catalase-positive and 9 (45%) tested positive in an oxidase test. Based on the results of biochemical tests (Table 3), a total of 20 isolates were identified as *G. anatis*, and 10 (5%) *E. coli* isolates were found during *G. anatis* isolation from 200 layers. Of these *E. coli* isolates, 6 (60%) were isolated from the lungs, 3 (30%) were isolated from the trachea and 1 (10%) was isolated from the heart.

Figure 1 – *G. anatis* β-hemolytic colonies on sheep blood agar.

Molecular Diagnosis

Molecular diagnosis of biochemically confirmed *G. anatis* isolates (n=20) exhibit the desirable PCR product of 790 bp and 1080 bp size of 16S-23S rRNA primers (Figure 2). The conventional PCR directed to detect 11 (2.2%) *G. anatis* from lung (2.5%), trachea (2%), and liver (1%) of 200 layers revealed, the results of which are presented in Table 4.

Table 3 – *G. anatis* biochemical test results.

Isolates	Catalase	Oxidase	MacConkey	Sucrose	Arabinose	Lactose	Glucose	Sorbitol	Trehalose	Maltose	Fructose	Mannitol	Urea	Indole	ONPG
1	+	-	-	+	-	-	-	-	-	+	-	-	-	-	+
2	+	-	-	+	-	-	-	-	-	+	-	-	-	-	+
3	+	-	-	+	-	-	+	-	-	+	-	-	-	-	+
4	+	+	-	+	-	-	-	+	-	+	-	-	-	-	+
5	+	-	-	+	+	-	-	+	-	+	+	-	-	-	+
6	+	-	-	+	+	-	-	+	-	+	+	+	+	+	+
7	+	-	-	+	+	-	-	+	-	+	+	+	+	+	+
8	+	+	-	+	+	-	-	+	+	+	+	+	+	+	+
9	+	-	-	+	+	-	+	+	-	+	+	+	+	+	-
10	+	+	-	+	+	-	+	-	+	+	+	+	-	+	-
11	+	+	-	+	+	-	+	-	+	+	-	+	-	-	-
12	+	+	-	+	-	+	+	-	+	+	-	+	+	+	-
13	+	+	-	+	-	-	+	+	-	-	-	-	-	+	-
14	+	-	-	+	+	-	-	-	-	-	-	-	+	+	-
15	+	-	-	+	-	-	+	-	-	-	-	-	+	+	+
16	+	+	-	+	-	+	+	-	-	+	-	+	-	-	-
17	+	+	-	+	-	-	-	-	-	+	+	-	-	-	+
18	+	-	-	+	+	-	-	-	-	+	+	-	+	-	+
19	+	+	-	+	+	+	-	+	-	+	+	-	+	+	+
20	+	-	-	+	+	-	+	+	-	+	+	+	+	-	-

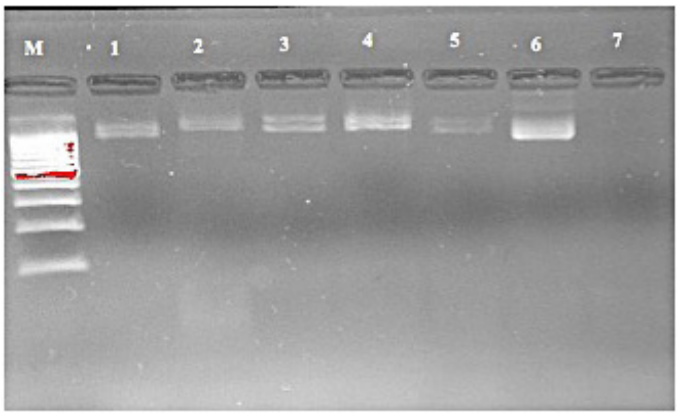

Figure 2 – PCR results of G.anatis (M=100bp marker; 1-5=positive isolates; 6= positive control; 7=negative control; 8-19=positive isolates; 20=positive control; 21= negative control).

Table 4 – PCR results obtained from tissues.

Positive flocks	Lung	Spleen	Heart	Liver	Trachea
6	2(%1)	-	-	1(%0.5)	1(%0.5)
7	1(%0.5)	-	-	-	1(%0.5)
10	-	-	-	-	-
11	-	-	-	-	1(%0.5)
14	-	-	-	1(%0.5)	1(%0.5)
23	2(%1)	-	-	-	-
24	1(%0.5)	-	-	-	-
31	-	-	-	-	-
Total	5(%2.5)	-	-	2(%1)	4(%2)

DISCUSSION

G. anatis is an infectious agent that has been isolated from broiler and egg-laying chickens with salpingitis and peritonitis in various countries around the world in recent years, and is associated with economic losses due to the resulting decline in egg yield (Bojesen *et al.*, 2003; Elbestawy *et al.*, 2018).

G. anatis can be found in European, African and Asian countries, but has also been reported in China, India, Japan, and North and South America (Singh *et al.*, 2016). No *G. anatis* infection has been reported in Turkey to date, and the present study is the first to report a prevalence rate of 10% in egg-laying chickens. It is thought that the reason why *G. anatis* has not been detected to date is due to the similarity of the symptoms of this infection to that of various respiratory tract infections, and particularly to the symptoms of fowl cholera, and the fact that the precise taxonomic classification of the bacteria was not established until 2003.

It has been reported that phenotypical characterization for *Gallibacterium* species (*G.* genomospecies 1 and 2) is difficult and time-consuming due to their heterogeneity (Alispahic *et al.*, 2011; El-Adawy *et al.*, 2018; Sing, 2016). The present study investigated the presence of *G. anatis* in tissues and organs collected from chickens showing symptoms of respiratory tract infection along with a decrease in egg yield. Conventional methods based on hemolysis and carbohydrate fermentation (Christensen *et al.*, 2003), and molecular methods based on the detection of 16S-23S rRNA sequences (Bojesen *et al.*, 2007) were preferred as the diagnostic tools. *G. anatis* was isolated and identified from 10% of the lung, spleen, heart, liver and trachea specimens obtained from 200 chickens. The rate of bacterial isolation on a material basis was 5% for lungs and 3.5% for trachea, which isolates particularly being identified in the respiratory tract organs, which is consistent with the findings reported in other studies (Bisgaard, 1977; Bojesen *et al.*, 2003; Mushin *et al.*, 1979). In their study, Bojesen *et al.* (2003) collected tracheal and cloacal swabs from infected flocks, and identified a high isolation rate for *G. anatis* in the tracheal swabs. Elbestawy *et al.*, (2018) identified six isolates of *G. anatis* (19.6%) in tracheal, ovarian and oviduct swabs obtained from egg-laying chickens with oophoritis, tracheitis, salpingitis and peritonitis. In a study conducted in China, Huangfu *et al.* (2012) collected tracheal, ovarian and oviduct samples and identified 33 (18.2%) isolates of *G. anatis*. In another study reported in Mexico, *G. anatis* isolates were identified from tracheal samples in 30%, and in egg follicules in 30% of 600 samples obtained from layer poultry houses (Chaveza *et al.*, 2017). *G. anatis* was detected in egg-laying chickens with symptoms of salpingitis in Iran (Ataei *et al.*, 2017). As was the case for Mexico and Iran, *G. anatis* was recently reported for the first time in Turkey (Ataei *et al.*, 2017; Chaveza *et al.*, 2017). Among the 31 investigated poultry houses located in the provinces of Afyonkarahisar, Gaziantep & Kütahya, only 8(25.80%) poultry houses were positive for the bacteria, all of which were located in Afyonkarahisar. It was considered that the high density of the egg-laying chicken population in this province compared to other provinces, and the fact that much of the sampling was particularly performed in this province, may explain the high isolation rate in Afyonkarahisar (Yumbir, 2018).

Molecular diagnostic methods have been widely used in the recent years for diagnosis and phenotyping, being fast, easy and with high specificity, sensitivity and reliability (Ataei *et al.*, 2017; Bojesen *et al.*, 2007). Similar to the studies of other researchers, the present study adopted the PCR method to confirm the identified *G. anatis* isolates and to further

examine the tissue and organ specimens that tested negative in the initial isolation tests (Bojesen *et al.*, 2010; Bisgaard *et al.*, 2009; Christensen *et al.*, 2003). Molecular confirmation of *G. anatis* was performed by using 16S rRNA-23S rRNA primers, which have previously been used in the literature and are known to be specific to *G. anatis* (Bojesen *et al.*, 2007). These primers are especially preferred for differentiating from other species in the *Pasteurellaceae* family that may cause diagnostic confusion (Christensen *et al.*, 2003). A conventional PCR confirmed 20 (10%) *G. anatis* isolates with bands at 790bp and 1080bp. A direct PCR analysis of organ and tissue samples revealed 11 (2.2%) *G. anatis*-specific bands. The PCR detection of *G. anatis* from 5 (2.5%) lungs, 2 (1%) hearts and 4(2%) trachea specimens, with simultaneous isolation of bacteria from the relevant specimens, is in parallel with the results of researchers who have conducted similar studies (Ataei *et al.*, 2017; Chavez *et al.*, 2017; Sorour *et al.,* 2015).

According to the information gathered from the poultry house owners, ventilation problems in the poultry houses where *G. anatis* was isolated and identified represented an important stress factor for the animals. It has previously been suggested that while *G. anatis* is found in the normal respiratory microflora of animals, it becomes the cause of an opportunistic respiratory tract infection when the immune system of the animal is compromised and/or due to stress and unfavorable changes in the care and nutritional intake of the animals (Bojesen *et al.,* 2003). The high rate of isolation from the lungs (5%) and trachea (7.5%) in the present study supports this hypothesis.

It was suggested that *G. anatis* could be the cause of both primary and secondary infections in animals, that *G. anatis* infections are often accompanied by *E. coli* infection, and that it is difficult to differentiate between these two microorganisms in animals with salpingitis and peritonitis (Bisgaard, 1977; Mirle *et al.*, 1991). In the present study, *E. coli* isolates 10 (5%) were recovered during *G. anatis* isolation from 200 chickens. In support of previous studies, *E. coli* was detected in respiratory tract organs, with 6 (60%) isolates recovered from the lungs and 3 (30%) isolates recovered from the trachea (Carlson & Whenham, 1968; Gross, 1961; Neubauer *et al.*, 2009).

It was concluded that to reduce the losses and to enhance productivity in poultry industry; other *Gallibacterium* species should be identified, the infection should be investigated in different age and breeding, the characteristics of the bacteria should be determined for future vaccines and additional studies to determine the sources of infection in terms of public health.

REFERENCES

Alispahic M, Christensen H, Hess C, Razzazi-Fazeli E, Bisgaard M, Hess M. Identification of *Gallibacterium* species by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry evaluated by multilocus sequence analysis. International Journal of Medical Microbiology 2011;301(6):513-522.

Ataei S, Bojesen AM, Amininajafi F, Ranjbar MM, Banani M, Afkhamnia M, *et al.*, First report of *Gallibacterium* isolation from layer chickens in Iran. Archives of Razi Institute Journal 2017;72(2):123-128.

Aubin GG, Haloun A, Treilhaud M, Reynaud A, Corvec S. *Gallibacterium anatis* bacteremia in a human. Journal of Clinical Microbiology 2013;51(11):3897-3899.

Bager RJ, Nesta B, Pors S.E, Soriani M, Serino L, Boyce JD, *et al.*, The fimbrial protein flfA from *Gallibacterium*anatis is a virulence factor and vaccine candidate. Infection and Immunity 2013;81(6):1964-1973.

Bisgaard M. Incidence of Pasteurella haemolytica in the respiratory tract of apparently healthy chickens and chickens with infectious bronchitis characterisation of 213 strains. Avian Pathology 1977;6(4):285-292.

Bisgaard M, Korczak BM, Busse HJ, Kuhnert P, Bojesen AM. Classification of the taxon 2 and taxon 3 complex of Bisgaard within *Gallibacterium* and description of *Gallibacterium* melopsittaci sp. nov., *Gallibacterium* trehalosifermentans sp. nov. and *Gallibacterium* salpingitidis sp. nov. International Journal of Systematic and Evolutionary Microbiology 2009;59(4):735-744.

Bojesen AM. *Gallibacterium* infection in chickens [thesis]. Denmark (DK): Department of Veterinary Microbiology the Royal Veterinary and Agricultural University; 2003.

Bojesen AM, Christensen JP, Nielsen OL, Olsen JE, Bisgaard M. Detection of *Gallibacterium*spp. in chickens by fluorescent 16S rRNA in situ hybridization. Journal of Clinical Microbiology 2003;41(11):5167-5172.

Bojesen AM, Nielsen OL, Christensen JP, Bisgaard M. In vivo studies of *Gallibacterium*anatis infection in chickens. Avian Pathology 2004;33(2):145-152.

Bojesen AM, Nielsen SS, Bisgaard M. Prevalence and transmission of haemolytic *Gallibacterium* species in chicken production systems with different biosecurity levels. Avian Pathology 2003;32(5):503-510.

Bojesen AM, Shivaprasad HL. Genetic diversity of *Gallibacterium* isolates from California turkey. Avian Pathology 2006;36(3):227-230.

Bojesen AM, Torpdahl M, Christensen H, Olsen JE, Bisgaard M. Genetic diversity of *Gallibacterium anatis* isolates from different chicken flocks. Journal of Clinical Microbiology 2003;41(6):2737-2740.

Bojesen AM, Vazquez ME, Bager RJ, Ifrah D, Gonzalez C. Antimicrobial susceptibility and tetracycline resistance determinant genotyping of *Gallibacterium anatis*. Veterinary Microbiology 2011;148(1):105-110.

Bojesen AM, Vazquez ME, Robles F, Gonzalez C, Soriano EV, Olsen JE, *et al.*, Specific identification of *Gallibacterium* by a PCR using primers targeting the 16S rRNA and 23S rRNA genes. Veterinary Microbiology 2007;123(1-3):262-268.

Carlson HC, Whenham GR. Coliform bacteria in chicken broiler house dust and their possible relationship to coli-septicemia. Avian Disease 1968;12(2):297-302.

Chaveza RFO, Barriosa RMM, Chaveza JFH, Mascarenoa JR, Escalantea JGAI, Yanesb MA. First report of biovar 6 in birds immunized against *Gallibacterium anatis* in poultry farms located in Sonora, Mexico. Veterinaria México Journal 2017;4(3):1-8.

Christensen H, Bisgaard M, Bojesen AM, Mutters R, Olsen JE. Genetic relationships among avian isolates classified as Pasteurella haemolytica, 'Actinobacillus salpingitidis' or Pasteurella anatis with proposal of *Gallibacterium anatis* gen. nov., comb. nov. and description of 31. additional genomospecies within *Gallibacterium* gen. nov. International Journal of Systematic and Evolutionary Microbiology 2003;53(Pt 1):275-287.

Clauer PJ. Why have my hens stopped laying? [cited 2017Apr 29]. Virginia: Poultry Extension Specialist, Animal and Poultry Sciences; 2009. Available from: http://www.pubs.ext.vt.edu/content.

El-Adawy H, Bocklisch H, Neubauer H, Hafez HM, Hotzel H. Identification, differentiation and antibiotic susceptibility of *Gallibacterium* isolates from diseased poultry. Irish Veterinary Journal 2018;71(5):1-10.

Elbestawy AR, Ellakany HF, S Abd El-Hamid H, Bekheet AA, Mataried NE, Nasr SM, *et al.*, Isolation, characterization, and antibiotic sensitivity assessment of *Gallibacterium anatis*biovar heamolytica, from diseased Egytian chicken flocks during the years 2013 and 2015. Poultry Science 2018;97(5):1519-1525.

Gross WB. The development of "air sac disease". Avian Disease 1961;5:431-436.

Huangfu H, Zhao J, Yang X, Chen L, Chang H, Wang X, *et al.*, Development and preliminary application of a quantitative PCR assay for detecting gtxA-containing *Gallibacterium* species in chickens. Avian Disease 2012;56(2):315-320.

Johnson TJ, Danzeisen JL, Trampel D, Nolan LK, Seemann T, Bager RJ, *et al.*, Genome analysis and phylogenetic relatedness of *Gallibacterium anatis* strains from poultry. PloS One 2013;8(1):1-12.

Jones KH, Thornton JK, Zhang Y, Mauel MJ. A 5-year retrospective report of *Gallibacterium anatis* and Pasteurella multocida isolates from chickens in Mississippi. Poultry Science 2013;92(12):3166-3171.

Lawal JR, Ndahi JJ, Dauda J, Gazali YA, Gadzama JJ, Aliyu AU. Survey of *Gallibacterium anatis* and its antimicrobial susceptibility pattern in village chickens (Gallus gallus domesticus) in Maiduguri, North-eastern Nigeria. International Journal of Veterinary Science and Medicine 2018;1(1):1-7.

Mirle C, Schöngarth M, Meinhart H, Olm U. Studies into incidence of Pasteurella haemolytica infections and their relevance to hens, with particular re- ference to diseases of the egg-laying apparatus. Monatsheftefuer Veterinaermedizin 1991;45:545-549.

Mushin R, Weisman Y, Singer N. Pasteurella haemolytica found in the respiratory tract of fowl. Avian Disease 1979;24(1):162-168.

Neubauer C, Souza-Pilz MD, Bojesen AM, Bisgaard M, Hess M. Tissue distribution of haemolytic *Gallibacterium anatis* isolates in laying birds with reproductive disorders. Avian Pathology 2009;38(1):1-7.

Paudel S, Alispahic M, Liebhart D, Hess M, Hess C. Assessing pathogenicity of *Gallibacterium anatis* in a natural infection model: the respiratory and reproductive tracts of chickens are targets for bacterial colonization. Avian Pathology 2013;42(6):527-535.

Paudel S, Liebhart D, Aurich C, Hess M, Hess C. Pathogenesis of *Gallibacterium anatis* in a natural infection model fulfils Koch's postulates: 2. Epididymitis and decreased semen quality are the predominant effects in specific pathogen free cockerels. Avian Pathology 2014;43(6):529-534.

Paudel S, Liebhart D, Hess M, Hess C. Pathogenesis of *Gallibacterium anatis* in a natural infection model fulfils Koch's postulates: 1. Folliculitis and drop in egg production are the predominant effects in specific pathogen free layers. Avian Pathology 2014;43(5):443-449.

Persson G, Bojesen AM. Bacterial determinants of importance in the virulence of *Gallibacterium anatis* in poultry. Veterinary Research 2015;46(1):1-11.

Rzewuska M, Karpinska E, Szeleszczuk P, Binek M. Isolation of *Gallibacterium* spp. from peacocks with respiratory tract infections. Medycyna Weterynaryjna 2007;63(11):1431-1433.

Sambrook J, Green MR. Molecular cloning: a laboratory manual. 4th ed. Cold Spring Harbor: Cold Spring Harbor Laboratory; 2012.

Sing SV. Studies on growth kinetics of *Gallibacterium anatis* in presence of deuterium oxide (D2O, heavy water) [thesis]. Izatnagar: Deemed University ICAR-Indian Veterinary Research Institute; 2016.

Singh SV, Singh BR, Sinha DK, Vinodh K, Prasanna VA, BhardwajM, *et al.*, *Gallibacterium anatis*: an emerging pathogen of poultry birds and domiciled birds. Journal of Veterinary Science and Technology 2016;7(3):1-7.

Sorour HK, Al Atfeehy NM, Shalaby AG. *Gallibacterium anatis* infection in chickens and ducks. Assiut Veterinary Medical Journal 2015;61(147):80-86.

Yum-Bir. Poultry sectoral data [cited 2018 Nov 6]. Ankara; 2017. Available from: http://www.yumbir.org/userfiles/file.

DECLARATIONS

Ethical approval and consent to participate

This study was approved by Animal Research Ethics Committee of Burdur Mehmet Akif Ersoy University (Protocol No. MAKU-HADYEK/ 2017-314) and the institutional ethics committee gave permission to collect samples from laying houses.

Consent for publication

Not applicable.

Availability of data and materials

We allow the use of data and materials upon request to the corresponding author.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

SY and OSY planned and designed the study. SY performed the experiments, SY and OSY contributed to the analysis and interpretation of data. SY and OSY

drafted the manuscript. All authors read and approved the final manuscript.

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