Research Article

Identification of an Immunogenic 38-40 kDa Protein in Indonesian *Gallibacterium anatis* Isolates

Raudatul Jannah[1], Agustin Indrawati[2*], Sri Murtini[2], Ryan Septa Kurnia[3], Christian Marco Hadi Nugroho[3], Muhammad Ade Putra[3]

[1]Master Program of Animal Biomedical Sciences, School of Veterinary Medicine and Biomedical Sciences, IPB University, Bogor, Indonesia; [2]Division of Medical Microbiology, School of Veterinary Medicine and Biomedical Sciences, IPB University, Bogor, Indonesia; [3]Indonesia Research and Diagnostic Unit, PT. Medika Satwa Laboratoris, Bogor, Indonesia.

Abstract | *Gallibacterium anatis* is a Gram-negative bacterium associated with salpingitis and peritonitis, which has recently emerged as a disease in poultry. Despite the identification of several virulence factors, the protein profile of Indonesian isolates remains uncharacterized. This study aimed to identify significant genes of *G. anatis* by quantitative PCR (qPCR) and to characterize its protein profiles by various extraction methods. SDS-PAGE was used to assess whole-cell protein and whole-cell protein extracted by heat, capsular, and sonication treatments to determine molecular weight. Western blotting was used to identify specific immunogenic proteins. The isolate grew well on blood agar, producing clear β-hemolytic zones, and qPCR confirmed the presence of three key virulence genes: *gyr*B, *flf*A, and *gtx*A. SDS-PAGE analysis revealed 34 visible protein bands in whole cells, while treated samples exhibited varying patterns, with sonication yielding the higest diversity. Western blotting detected a strong immunoreactive protein within the 38-40 kDa range, confirming its immunogenicity and indicating that it may be surface exposed despite bioinformatic prediction of a cytoplasmic location. These findings suggest the presence of a potentially moonlighting protein that may contribute to host immune recognition. The characterize of this immunogenic protein provides valuable insight into potential antigenic targets for vaccine or diagnostics development against *G. anatis* infection in poultry.

Keywords | *Gallibacterium anatis*, Emerging disease, Protein profiling, Immunogenic

Received | October 20, 2025; **Accepted** | November 19, 2025; **Published** | December 03, 2025
***Correspondence** | Agustin Indrawati, Division of Medical Microbiology, School of Veterinary Medicine and Biomedical Sciences, IPB University, Bogor, Indonesia; **Email:** raudajnnhraudatul@apps.ipb.ac.id
Citation | Jannah R, Indrawati A, Murtini S, Kurnia RS, Nugroho CMH, Putra MA (2025). Identification of an immunogenic 38-40 kDa protein in Indonesian *Gallibacterium anatis* isolates. Adv. Anim. Vet. Sci., 13(12):2592-2597.
DOI | https://dx.doi.org/10.17582/journal.aavs/2025/13.12.2592.2597
ISSN (Online) | 2307-8316

INTRODUCTION

Infection of *G. anatis* has been reported in many countries, including Iran, China, and India. This bacterium is considered as an emerging pathogen in poultry, as its pathogenicity is still debated due to its presence as part of the normal microbiota in healthy chickens (Abd El-Ghany *et al.*, 2023). *G. anatis* infection is commonly reported in layer chickens; however, it can also lead to respiratory disorders, diarrhea, and weight loss in broilers. In hens, it is associated with oophoritis, peritonitis, and salpingitis, while in roosters, it can cause epididymitis and reduced semen quality (Krishnegowda et al., 2020; Abd El-Ghany *et al.*, 2023). According to previous studies by (Paudel *et al.*, 2013), this pathogen poses a significant threat to poultry health and productivity across different production systems.

Transmission of *G. anatis* occurs horizontally through the respiratory system, often via aerosols. In addition, vertical transmission may also occur in embryonated eggs through the trans-ovarian route, whereby this bacterium invades and ruptures ovarian follicles. This leads to abnormal egg formation and a reduced egg production by approximately 20–30% (Krishnegowda et al., 2020). The pathogenicity of *G. anatis* largely depends on the expression and activity of virulence-associated proteins, which enable the bacteria to interact with the host. For example, outer membrane proteins (OMPs) play a critical role in modulating the host immune system and facilitating bacterial survival within the host environment (Brüssow, 2024).

Gallibacterium anatis has been increasingly reported in poultry farms across Indonesia, where differences in management systems, biosecurity, and host genetics may influence bacterial diversity and virulence expression. However, no studies have characterized the protein or virulence profiles of local isolates. Such characterization is essential because regional strains may harbor distinct antigenic or virulence-associated proteins compared to those reported in other countries, potentially affecting vaccine effectiveness and diagnostic accuracy. This study was therefore designed to characterize the protein profiles of Indonesian *G. anatis* isolates and to identify immunogenic components that could contribute to understanding their pathogenic mechanisms and inform the development of locally relevant control strategies.

MATERIALS AND METHODS

Characterization of *G. anatis* local isolates

Bacteria were characterized by biochemical and molecular methods. Bacterial samples is archive isolates from PT. Medika Satwa Laboratoris, which were obtained from commercial poultry in Bogor City, West Java, Indonesia. Archive isolates and field samples were obtained from the respiratory organ and tracheal swabs from layer chickens with respiratory disorder. Isolates were sub-cultured on blood agar and incubated at 37°C overnight microaerophilically (Krishnegowda et al., 2020). After culturing on media, each colony showed a typical colonial appearance and was subjected to staining by Gram's stain, and biochemical identification was examined for catalase and oxidase. Molecular characterization was conducted by qPCR to confirm with primers complementary to the general *G. anatis*, and the virulent factor, such as *gyr*B, *flf*A, and *gtx*A (Table 1), were used as described by (Huangfu *et al.*, 2018), with modification on qPCR kit that was used and total volume in the mixture of samples.

The reaction was carried out using SeniFAST SYBR® Lo-ROX Kit in total volume of 20 µL with 10 µL SYBR, 0,8 µL primer, 2,4 µL sample and 6 µL of distilled water under the following qPCR conditions: 95 °C for 2 min and 40 cycles of 95 °C for 5 s denaturation, annealing in 60 °C for 10 s and extension step in 72 °C for 15 s (Wang *et al.*, 2016). In the qPCR test, the positive control from the virulence gene was included, respectively.

Table 1: List of primer for qPCR of *Gallibacterium anatis.*

	Primer qPCR
*gtx*A	F: TGG GAC ATT CTA TCG CAA ACC R: AAA GGC TTA ACA CAT CGC TGA
*flf*A	F: CACCATGGGTGCATTTGCGGATGATCC R: TATTCGTATGCGATAGTATAGTTC
*gyr*B	F: CGATTGTGTCCGTTAAAGTGC R: TGCAAACGCTCACCAACTG

Protein extraction from *G. anatis*

The protein extraction was prepared from whole cells without extraction and whole cells with several extraction methods, including heat treatment, sonication, and capsular extraction. The procedures for each extraction method were as follows, based on a study by (Afzal *et al.*, 1992), with modifications on heated temprature.

- Sample heated at 96 °C and 56 °C. Suspended 1x10^8 CFU/mL sample in normal saline and was heated at 96°C and 56°C for 1 hour in a dry water bath. The suspension was centrifuged at 5000 x g for 1,5 hours, and the supernatant was used for the protein sample.
- Capsular sample. 1x10^8 suspended in 2,5% sodium chloride and shaken in a water bath at 37 °C for 4,5 hours at 80 rpm. The suspension was centrifuged at 5000 x g for 4,5 hours, and the supernatant dialysed against normal saline at 4 °C for 72 hours.
- A bacterial suspension was prepared by resuspending 1x10^8 CFU/mL of bacteria in normal saline. The suspension was then sonicated at 40.000 Hz for 30 minutes. Following sonication, the suspension was centrifuged at 5000 x g for 1.5 hours.

Protein concentration was determined by Bradford assay and UV spectrophotometry (UV-Vis Spectrophotometry (Genesys, Thermo Scientific, USA) at a wavelength of 260 and 280. Using bovine serum albumin as a standard (Jie *et al.*, 2019).

Protein characterization by SDS-PAGE and western blot

All five protein samples of *G. anatis* were subjected to SDS-PAGE by the method reported by (Desem *et al.*, 2023) with modification. Each protein sample (supernatant) was diluted 1:4 ratio with sampel buffer and then incubated at 98 °C for 5 minutes. Samples containing 10 µL per well were loaded. They were electrophoretically separated by 10% resolving gel and 4% stacking gel for 2 hours at 90

V, along with the known molecular weight protein marker ranging from 10 kDa to 245 kDa. Then the proteins were electrically transferred to a nitrocellulose membrane. The membrane was blocked with PBS containing 5% skim milk for 1 hour with a shaker incubator at room temperature.

The membrane was washed three times with PBS-Tween 20 and then incubated with primary antibody against *G. anatis*. Polyclonal antisera against *G. anatis* were generated in chickens experimentally immunized with whole-cell bacterial antigen, collected two weeks after the booster and used as the primary antibody. The serum was diluted at 1:2000 in TBS-Tween Casein prior to incubation for 1h with a shaker incubator at room temperature. The anti-chicken IgG-Peroxidase conjugate diluted at 1:8000 with PBS-Tween casein was used as a secondary antibody and incubated after the incubation of the primary antibody. Finally, the membrane was soaked in 3,3'diaminobenzidine (DAB) substrate for around 3 minutes and was photographed by Bio-Vision (Vilber E-box, France) and was analysed by Vilber Bio-Vision software.

Protein classification

The classification of proteins was predicted using the PSLpred (http://crdd.osdd.net/raghava/pslpred/), and the molecular weight was compared based on the online web server Expasy's ProtParam (https://web.expasy.org/protparam/) with the protein sequence obtained from UniProt (https://www.uniprot.org/) (Rajapaksha *et al.*, 2022).

Data analysis

The results of this study are presented descriptively in the form of Tables and Figures.

RESULTS

Characterization of *G. anatis* local isolates

According to the blood agar characterization (Figure 1), the following are circular, raised colonies with an entire margin of 1.0-2.0 in diameter, opaque, and produce a wide β-hemolytic zone. Based on Gram stain, the bacterial colonies showed a red coloration in bacterial cells. The biochemical assay performed on *G. anatis* showed positive results for catalase and oxidase activities. The positive catalase reaction is attributed to the enzyme catalase, which degrades hydrogen peroxide into H_2O and O_2, resulting in bubble formation.

The qPCR analysis revealed that the grown colonies were positive as *G. anatis,* with the Ct value of the *gyr*B gene in the sample was 34.48, slightly higher than the positive control (31.73). The Ct value for the *gtx*A gene in the sample was 12.52, lower than the positive control (14.15), indicating a high copy number of this virulence gene. Furthermore, the *fif*A gene was also detected in the sample with a Ct value of 15.97, which was close to that of the positive control (13.49), suggesting potential expression of adhesin factors in the tested samples (Bager *et al.*, 2013) (Figure 2).

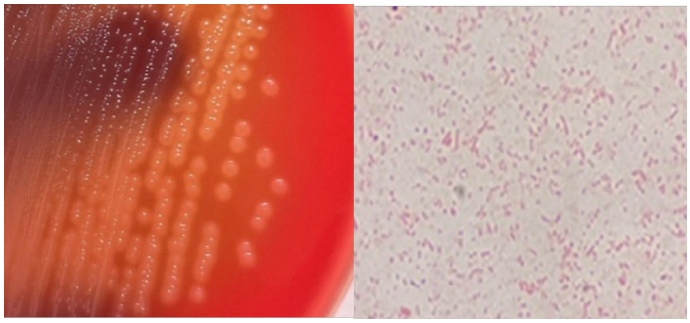

Figure 1: Colony on blood agar and Gram staining.

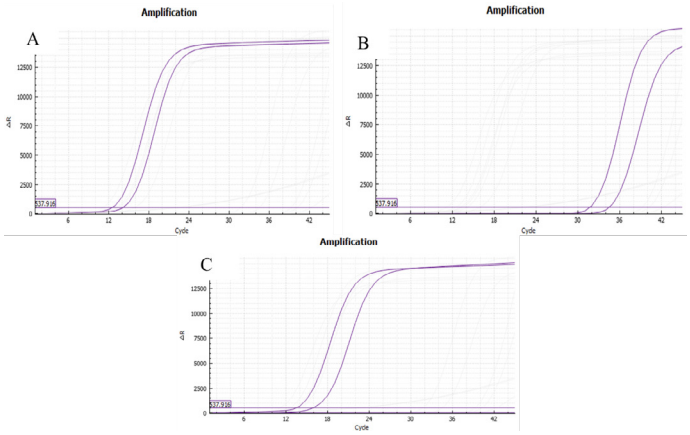

Figure 2: qPCR results.

Protein characterization

The results of the protein concentration from protein samples were obtained using two different methods. The largest concentration was obtained from sonication treatment of 0.517 and 1.421 mg/mL, respectively. Heating at 96 and 56 °C from Bradford yielded 0.284 and 0,254 mg/mL from UV spectrophotometry of 605 and 569 µL/mL. The protein concentration in the capsular treatment was obtained from the Bradford assay, which was 0,169 and 0.041 mg/mL by UV spectrophotometry (Figure 3).

Based on the SDS-PAGE characterization results (Figure 3a), approximately 34 visible bands with different intensities were revealed from whole cells without treatment. Protein bands obtained from SDS-PAGE ranged from approximately 8-161 kDa across treatment, with sonication yielding the broadest molecular weight range compared to heat and capsular extractions. SDS-PAGE also showed a unique band from the whole cell protein, with a MW of 23.9 and 14 kDa, which were observed only in the whole cell of *G. anatis*. The dominant protein of *G. anatis*, which appeared in all five protein samples, was observed with

molecular weights of 60.11 and 76.37. In the capsular treatment, fewer protein bands were obtained, with the presence of specific surface proteins with smaller molecular weights of 8.71 kDa and the highest molecular weights of 148.4 kDa. Proteins with molecular weights of 105.43, 91.24, 48.24, 43.12, and 24.95 kDa appeared at heating at 56 ˚C but not at 96 ˚C. On the other hand, proteins with molecular weights of 148.41, 99.50, 52.87, 44.28, and 26.52 kDa appeared at heat treatment at 96 ˚C, indicating that this treatment tends to release thermostable proteins.

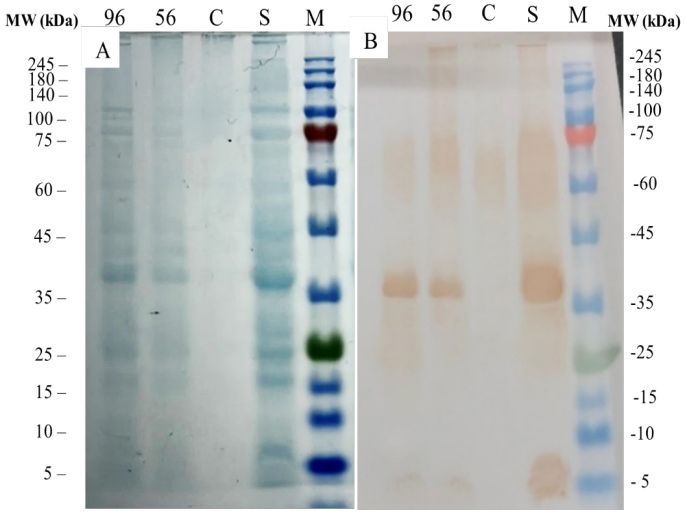

Figure 3: Protein profile.

Protein from sonication treatment showed a more dominant protein band than other treatments. The range of molecular weights varied widely from large to small. Proteins with 45.47 and 11.15 kDa molecular weights were specifically detected only in sonicated proteins. From the results of antigenic protein analysis by western blotting from heating treatments at 56˚C, 96˚C, and sonication, the antigenic protein was in the molecular weight range of 38-40 kDa.

Protein classification

Based on reference proteins of similar molecular weights in available databases, the prediction results of protein classification using PSLpred indicated that cytoplasmic proteins dominated the capsular proteins. Heat treatment was shown to influence the diversity of protein localization. At 56 ˚C, a heterogeneous distribution was observed, including two extracellular proteins, two inner membrane proteins, two outer membrane proteins, and several cytoplasmic proteins. At 96 ˚C, cytoplasmic proteins predominated, while sonication released a broad spectrum of proteins with diverse subcellular localizations, reflecting the disruptive nature of this extraction method. However, these predictions remain hypothetical since the actual sequences of the proteins were not determied.

DISCUSSION

Gallibacterium anatis has become a serious threat in poultry production, and the infection of *G. anatis* is confirmed through agent isolation, characterized by phenotypic and genotypic methods. In the present study, phenotypic results were obtained from Gram-negative bacteria that produce the enzyme cytochrome oxidase for the oxidase test, which oxidizes tetramethyl-p-phenylenediamine dihydrochloride into indophenol (Yaman, 2019). Most organisms produce catalases, peroxiredoxins, and superoxide dismutases, which are enzymes that react with harmful oxidants and convert them to harmless products by neutralizing them to protect before they cause damage to cellular components (Ezraty *et al*., 2017). The genotypic character was identified virulence factor by qPCR. The isolates were positive by qPCR, including the gyrB gene, which plays a crucial role in synthesizing DNA replication enzymes, as it encodes the ATPase domain of DNA gyrase (Deng *et al*., 2020). Furthermore, the *gtxA* gene is a key determinant in the hemolytic activity of *G. anatis*, exhibiting leukotoxic properties against avian macrophages. *Gtx*A contains two functional domains: A C-terminal domain homologous to RTX toxins responsible for hemolytic activity, and an N-terminal domain associated with leukotoxic activity (Krishnegowda et al., 2020). *G. anatis* has the capability to adhere to the epithelial cells of chicken by short fimbriae that seem to be type IV-like pili. Type IV fimbriae are appendages participating in intracellular motility, microcolony formation, colonization, and the secretion of proteases by host tissues (Craig and Juliana, 2008).

SDS-PAGE was used ini this study primarily as a comparative and preliminary screening tool to evaluate the efficiency of different extraction methods rather than to quantify individual proteins. Among the tested extraction methods, sonication yielded the highest protein diversity and concentration, indicating its efficiency in releasing both cytoplasmic and membrane-associated proteins. The sonication method produces a cavitation effect that creates bubbles through ultrasonic waves that can implode and effectively break down bacterial cell walls. The ability of the sonication method to release both cytoplasmic and membrane-associated proteins provides a more comprehensive antigen repertoire for immunological screening. This has important implications for antigen discovery, as complex protein mixtures increase the likelihood of identifying conserved epitopes relevant to field immunity.

The present study identified a strongly immunogenic 38-40 kDa protein in Indonesian *Gallibacterium anatis* isolates through western blotting. The detection of a strong immunogenic signal in the 38-40 kDa range suggests that this protein may play a role in the interaction between *G.*

anatis and the host immune system. Proteins of similar molecular weights in related Pasteurellaceae species, such as OmpA and FbpA, are known to mediate adhesion, host cell recognition, and induction of antibody responses (Mosier *et al*., 1998). Therefore, it is plausible that the 38-40 kDa protein in *G. anatis* serves a comparable biological function.

Although the 38-40 kDa protein has not been previously reported in *G. anatis*, its molecular weight overlaps with conserved bacterial proteins such as OmpA or heat shock proteins. However, these findings present a biological paradox, as cytoplasmic proteins are typically not accessible to the host immune system. One possible explanation is that the existence of "moonlight" proteins, which are cytoplasmic enzymes that can be translocated to the bacterial surface and perform additional extracellular functions, such as adhesion or immune evasion (Singh and Bhalla, 2020). Such dual localization has been reported in other Gram-negative bacteria and may also occur in *G. anatis*. Alternatively, the apparent cytoplasmic profile could result from partial cell lysis during extraction, particularly with sonication, allowing intracellular proteins to become exposed and recognized by antibodies during the western blot analysis (Martin, 2014).

Interestingly, bioinformatic prediction suggested the most immunogenic proteins, including the 38-40 kDa band, were cytoplasmic. This finding strengthens the hypothesis of moonlighting behavior or potential methodological effects during extraction. Nonetheless, this dual localization has been well documented in Gram-negative pathogens and may also occur in *G. anatis*. Another explanation is methodological; mechanical disruption during sonication or heating may cause partial lysis of bacterial cells, releasing cytoplasmic proteins that can bind to membranes or remain associated with the cell fragment, thereby appearing immunogenic during in vitro assays (Khan *et al*., 2017). In addition, the consistent detection of this 38-40 kDa antigen across multiple extraction treatments supports the hypothesis that it is stably expressed and potentially surface-associated. Future investigation employing surface proteomics, immunogold labeling, or cell fractionation with protease shaving will be necessary to confirm its true subcellular localization and functional role.

The identification of these immunogenic proteins represents a promising first step in defining specific antigenic markers in *G. anatis*. However, further molecular identification, such as through LC-MS/MS, is required to confirm its amino acid sequence and homology to known bacterial proteins. Clarifying this will provide a crucial insight into whether the 38-40 kDa band represents a true virulence-associated antigen or a cross-reactive conserved protein. Additionally, serological testing using sera from naturally infected chickens will help validate its diagnostic relevance and confirm whether this antigen elicits a consistent immune response *in vivo*. Ultimately, these findings will bridge the gap between protein characterization and applied immunology, providing a foundation for the development of improved diagnostic tools and subunit vaccines for controlling *G. anatis* infection in poultry.

CONCLUSION

This study provides the first comprehensive characterization of *Gallibacterium anatis* protein profiles from Indonesian isolates using multiple extraction methods combined with SDS-PAGE and Western blot analysis. The isolates carried key virulence genes and confirming their pathogenic potential. Among extracted proteins, a consistently detected 38-40 kDa immunogenic protein emerged as the most significant findings. Although protein prediction classified this protein as cytoplasmic, its strong immunoreactivity suggests that it may be surface-associated or function as a moonlighting protein. These results highlight the 38–40 kDa antigen as a promising target for further identification and evaluation as a potential diagnostic or vaccine candidate against *G. anatis* infection in poultry.

ACKNOWLEDGMENTS

The authors gratefully acknowledge IPB University and PT Medika Satwa Laboratoris for their support and collaboration during this research.

NOVELTY STATEMENT

This study provides the first characterization of protein profiles from Indonesian *Gallibacterium anatis* isolates, combining several extraction methods with SDS-PAGE and Western blot analysis. Most importantly, it reports the first identification of a consistently immunogenic 38–40 kDa protein in local isolates, a potential moonlighting protein not previously described for *G. anatis*. The discovery of this offers new insight into region-specific virulence components and provides the first foundational evidence supporting its development as a diagnostic or vaccine candidate for controlling *G. anatis* infection in poultry

AUTHOR'S CONTRIBUTION

The authors gratefully acknowledge the contributions of all team members. RJ, RSK, SM, and AI, CMHN, and MAP conceptualized the study, designed the methodology, and led the investigation. RJ and RSK are involved in protein analysis and drafting the manuscript. CMHN and MAP developed and optimized the data collection tools. AI, SM and RSK contributed to validation, supervision, and

critical review manuscript. All authors read and approved the final version of the manuscript.

Animal rights statement

All animal experiments were conducted according to ethical standards and with the approval No. 298/KEH/SKE/II/2025 of the Animal Ethics Committee, School of Veterinary Medicine and Biomedical Sciences, IPB University, Bogor, Indonesia.

Generative AI and AI-assisted technology statement

Generative artificial intelligence (AI) tools, specifically ChatGPT (OpenAI), were utilized solely for language refinement, including improving grammar, wording, and readability of the manuscript. No AI tools were employed in the conception of the study, data acquisition, data analysis, interpretation of results, or formulation of scientific conclusions. All intellectual content, including experimental design and interpretation, is entirely the work of the authors. All AI-assisted modifications were carefully reviewed and validated by the authors to ensure accuracy, integrity, and compliance with ethical publication standards.

Conflict of interest

The authors have declared no conflict of interest.

REFERENCES

Abd El-Ghany WA, Algammal, A. M., Hetta, H. F., Elbestawy, A. R. (2023). *Gallibacterium anatis* infection in poultry: A comprehensive review. Trop. Anim. Health Prod., 55(6). https://doi.org/10.1007/s11250-023-03796-w

Afzal M, Muneer R, Akhtar S (1992). Serological evaluation of *Pasteurella multocida* antigens associated with protection in buffalo calves. Rev. Sci. Tech. (Int. Off. Epizoot.), 11(3): 917–923. https://doi.org/10.20506/rst.11.3.634

Bager RJ, Nesta, B., Pors, S. E., Soriani, M., Serino, L., Boyce, J. D., Adler, B., Bojesen, M (2013). The fimbrial protein FlfA from *Gallibacterium anatis* is a virulence factor and vaccine candidate. 81(6): 1964–1973. https://doi.org/10.1128/IAI.00059-13

Brüssow H (2024). The antibiotic resistance crisis and the development of new antibiotics. Microbial Biotechnol., 17(7): 1–17. https://doi.org/10.1111/1751-7915.14510

Craig L, Juliana L (2008). Type Iv Pili: Paradoxes in form and function, 18(2): 267–277. https://doi.org/10.1016/j.sbi.2007.12.009

Deng Y, Xu, L., Chen, H., Liu, S., Guo, Z., Cheng, C., Ma, H., Feng, J. (2020). Prevalence, virulence genes, and antimicrobial resistance of Vibrio species isolated from diseased marine fish in South China', Scientific Reports, pp. 1–8. https://doi.org/10.1038/s41598-020-71288-0

Desem MI, Handharyani, E., Setiyono, A., Safka, S., Subekti, D. T., Ekawasti, F. (2023). Morphology, biochemical, and molecular characterization of *Pasteurella multocida* causing hemorrhagic septicemia in Indonesia. https://doi.org/10.1155/2023/7778707

Ezraty B, Gennaris, A., Barras, F., Collet, J. (2017). Oxidative stress, protein damage and repair in bacteria. Nat. Publ. Group [Preprint]. https://doi.org/10.1038/nrmicro.2017.26

Huangfu H, Xu, W., Wang, H., Dong, Q., Guo, H., Sun, Y., Li, Y., Gao, W., Wang, W., Zhang, J., Shi, J., Pan, H., Li, C., Wang, L., Huangfu, H., Xu, W., Wang, H., Dong, Q., Guo, H., Li, Y. (2018). Detection of *Gallibacterium anatis* by TaqMan fluorescent quantitative PCR. Avian Pathol., 47(3): 245–252. https://doi.org/10.1080/03079457.2017.1416590

Jie X, Liu, G., Zhang, M., Zhao, J., Li, H., Yang, Z., Bai, H., Liang, P., Lu, Y. (2019). Science of the total environment membrane-sensitive bacterial DNA extractions and absolute quantitation of recovery efficiencies. Sci. Total Environ., (xxxx): 135125. https://doi.org/10.1016/j.scitotenv.2019.135125

Khan IK, Bhuiyan M, Kihara D (2017). DextMP: Deep dive into text for predicting moonlighting proteins. https://doi.org/10.1093/bioinformatics/btx231

Martin ACR (2014). Structural biology of moonlighting: Lessons from antibodies. 42: 1704–1708. https://doi.org/10.1042/BST20140211

Mosier D, Iandolo, J., Rogers, D., Uhlich, G., Crupper, S (1998). Characterization of a 54-kDa heat-shock-inducible protein of *Pasteurella haemolytica*, pp. 67–73. https://doi.org/10.1016/S0378-1135(98)00148-5

Krishnegowda, N. D., Dhama, K., Kumar Mariappan, A., Munuswamy, P., Iqbal Yatoo, M., Tiwari, R., Karthik, K., Bhatt, P., Reddy, M. R. (2020). Etiology, epidemiology, pathology, and advances in diagnosis, vaccine development, and treatment of Gallibacterium anatis infection in poultry: a review. Veterinary Quarterly, 40(1), 16–34. https://doi.org/10.1080/01652176.2020.1712495

Paudel S, Alispahic, M., Liebhart, D., Hess, M., Hess, C. (2013). In a natural infection model: The respiratory and reproductive tracts of chickens are targets for bacterial colonization Assessing pathogenicity of *Gallibacterium anatis* in a natural infection model: The respiratory and reproductive tracts of chickens are targets for bacterial colonization. Avian Pathol., 42(6): 527–535. https://doi.org/10.1080/03079457.2013.843160

Rajapaksha LGTG, Gunasekara CWR, Alwis PSD (2022). *In silico* detection and characterization of novel virulence proteins of the emerging poultry pathogen *Gallibacterium anatis*, 20(4): 1–9. https://doi.org/10.5808/gi.22006

Singh N, Bhalla N (2020). Moonlighting proteins, pp. 265–285. https://doi.org/10.1146/annurev-genet-030620-102906

Wang C, Robles, F., Ramirez, S., Riber, A. B., Bojesen, A. M. (2016). Culture-independent identification and quantification of *Gallibacterium anatis* (*G. anatis*) by real-time quantitative PCR. Avian Pathol., 45(5): 538–544. https://doi.org/10.1080/03079457.2016.1184743

Yaman SSYO (2019). First report in Turkey.