Research Article

BASJVETRES

BASRAH JOURNAL OF VETERINARY RESEARCH,2024, 23(2):149-161
https://bjvr.uobasrah.edu.iq/

Polymerase Chain Reaction for the Detection of Histamine-Producing *Histophilus somni* Isolated from Basra Cattle

Jasim R.M.[1], Abdullah F.A.[1], Mustafa J.Y[2]

1- Department of Microbiology, College of Veterinary Medicine, University of Basrah. Basrah, Iraq.

2- Department of Public Health, College of Veterinary Medicine, University of Basrah. Basrah, Iraq.

Corresponding Author Email Address: fawzia.abdullah@uobasrah.edu.iq

ORCID ID: https://orcid.org/0000-0002-1217-3967

DOI: https://doi.org/10.23975/bjvr.2024.149689.1086

Received: 15 May 2024 Accepted: 26 June 2024.

Abstract

This study sought to examine the significance of *Histophilus somni* as a producer of histamine and a respiratory tract pathogen. Conventional bacteriological and molecular techniques were used to examine 200 nasal swab samples for the presence of *Histophilus somni* and its ability to produce histamine. Out of 200 cattle nasal swabs tested, 25% (50/200) tested positive for conventional biochemical properties and culture. *Histophilus somni* was found in 70% (35/50) of the isolates that tested positive for the PCR test after culturing. It was found that 20% (7/35) of the 35 H. somni positive PCR isolates produced positive products when the histidine decarboxylase gene was amplified. Cattle that were infected in the first age group (less than one year) had higher percentages of culture (72%; 36/50) and positive PCR results (80%; 28/35), based on their age. Males demonstrated higher culture (82%;41/50) and PCR (85%; 30/35) positivity percentages regarding the impact of sex. There was a statistically significant difference ($P<0.05$) between the genders and between the two age groups.

Keywords: PCR, *Histophilus somni,* Histamine.

Introduction

A Gram-negative bacterium called *Histophilus somni* (HS) primarily infects cattle, while it can also sporadically infect small ruminants (1). Although it has been demonstrated that weaned calves appear to be at increased risk of infection, animals of any age may be impacted (2). Various strains of HS have been recovered from urogenital secretions, even though it is thought to be a commensal bacterium of the nasal system. These strains may be the cause of venereal transmission (3). Vasculitis is the characteristic symptom of HS, which is linked to a disease complex collectively known as histophilosis. This illness is now known as the *H.Somni* disease complex, or HSDC (4;5). A study by Headley *et al.* in 2023 discovered that HS and ovine gammaherpesvirus 2 nucleic acids were found in cattle from the southern part of Brazil that had septicemic signs and neurological symptoms of *H. somni* sickness (6). Disease of the bovine respiratory system is the most prevalent illness around the world that necessitates the use of antibiotics in industrial calf production. This bovine respiratory disease (BRD) is connected to several viruses and harmful microorganisms., including *Mycoplasma bovis*, *Pasteurella multocida* (Pm), *Mannheimia haemolytica* (Mh), and Hs. Both sick and healthy cattle can harbor the majority of these infections (7). The pathogenesis of BRD has been linked to an initial viral infection followed by bacterial colonization of damaged epithelium, which results in a secondary infection (8). Furthermore, Hs generates exopolysaccharides and histamines that are involved in the disease's etiology (9).

Histamine is a biogenic amine with health effects. It functions physiologically through immunomodulation to alter blood vessel permeability, mucus secretion, and signal transmission in neurons (10). Histidine, an amino acid, is decarboxylated by the enzyme histidine decarboxylase to generate histamine (11). In 2021, Mou et al. found 117 possible species of bacteria that release histamine. These bacteria belonged to the Verrucomicrobiota and Fusobacteriota groups (12). Since histidine decarboxylation takes place within the bacterial cytoplasm, histidine decarboxylation requires the Histidine/Histamine antiporter, which enters cells via histidine and exits them with histamine (13).

Most studies on exogenously produced histamine have focused on food-borne diseases caused by histamine in milk and fish (14, 15). For instance, consuming meals contaminated with a lot of histamine may cause respiratory, gastrointestinal, and neurological conditions (16). The main source of histamine found in food products is histamine-secreting bacteria, which produce histamine together with other amines to keep their cytoplasmic pH neutral and enable them to live in acidic environments (17). For instance, it has been discovered that *Lactobacillus vaginalis* produces histamine to keep the cytosolic pH at the proper level in acidic environments (18). After diagnosing *H. somni* based on the gross lesion, bacterial culture and molecular tests are carried out. (19). Many histamine-producing bacteria have provided information about the molecular structure of

the genes that regulate histamine synthesis. Because of this understanding of molecules, molecular techniques have been developed to identify bacteria that can make histamine (20) quickly. Many studies have used PCR technology to detect the histamine gene in the bacteria that produce it (21- 23). This study aimed to understand the relationship between histamine secreting H. somni and respiratory disease.

Materials and Methods

Nasal samples from 200 symptomatic animals were collected. Females (n = 23) and males (n = 177) were investigated in this study; their ages ranged from 2 months to 4 years. The symptomatic animals complain of respiratory distress, coughing, and nasal discharge. Study samples were collected in Basrah provinces from November 2022 to March 2023. Clinical samples were taken from three animal sources: Al Zubair district's housekeeping cattle, slaughterhouse, and agriculture college farm. All nasal swab samples were immersed in sterile tubes containing phosphate-buffered saline (pH 7.2) and then transported on ice baths to the microbiology laboratory of the Veterinary Medicine College of Basrah University, Iraq. On arrival, the collected samples were inoculated into brain-heart infusion broth supplemented with 0.1% tris-base. The samples underwent a 24-hour incubation period at 37 °C.

Bacterial isolation and growth condition

H. somni was grown on Columbia agar base (Liofilchem/Italy) with 5% sheep blood, incubated in 5% CO_2 at 37 °C, and examined after 24–48 h of incubation. The colonies were identified depending on morphological characteristics, alpha hemolytic pattern, and Gram's staining reaction. The representative colonies that did not show Gram's staining and looked like coccobacillus under a microscope were grown on Brain Heart Agar plates (Oxoid, UK) and kept warm at 37 °C for 24 hours. Eventually, identification of the agent was done based on biochemical tests such as catalase, oxidase, and sugar fermentation (glucose, lactose, sucrose, mannitol, and mannose). Samples were considered positive for *H. somni* when the isolates showed fermentation of glucose (strong yellow discoloration of media) (24).

DNA extraction and PCR

Nucleic acids (DNA) were extracted from the pure culture of nasal swabs after overnight culturing on Columbia blood agar. A single colony was isolated by a wire loop and inoculated into a brain heart infusion broth tube, followed by proliferation after overnight in a 5% CO2 incubator with 1 ml of culture added to a 1.5 centrifuge tube and extraction, as described in the in the Wizard® Genomic DNA Purification Kit (Promega/USA) and recommended protocols. The concentration of DNA was determined using Nanoodrop (Quawell, USA). Two different PCR procedures were applied. In the first procedure, DNA samples were tested using 16S rDNA-specific PCR for the *Histophilus genus* (25), and in the second, they were tested using specific PCR assays for the *Histophilus somni* (26). The PCRs were performed in a PCR

thermocycler apparatus (TEKNE TC-3000G, USA) in a reaction volume of 25 µl containing: 5 µl of DNA template, 1 µl each primer, 5.5 µl nuclease-free water, and 12.5 µl master mix (Promega, USA). The amplified products were detected by staining with ethidium bromide (0.5 mg/ml) after electrophoresis at 70 V for 1 h in 1.5% agarose gels for Histophilus genus and species genes and 1.8% agarose gels for histidine decarboxylase. The expected amplicon bands were visualized and photographed under a UV transilluminator (EDVOTEK.UK) and gel documentation system (Gel Doc, ATP Co).

The primers for the amplification of *16S rDNA* specific PCR for Bacteria universal primers 27 F5'-AGAGTTTGATCCTGGC-3' and R 5'-GGTTACCTTGTTACGACTT-3, which amplified DNA fragments of 1500 bp, The PCR conditions consisted of initial denaturation at 94°C for 5 min, 30 cycles each of denaturation at 94°C for 30sec, annealing at 51.8°C for 45sec and 72°C for 30sec and a final extension of 72°C for 10 min. PCR employing specific primers of *H. somni* gene was used to amplify a fragment of 313 bp (forward 5'--GAAGGCGATTAGTTTAAGAG-3' and reverse 5'-ACTCGAGCGTCAGTATCTTC-3'). The PCR circumstances consisted of initial denaturation at 94°C for 2 min, 35 cycles each of denaturation at 94°C for 30sec, annealing at 49°C for 30sec and 72°C for 1 min, and a final extension of 72°C for 6 min..

The primers for the amplification of the histidine decarboxylase coding gene (*hdc*) in Gram negative strains were 106: 5', AAYTCNTTYGAYTTYGARAARGARG-3' and 107: 5'-ATNGGNGANCCDATCATYTTRTGNCC-3' (Y = C or T; R = A or G; W = A or T; D = G, A or T; N = A, C, G or T) (26). which amplified DNA fragments of 534 bp. The PCR conditions consisted of initial denaturation at 94°C for 10 min, 35 cycles each of denaturation at 94°C for 45sec, annealing at 58°C for 1min and 72°C for 1 min, and a final extension of 72°C for 5 min.

Statistical analysis

All of the data was gathered and cleaned using Microsoft Excel version 2016. At a significance level of 5%, the Fisher Exact test was utilized to determine whether any association existed between the outcomes.

Results

Bacterial isolation of *Histophilus somni*

The samples were deemed positive for *H somni* upon observing bacterial growth, and it was proved by colonies characteristics, hemolytic pattern on blood agar (Figure-1a) and conventional biochemical properties. all 50 isolates showed positive glucose fermentation, oxidase and catalase negative. Depending on culture and conventional biochemical properties, 25% (50/200) of tested cattle nasal swabs were positive (Table-1).

After culturing, the PCR assay identified 70% (35/50) of isolates that were positive for culture and were confirmed to belong to the Histophilus genus (Table 1). (Figure-1b) 16S rDNA produced separate bands with a

molecular size of 1500 bp when used in genus-specific PCR to detect the Histophilus genus. All Histophilus genus-positive isolates (n = 35) obtained positive results with a molecular size of nearly 313 bp, according to H. somni-specific PCR amplification (Figure 1c). Consequently, 70% (35/50) of the culture-positive isolates overall were determined to be H. somni in cattle (Table 1).

Histidine decarboxylase gene-specific PCR amplification of the 35 H somni positive PCR, 20% (7/35) of positive products with an approximate molecular size of 534 bp were generated by the H somni isolates. (Table-1;Figure-1d). All the PCR/Hs and PCR/hdc positives were culture-positive, while culture-positive and negative isolates showed negative PCR/Hs or PCR/hdc results.

Table-1: Percentage of positive cattle nasal swabs with *Histophilus somni* (Hs) in relation to culture, PCR and Histidine decarboxylase *(hdc).*

Diagnostic test	Positive n. (%)	Negative n.(%)	Total
Culture	50(25)	150(75)	200
PCR/Hs	35(70)	15(30)	50
PCR/ *hdc*	7(20)	28(80)	35

Table-2: Frequency of combination among diagnostic test.

Diagnostic tests combination	+Ve Number	Percentage %
PCR/Hs+/culture+	35	70
PCR/Hs +/culture-	0	0
PCR/Hs –/culture+	15	30
PCR/Hs –/culture-	0	0
PCR/Hs +/culture+/PCR/ *hdc*+	7	20
PCR/Hs -/culture+/ PCR/*hdc*+	0	0
PCR+/culture-/*hdc*+		

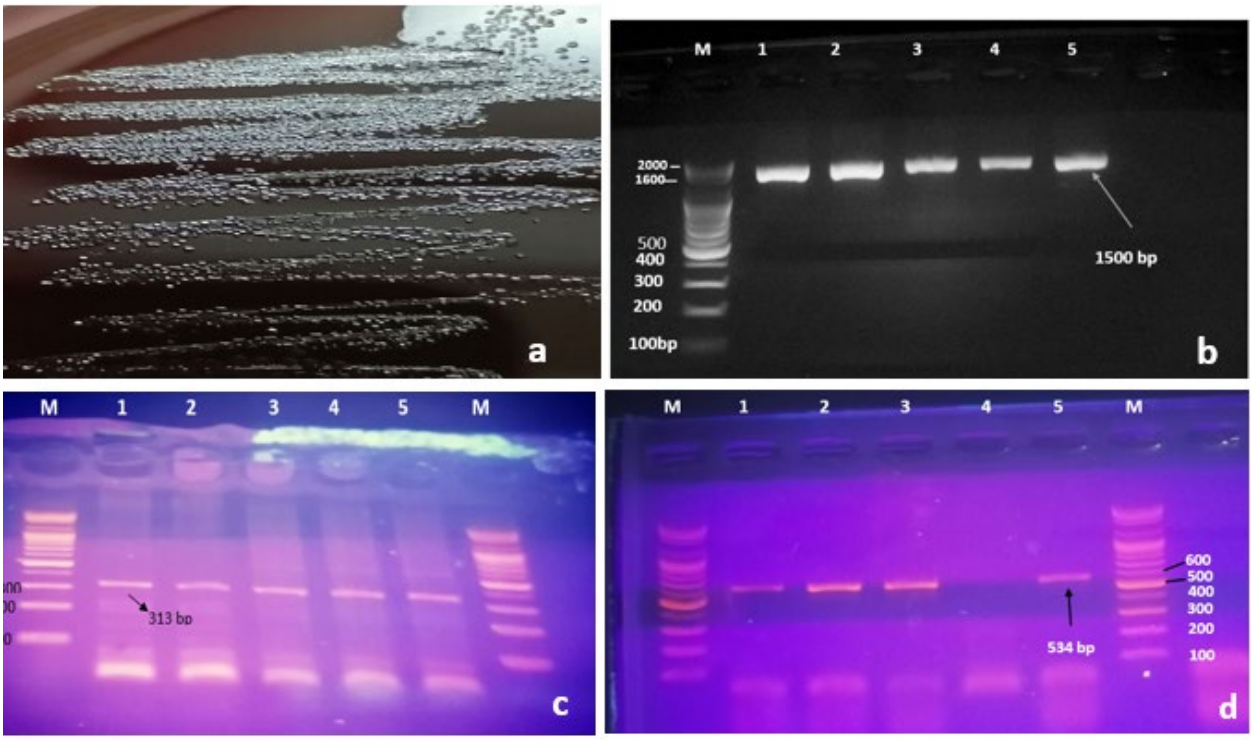

Figure-1: (a) *Histophilus somni* colonies and blood hemolysis on blood agar medium. (b) The 16S gene was detected using an amplicon size of 1500 bp and a *Histophilus* genus-specific primer on an Agarose gel using a standard polymerase chain reaction. Lane 1–5, positive samples; Lane (M) marker in molecular weight, 1000–2000 bp (c) *Histophilus somni* species was detected using an agarose gel of traditional polymerase chain reaction at an amplicon size of 313 bp. Lane M (Molecular weight marker, 100-1000 bp) was positive, whereas Lanes 1–5 were negative (d) The histidine decarboxylase (hdc) gene at amplicon size of 534 bp was found on an agarose gel in a standard polymerase chain reaction. Lane M, or molecular weight marker, spans 100-1000 bp, while Lanes 1-5 show positive amplicons. Lane M (100–1000 bp DNA marker).

Some data of epidemiology

The outcomes of the various *Histophilus somni* that were isolated from the studied cow nasal swabs over the course of several months of research are illustrated in Table 3. The overall percentage of *Histophilus somni* infection in cattle was 17.5%, and a higher infection percentage (24.4%) was observed in February**.** Depending on age and sex of the cattle, infected cattle in the first age group (<1 year**)** had a higher percentage of culture (72%; 36/50) and PCR-positive results (80%; 28/35). Concerning the effect of sex, males showed a higher culture (82%; 41/50) or PCR (85%; 30/35) positivity percentage. The result of the Fisher Exact test showed a significant statistical difference (P<0.05) between the two age groups as well as between males and females (Tables 4)

.

Table-3: Number and percentage of positive samples with *Histophilus somni* in relation to months of study.

Months	Number of samples	Number of positive	Percentage of positive
November	25	2	8
December	30	1	3.3
January	55	10	18.2
February	90	22	24.4
Total	200	35	17.5

Table-4: Number and percentage of culture or PCR positive samples with *Histophilus somni* in relation to cattle age and sex.

Variables	Culture		PCR		P value
Age(year)	**Number of positive**	**Percentage of positive**	**Number of positive**	**Percentage of positive**	
<1 year	36	72	28	80	**<0.05**
≥1 year	14	28	7	20	
Total	50	100	35	100	
Sex					
Male	41	82	30	85.7	**<0.05**
Female	9	18	5	14.3	
Total	50	100	35	100	

Discussion

The economic losses resulting from decreased productivity and culling are primarily caused by respiratory diseases in cow herds. This disease can affect cattle of any age or sex, but it is particularly dangerous to calves (28). According to Cengiz *et al.* (29): *P. multocida*, *M. haemolytica*, *H. somni*, and *M. bovis* are significant opportunistic pathogens responsible for animal deaths and economic losses. Apart from infections, some predisposing conditions, such as inadequate food, overcrowding in poorly ventilated barns, and additional infectious diseases, increased the chance of infection. Transmission typically occurs horizontally within these herds (30). Many last studies were concentrated on the detection of *H. somni* in *the respiratory* tract tissue of cattle (31, 32; 10).

In this study, out of 200 cows, only 50 (25%) had positive results for bacterial culture, even though most of the cows had clinical involvement of a respiratory tract infection. This percentage of positive animals was reported to be higher than that of (29), who detected *H. somni* in the lungs of six (7.3%) cows (25). The decreased culture detection rate for *H. somni* emphasizes how challenging it was to isolate

this organism for the current studies. The explanation for this finding might be attributed to the study of (33), who discovered that *Proteus spp*., *P. multocida*, *Klebsiella*, *Escherichia coli*, and *H. somni* may easily overgrow each other. These organisms were identified in many *H. somni* PCR-positive and culture-negative cases. The current results also revealed that a larger percentage of positive animals was found by the PCR assays (70%) compared to culture (25%). *According to the current findings, 33% identified H. somni in 23.3% of cattle through PCR and 4% through culture (28).*

According to (34), the fact that antibiotics are often used on farms to treat respiratory tract infections may not be surprising considering the larger number of positive PCR results obtained compared to the diagnostic usage of culture. One benefit of PCR is its capacity to identify dead and growth-inhibited bacteria after antibiotic treatment (34).

Because these bacteria can cause food poisoning, it is critical to identify them in food as soon as possible. To stop the buildup of biogenic amines in food items, it is crucial to employ techniques for the early and quick detection of these microorganisms. As an alternative to conventional culture techniques, molecular approaches are increasingly being used to identify and recognize food-borne bacteria. Biogenic amines are low-molecular-weight chemical bases with biological action. Even though biogenic amines, like histamine, are necessary for numerous vital processes in humans and animals, eating foods high in these amines can have toxicological consequences (35). Various food items, such as dairy and animal products, contain biogenic amines (36–41). The bulk of amines in most foods containing amines are produced by decarboxylation of the respective amino acids using enzymes particular to the food's microorganisms; even though bacteria do not generally include amino acid decarboxylases, several different genera of bacteria can decarboxylate one or more amino acids. Microorganisms' capacity to decarboxylate amino acids varies greatly, though. In addition to the species, it relies on the strain and the surrounding circumstances (42).

There is a molecular detection technology that can be used to find the gene that makes histidine decarboxylase (hdc). HDC helps histidine decarboxylate, which turns into histamine. In numerous earlier investigations, the hdc gene in bacteria with gram-negative status was amplified using oligonucleotide primers (27; 22). The PCR assay of 35 culture+ and PCR+ H somni isolates showed histamine-positive results that were consistent with current results. (22) reported that after screening a total of 500 isolates on selective medium, only 15 isolates showed histamine-positive results based on PCR assays (22).

Production of histamine by *H. somni* isolates in this study was confirmed by (43) and (44), who reported that *H. somni* can synthesize and secrete histamine. Using uniplex-PCR techniques, genes encoding a single kind of *bioamins* have been used in this study, depending on primers 106 and 107 with an approximate molecular size of

534 bp. (27) found that a reliable uniplex-PCR approach for the identification of gram-negative bacteria that produce histamine is comprised of primers 106 and 107. In conclusion, the PCR method demonstrated the potential effectiveness of identifying H. somni as a histamine producer and a respiratory tract pathogen.

Conflicts of interest

The authors declare that there is no conflict of interest.

Ethical Clearance

This work is approved by The Research Ethical Committee.

References

1-Headley SA, Pereira AHT, Balbo LC, Di Santia GW, Bracarense APFRL,Filho LFCC, Schade J, Okano W, Pereira PFV, Morotti F, Preto-Giordano LG, Marcasso RA, Alfieri AF, Lisboa JAN, Alfieri AA (2018) Histophilus somni-associated syndromes in sheep from Southern Brazil. Brazilian *J Microbiol 49*:591–600.https://doi.org/10.1016/j.bjm.2017.12.008.

2-Murray GM, More SJ, Sammin D, Casey MJ, McElroy MC, O'Neill RG, Byrne WJ, Earley B, Clegg TA, Ball H, Bell CJ, Cassidy JP (2017) Pathogens, patterns of pneumonia, and epidemiologic risk factors associated with respiratory disease in recently weaned cattle in Ireland. *J Vet Diagn Invest 29:*20–34. https:// doi. org/ 10. 1177/ 10406 38716 674757.

3-Givens MD (2018) Review: risks of disease transmission through semen in cattle. *Animal 12*:s165–s171. https:// doi. org/ 10. 1017/ S1751 7311180007 08.

4-O'Toole D and Sondgeroth KS (2016) Histophilosis as a natural disease. *Curr Top Microbiol Immunol 396*:15–48.

5-O'Toole D, Hunter R, Allen T, Zekarias B, Lehmann J, Kim KS, Grab D, Corbeil LB (2017) Effect of Histophilus somni on heart and brain microvascular endothelial cells. *Vet Pathol 54*(4):629–639.

6- Headley, S. A.; Fritzen, J. T. T.; Silva, D. C. ; Ana Aparecida Correa Xavier; Carolina Yuka Yasumitsu; Flavia Helena Pereira Silva; Alfieri, A. F.; Soethe, A. M. and Alfieri, A. A. (2023) Histophilus somni disease conditions with simultaneous infections by ovine gammaherpesvirus 2 in cattle herds from Southern Brazil *Brazilian Journal of Microbiology 54*:1169–1179.https://doi.org/10.1007/s42770-023-00915-5.

7-Pansri, P., Katholm, J., Krogh, K. M., Aagaard, A. K., Schmidt, L. M. B., Kudirkiene, E., ... & Olsen, J. E. (2020). Evaluation of novel multiplex qPCR assays for diagnosis of pathogens associated with the bovine respiratory disease complex. *The Veterinary Journal, 256,* 105425.

8- Sudaryatma P. E., Mekata H., Kubo M., Subangkit M., Goto Y., Okabayashi T. (2019). Co-infection of epithelial cells established from the upper and lower bovine respiratory tract with bovine respiratory syncytial virus and bacteria. *Vet. Microbiol. 235* 80–85.

9-Corbeil, L.B. 2007. Histophilus somni host-parasite relationships. *Anim. Health Res.Rev. 8:* 151–160.

10-O'Mahony L, Akdis M, Akdis CA.(2011). Regulation of the immune response and inflammation by histamine and histamine receptors. *J Allergy Clin Immunol.;128*(6):1153–62. https://doi.org/10.1016/j.jaci.2011.06.051.

11-Shahid, M., Tripathi, T., Sobia, F., Moin, S., Siddiqui, M., & Khan, R. A. (2009). Histamine, histamine receptors, and their role in immunomodulation: an updated systematic review. *The Open Immunology Journal,* *2*(1). https://doi.org/10.2174/1874226200902010009.

12-Mou, Z., Yang, Y., Hall, A. B., & Jiang, X. (2021). The taxonomic distribution of histamine-secreting bacteria in the human gut microbiome. *BMC genomics, 22*, 1-11.

13. Pishko, E. J., Potter, K. A., & Robertus, J. D. (1995). Site-directed mutagenesis of intersubunit boundary residues in histidine decarboxylase, a pH-dependent allosteric enzyme. *Biochemistry, 34*(18), 6069-6073.https://doi.org/10.1021/bi00018a009.

14-Visciano, P., Schirone, M., & Paparella, A. (2020). An overview of histamine and other biogenic amines in fish and fish products. *Foods, 9* (12), 1795. https://doi.org/10.3390/foods9121795.

15-Moniente, M., García-Gonzalo, D., Ontañón, I., Pagán, R., & Botello-Morte, L. (2021). Histamine accumulation in dairy products: Microbial causes, techniques for the detection of histamine-producing microbiota, and potential solutions. *Comprehensive Reviews in Food Science and Food Safety, 20*(2), 1481-1523. https://doi.org/10.1111/1541-4337.12704.

16- Alvarez, M. A., & Moreno-Arribas, M. V. (2014). The problem of biogenic amines in fermented foods and the use of potential biogenic amine-degrading microorganisms as a solution. *Trends in food science & technology, 39*(2), 146-155. https://doi.org/10.1016/j.tifs.2014.07.007.

17- Krulwich, T. A., Sachs, G., & Padan, E. (2011). Molecular aspects of bacterial pH sensing and homeostasis. *Nature Reviews Microbiology, 9*(5), 330-343. https://doi.org/10.103 8/nrmicro2549.

18-Diaz, M., Del Rio, B., Ladero, V., Redruello, B., Fernández, M., Martin, M. C., & Alvarez, M. A. (2020). Histamine production in Lactobacillus vaginalis improves cell survival at low pH by counteracting the acidification of the cytosol. In*ternational journal of food microbiology, 321,* 108548.https://doi.org/10.1016/j.ijfoodmicro.2020.108548.

19-Guzman-Brambila C, Rojas-Mayorquin AE, Flores-Samaniego B, Ortuno-Sahagun D (2012) Two outer membrane lipoproteins from Histophilus somni are immunogenic in rabbits and sheep and induce protection against bacterial challenge in mice. *Clin*

Vaccine Immunol 19:1826. https:// doi. org/ 10. 1128/ CVI. 00451- 12.

20-Landete, J. M., De Las Rivas, B., Marcobal, A., & Munoz, R. (2008). Updated molecular knowledge about histamine biosynthesis by bacteria. *Critical reviews in food science and nutrition, 4*8(8), 697-714.

21-Takahashi, H., Kimura, B., Yoshikawa, M., & Fujii, T. (2003). Cloning and sequencing of the histidine decarboxylase genes of gram-negative, histamine-producing bacteria and their application in detection and identification of these organisms in fish. *Applied and Environmental Microbiology, 69(*5), 2568-2579.

22-Wongsariya, K., Bunyapraphatsara, N., Yasawong, M., & Chomnawang, M.T. (2015). Development of molecular approach based on PCR assay for detection of histamine producing bacteria. *Journal of Food Science and Technology, 53*, 640-648.

23-Kung, H. F., Lee, T. M., Lin, C. S., Hong, T. Y., & Tsai, Y. H. (2012). Polymerase chain reaction for the detection of histamine-producing bacteria isolated from Taiwanese foods. *Journal of Food and Drug Analysis, 20*(1), 6.

24-Barrow, G.I., Feltham, R.K.A.(1993). Cowan and Steel's Manual for the Identification of 2 Medical Bacteria 3rd ed. Cambridge University Press, New York, pp. 24., 26-27., 36.

25-Miyoshi, T., Iwatsuki, T. and Naganuma,T.(2005). Phylogenetic characterization of 16S rRNA gene clones from deep- groundwater microorganisms that pass through 0.2-micrometer-pore-sizefilters.*Appl. Environ.Microbiol. 71*:1084-1088.

26-VF Saunders, LA Reddacliff, T Berg, M Hornitzky (2007). Multiplex PCR for the detection of Brucella ovis, Actinobacillus seminis and Histophilus somni in ram semen. *85*(1) 72-77.https://doi.org/10.1111/j.1751-0813.2006.00098.x.

27-De las Rivas, B.; Marcobal, A and Munoz, R. (2005).Improved multiplex-PCR method for the simultaneous detection of food bacteria producing biogenic amines. *FEMS Microbiology Letters 244:*367–372.

28-Headley, S. A., Oliveira, V. H., Figueira, G. F., Bronkhorst, D. E., Alfieri, A. F., Okano, W., & Alfieri, A. A. (2013). Histophilus somni-induced infections in cattle from southern Brazil. *Tropical animal health and production, 45*, 1579-1588.

29- Cengiz , S; Adıgüzel, M. . and Dinç Gökçen (2021). Detection of Pasteurella multocida, Mannhemia haemolytica, Histophilus somni and Mycoplasma bovis in cattle lung. *Rev Mex Cienc Pecu ;12*(3):710-720.

30-Kurcubic, V. S., Djoković, R. D., Ilić, Z. Z., Stojković, J. S., Petrović, M. P., & Caro Petrović, V. (2014). Modern approach to the enigma of bovine respiratory disease

complex: *A review. Pakistan Veterinary Journal, 34*(1), 11-17.

31-Aebi, M., van den Borne, B. H., Raemy, A., Steiner, A., Pilo, P., & Bodmer, M. (2015). Mycoplasma bovis infections in Swiss dairy cattle: a clinical investigation. *Acta Veterinaria Scandinavica, 57*, 1-11.

32-Bandara, A. B. ;Ziwei Zuo;Kelly McCutcheon;Siddharth Ramachandran; James R. Heflin and Thomas J. Inzana(2018).Identification of Histophilus somni by a nanomaterial optical fiber biosensor assay. *Journal of Veterinary Diagnostic Investigation 30*(6) 821–829.

33-Bell, C. J.; Paul Blackburn; Mark Elliott, Tony I. A. P. Patterson, Sean Ellison, Angela Lahuerta-Marin, Hywel J. Ball (2014) Investigation of polymerase chain reaction assays to improve detection of bacterial involvement in bovine respiratory disease. *Journal of Veterinary Diagnostic Investigation, 26*(5) 631–634.

34-Tegtmeier, C., Angen, Ø., Grell, S. N., Riber, U., & Friis, N. F. (2000). Aerosol challenge of calves with *Haemophilus somnus* and *Mycoplasma dispar*. *Veterinary microbiology, 72*(3-4), 229-239.

35-Lehane, L. and Olley, J. (2000) Histamine fish poisoning revisited. *Int. J. Food Microbiol. 58,* 1–37.

36- Silla, M.H. (1996) Biogenic amines: their importance in foods. *Int. J. Food Microbiol. 29,* 213–231.

37-Ibrahim, H. k.; Abdulelah A. Almayah and Awatif H. Issa (2017).Virulence factors and antibiotic susceptibility patterns of Klebsiella pneumonia strains Histamine producing bacteria isolated from sputum. *Journal of Medical Research, 1*(4).

38-Issa, A. H. , Saewan, S. A.& Abdulrahman, B. A.(2018) Studying of Histamine Concentration and Quality Parameters in Some Local Soft cheese and Imported Processed Cheese Types in Basrah Markets. *Special Issue, 2nd Int. Conference of Agricultural Sciences*

39-Ibrahim, H. k.; Abdulelah A. Almayah and Awatif H. Issa (2017). Molecular Detection of Environmental Morganella morganii as Histamine Producing Bacteria . *Donnish Journal of Medicine and Medical Sciences 4*(2) pp. 008-013.

40-Al-Noor, J. M.; Najim, S. M.; Al-Tameemi, R. A. (2023) Detection and Levels of Some Mycotoxins and Biogenic Amines in Fish Diets and Feed Ingredients from Basrah, Iraq. *Egyptian Journal of Aquatic Biology & FisheriesVol. 27*(4): 997 – 1012.

41-Saewa, S. A.; Khidhir,Z.K ,; Al Bayati M. M.(2021) The impact of storage duration and conditions on the formation of biogenic amines and microbial content in poultry meat. *Iraqi Journal of Veterinary SciencesVolume 35,(* 1*),* Pages 183-188

42-Veciana-Nogues, M.T., Bover-Cid, S., Marine´-Font, A. andVidal-Carou, M.C. (2004) Biogenic amine production by Morganella morganii and Klebsiella oxytoca

in tuna. Eur. *Food Res.Technol. 218*, 284–288.

43. Ruby, K. W., Griffith, R. W., & Kaeberle, M. L. (2002). Histamine production by Haemophilus somnus. *Comparative immunology, microbiology and infectious diseases, 25*(1), 13-20.

44. Corbeil, L. B., Arnold, K. F., Kimball, R., Berghaus, L., & Gershwin, L. J. (2006). Specificity of IgG and IgE antibody responses to Haemophilus somnus infection of calves. *Veterinary immunology and immunopathology, 113*(1-2), 191-199**.**

تفاعل البلمرة المتسلسل للكشف عن بكتيريا الهستوفيلس سومني المنتجة للهستامين المعزولة من أبقار البصرة

رغد مالح جاسم[1], فوزية علي عبد الله[1] , جلال ياسين مصطفى[2]

1- فرع الاحياء المجهرية، كلية الطب البيطري، جامعة البصرة، البصرة، العراق.

2- فرع الصحة العامة، كلية الطب البيطري، جامعة البصرة، البصرة، العراق.

الخلاصة

سعت هذه الدراسة الى دراسة أهمية بكتيريا *Histophilus somni* كمنتج للهيستامين وممرض للجهاز التنفسي. استخدمت التقنيات البكتريولوجية التقليدية و الجزيئية لفحص 200 عينة من مسحاة الانف للتأكد من وجود بكتيريا *Histophilus somni* و قدرتها على انتاج الهيستامين من بين 200 مسحة من انف الماشية التي تم اختبارها، كانت نتيجة 25% (50 / 200) منها ايجابية بالنسبة للخصائص البيوكيميائية التقليدية والزرع الجرثومي. وجدت بكتيريا *Histophilus somni* في 70 % (35 / 50) من العزلات التي اظهرت نتيجة ايجابية في اختبار PCR الذي اجري بعد عزلها على الاوساط الزرعية. من بين 35 عزلة *H. Somni* لها نتائج ايجابية في اختبار PCR كانت 20 % (7 / 35) منها لها نتائج ايجابية في تضخيم PCR الخاص بجين الهيستيدين ديكاربوكسيلليز. الماشية المصابة في الفئة العمرية الاولى (اقل من سنة واحدة) كانت لديها نسب اعلى من النتائج الايجابية للاستزراع (72% ، 36 / 50) و اختبار PCR (80 % ، 28 / 35). فيما يتعلق بتأثير الجنس اظهر الذكور نسب ايجابية اعلى للاستزراع (82 % ، 41 / 50) و اختبار PCR (85 % ، 30 / 35) .

الكلمات المفتاحية: سلسلة تفاعل البلمرة، الهستوفيلوس سومني، الهيستامين.