Assiut Veterinary Medical Journal   Assiut Vet. Med. J. Vol. 70 No. 183 October 2024, 531-543

Assiut University website: www.aun.edu.eg

ASSOCIATION OF IFN-Γ AND IL-4 CYTOKINES WITH TYPE I HYPERSENSITIVITY CAUSED BY *HISTOPHILUS SOMNI* - LIPOPOLYSACCHARIDE IN CATTLE

JASIM R.M.[1]; ABDULLAH F.A.[1] AND MUSTAFA J.Y [2]

[1] Microbiology Department, College of Veterinary Medicine, University of Basrah, Basrah, Iraq.
[2] Department of Public Health, College of Veterinary Medicine, University of Basrah, Basrah, Iraq.

Received: 21 August 2024; **Accepted:** 18 September 2024

ABSTRACT

A total of 200 cattle of different ages, sexes, and breeds, suffering from respiratory symptoms, including 10 apparently healthy cattle, were examined clinically and bacteriologically for the isolation and identification of *Histophilus somni*. Enzyme-Linked Immunosorbent Assay (ELISA) was used to determine the levels of Bovine Interferon gamma (IFN-γ), Bovine Interleukin-4 (IL-4), and Immunoglobulin E (IgE) in the sera of the studied animals. Conventional bacteriological testing revealed that 25% (50/200) of the nasal swabs tested positive for *H. somni*. After culturing, the 16S rDNA and *H. somni*-specific PCR, gene sequencing, and phylogenetic analysis identified that 70% (35/50) of the isolates were confirmed to belong to *Histophilus somni*, with one isolate submitted to NCBI GenBank, receiving accession number OR100605. The seropositivity against *H. somni* LPS was estimated by an indirect ELISA test. The results showed that 35 (70%) of the cattle suffering from respiratory symptoms were seropositive for *H. somni* LPS. The LPS-specific IgE level, when compared between cattle with negative *H. somni* molecular results (0.429 ± 0.0139) and those with positive molecular results (0.733 ± 0.0227), showed a statistically significant ($p < 0.05$) increase in the mean Optical Density (OD) values. The association of IFN-γ and IL-4 with LPS-specific IgE antibodies was determined by indirect ELISA. The results indicated a significant ($p < 0.05$) increase in the mean IL-4 concentration in the sera of LPS-specific IgE seropositive cattle (200.151 ± 70.905) compared to seronegative animals (118.626 ± 27.642), with no statistically significant association ($p > 0.05$) between seropositivity and IFN-γ concentration. The results of this study suggest that *H. somni* LPS may induce a hypersensitized state in cattle. Additionally, this study suggests that Iraqi cattle may have elevated IgE levels against *H. somni* LPS.

Key words: LPS, *Histophilus somni*, cytokines

INTRODUCTION

The term "bioaerosol" refers to dust particles that consist of or include biological materials. The dust can contain mold spore-derived mycotoxins, bacteria, viruses, and/or bacterial endotoxins. Many bioaerosol substances can cause respiratory inflammation, allergies, malignancies, and infectious diseases when inhaled (Clark, 2017). Bacteria play a dual role in allergy. While they are often associated with protection, certain bacterial species can

Corresponding author: Jasim R.M.
E-mail address: pgs.raghad.malih@uobasrah.edu.iq
Present address: Microbiology Department, College of Veterinary Medicine, University of Basrah, Basrah, Iraq.

promote the development and exacerbation of allergic inflammation. Notably, IgE antibodies specific to bacterial antigens have been found in the sera of allergic individuals, suggesting that some bacterial factors act as allergens, eliciting a type 2 immune response (Nordengrün *et al*., 2018).

Because lipopolysaccharide (LPS) has been linked to several livestock diseases, it is frequently used in studies on cattle health. LPS, a membrane component found in Gram-negative bacteria, plays a significant role in this research (Vargas and Marino, 2016; Bilal *et al*., 2016; Guo *et al*., 2017; Herry *et al*., 2016). The innate immune system can identify LPS as a pathogen-associated molecular pattern (PAMP) through various pattern-recognition receptors (PRRs). Recognition of LPS triggers the innate immune response, leading to inflammation characterized by the production and release of pro-inflammatory cytokines and chemokines into the bloodstream. Several circulatory biomarkers of LPS-induced inflammation in animals include pro- and anti-inflammatory cytokines, chemokines, microRNAs (Naylor *et al*., 2020), and populations of white blood cells (WBC) (Hadfield *et al*., 2018).

The immunological response is mounted to varying degrees by distinct bacterial strains. Some bacteria, including *Yersinia pestis*, modify the degree of acylation of lipid A during infection to create low-immunogenicity lipoproteins (LPP) in vivo. In contrast, lipid A produced by *E. coli* or *Salmonella* is highly immunogenic. Higher LPS gene abundance in the gut microbiota is linked to TLR4-dependent proinflammatory gene expression, eliciting a mixed type 2/type 3 response in mice (Campbell *et al.*, 2024). Interestingly, bacteria use the synthesis of low-immunogenic LPS as a tactic to enhance intracellular survival and evade the host immune system (Maldonado *et al*., 2016). Additionally, *H. somni*'s LOS can undergo multiple alterations, resulting in greater diversity and continuous adjustment of the bacterium's antigen expression, enabling it to evade the host immune system. Furthermore, *H. somni* can add neuraminic acid to its LOS. This sialylation process can increase resistance to the bactericidal effect of serum by preventing antibodies from binding to specific epitopes (Inzana *et al.*, 2015).

The influence of microbiota on the development of allergies is a prominent topic. Moreover, the microbiota has been considered an important target for allergy treatment and prevention. Novel intervention strategies targeting the microbiome will be critical for the control of allergic respiratory diseases (*Zubeldia-Varela et al.*, 2022). The balance between the immune system and the resident microbiota is crucial for maintaining health. Dysbiosis, caused by population disturbances and imbalances in the microbiota, has been associated with allergic diseases (*Pascal et al.*, 2018; *Rodriguez-Coira et al.*, 2021).

Haemophilus somnus was the previous name for the Gram-negative coccobacillus *Histophilus somni* (HS) (Corbeil, 2015). It is the sole member of the *Histophilus* genus (Angen, 2015). The description of immunological diseases such as HS-LPS type I hypersensitivity or allergy in Iraqi cattle has not been reported, making the present study the first to document this disease in Iraq. Chronic inflammatory illnesses known as allergies are caused by immunological dysregulation in response to certain environmental substances called allergens. Allergens are substances that typically induce the host to produce IgE antibodies (*Breiteneder and Chapman*, 2014; *Nordengrün et al.*, 2018). Epidemiological evidence suggests that specific bacterial species can cause or aggravate allergies through colonization or infection (*Edwards et al.*, 2012). Bacteria can exacerbate asthma symptoms either on their own or in combination with viruses such as respiratory syncytial virus or rhinovirus (*Darveaux and Lemanske*, 2014).

Exposure to allergens can lead to hyperreactivity in various cells, resulting in the recruitment of eosinophils and activation of mast cells, dendritic cells (DCs), epidermal keratinocytes, lymphocytes, and goblet cells in various parts of the airways. These activated immune cells initiate a cascade of local and systemic inflammatory reactions (*Gupta et al.*, 2017). It is hypothesized that many of the signs and symptoms of respiratory allergy are caused by uncontrolled inflammation (*Chowdhury et al.*, 2019). Cytokines produced by these immune cells contribute to the development of hypersensitive diseases, with subtypes of T helper cells (Th1 and Th2) playing a critical role in the development and progression of allergic rhinitis (AR). These immune cells produce a number of cytokines that are involved in pro-inflammatory responses, but also in anti-inflammatory effects, depending on the balance of these cytokines and biomolecules (*Gupta et al.*, 2017).

It is well established that respiratory allergies are associated with an enhancement of Th2 lymphocyte responses, as evidenced by elevated levels of Th2-profile cytokines in the nasal mucosa, including interleukin (IL)-4, IL-5, and IL-13 (*Segundo et al.*, 2009), which play a key role in the pathogenesis of AR. In addition to the initial Th2/Th1 concept, other cytokines such as Th17 cell cytokines (IL-17, IL-22, and IL-21), IL-3, granulocyte/macrophage colony-stimulating factor (GM-CSF), and tumor necrosis factor-α (TNF-α) significantly contribute to the pathogenesis of respiratory allergies (*Gupta et al.*, 2017). Localized infiltration of eosinophils, linked to the suppression of interferon-γ (IFN-γ) production, further promotes the inflammatory process. These altered immune responses are responsible for the symptoms of the disease (*Segundo et al.*, 2009).

Therefore, the purpose of this study was to investigate the association of IFN-γ and IL-4 cytokines with Type I Hypersensitivity caused by *Histophilus somni* lipopolysaccharide in cattle.

MATERIALS AND METHODS

Sampling and bacterial isolation

The isolation and identification of *H. somni* were performed on nasal swabs from 200 cattle of different ages, sexes, and breeds suffering from respiratory symptoms. Additionally, 10 apparently healthy cattle were subjected to LPS-based IgE-ELISA testing as a control group. The investigation was carried out between November 2022 and March 2023. Nasal swabs were collected from 23 females and 177 males suffering from respiratory manifestations in various localities of Basra Governorate. Sterile cotton swabs were used to obtain nasal samples from the affected cattle. The swabs were placed in brain-heart infusion broth containing 0.1% tris-base and 0.1% L-cysteine. The samples, kept in an ice box, were immediately transported to the laboratory. Upon arrival, the samples were incubated for 24 hours at 37°C (*Talan et al.*, 1989), then cultured on a solid Columbia agar base supplemented with 5% sheep blood. They were incubated at 37°C with 5% CO2 and inspected 24–48 hours later (*Kilian and Biberstein*, 1984).

Identification of the agent was conducted 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 glucose fermentation (strong yellow discoloration of the media) and a positive oxidase test (*Barrow and Feltham*, 1993).

PCR detection of a bacterial isolate

DNA extraction

One milliliter was taken from each of the 50 positive cultures in brain-heart infusion broth. After centrifuging for ten minutes at 12,000 rpm, the pellet was used to extract genomic DNA according to the

manufacturer's instructions (Wizard® Genomic DNA Extraction Kit; Promega, USA). The isolated DNA was confirmed on a 1% agarose gel stained with ethidium bromide (0.5 mg/ml). The concentration and purity of the isolated DNA were determined using a Nanodrop spectrophotometer (Quawell, USA) with a 260/280 nm calibration.

PCR amplification

Two different PCR procedures were applied to 50 DNA extracts. In the first procedure, DNA samples were tested by specific PCR assays for *Histophilus somni* (*Saunders et al.*, 2007), and in the second PCR, a 16S rDNA universal primer for bacteria was used (*Miyoshi et al.*, 2005). The PCRs were performed using a PCR thermocycler (TEKNE TC-3000G, USA) in a reaction volume of 25 µl containing: 5 µl of DNA template, 1 µl of each primer, 5.5 µl of nuclease-free water, and 12.5 µl of 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 hour in 1.5% agarose gels for *Histophilus* genus and species genes. The expected amplicon bands were visualized and photographed under a UV transilluminator (EDVOTEK, UK) and gel documentation system (Gel Doc, ATP Co).

The amplification of the 16S rDNA-specific PCR relied on universal bacterial gene primers 27 F 5'-AGAGTTTGATCCTGGC-3' and R 5'-GGTTACCTTGTTACGACTT-3', which amplified DNA fragments of 1500 bp. The PCR conditions consisted of an initial denaturation at 94°C for 5 min, followed by 30 cycles of denaturation at 94°C for 30 sec, annealing at 51.8°C for 45 sec, and extension at 72°C for 30 sec, with a final extension at 72°C for 10 min. PCR using specific primers for the *H. somni* gene amplified a fragment of 313 bp (forward 5'-GAAGGCGATTAGTTTAAGAG-3' and reverse 5'-ACTCGAGCGTCAGTATCTTC-3'). The PCR conditions for *H. somni* consisted of an initial denaturation at 94°C for 2 min, followed by 35 cycles of denaturation at 94°C for 30 sec, annealing at 49°C for 30 sec, and extension at 72°C for 1 min, with a final extension at 72°C for 6 min.

DNA sequencing and phylogenetic analysis

Sequence alignment and DNA sequencing were used to confirm the species identification of *Histophilus somni*. One PCR amplicon from the 16S rDNA PCR-positive *Histophilus somni* was sent to the Macrogen Company laboratory in Korea. DNA sequences were assembled in both directions using the forward and reverse primers used in the PCR.

A BLAST® analysis (Basic Local Alignment Search Tool) was initially performed to establish sequence identity with GenBank accessions. The sequence alignments were thoroughly checked manually to determine the percentages of similarities and discrepancies. A comparison was made between the sequence alignment of the currently identified bacterial isolate and those of previously published species of *Histophilus somni* (*Histophilus somni* strain 8025 16S ribosomal RNA). Phylogenetic analyses were conducted using maximum likelihood, neighbor-joining, and maximum parsimony methods in MEGA6.

Extraction of LPS

LPS was extracted from PCR and gene sequence-confirmed *H. somni* isolates using the hot phenol-water method, with slight modifications from the procedure previously described by Inzana (1983). In brief, bacterial suspensions (10^8 colony-forming units/mL) were centrifuged at 10,000×g for 5 minutes. The pellets were washed once with 1 mL of PBS at pH 7.4 (8.5 g Sodium chloride (NaCl), 0.23 g sodium dihydrogen phosphate (NaH_2PO_4), and 2.17 g di-sodium hydrogen phosphate heptahydrate ($Na_2HPO_4 \cdot 7H_2O$). The washed cells were

resuspended in 300 µL of distilled water and transferred to a glass vial.

In the next step, an equal volume of hot (65-70°C) 90% phenol was added to the bacterial cell suspension. The vial was placed in a beaker of water on a hot plate/stirrer set to 65-70°C, followed by vigorous stirring for 15 minutes. The suspensions were then cooled on ice and centrifuged at 1,000×g for 10 minutes. Supernatants were transferred to 15 mL conical centrifuge tubes, and the phenol phases were re-extracted with 300 µL distilled water. The mixture was stirred for 15 minutes, cooled on ice, and centrifuged at 1,000×g for 10 minutes. Supernatants were transferred to new 15 mL conical centrifuge tubes. The aqueous phases in the 15 mL conical centrifuge tubes were pooled, and the sodium concentration of the collected aqueous phases was adjusted to 0.5 M by adding one-tenth the volume of Sodium chloride solution (5 M NaCl) (29.22 g NaCl / 100 mL distilled water).

Ten volumes of 95% ethanol were added to the extracts, and samples were stored at -20°C overnight to precipitate LPS. The tubes were then centrifuged at 2,000×g at 4°C for 10 minutes, and the pellets were resuspended in 1 mL distilled water. Extensive dialysis against double-distilled water at 4°C was carried out until the residual phenol in the aqueous phases was completely eliminated. The partially purified LPS product was placed in a Petri dish and stored at 4°C until completely dry.

ELISA for Specific IgE detection

This assay was performed on 50 serum samples from cattle suffering from respiratory manifestations, in addition to 10 apparently healthy cattle. As described by Bahr *et al.* (1980), the *H. somni* LPS (0.4 g/mL) antigen was coated onto the wells of a micro-ELISA plate in 100 µL carbonate/bicarbonate buffer, pH 9.6, and incubated overnight at 4°C. After coating, the wells were washed with phosphate-buffered saline (PBS), pH 7.4, containing 0.05% Tween 20, to block uncoated sites and reduce non-specific binding. This buffer was also used as the diluent for sera and conjugates.

Undiluted sera were added to the sample wells and incubated at room temperature for 2 hours. After further washes with PBS/Tween, undiluted conjugates specific for bovine IgE were added and incubated for 1 hour at 4°C. Following additional washes, 50 µL of each chromogen solution A and B was added to each well, mixed gently, and incubated at 37°C for 30 minutes. To terminate the reaction, 50 µL of stop solution was added to each well. The absorbance values were then read immediately at 495 nm. The mean value of optical density (OD_{495}) plus 3 standard deviations of the mean, calculated from a group of 10 apparently healthy cattle tested as negative controls, was regarded as the negativity cutoff. Thus, all sera producing an OD_{495} higher than 0.46 were scored as positive for the presence of LPS-specific IgE (Difelice *et al.*, 1994).

ELISA for IFN-γ and IL-4 measurement

Bovine interferon-gamma (IFN-γ) and bovine interleukin-4 (IL-4) were measured using the bovine IFN-γ and IL-4 ELISA kits (SunLong Biotech Co., LTD, China), following the manufacturer’s instructions. The same procedure was performed for the measurement of both IFN-γ and IL-4. The standard concentrations used were 180 pg/mL, 120 pg/mL, 60 pg/mL, 30 pg/mL, and 15 pg/mL. Quantities of IFN-γ and IL-4 in the serum were measured with ELISA using 96-well microplates coated with anti-bovine IFN-γ or IL-4 antibodies. After dilution, serum samples (50 µL/well) and standards (50 µL/well) were incubated for 30 minutes at 37°C. Subsequently, HRP-conjugate reagent (50 µL/well) was added. Then, from each chromogen solution A and B, 50 µL/well were added and incubated at 37°C for 15 minutes. The chromogenic process was stopped with 50 µL of stop solution, and the optical density values were read with a plate reader at 450 nm (Micro

ELISA auto reader, Biotek, USA). The average of duplicate readings was calculated for each standard to interpret the ELISA results, and to create a standard curve. The values for the test samples were extrapolated from the standard curve.

Statistical analysis

All data were gathered and cleaned using Microsoft Excel version 2016. A t-test was utilized to determine whether any association existed between the outcomes at a significance level of 5%. Statistical analysis was conducted using SPSS software version 22.

RESULTS

Bacteriological and molecular identification

The samples were considered positive for *H. somni* upon observation of bacterial growth, confirmed by colony characteristics, hemolytic patterns on blood agar, and conventional biochemical properties. All 50 isolates were catalase-negative, while positive results were observed in the oxidase and glucose fermentation tests. Based on culture and conventional biochemical properties, 25% (50/200) of the tested cattle nasal swabs were positive (Table 1).

After culturing, the results of the 16S rDNA-PCR assay revealed that 70% (35/50) of the culture-positive isolates belonged to the *Histophilus* spp. The 16S rDNA produced distinct bands with a molecular size of 1500 bp (Table 1, Figure 1a). According to the specific PCR amplification for *H. somni*, all 16S rDNA-PCR positive isolates (n = 35) showed positive results with a molecular size of approximately 313 bp (Figure 1b). Consequently, 70% (35/50) of the overall culture-positive isolates were determined as *H. somni* in cattle (Table 1).

The amplified and purified PCR product of one isolate was subjected to sequencing analysis, and the sequence was subsequently submitted to NCBI GenBank, receiving the accession number OR100605. A phylogenetic tree (Figure 2) was constructed based on the 16S gene sequence of one purified strain of *H. somni* from the Iraq-Basrah isolates and other GenBank accessions. The new strain of Iraqi *H. somni* shared common ancestors with previously reported strains of *H. somni* from Japan (AB176899) and China (OP756060).

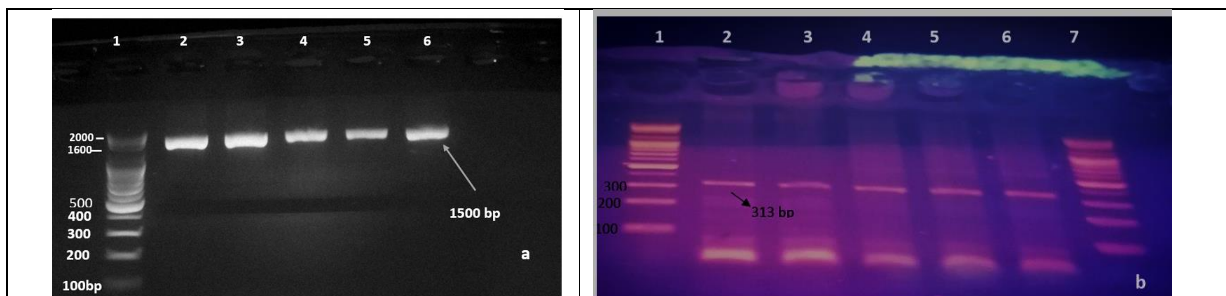

Figure 1: (a) The 16S universal gene was detected as an amplicon of 1500 bp on an agarose gel after polymerase chain reaction (PCR). Lane 1 shows the molecular weight marker, ranging from 1000 to 2000 bp, while Lanes 2–6 represent positive samples. (b) The *Histophilus somni* species was detected on another agarose gel using PCR, with an amplicon size of 313 bp. Lane 1 and Lane 7 show the molecular weight marker (100–1000 bp), while Lanes 2–6 represent positive samples.

Specific IgE response to *H. somni* LPS

The new Iraqi strain of *H. somni* (OR100605) was used as the source of antigen in this test. The seropositivity against *H. somni* LPS was estimated using an indirect ELISA test, and the results are displayed in Table (2). In this table, 35 (70%) of the cattle suffering from respiratory manifestations were seropositive for *H. somni* LPS. The LPS-

specific IgE level was represented by the mean ± SD of the OD values. When compared to animals with negative *H. somni* molecular results (0.429 ± 0.0139), cattle sera with positive results (0.733 ± 0.0227) showed a statistically significant increase in the means of OD values ($p < 0.05$).

Table 1: Distribution of *H somni* infection according to cultural, *16S rRNA* and *H. somni* gene PCR results of the examined nasal samples.

Diagnostic test	Positive n. (%)	Negative n. (%)	Total
Culture	50(25)	150(75)	200
PCR/*16S rRNA*	35(70)	15(30)	50
PCR/ H somni	35(70)	15(30)	50

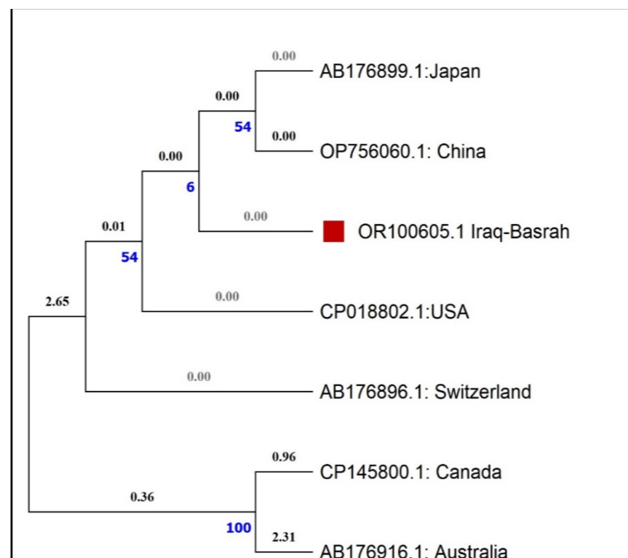

Figure-2: Phylogenetic analysis of *H. somni* isolates based on 16S gene sequences revealed that the OR100605 strain under study had common ancestors with previously reported *Histophilus somni* strains, including those from Japan (AB176899) and China (OP756060), as well as CP018802.1 and AB176896.1 strains isolated in the USA and Switzerland, CP145800.1 isolated in Canada, and AB176916.1 from Australia.

Table 2: ELISA results of LPS-specific IgE antibodies in bovine sera.

PCR results	Tested n. (%)	ELISA results				
		Mean ±SD (OD value)	SE	95% CI	t statistic	P value
PCR positive	35(70)	0.733±0.0227	0.009	0.2845 to 0.3211	33.309	0.0001
PCR negative	15(30)	0.429±0.0139				
Total	50					

Standard Error (SE); Optical density (OD); Confidence Interval (CI)

Association of IFN-γ and IL-4 with LPS-specific IgE antibodies

The concentrations of IFN-γ and IL-4 in bovine sera were compared based on LPS-specific IgE seropositivity. A significant increase ($p < 0.05$) in the mean IL-4 concentration was observed in the sera of seropositive cattle (200.151 ± 70.905) compared to seronegative animals (118.626 ± 27.642). However, no statistically significant difference ($p > 0.05$) was found between seropositive and seronegative animals in the case of IFN-γ (Table 3).

Table 3: IFN-Y and IL-4-based ELISA results in relation to bovine clinical status

ELISA results	Cytokines	Tested n.	Mean ±SD (pg/ml)	SE	95% CI	t-statistic	P value
Seropositivity	IFNY	35	155.791± 94.2996	26.28	- 61.083 to 44.583	--0.314	0.7549
Seronegativity		15	83.43±18.01				
Seropositivity	IL4	35	200.151±70.905	18.984	- 119.694 to -43.356	-4.294	0.001
Seronegativity		15	118.626±27.642				

Standard Error (SE); Confidence Interval (CI)

DISCUSSION

Cattle suffer immediate hypersensitivity as a result of an allergic reaction caused by IgE cross-linking to receptors on mast cells and basophils after exposure to an appropriate antigen. The clinical manifestations of allergic reactions, such as tachypnea, fever, itchy eyes, salivation, edema, depression, and anorexia, are brought on by the degranulation of these cells (Ruby *et al.*, 1999). In the current study, the ability of *H. somni* to induce an immediate hypersensitive state in cattle was investigated, and the immune response associated with this state was measured. The results revealed that, out of 200 cattle, only 50 (25%) had positive results for bacterial culture, despite the fact that most of these cattle exhibited respiratory manifestations. This finding might be explained by a study by Klima *et al.* (2014), which discovered that *H. somni* can be challenging to identify due to its fastidious growth requirements and the frequent co-occurrence of other bacterial infections, such as *M. haemolytica* or *P. multocida*. The excessive growth of these organisms often prevents the isolation of *H. somni* from culture. In contrast to culture results, PCR assays showed a larger percentage of positive animals (70%). The difference between culture and PCR results may be explained by Tegtmeier *et al.* (2000), who stated that one advantage of PCR is its ability to identify dead, as well as growth-inhibited bacteria following antibiotic treatment. Antibiotics are frequently utilized on farms for treating respiratory tract illnesses. Based on *H. somni* LPS-specific IgE ELISA, the percentage of type I hypersensitivity in cattle suffering from respiratory manifestations was 70%. The LPS-specific IgE level was represented by the mean ± SD of the optical density (OD) values. When compared to animals with negative *H. somni* molecular results, cattle sera with positive results showed a statistically significant ($p < 0.05$) increase in the mean OD values. It should be clarified that the *H. somni* gene, 16S rDNA-PCR, 16S rDNA gene sequencing, and phylogenetic analysis confirmed that these isolates belonged to *Histophilus somni*, with the sequence submitted to NCBI GenBank under accession number OR100605. Phylogenetically, the 16S rDNA gene sequence of one purified strain of *Histophilus somni* from the Iraq-Basrah isolates had common ancestors with previously reported strains of *Histophilus somni*. Therefore, the current results may

reflect the importance of natural *Histophilus somni* infection as a causative agent of respiratory allergy in cattle. Respiratory allergies have not been extensively studied in cattle; early reports are largely restricted to reactions associated with *Micropolyspora faeni* and bovine respiratory syncytial virus (Gershwin *et al.*, 1990). The potential involvement of a type-1 hypersensitivity component in bovine respiratory disease caused by *Haemophilus somnus* was investigated by Ruby *et al.* (1999), who used sensitized live *H. somnus* organisms. H. somnus-sensitized calves had the highest levels of anti-*H. somnus* IgE antibodies (Nordengrün *et al.*, 2018). Gershwin *et al.* (2005) examined the impact of co-infection in calves with BRSV and *H. somnus*. In that study, calves infected with both agents had the highest clinical scores and IgE anti-*H. somnus* levels compared to calves infected with BRSV or *H. somnus* alone. Additionally, Abbas *et al.* (2005) confirmed the association of *Escherichia coli* with the development of respiratory allergies in humans. Campbell *et al.* (2024) investigated the role of gut microbiota in promoting cow milk allergy-associated microbiota in mice.

Dysregulated immune responses to specific substances, known as allergens, result in allergies, which are chronic inflammatory illnesses. Allergens are substances that typically stimulate the host to produce IgE antibodies (Breiteneder and Chapman, 2014). Accordingly, this study utilized *H. somnus* LPS as an allergen and assessed its associated immune function. Few Iraqi studies have explored the effects of bacterial LPS on human and animal immune responses (Abbas *et al.*, 2005; Al-Amery *et al.*, 2008; Al-Aalim *et al.*, 2021). Additionally, according to Matsuura (2013), among the components of bacterial cell walls, LPS is often regarded as the most potent immune stimulant.

The association of IFN-γ and IL-4 with LPS-specific IgE antibody responses was investigated using an ELISA test. The results indicated a significant ($p < 0.05$) increase in the mean IL-4 concentration in the sera of LPS-specific IgE seropositive cattle compared to seronegative animals, with no statistically significant association between seropositivity and IFN-γ concentration. Few reports have investigated the role of cytokines in determining immune responses. Abdoli and Najafian (2014) noted that the analysis of Th1 and Th2 responses is possible through the detection of specific cytokines secreted by cells involved in each response. For example, Th1 responses can be determined by analyzing interferon-gamma (IFN-γ) or interleukin-2 (IL-2) levels, while Th2 responses can be determined by analyzing levels of interleukin-4 (IL-4) or interleukin-5 (IL-5) (Abdoli and Najafian, 2014). Wills-Karp *et al.* (2012) reported that the central feature of allergies is type 2 inflammation, characterized by increased numbers of Th2 cells, which release IL-4, IL-5, IL-9, and IL-13 upon allergen exposure, as well as by allergen-specific IgE, mast cell activation, and tissue infiltration by eosinophils. However, other types of helper T cells and their cytokines may also be involved (Farahani *et al.*, 2014). Furthermore, because IgE antibodies have been associated with increased respiratory disease and IgG antibodies with decreased clinical signs, Corbeil *et al.* (2006) hypothesized that differences in the specificity of IgE and IgG responses may aid in identifying protective versus immunopathogenic antigens. These researchers also reported that the immunodominant antigen for IgE differs from that for IgG (Corbeil *et al.*, 2006).

Few reports have investigated the role of bacterial LPS in promoting allergic responses. Reynolds *et al.* (2013) reported that lung disease has been strongly associated with endotoxins and an exposure-response relationship. In addition, Vatanen *et al.* (2016) indicated that the presence of *Bacteroides* species LPS in fecal samples from Northern European infants is associated with higher levels of food allergies. Gomes *et al.* (2017) indicated that low-dose LPS exposure is correlated with

autoimmune diseases and allergies, while high concentrations of LPS in the blood lead to metabolic syndrome. In conclusion, the results of this study suggest that *H. somni* LPS is capable of inducing a hypersensitized state in these cattle. This study also indicates that Iraqi cattle had IgE levels against *H. somni* LPS, with a significant increase in IL-4 concentration observed in the sera of LPS-specific IgE seropositive cattle.

REFERENCES

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.

*Saunders VF, Reddacliff LA, Berg T, Hornitzky M. (*2007*):* Multiplex PCR for the detection of Brucella ovis, Actinobacillus seminis and Histophilus somni in ram semen. Aust Vet J, 85: 72–77.

Zubeldia-Varela, E.; Barker-Tejeda, TC.; Obeso, D; Villaseñor, A; Barber, D. and Pérez-Gordo, M. (2022): Microbiome and Allergy: New Insights and Perspectives. J Investig Allergol Clin Immunol 2022; Vol. 32(5): 327-344

Pascal, M.; Perez-Gordo, M.; Caballero, T.; Escribese, MM.; Lopez Longo MN.; Luengo O.; Manso, L.; Matheu, V.; Seoane, E.; Zamorano, M.; Labrador, M. and Mayorga, C. (2018): Microbiome and Allergic Diseases. Front. Immunol. 9:1584. doi: 10.3389/fimmu.2018.01584.

Rodriguez-Coira, J.; Villaseñor, A.; Izquierdo, E.; Huang, M.; Barker-Tejeda, TC.; Radzikowska, U.; Sokolowska, M. and Barber, D. (2021): The Importance of Metabolism for Immune Homeostasis in Allergic Diseases. Front. Immunol. 12:692004. doi: 10.3389/fimmu.2021.692004 97. Fan Y, Pedersen O. Gut microbiota in human metabolic health and disease. Nat Rev Microbiol. 2021; 19: 55-71.

Clark, E. (2017): Bioaerosol Exposures from Three Utah Cattle Operations. MSc. report, University of Montana.

Vargas, N. and Marino, F. (2016): Heat stress, gastrointestinal permeability and interleukin-6 signaling – Implications for exercise performance and fatigue. Temperature, 3(2), 240–251. https://doi.org/10.1080/23328940.2016.1179380.

Bilal, M.S.; Abaker, J.A.; Ul Aabdin, Z.; Xu, T.; Dai, H.; Zhang, K.; Liu, X. and Shen, X. (2016): Lipopolysaccharide derived from the digestive tract triggers an inflammatory response in the uterus of mid-lactating dairy cows during SARA. BMC Veterinary Research, 12, 284.

Barrow, G.I. and 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.

Guo, J.; Chang, G.; Zhang, K.; Xu, L.; Jin, D.; Bilal, M.S. and Shen, X. (2017): Rumen-derived lipopolysaccharide provoked inflammatory injury in the liver of dairy cows fed a high-concentrate diet. Oncotarget, 8(29), 46769–46780.

Herry, V.; Gitton, C.; Tabouret, G.; R_ep_erant, M.; Forge, L.; Tasca, C.; Gilbert, F.B., Guitton, E.; Barc, C.; Staub, C.; Smith, D.G.E.; Germon, P.; Foucras, G. and Rainard, P. (2017): Local immunization impacts the response of dairy cows to Escherichia coli mastitis. Scientific Reports, 7(1), 3441.

Naylor, D.; Sharma, A.; Li, Z.; Monteith, G.; Sullivan, T.; Canovas, A.; Mallard, B.A.; Baes, C. and Karrow, N.A. (2020): Short communication: Characterizing ovine serum stress biomarkers during endotoxemia. Journal of Dairy Science, 103(6), 5501–5508.

Hadfield, J.M.; Bowdridge, E.C.; Hol_askov_A.I.; Elsasser, T.H. and Dailey, R.A. (2018): Breed-specific differences in the immune response to lipopolysaccharide in ewes. Journal of Animal Science, 96(10), 4220–4228.

Campbell, E.; Lauren A. Hesser; Roberto Berni Canani;, Laura Carucci; bLorella Paparo; Robert T. Patry and Cathryn R. Nagler (2024): A Lipopolysaccharide-Enriched Cow's Milk Allergy Microbiome Promotes a TLR4-Dependent Proinflammatory Intestinal Immune Response.J Immunol 212 (4): 702–714.

Maldonado, RF.; Sá-Correia I. and Valvano, MA. (2016): Lipopolysaccharide modification in Gram-negative bacteria during chronic infection. FEMS Microbiol Rev. Jul; 40(4): 480-93. [PMC free article] [PubMed].

*Inzana, T.J. (*2015): The Many Facets of Lipooligosaccharide as a Virulence Factor for Histophilus somni. In: Inzana, T. (eds) Histophilus somni. Current Topics in Microbiology and Immunology, vol 396. Springer, Cham.

Corbeil. LB. (2015) Host immune response to Histophilus somni. In: Inzana T (ed) Histophilus somni. Current Topics in Microbiology and Immunology, vol 396. Springer, Switzerland, pp 109–29.

Angen, Ø. (2015): Taxonomy of Histophilus somni. In: Inzana. T (ed) Histophilus somni. Current Topics in Microbiology and Immunology, vol 396. Springer, Switzerland, pp 1–4.

Breiteneder, H. and Chapman, M.D. (2014): Allergen nomenclature, allergens and allergen immunotherapy: Subcutaneous, sublingual and oral.

Ruby, K.W. (1999): Immediate (type-1) hypersensitivity and Haemophilus somtms. PhD thesis, Graduate Faculty. Iowa State University.USA.

Nordengrün, M.; Michalik, S.; Völker, U.; Bröker, B.M. and Gómez-Gascón, L. (2018): The quest for bacterial allergens. International Journal of Medical Microbiology 308 738–750.

Edwards, M.R.; Bartlett, N.W.; Hussell, T.; Openshaw, P. and Johnston, S.L. (2012). The microbiology of asthma. Nature Reviews Microbiology, 10(7), 459-471.

Darveaux, J.I. and Lemanske, Jr.R.F. (2014): Infection-related asthma. The Journal of Allergy and Clinical Immunology: In Practice, 2(6), 658-663.

Talan, D.A.; Staatz, D.I.A.N.A.; Staatz, A.N.D.R.E.W. and Overturf, G.D. (1989): Frequency of Staphylococcus intermedius as human nasopharyngeal flora. Journal of Clinical Microbiology, 27(10), 2393.

Kilian, M. and Biberstein, EL. (1984): Genus II Haemophilus. In: Krieg NR (Editor). Bergey's Manual of Systematic Bacteriology (volume 1). Baltimore, MD: Williams & Wilkins, pp.558-569.

Inzana, T.J. (1983): Electrophoretic heterogeneity and interstrain variation of the lipopolysaccharide of Haemophilus influenzae. J Infect Dis 148(3): 492-499.

Bahr, G.M.; Rook, W.A.; Moreno, E. and Lydyard, P.Z. (1980). Use of the ELISA to screen for any thymocyte and anti B2 microglobulin antibodies in leprosy and SLE. J. Immuno. 41: 865 - 873.

Difelice, G.; Calufia, M.; Dipaola, R. and Pini, C. (1994): Allergens of Arizona cypress (Cuprssusarizonica) pollen; characterization of pollen extract and identification of allergenic components. J. Allergy. Clin. Immunol. 94 : 547-555.

Klima, C.L.; Zaheer, R.; Cook, S.R.; Booker, C.W.; Hendrick, S.; Alexander, T.W. and McAllister, T.A. (2014): Pathogens of bovine respiratory disease in North American feedlots conferring multidrug resistance via integrative conjugative elements. Journal of Clinical Microbiology, 52(2), 438-448.

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

Gershwin, LJ.; Dungworth, DL. and Friebeashauser, BCE. (1990): Immunoglobulin E responses and lung patholog\' resulting from aerosol exposure of calves to respiratorv' syncytial virus and Micropotyspora faeni hii arch Allergy Appl Immunol 92:293-300.

Gershwin, LJ.; Berghaus, LJ.; Arnold, K.; Anderson, ML. and Corbeil, LB. (2005): Immune mechanisms of pathogenetic synergy in concurrent bovine pulmonary infection with Haemophilus somnus and bovine respiratory syncytial virus. Veterinary Immunology and Immunopathology 107: 119–130.CrossRefGoogle ScholarPubMed.

Abbas, W.H. (2005): The Role Of Candida albicans And Escherichia coli Lipopolysaccharide Allergens As A Causative Agents Of Type I Hypersensitivity.MSc thesis College of Medicine – University of Basrah.

Al-Amery, M.A.Y.; Faisal G. Habasha Khalil H.Z. Al-Joboury (2008): THE Hematological Effect Of Salmonella Typhimurium Endotoxin In Rabbits. Bas. J. Vet., Res., Vol.7, No.2.

Al-Aalim, A.M.; Al-Iedani, A.A. and Hamad, M.A. (2021): The effect of a lipopolysaccharide extracted from Escherichia coli on some innate immunity indices of albino rats. Kufa Journal For Veterinary Medical Sciences Vol. (12) No. (2).

Matsuura, M. (2013): Structural modifications of bacterial lipopolysaccharide that facilitate gram-negative bacteria evasion of host innate immunity. Frontiers in immunology, 4, 109.

Abdoli, R. and Najafian, N. (2014): T Helper cells fate mapping by co-stimulatory molecules and its functions in allograft rejection and tolerance. Int J Org Transplant Med,. 5(3): p. 97-110.

Wills-Karp, M.; Rani, R.; Dienger, K.; Lewkowich, I.; Fox, J.G.; Perkins, C. and Herbert, D. B.R. (2012): Trefoil factor 2 rapidly induces interleukin 33 to promote type 2 immunity during allergic asthma and hookworm infection. Journal of Experimental Medicine, 209(3), 607-622.

Farahani, R.; Sherkat, R.; Hakemi, M.G.; Eskandari, N. and Yazdani, R. (2014): Cytokines (interleukin-9, IL-17, IL-22, IL-25 and IL-33) and asthma. Advanced biomedical research, 3(1), 127.

Corbeil, L.B.; Arnold, K.F.; Kimball, R.; Berghaus, L. and 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.

Reynolds, S.J.; Nonnenmann, M.W.; Basinas, L.; Davidson, M.; Elfman, L.; Gordon, J.; Kirychuck, S.; Reed, S.; Schaeffer, J.W.; Schenker, M.B.; Schlunssen, V. and Sigsgaard, T. (2013): Systematic Review of Respiratory Health Among Dairy Workers. Journal of Agromedicine, 18: 3, 219-243. DOI: 10.1080/1059924X.2013.797374.

Vatanen, T.; Kostic, A.D.; D'hennezel, E.; Siljander, H.; Franzosa, E.A.; Yassour, M. and Xavier, R.J. (2016): Variation in microbiome LPS immunogenicity contributes to autoimmunity in humans. Cell, 165(4), 842-853.

Gomes, J.M.G.; De Assis Costa, J. and Alfenas, R.D.C.G. (2017): Metabolic endotoxemia and diabetes mellitus: A systematic review. Metabolism, 68, 133-144.

Segundo, G.R.; Gomes, F.A.; Fernandes, K.P.; Alves, R.; Silva, D.A. and Taketomi, E.A. (2009). Local cytokines and clinical symptoms in children with allergic rhinitis after different

treatments. *Biologics: Targets and Therapy, 469-474.*

Chowdhury, N.I.; Chandra, R.K.; Li, P.; Ely, K. and Turner, J.H. (2019, May): Investigating the correlation between mucus cytokine levels, inflammatory cell counts, and baseline quality-of-life measures in chronic rhinosinusitis. In *International Forum of Allergy & Rhinology* (Vol. 9, No. 5, pp. 538-544).

Gupta, R.K., Gupta, K. and Dwivedi, P.D. (2017): Pathophysiology of IL-33 and IL-17 in allergic disorders. Cytokine & Growth Factor Reviews, 38, 22-36.

ارتباط السيتوكينات IFN-γ و IL-4 مع فرط الحساسية من النوع الأول الناجم عن عديد السكاريد الشحمي لبكتريا الهستوفلس سومني في الابقار

رغد مالح جاسم [١] ،فورية علي عبد الله [١] ، جلال ياسين مصطفى [٢]

١-فرع الاحياء المجهرية ،كلية الطب البيطري ، جامعة البصرة ،العراق.
٢-فرع الصحة العامة ، كلية الطب البيطري ،جامعة البصرة، العراق.

Email: pgs.raghad.malih@uobasrah.edu.iq Assiut University website: www.aun.edu.eg

تم فحص ٢٠٠ رأس من الأبقار من مختلف الأعمار والجنس والسلالات تعاني من أعراض تنفسية بالإضافة إلى ١٠ أبقار سليمة ظاهريا تم فحصها سريريا وجرثوميا لعزلها وتحديد هويتها هستوفلس سومني. تم استخدام تقنية الاليزا لتقدير الانترفيرون جاما البقري (IFN-γ) والإنترلوكين-٤ البقري (IL-4) والجلوبيولين المناعي E (IgE) في أمصال الحيوانات المدروسة. كشف الاختبار البكتريولوجي التقليدي أن ٢٥% (٥٠/٢٠٠) من مسحات الأنف التي تم اختبارها أظهرت نتائج إيجابية بعد الزراعة. تم تحديد H. somni- PCR و16S rRNA وتسلسل الجينات وتحليل النشوء والتطور أن ٧٠٪ (٣٥/٥٠) من العزلات تم تأكيد انتمائها إلى Histophilus somni وتم تقديم عزلة واحدة إلى NCBI GenBank برقم الانضمامOR100605. تم تقدير الإيجابية المصلية ضد H. somni- LPS بواسطة اختبار ELISA غير المباشر. وأظهرت نتائج هذا الاختبار أن ٥٠/٢٠٠ (٢٥%) من الأبقار التي تعاني من المظاهر التنفسية كانت إيجابية مصليا ضد H. somni LPS.أظهر مستوى IgE الخاص بـ LPS عند مقارنته بالماشية ذات النتائج الجزيئية H somni السلبية (0.429±0.0139)، والحيوانات ذات النتائج الجزيئية الإيجابية(0.733±0.0227) زيادة ذات دلالة إحصائية($p < 0.05$) . تم تحديد ارتباط IFN-γو IL-4 مع الأجسام المضادة IgE الخاصة بـ LPS بواسطة ELISA المباشر.أشارت نتائج هذا الاختبار إلى أن زيادة معنوية ($P < 0.05$) في وسائل تركيز IL-4 قد لوحظت في أمصال الأبقار إيجابية المصل IgE الخاصة بـ LPS (200.151 ± 70.905) مقارنة بالحيوانات سلبية المصل (118.626±27.642) ولا يوجد دلالة إحصائية كبيرة على وجود علاقة ($p < 0.05$) بين الإيجابية المصلية وتركيز IFN-Y. تشير نتائج هذه الدراسة إلى أن H. somni LPS قد يكون قادرًا على إحداث حالة فرط الحساسية في الماشية. تشير هذه الدراسة أيضًا إلى أن الماشية العراقية قد تحتوي على مستويات IgE ضد H. somni LPS.