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Study of some aspects of otitis externa in calves in Mosul city

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Abstract

In this study 60 local calves breed (30 animals of with otitis externa and 30 without otitis externa) of both sexes, three weeks to six months old were examined clinically and bacteriologically for isolaltion and identification of the bacterial agents from external otic canal. The main clinical signs observed in animals with otitis externa included auricular discharge (unilateral in 23 and bilateral in 7 calves), cough, nasal discharge, anorexia, and emaciation. Results revealed isolation of (9) bacterial species from external otic canal in both calves with otitis externa and without otitis externa. The predominant species found in calves with otitis externa were *Pasteurella multocida, Mannheimia hemolytica* and *Staphylococcus aureus*, while in the calves without otitis externa were *Escherichia coli, Klebsiella pneumonia*, and *Staphylococcus epidermis.* Results also indicated that the most bacterial isolated from calves with otitis externa were resistance to the bactericidal effect of the calf normal serum and produced hydroxymate siderophore in comparison with bacterial isolated from calves without otitis externa.

Keywords: Otitis externa, Calves, Hydroxymate Siderophore.
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دراسة بعض جوانب التهاب الاذن الخارجية في العجول في مدينة الموصل

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الخلاصة

تم في هذه الدراسة الفحص السريري والجرثومي على ٦٠ عجلا محليا من كلا الجنسين، وباعمار تراوحت بين ثلاثة اسابيع الى ستة اشهر، لغرض العزل الجرثومي من قناة الاذن الخارجية في كل من العجول التي اظهرت علامات التهاب الاذن الخارجية (عددها ٣٠)، او السوية سريريا (عددها ٣٠). كانت اهم العلامات السريرية الظاهرة على الحيوانات المصابة بالتهاب الاذن الخارجية هي ظهور النضح الاذني (من اذن واحدة في ٢٣ حيوانا، ومن كلا الاذ نين في ٧ حيوانا ت)، والسعال مع النضح الانفي، وفقدان الشهية، والهزال. اشارت النتائج الى عزل تسعة انواع جرثومية من قناة الاذن الخارجية في كل من العجول المصابة بالتهاب الاذن الخارجية والسوية سريريا. كانت اهم الانواع الجرثومية المعزولة من العجول المصابة هي الباستوريلا مالتوسيدا، و الباستوريلا الحالة للدم، والمكورات العنقودية الذهبية، بينما كانت في العجول غير المصابة بالتهاب الاذن الخارجية هي الايشيريكيا القولونية، والكلبسيلا الرئوية، و المكورات العنقودية الذهبية. كما اشارت نتائج الدراسة الى ان معظم العزلات الجرثومية المعزولة من العجول المصابة بالتهاب الاذن الخارجية كانت مقاومة لتأثير لمصل العجول الطبيعي القاتل للجراثيم، وان معظمها كانت منتجة للهيدروكسيمات سيدروفور مقارنة بالعزلات الجرثومية المعزولة من الحيوانات غير المصابة بالتهاب الاذن الخارجية.

Introduction

Otitis externa is an inflammatory process of the external ear, and it is one of the most common disease processes seen in small animal practice (1,2). It is not a very commonly seen a clinical disease in adult cattle, however calves can develop otitis as a sequele to respiratory infections (3,4). Otitis externa has a multifactorial etiology, and bacteria play an important role in otic disease (4). Most of the bacteria incriminated in ear infections including

Stapylococcus, Pseudmonas,Escherichia and *Proteus species*, and can be recovered on occasion, usually in small numbers from healthy ears (4-6). In otitis externa otic exudates in conjunction with the isolation of a particular bacterial species in large numbers are of significance in most cases and may indicate the presence of a pathogens (3,4,7).

Iron is one of the most important nutrients of bacteria, because of its essential metabolic role (8,9). To obtain iron, pathogenic bacteria possess high affinity iron uptake systems which consist in part of outer membrane proteins and lipopolysaccharides expressed under conditions of iron limitation (9,10). Most aerobic, facultative anaerobic, and saprophytic microorganisms have the ability to produce or to use high - affinity iron - binding compounds, termed siderophores, that are capable of chelating ferric iron and allow its assimilation through cell surface receptors (11). Therefore, the ability to produce and utilize siderophores has been frequently linked to the virulence of certain pathogenic bacteria (12). Siderphores are broadly grouped into two classes, namely, hydroxamates and catecholates, according to the chemical group that is involved in forming the iron lingands (13,14).

In Iraq there is very little records on the aspects of otitis externa in calves, in addition the normal flora inhabiting the external otic canal of healty calves has not yet been studied sufficiently.Knowledge of the composition of normal bacterial flora in external otic canal is an important factor in determining the etiological, and epidemiological factors which associated with otitis externa. The present study was therefore designed to reveal the importance of some bacteria in both calves with and without otitis externa by determination of their virulence factors with refernce to serum resistance as well as production of siderophore.

Materials and methods

Animals

Atotal of 60 local calves breed of both sexes, three weeks to six months old were used in this study. Routine clinical examinations were carried out for each animal. According to the clinical examinations the animals can be divided equally into two groups, the first group was included animal with signs of the otitis externa, whereas animals of the second group were included clinically normal animals.

Samples

Both external ears canals were swabbed with sterile cotton tipped applicators. A120 swabs were inoculated (cultured aerobically) on peptone water and incubated at 37 C° for 24 - 48 hours. Routine bacterial culture was performed in all samples by streaked on nutrient agar, blood agar, chocolate agar and MacConkeys agar plates and incubated at 37 C° for 24 - 48 hours.The isolated colonies were identified morphologically, culturally and biochemically (15,16).

Determination of serum resistance

For determination of serum resistance of the isolates a pooled serum of ten health calves were used. The sera were sterilized by 0.22 mm Millipore membrane filter and kept at 30 C°. Serum resistance was conducted according (17). To 100 µl of 24 hours old culture of each of the isolates inoculated in 5 ml tryptic soy broth. After incubation at 37o C, their optical densities were adjusted to at 540 nm wave length using spectrophotometer (CECIL CE 1010, England).

Twenty five microliters were mixed with 2.5 ml of sterile sheep serum. For determination of their count aliquots were serially diluted before and after one hour incubation at 37 C° in an orbital shaking water bath. The viable bacterial count of each isolate was determined by computing the average of a replicate of 2 successive dilutions. The isolate was determined as serum resistant when its count was increased or as serum sensitive when the count was decreased.

Determination of hydroxymate siderophore production

Determination of hydroxymate siderophore production was based on the technique described by (18). To 1 ml of the supernatant bacterial culture in its stationary phase, 1 ml H2So4 (3 M) was added. After 4 hours hydrolysis time 120 C°, 1.55 ml of 35% CH3CooNa, 1 ml of each of 1% sulfanilic acid, 1.3% iodine (for oxidation) 2 % sodium arsenite and 0.3% α-naphtylamin were added. Development of red coloration (in darkness) after 30 minutes would indicate the hydro-xylmate siderophore production. The data were analyzed statistically using two ways analysis of variance, the level of significance was at $P< 0.05$, by using computerized statistical program (SPSS, 2005).

Results

The main clinical signs appeared in animals with otitis externa included unilateral auricular discharge in 23 calves and bilateral in others, (thick white yellowish, thick or thin yellowish with blood), cough, nasal discharge, anorexia, and emaciation (Table 1). As shown in Table 1, a significant increased in body temperature, respiratory, and heart rates have been detected.

Results also showed that the percentage of the infected swabs were 53%, and 30 % in calves with and without otitis externa respectively (Table 2).

Table 1: Clinical and vital signs of the calves with and without otitis externa.

Clinical signs		
Signs	Frequency	Percentages
Auricular discharge	30	100
Unilateral	23	67.6
Bilateral	7	23.3
Cough	21	70.0
Nasal discharges	12	40.0
Anorexia	7	23.3
Emaciation	6	20.0
Vital signs		
Clinical parameters	Animal with otitis externa	Animal without otitis externa
Temperature C°	39.0 ± 2. 5*	37.0 ± 3.1
Respiratory rate / min.	43.0 ± 1.4*	27.0 ± 1.7
Heart rate / min	131 ± 7.3*	90.0 ± 6.4

* Significantly P<0.05, Values are mean ± SE.

Table 2: The percentages of infected swabs from external ear canal in calves.

Animal groups	Numbers of examined swabs	Numbers of infected swabs	Percentages
With otitis externa	60	32	53
Without otitis externa	60	18	30
Total	120	50	41.6

Results of bacterial isolation indicated that only (9) bacterial species were isolated from external otic canal. The predominant species found in calves with otitis externa were *Pasteurella multocida* (23.5 %), followed by *Mannheimia hemolytica* and *Staphylococcus aureus* (19.6 %), while *Pseudomonas aerouginosa*, *Staphylococcus epidermis*, *Escherichia coli*, *Klebsiella pneumonia,* and *Proteus spp* were isolated at (17.6, 11.7, 3.9, 1.9, 1.9 %) respectively (Table 3, 4).

More over the most bacterial species were isolated from calves without otitis externa were *Escherichia coli,* and *Klebsiella pneumonia* (23.2 %), followed by *Staphylococcus epidermis* (17.9 %), *Proteus spp* (14.3), *Streptococcus spp* (8.9 %), but *Pasteurella multocida* and *Mannheimia hemolytica* were isolated at (5.3 %). From total of 120 ear swabs, 107 isolates showed that the mixed isolation was more frequently than single isolation (Table 3, 4).

Results of resistance of the bacterial isolates from external ear canal of calves to normal bactericidal effect of normal calf serum revealed that 31 bacterial isolates from the total numbers (107 bacterial isolates) were resistance to normal calf, other bacterial isolates (76) were sensitive to normal calf serum. *Pasteurella multocida, Mannheimia haemolytica, Staphylococcus aureus*,, *Pseudomonas aeruginosa,* were resistance to normal calf serum at 75.0 %, 70.0%, 70.0%, and 55.5% respectively. While only 50.0 % of *E. coli* and *Klebsiella pneumonia* isolates were resistance to normal serum (Table 5). Resistance to normal calf serum was detected in 33.3 % of *Staphylococcus aureus* isolated from calves without otitis externa (Table 5).

The most isolates of *Klebsiella pneumonia*, *Pasteurella multocida* 100 %, 83.3 %, and 80.0 % of the *Mannheimia haemolytica* and *Staphylococcus aureus* isolates respectively were produced siderophore hydroxymate., while 77.7 % of *Pseudomonas aeruginosa* isolates were produced siderophore, and only 50.0 % of the E. coli isolates were produced siderophore hydroxymate (Table 6).

Table 3: Bacterial species isolates from otic ear canal in calves.

Bacterial species	Animal with otitis externa			Animal without otitis externa		
	Numbers (%)	Single isolation	Mixed isolation	Numbers (%)	Single isolation	Mixed isolation
Pasteurella multocida	12 (23.5)	8	4	3 (5.3)	----	3
Mannheimia hemolytica	10 (19.6)	6	4	3 (5.3)	----	3
Staphylococcus aureus	10 (19.6)	8	2	1 (1.8)	1	----
Pseudomonas aerouginosa	9 (17.6)	5	4	----	----	----
Staphylococcus epidermis	6 (11.7)	----	6	10 (17.9)	----	10
Escherichia coli	2 (3.9)	----	2	13 (23.2)	----	13
Klebsiella pneumonia	1 (1.9)	----	1	13 (23.2)	----	13
Proteus spp.	1 (1.9)	----	1	8 (14.3)	----	8
Streptococcus spp.	----	----	----	5 (8.9)	----	5
Total	51	27 (52.9)	24 (47.0)	56	1 (1.8)	55 (98.2)

Table 4: Biochemical reactions characteristic of bacterial species isolates from otic ear canal in calves.

Bacterial species	IMVC				Catalase production	Oxidase production	Motility	Urase production	Coagulase production	Lactose fermentation
	Indole production	Methelene production	Voges proskauer	Citrate utilization						
Pasteurella multocida	+	/	/	/	/	+	/	−	/	−
Mannheimia hemolytica	−	/	/	/	/	+	/	−	−	−
Staphylococcus aureus	/	/	+	/	+	−	/	±	+	−
Pseudomonas aerouginosa	−	−	−	+	+	+	+	+	/	−
Staphylococcus epidermis	/	/	/	/	+	−	/	+	−	/
Escherichia coli	+	+	−	−	+	−	+	−	/	+
Klebsiella pneumonia	−	−	+	+	+	−	−	+	/	+
Proteus spp.	−	+	−	+	+	−	+	+	/	−
Streptococcus spp.	/	/	−	/	−	−	/	/	−	+

+: mean positive reaction. −: mean negative reaction. /: not tested.

Table 5: Resistance of bacterial isolates from external ear canal to the normal bactericidal effect of calf serum.

Bacterial species	Number of resistance isolates (%)	
	Animal with otitis externa	Animal without otitis externa
Pasteurella multocida	9 (75.0)	−−−−
Mannheimia hemolytica	7 (70.0)	−−−−
Staphylococcus aureus	7 (70.0)	1 (33.3)
Pseudomonas aerouginosa	5 (55.5)	−−−−
Staphylococcus epidermis	---	−−−−
Escherichia coli	1 (50.0)	−−−−−
Klebsiella pneumonia	1 (100)	−−−−−
Proteus spp.	−−−	−−−−
Streptococcus spp.	−−−	−−−−
Total	30	1

Table 6: Types and numbers of bacterial isolates produced hydroxymate siderophore.

Bacterial species	Number of isolates produced (%)	
	Animal with otitis externa	Animal without otitis externa
Pasteurella multocida	10 (83. 3)	−−−−
Mannheimia hemolytica	8 (80.0)	−−−−
Staphylococcus aureus	8 (80.0)	1 (33.3)
Pseudomonas aerouginosa	7 (77.7)	−−−−
Staphylococcus epidermis	−−−−	−−−−
Escherichia coli	1 (50.0)	−−−−
Klebsiella pneumonia	1 (100)	−−−−
Proteus spp.	−−−−	−−−−
Streptococcus spp.	−−−−	−−−−
Total	35	1

Discussion

The present study aimed to determine the clinical signs, isolation and identification of the bacterial species of otitis externa in calves, in addition to evaluate their serum resistance of normal calf serum and production of hydroxymate siderophore from bacterial isolates (for determination of bacterial virulence of the isolated bacteria). The main clinical signs observed included auricular discharge, cough, nasal discharge, anorexia, and emaciation. These sings were attributed to the chronic otitis externa and/or upper respiratory infection (7). Ear infection in calves has been associated with concurrent respiratory diseases and mixed infection (4). Otitis externa is an acute or chronic inflammation of the epithelium of the external ear canal. It may develop any where from the tympanic membrane to the pinna. It is variably characterized by erythema, edema, increased sebum or exudate, and desquamation of the epithelium (19).

Otitis externa has numerous causes, and they are categorized as predisposing factors and primary causes.Predisposing factors alone do not cause otitis externa, but increase the risk of an animal developing otitis (7, 19).

Examples of predisposing factors are ear conformation, climatic variation, excessive cleaning, excessive cerumen production, obstructive ear diseases (polyp or neoplasm and systemic disease, such as immunosuppresion (20). Studies of cattle with otitis externa in tropical regions found that poor horn conformation can be a predisposing factor because the horn can either compress the external canal or occlude its entrance (21).

Chronic inflammation can also damage the normal epithelial migration pattern, which will affect the normal clearing mechanism of the ear. Finally, middle ear disease, although frequently the result of otitis externa, can act as a nidus for infection if left untreated (22).

Results of the bacterial isolation indicate that only (9) bacterial species were isolated from external otic canal. The bacterial isolates in calves with and without otitis externa were *Pasteurella multocida,* followed by *Mannheimia hemolytica Staphylococcus aureus*, *Pseudomonas aerouginos*a, *Staphylococcus epidermi*s, *Escherichia coli*, *Klebsiella pneumonia, Proteus spp*, and *Streptococcus spp*. The principal reported agents of otitis in calves are bacteria such as *Actinomyces spp*., *Corynebacterium pseudotuberculosis*, *Escherichia coli*, *Haemophilus somnus*, *Pasteurella multocida*, *Mannheimia haemolytica*, *Pseudomonas spp*., *Streptococcus spp*. and *Mycoplasma bovis* (4). It is normal to culture *Staphylococcus intermedius* and *Malassezia* yeast from healthy canine ears in low numbers (23). Many bacterial species commonly inhabit the ear canal and can become secondary opportunistic invaders when conditions are favorable (24,25), and it probably ascended from the pharynx through the auditive tubes into, the tympanic cavities.

The results revealed that the most bacterial isolates were resistance to the bactericidal effect of the normal serum included *Pasteurella multocida, Mannheimia haemolytica, Staphylococcus aureus*,, *Pseudomonas aeruginosa aeruginosa*. While the most bacterial isolates were produced hydroxymate siderophore included *Klebsiella pneumonia*, *Pasteurella multocida, Mannheimia haemolytica* and *Staphylococcus aureus.* The serum resistance beside the hydrooxymate sidero-phore and other factors are counted as strong indicators for bacterial virulence (8-10). Previous studies showed that iron acquisition may be important in the diseases process (11-14). The bacterium is known to produce a siderophore-mediated iron uptake system, which may be one of the ways that the bacterium obtains iron for its growth in vivo from the host iron-binding proteins (26). In many pathogenic bacteria this mechanism consists of 2 elements, the ferric chelating siderphore itself and a cell-associated receptor that processes the ferrisiderophore for delivering the metal to the cytoplasm (27). Many bacteria including numerous human pathogens, synthesize small molecules known as siderophores to scavenge iron. Enterobactin, a siderophore produced by enteric bacteria, is surprisingly ineffective as an iron-scavenging agent for bacteria growing in animals because of its hydrophobicity and its sequestration by the mammalian protein siderocalin, a component of the innate immune system. However, pathogenic strains of *Escherichia coli* and *Salmonella* use enzymes encoded by the *iroA* gene cluster to tailor enterobactin by glycosylation and linearization (26-28). The resulting modified forms of enterobactin, known as salmochelins, can evade siderocalin and are less hydrophobic than enterobactin, restoring this siderophore's iron-scavenging ability in mammals (28). The encapsulated avian strains of *Pasteurella multocida* were recorded to resist the bactericidal action of serum, whereas the uncapsulated were not (17).

The Coagulase-negative *Staphylococcus spp.* could not produce siderophore, and therefore the ability of siderophore mediated iron uptake would contribute to the increased pathogenesity of Staphylococcus aureus (29).

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