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Clinical and microbiological study of otitis externa in sheep

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Abstract

In this study one hundred Awassi sheep were examined clinically and bacteriologically for isolation and identification of the bacterial agents of otitis externa in sheep. The main clinical signs appeared included weakness, pale mucus membrane, auricular discharge, cough, anorexia, emaciation, and nasal discharge. Results revealed isolation of bacteria from (45%) examined swabs. The most being from right ear. Younger animals were more frequently infected than older animals. *Pseudomonas aeruginosa*, *Staphylococcus aureus, Mannheimia haemolytica*, *Staph. epidermidis, Pasteurella multocida, Streptococcus spp., Acintobacter spp., Escherichia coli* and *Klebsiella pneumonia* were isolated. The results revealed that the most bacterial isolates were resistance to the bactericidal effect of the normal serum included *Streptococcus pneumonia, Staphylococcus aureus, Mannheimia haemolytica.* While the most bacterial isolates were produced hydroxymate siderophore included *Staphylococcus aureus, Mannheimia haemolytica, Streptococcus pneumonia.* The obtained results indicated to the importance of determination of serum resistance as a bacterial virulence factor in otitis externa in sheep.

***Keywords*:** Otitis externa; Bacterial infection; sheep

دراسة سريرية وجرثومية لالتهاب الاذن الخارجية في الضأن

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الخلاصة

تم في هذه الدراسة فحص مائة رأس من الضان العواسية, سريريا وجرثوميا وذلك لعزل وتشخيص المسببات الجرثوميه لالتهاب الاذن الخارجيه في الضان. اظهرت النتائج ان الضعف, وشحوب الاغشيه المخاطيه, ووجود النضح من الاذن هي من اهم العلامات السريريه الملاحظه على الحيوانات المصابه, تلاها السعال، وفقدان الشهيه والهزال, والنضح الانفي. اشارت النتائج الى عزل الجراثيم بنسبه ٤٥% من العينات المفحوصه, وان معظم العزلات كانت من الاذن اليمنى, كما ان نسبه الاصابه في الحيوانات الصغيره العمر يكون اكثر من الحيوانات الكبيره. عزلت جراثيم الزوائف القيحيه, والمكورات العنقوديه الذهبيه, والباستوريلا المحلله للدم, والمكورات البشرانية, والباستوريلا مالتوسيدا, والمكورات السبحية, والاسينتوباكيتريم القيحية, والايشيريكيا القولونيه, والكلبسيلا الرئويه. أظهرت النتائج أن اكثر العزلات الجرثوميه المقاومه للتأثير القاتل للمصل الطبيعي للضان كانت من جراثيم المكورات السبحية, والمكورات العنقوديه الذهبيه, والباستوريلا المحلله للدم. في حين ان اكثرالعزلات الجرثوميه المنتجه للهيدروكسيمات سيدروفو كانت من جراثيم المكورات العنقوديه الذهبيه, والباستوريلا المحلله للدم, والمكورات السبحية. اثبت النتائج اهميه تحديد مقاومه المصل, وانتاج الهيدروكسيمات سيدروفور لتحديد فوعه الجراثيم المعزوله المسببه لالتهاب الاذن الخارجيه في الضان.

Introduction

Otitis externa is an inflammation of the external auditory canal (1). Otitis is common in calves and pigs but young and adult of all species are susceptible (2), especially in lambs (1,3). Although affected animals remain alert, strong, while in cases of bilateral ear infection a tremor observed, resembling that seen with cerebellar diseases (4). Clinical signs such as an exudates in conjunction with the isolation of a particular bacterial species in large number, are of significance in most cases and may indicate the presence of pathogen (4, 5). In advanced clinical cases there can be irreversible and fatal neural lesion (2). Ear infection in calves and lambs has been associated with concurrent respiratory diseases and mixed infection (1,5). Otitis externa has a multifactorial etiology and bacteria play an important role in otic diseases (1,3,6). Most of the bacteria incriminated in ear infections including *Staphylococcus spp, Pseudo-monas spp, Escherichia spp, Mannheimia haemolytica, Pasteurella multocida and Proteus spp* can be recovered occasionally, usually in small numbers from healthy ears (1,4,6-8). Iron is essential for almost all living organisms, including bacteria. The ability of pathogenic bacteria to acquire iron in hosts is absolutely essential for bacterial growth and infection (9,10). In animal hosts, iron is usually bound to proteins such as transferrin and lactoferrin in extracellular fluid and to ferritin, hemoglobin, and heme-containing enzymes in cells (11,12). To utilize such complexes as iron sources, bacteria generally possess some sophisticated mecha-nisms, which include an iron uptake system mediated by high-affinity iron chelators called siderophores and a system for heme uptake via specific receptors (11,13). The siderophore defined as low iron induced virtually ferric specific ligands, is widely distributed in microbial species (14), where they function in the sequestration and transport of iron. Siderophore is a component of the virulence of clinical isolates *E. coli* and certain other microorganisms pathogenic to man and animals. The serum resistance beside the hydrooxymate siderophore and other factors which are counted as strong indicators for bacterial virulence (14-16). In Iraq there is very little records on the prevalence of otitis externa in sheep. The present study was, therefore designed to reveal the importance of some bacteria in sheep otitis externa by the determination of their virulence factors with refernce to serum resistance as well as production of siderophore.

Materials and Methods

Animals

A total of 100 Awassi sheep of both sexes (40 male, and 60 female), 1 month -1 year old were used in this study. The number of the animals examined included 53 sheep were presented for treatment from different diseases and 47 animals referred to the Veterinary Clinic from farms around Mosul city. The routine clinical examinations were carried out for each animals (2).

Samples

Both external ears canals were swabbed with sterile cotton–tipped applicators. Swabs were inoculated on peptone water and incubated at 37Cº for 24 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 37Cº for 24-48 hours. The isolated colonies were identified morphologically, culturally and biochemically (17, 18, 19).

Determination of serum resistance

For determination of serum resistance of the isolates a pooled serum of ten healthy sheep were used. The sera were sterilized by 0.22 mm Millipore membrane filter and kept at 30º C. Serum resistance was conducted according (15). In brief, 100 µl of 24 hours old culture of each of the 120 isolates inoculated in 5 ml tryptic soy broth .After incubation at 37º C, their optical densities were adjusted to 0.3 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 1 hour incubation at 37ºC 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 stain 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 (20). In brief, to 1 ml of the supernatant bacterial culture in its stationary phase, 1 ml H2So4 (3 M) was added. After 4 hours hydrolysis time 120º 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 analysis of variance, the level of significance was at $P< 0.05$.

Results

Clinical examination revealed that 60 (80%) of animals were suffered from general weakness, while 66.7% of them showed pale mucus membrane, auricular discharge (thick white yellowish, thick or thin yellowish with blood), cough, anorexia and emaciation, and bilateral nasal discharge appeared only on the 53.3% from affected animals (Table1). The rest of animals (25) were clinically healthy.

The results also shows that the percentage of the infected swabs were (45%), (12 bacterial isolates from healthy animals, sub clinical cases), 60 (30%) from right ear canal, 30 (15%) from left ear canal. (Table 2) .Younger animals were more frequently infected (37.5%) than older animals (7.5%) (Table 3).

Table 1: Clinical sings in sheep suffered from otitis externa.

Clinical signs	Number of affected animals	%
Weakness	60	80
Pale mucus membrane	50	66.7
Auricular discharge	50	66.7
Cough	40	53.3
Anorexia and emaciation	40	53.3
Bilateral nasal discharge	40	53.3

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

Site of ear swabs	Number of examined swabs	Number of infected swabs	%
Right ear	100	60	30*
Left ear	100	30	15
Total	200	90	45

* Significantly P< 0.05.

Table 3: Age and percentage of the infected sheep with otitis externa.

Age	Number of examined swabs	Number of infected swabs	%
1 – 6 month	100	75	37.5*
6 month-1 year	100	15	7.5
Total	200	90	45.0

* Significantly P< 0.05.

The bacterial species isolated from external ear canal included *Pseudomonas aeruginosa* were most prevalent (26.7%), followed by *Staphylococcus aureus*, and *Mannheimia haemolytica* (16.7%), but *Staph. epi-dermidis* and *Pasteurella multocida* were isolated at (12.5%), while *Streptococcus spp, Acintobacter spp., Escherichia coli* and *Klebsiella pneumonia* were isolated at (5.8, 4.2, 2.5%) respectively. From total of 200 ear swabs, 120 isolates showed that the single isolation was more frequently than mixed isolation. (Table 4 and 5).

The results of resistance of the bacterial isolates from external ear canal of sheep to normal bactericidal effect of sheep normal serum revealed that 73 bacterial isolates from the total numbers (120 bacterial isolates) were resistance to normal sheep serum. *Streptococcus spp, Staphylococcus aureus, Mannheimia haemolytica, Pseudomonas aeruginosa, Pasteurella multocida* were resistance to normal sheep serum at 85.7%, 80.0%, 70.0%, 62.5%, 60.0% respectively. While only 30 % of *E. coli* and *Klebsiella pneumonia* and 13.0% of *Staph. epidermidis* isolates were resistance to normal serum (Table 6).

Table 4: Bacterial species isolates from ear canal in sheep.

Bacterial species	Number of isolates	%	Single isolation	Mixed isolation
Pseudomonas aeruginosa	32	26.7	28	4
Staphylococcus aureus	20	16.7	17	3
Mannheimia haemolytica	20	16.7	18	2
Staph. epidermidis	15	12.5	13	2
Pasteurella multocida	15	12.5	14	1
Streptococcus spp.	7	5.8	4	3
Acintobacter spp.	5	4.2	4	1
Escherichia coli	3	2.5	2	1
Klebsiella pneumonia	3	2.5	3	-
Total	120	100	103(85.8*)	17(14.2)

* Significantly P< 0.05.

Table 5: Biochemical reactions characteristic of bacterial species isolates from ear canal in sheep.

Bacterial species	Catalase production	Oxidase production	IMVC				Motility	Urase production	Coagulase production	Lactose fermentation
			Indole production	Methelene red	Voges-Proskauer	Citrate utilization				
Pseudomonas aeruginosa	+	+	-	-	-	+	+	+	/	-
Staphylococcus aureus	+	-	/	/	+	/	/	±	/	-
Mannheimia haemolytica	/	+	-	/	/	/	/	_	-	-
Staph. epidermidis	+	-	/	/	/	/	/	+	-	/
Pasteurella multocida	/	+	+	/	/	/	/	-	/	-
Streptococcus spp.	-	-	/	/	-	/	/	/	-	+
Acintobacter spp.	+	-	-	/	/	+	-	±	/	+
Escherichia coli	+	-	+	+	-	-	+	-	/	+
Klebsiella pneumonia	+	-	-	-	+	+	-	+	/	+

+ : mean positive reaction. **- :**mean negative reaction.

Table 6: Resistance of bacterial isolates from external ear canal to the normal bactericidal effect of sheep serum.

Bacterial species	Number of resistance isolates	%	Number of sensitive isolates	%
Streptococcus spp.	6	85.7	1	14.3
Staphylococcus aureus	16	80.0	4	20.0
Mannheimia haemolytica	14	70	6	30.0
Pseudomonas aeruginosa	20	62.5	12	37.5
Pasteurella multocida	10	60.0	5	40.0
Acintobacter spp.	3	60.0	2	40.0
Escherichia coli	1	30.0	2	70.0
Klebsiella pneumonia	1	30.0	2	70.0
Staph. Epidermidis	2	13.0	13	87.0
Total	73	60.8	47	39.2

The most isolates of *Staphylococcus aureus* 95.0%, and 80.0%, 71.4% of the *Mannheimia haemolytica* and *Streptococcus spp.* isolates respectively were produced hydroxymate siderophore. A 66.7% of *Pasteurella multocida*, 65.6% of *Pseudomonas aeruginosa*, 60% of the *Acintobacter spp.* isolates were produced sidero-phore, while only 33.3% of the *E. coli* and *Klebsiella pneumonia* isolates produced siderophore (Table 7).

Table 7: Types and numbers of bacterial isolates produced hydroxymate siderophore.

Bacterial species	Number of isolates produced	%
Staphylococcus aureus	19	95.0
Mannheimia haemolytica	16	80.0
Streptococcus spp.	5	71.4
Pasteurella multocida	10	66.7
Pseudomonas aeruginosa	21	65.6
Acintobacter spp.	3	60.0
Escherichia coli	1	33.3
Klebsiella pneumonia	1	33.3
Staph. epidermidis	-	-
Total	76	60.3

Discussion

The present study aimed to determine the clinical signs, isolation and identification of the bacterial species of otitis externa in sheep , in addition to evaluate their serum resistance of normal sheep serum and production of hydroxymate siderophore from bacterial isolates (for determination of bacterial virulence of the isolated bacteria). The main clinical signs appeared included general weakness, pale mucus membrane, auricular discharge, cough, anorexia, and emaciation, nasal discharge. This sings were attributed to the chronic otitis and/or upper respiratory infection (2). Ear infection in calves and lambs has been associated with concurrent respiratory diseases and mixed infection (1,4,21).

Pugh (1) reported that little is known about otitis in goat and sheep compared with the information available on cattle and horses, and many factors can predispose sheep to otitis externa including the anatomic orientation of the ear canal itself. The vertical canal slopes medially into horizontal orientation on the outside of the tympanic membrane, this prevent drainage of debris leads to accumulate it. The skin lining of the external ear canal has a large numbers of glands, these include modified a porcine glands which produce large amounts of sec-retions which provide good suitable media for irritation and infection (1, 2). The results revealed isolation of a percentage of 45% from examined samples, most of isolates were from right ear. Many bacterial species commonly inhabit the ear canal, and can become secondary opportunistic invaders when conditions are favorable (1,4,6), and it probably ascended from the pharynx through the auditive tubes into, the tympanic cavities (21). In this study *Pseudomonas aeruginosa* was prevalent bacteria, followed by *Staphylococcus aureus, Mannheimia haemolytica*, while *Escherichia coli* and *Klebsiella pneumonia* were isolated in lower percentage (1,3,4,6) recorded many pyogenic bacterial species causes otitis externa in animals. The results revealed that the most bacterial isolates were resistance to the bactericidal effect of the normal serum included *Streptococcus pneumonia*, *Staphylococcus aureus, Mannheimia haemolytica.* While the most bacterial isolates were produced hydroxymate siderophore included *Staphylococcus aureus, Mannheimia haemolytica, Streptococcus pneumonia*. The virulence of bacterial isolates from external ear swabs was correlated to some other different factors like the hemolytic activity and aerobatic production (22,23). The serum resistance beside the hydrooxymate sidero-phore and other factors are counted as strong indicators for bacterial virulence (14,15,16). The encapsulated avian strains of *Pasteurella multocida* were recorded to resist the bactericidal action of serum, whereas the uncapsulated were not (15). The Coagulase-negative *Staphylococcus* could not produce siderophore, and therefore the ability of siderophore mediated iron uptake would contribute to the increased pathogenesity of *Staphylococcus aureus* (24). However, siderophore production may be increased in the presence of some antibiotic even in their minimum inhibitory concentration (25).

In conclusion, the present study indicated, the importance of some bacteria in sheep otitis and the determination of their virulence factors with reference to serum resistance as well as the production of siderophore is important for better understanding of the bacterial infection in sheep..

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