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PUBLIC HEALTH SIGNIFICANCE OF OTITIS MEDIA IN RABBITS

(With 2 Tables 3 Figures)

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الأهمية الصحية لالتهاب الأذن الوسطى في الأرانب

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كانت النسبة الكلية للأرانب المصابة بالتهاب الأذن الوسطى ١٩,٤% في ستة تجمعات لتربية الأرانب خلال فصل الصيف عام ٢٠٠٣. أوضح الفحص الميكروسكوبي وجود طفيل حلم الجرب (سوربتس كانيكيولاى) في ٧٠% من الحالات المريضة بينما أوضح الفحص البكتريولوجى عزل المكور العنقودي من ٦٤,٧% من الحالات بالإضافة إلى أنواع أخرى تم عزلها وهى الباستريللا ملتوسيدا (١٤,٧%) والبورديتللا والميكروب القولوني في (١١,٨%) من الحالات. عند دراسة البروتين الخلوي السطحي عن طريق الفصل الكهربائي اتضح التشابه التام بين كل عزلات المكور العنقودى فى الحلقات البروتينية ذات نفس الوزن الجزيئى بينما اختلف فى المعزولات الميكروبية الأخرى. عند توصيف البلاسميد إتضح عدم وجوده فى عترات الميكروب المكور العنقودى بينما تم توصيف بلاسميد واحد (٣,٤ ميجا دالتون) من عزلات الباستريللا والميكروب القولونى المختلفة. عند دراسة حساسية العترات المعزولة للمضادات الميكروبية المختلفة ، اتضح التأثير العالى لمركبات السبروفلوكساسين والإنروفلوكساسين وتلاهم بعد ذلك الفليموكوين واللنكوسبكتين وظهرت المقاومة العالية ضد مركبات الأمبسيللين والسلفا والأسبكتينوميسين بينما كانت التأثيرات بدرجة متوسطة مع مركبات الكيتاموكس والجنتاميسين والإكسينيل والأموكساسيللين وعند إستخدام مركب السبروفلوكساسين بمعدل ١٥ مجم / كجم من وزن الحيوان فى ماء الشرب لمدة ٤ أيام مع حقن الأيفرمكتين تحت الجلد بمعدل ٥٠٠ ميكروجرام / كجم من وزن الحيوان تم شفاء الحالات المريضة التى تم علاجها فى إحدى التجمعات المصابة فى هذة الدراسة.

SUMMARY

The total percentage of affected rabbits with otitis media among six rabbit breeding colonies were 40 out of 206 (19.4%). This affection was observed during summer season 2003. The microscopical examination of ear scrabings revealed demonstration of psoroptes mites (*Psoroptes*

cuniculi) frequently, 28/40 (70%) of diseased cases. Bacterial examination of diseased cases showed *Staphylococcus areus*, 22/34 (64.7%). Another associating bacterial agents were *Pasteurella multocida*, 5/34 (14.7%), *Bordetella bronchiseptica* and *Escherchia coli*, 4/34 (11.8%). The surface protein patterns of whole cells of the isolates were studied using sodium dodecyle sulphate polyacrylamide gel electrophoresis. Electrophoretic patterns showed complete similarity among staph. isolates especially higher molecular weight protein bands. Non of the staph. isolates were carrying plasmids, while Gram negative bacteria (*P. multocida* and *E. coli*) carrying single plasmid of the same molecular weight (3.5 Mda). Ciprofloxacin and enrofloxacin showed complete antimicrobial susceptibility among all tested bacterial species, followed by flumequine and lincospectin. Resistance was observed especially against ampicillin, sulfadimethoxine-trimethoprim and spectinomycin, while intermediate susceptibility was demonstrated with kitamox, gentamicin, exenel and amoxacillin. Ciprofloxacin which gave the best results at antimicrobial susceptibility testing was used for treatment of diseased rabbit colony. Ivermectine (single dose 500 µg/kg. b. w., S/C) was used with ciprofloxacin (15 mg/kg b.w.) in drinking water for 4 days gave complete recovery of causative parasitic and bacterial infection.

Key words: Rabbits, Otitis media, Zoonoses

INTRODUCTION

Rabbit-keeping is now practiced in most parts of the world, mainly for meat production, occasionally for fur production, in addition it is used for educational and research purposes. Rabbits can assist in solving the problem of meat production in Egypt owing to the possibility of feeding on forage, fibrous plant materials and agricultural by-products in addition short gestation period and rapid growth. An important prerequisite to development of a viable rabbit meat industry is established of steady supplies of product throughout the year (McNitt and Moody, 1990).

One from the most important factors affecting rabbit productivity and efficiency is the rabbit diseases, via decreasing production and increasing mortality and production costs (Bhasin and Singh, 1995).

Infection with *Pasteurella multocida* (*P. multocida*) is a significant cause of clinical disease in rabbits especially infection of the

upper respiratory tract, middle ears, lungs, paranasal sinuses, reproductive organs, kidneys, liver, lymph nodes, and bones (Beeb *et al.*, 1990; Delong and Manning, 1994). The principal sign of chronic enzootic pasteurellosis in rabbits is recurrent purulent rhinitis (snuffles), which often results in sequelae such as conjunctivitis, otitis media, sinusitis, subcutaneous abscesses, and chronic bronchopneumonia (Fox *et al.*, 1971; Shewen and Rice Conlon, 1993). *P. multocida* must be considered as an opportunistic pathogen in man, although it has some invasive properties. The organism is introduced into humans on numerous occasions via animal bites, scratch injuries and mucous membranes. An indirect route would be through the oral route or by inhalation (Frederiksen, 1989; Shewen and Rice Conlon, 1993).

Mange one of the most important economic disease affecting rabbits, it is incriminated in delayed growth and lowered resistance against all kinds of infections (Hoop *et al.*, 1993). The zoonotic importance of mites should not be neglected where mange was transmitted from animals to man by direct contact. Human parasitism by mites develops human acariasis which usually involves the skin (Hendrix, 2002).

Transfer of antibiotic resistance genes in natural environment can occur between gram positive and gram negative bacteria and plasmid conjugation is a mechanism of transfer of genetic information with a very broad host range (Patrice Courvalin, 1994).

The purposes of this study were designed to study the causative agents of otitis media in rabbit, to obtain information on sensitivity of bacterial isolates to chemotherapeutic agents to enhance clinical success in treating and possibly preventing the disorder, to compare microbiological differences between the isolates, to compare protein patterns of whole cells among similar isolates by using sodium dodecyle sulphate polyacrylamide gel electrophoresis (SDS-PAGE), and to study plasmid profile of the bacterial isolates.

MATERIALS and METHODS

Rabbits:

Forty affected bucks and does out of 206 were examined in six rabbit breeding colonies of different breeds (California, New Zealand, Papion, Flemish and native breed) were the subjects of the observations in this study at Assiut and El-Menia Governorates. The present affection was noticed between June and September 2003.

Examination and identification of organisms

Bacterial examination:

Samples from the middle and external ear were obtained by swabbing the mucus membranes from clinically diseased rabbits. The swabs were out plated after sterile saline soaking on brain heart infusion (BHI) agar plates for examination of colonial morphology before enrichment broth culture. Each type of colonies was cultured in BHI broth for 24 h at 37 ° C then streaked on BHI agar. Gram's staining were done for each colony type. Gram negative bacilli were subcultured on dextrose starch agar (DSA), MaConkey's agar, semisolid agar tubes, and blood agar plates. The plates were incubated for 24 h at 37 ° C. Each colony type on the plate was identified. Identification was based on standard criteria including morphology, motility, haemolytic activity and biochemical characteristics including catalase, indole production, methyl red, citrate utilization, urease, triple sugar iron agar slant, oxidase and coagulase (Holt *et al.*, 1994).

Parasitic examination:

Scraping from internal side of the ear and deeply to the middle ear were obtained from each affected case as in alkali maceration technique. The scraps were soaked in 10% potassium hydroxide in a clean test tubes, heated not boiled to avoid destruction of mites. Test tubes were centrifugated for 5 minutes at 1500 r.p.m, the supernatant fluid was poured off and the sediment was placed on a clean glass slide. The parasites were visualized under microscopy, photographed (Fig. 1. D) and morphologically identified by the help of description, illustrations and keys given by Soulsby (1982), Hendrix (2002).

Whole cell protein profile:

This was carried out using SDS-PAGE. This procedure was done to study the similarity among isolated bacteria within the species. Colonies from a 24-h BHI agar plate culture of each strain were inoculated into tryptose phosphate broth (Difco). After incubation for 24 h at 37 ° C, the broth culture was centrifugated at 12,000 ×g for 20 min. The bacterial cells were washed three times with phosphate buffered saline (PBS) and were suspended in 62.5 mM Tris hydrochloride buffer (pH 6.8) containing 5% 2-mercaptoethanol, 2% sodium dodecyle sulphate, 10% glycerol, and 0.002% bromophenol blue. SDE-PAGE were performed in (Mini-Protein II cell, Bio-Rad, USA) using acrylamide slab gels consisted of 4% stacking gel and 10% separating gel by the method of Laemmli (1970). Running condition were 45 Volt

(V) for 30 minutes and 120 V for 90 minutes. The gel was stained overnight using 0.1% Coomassie brilliant blue R-250 (Pharmacia, LKB Biotechnology, Sweden). Prestained molecular weight was used (Bio-Rad) (Molecular weights: 180, 116, 84, 58, 48.5, 36.5 and 26.5 kDa). Approximate molecular masses were determined by comparing migration patterns of samples with that of prestained marker (Sigma). Results are shown in (Fig. 2).

Plasmid profile:

A single colony from 18 h DSA plates was inoculated in Luria Bertini (LB) broth. The broth culture (12 h) were used for alkaline lysis extraction of plasmid DNA (Birnboim and Doly, 1979). The final ethanol precipitated plasmid DNA was kept in Tris-EDTA buffer (pH 8.0) for electrophoresis. Electrophoresis was done in horizontal 0.7% agarose ethidium bromide stained gel system (Pharmacia). The markers used were plasmids of *E coli* V 517 (Molecular weights: 35.8, 4.8, 3.7, 3.4, 2, 1.8 and 1.4 Mda) and 100 bp-ladder marker (2400 bp). The results were obtained by U.V. transillumination (Fig. 3).

Antimicrobial susceptibility:

The antibioitic susceptibility of isolates was determined using the disk diffusion method (Howe and Linton, 1976). The isolates were tested for sensitivity to antibiotics as listed in Table 2 were enrofloxacin, ciprofloxacin, flumequin, lincospectin, spectinomycin, kitamox, gentamicin, exenel, amoxacillin, ampicillin and sulphadimethoxine-trimethoprime combination. Results were illustrated in Table 2.

Treatment of diseased colony:

This experiment was carried out on rabbit colony No. 2 in table (1). The rabbits were inoculated with single dose of ivermectin (500 µg/kg. b. w., S/C) and ciprofloxacine (15 mg / kg b.w.) in drinking water for 4 days. Reisolation trial were tried after treatment course at 1st and 2nd week respectively.

Spraying:

The acaricide used was diazinon on rabbitary of rabbit colony No. 2 and applied at dilution recommended by the manufactor.

RESULTS

This affection appeared on adult male and female rabbits. Clinical signs were noticed as torticollis or lateral deviation of head and neck. Dermatitis around eyes and nose as well as crusts formation and discharges from the inner side of external and middle ear. The total

percentage of affected rabbits among six rabbit breeding colonies were 40 out of 206 (19.4%). This affection was observed during summer season 2003. Results are shown in Table (1), and Fig. (1.B). Affection percentage was ranged between 15-26.5%.

Bacterial isolation:

Ear swabs from clinically diseased rabbits were cultured for aerobic bacteria. The prevalence of organisms isolated is given in Table 1. *Staphulococcus areus (Staph. areus), Bordetella bronchiseptica (B. bronchiseptica), Esherichia coli (E. coli)* and *P. multocida* were the most common organisms isolated. Most of the rabbits harbored more than one species of organism.

Most of diseased cases showed *Staph. areus* isolation 22/34 (64.7%). Another associating agents were *P. multocida* 5/34 (14.7%), *B. bronchiseptica* and *E. coli* 4/34 (11.8%).

Parasitic examination:

The microscopical examination of ear scrabings revealed demonstration of psoroptes mites (*Psoroptes cuniculi*) frequently from 28 (70%) of 40 diseased cases (Fig. 1. A, C, D).

Whole cell protein profile:

SDS-PAGE of whole cell or cell surface proteins showed complete similarity among Staphylococcus isolates especially at higher molecular weight protein bands. Another associating gram negative bacterial isolates were differ in surface protein profile. Results are shown in (Fig. 2).

Plasmid profile:

Non of the staphylococcus isolates were carrying plasmids, while Gram negative bacteria (*P. multocida* and *E. coli*) carrying single plasmid of the same molecular weight (3.5 Mda) (Fig. 3).

Antimicrobial susceptibility:

Ciprofloxacin and enrofloxacin showed complete antimicrobial susceptibility among all tested bacterial species, followed by flumequine and lincospectin. Resistance was observed especially against ampicillin, sulfadimethoxine-trimethoprim and spectinomycin. Intermediate susceptibility was demonstrated with kitamox, gentamicin, exenel and amoxacillin (Table 2).

Treatment:

Ciprofloxacin which gave the best results at antimicrobial susceptibility testing was used for treatment of one of rabbit colony (colony No. 2. in Table 1). Single dose of ivermectin (500 µg/kg. b. w.,

S/C) was used, at the same time with ciprofloxacin (15 mg/kg b.w.) in drinking water for 4 days in addition to spraying gave complete recovery of parasitic and bacterial infection. Reisolation was negative after the end of treatment.

DISCUSSION

The total percentage of otitis media in rabbits among six rabbit breeding colonies were 19.4% in the present study while the prevalence of otitis media at necropsy in conventionally managed rabbit colonies can approach 33% whereas the number of animals with torticollis is usually under 5% (Synder, *et al.*, 1973). The present results indicate that the most of diseased cases showed *Staph. areus* isolation, 64.7%. Another associating agents were *P. multocida*, 14.7%, *B. bronchiseptica* and *E. coli*, 11.8%. *Staph. areus* is a bacterial pathogen notorious for producing both localized and disseminated infections in humans (Lee *et al.*, 1987).

Clinical signs are usually absent in rabbits with otitis media. If the inflammatory process extends to the inner ear, torticollis can occur (Fox *et al.*, 1971; Synder *et al.*, 1973). In both clinical and subclinical cases of otitis media, *P. multocida* is usually isolated in pure culture, but *B. bronchiseptica* and Staphylococci may also be found in 5 to 10% of cases (Fox *et al.*, 1971).

It is generally considered that *P. multocida* infects rabbits via the respiratory route first, and then spreads to the middle and inner ear through the ecustachian tube or via the blood vascular route and to various organs (Nakagawa *et al.*, 1986).

P. multocida infection in man can be considered as a zoonosis. The organism can cause a dermatitis in humans, also arthritis, meningitis, cerebral abscesses, peritonitis, appendicitis, pneumonia and septicaemia. Transmission of *P. multocida* from man to man might occur among patients with respiratory tract disease (Frederiksen, 1989; Shewen and Rice Conlon, 1993).

Bortetellae are highly communicable pathogenic, obligatory parasites of man and animals, and worldwide in distribution. They are transmitted by intimate exposure to expired droplets. The bacteria localize and multiply among the cilia of the epithelial cells of the respiratory tract (Pittman and Wardless, 1981).

Foodstuffs especially those of animal origin, rabbit meat, may be regarded as the primary source of food poisoning bacteria, *E. coli* is

judged as a significant food pathogen constituting a public health hazard and recognized as the primary cause of haemorrhagic diarrhoea in man (Karmali, 1989).

Mange represents one of the major problems affecting health and impairing the profitable productivity of rabbits, hence mites infestations causing annoyance, weakness and anemic conditions, as well as may seriously causes economic losses in meat and fur production (Madsen, 1986). The results obtained point out that 70% of diseased rabbits in this study were infested with psoroptes mites (*Psoroptes cuniculi*). A finding that coincides with that reported by (Abd El-All, 1990) who mentioned that the rate of infestation was (60.2%) and (64.6%) in native and foreign breeds respectively in farms of poor hygienic level. In addition, Madsen, 1986, established that the ear mange caused by psoroptes is the most important parasitic disease of the domestic rabbits. In contrast to the results obtained by Hegazi, 1978 and Rai, 1988 who recorded that the infestation rate with psoroptic mange in rabbits was 11.1% and 7.16% respectively. Increased infestation rate may be due to bad hygiene and impaired animal husbandry as well as ecological and climatological variations.

Our study concluded that the infestation with psoroptic acarine appeared to propagate mostly in summer among aged rabbits than young ones. These results agree with Hegazi (1978) and Abd El-All (1990). While the breeds of higher susceptibility among dams to mange were New Zealand breeds than other breeds. These results agreed with those of Hoop *et al.*, (1995). In contrast, Hegazi, 1978, mentioned that Bouscat rabbits were the most susceptible breeds to psoroptic acarine (9.82%). He also reported that infection of rabbits with psoroptic acarine reaches its maximum during May, June, July, with its peak in August, while Maske and Rurrah (1981) found that psoroptic mites thrived at temperature ranged between 0.6-46°C indicating that the animals remained vulnerable to infection allover the year.

Human parasitism by mites develops human acariasis which usually involves the skin. It was concluded that the described type of acarine resembled in a great extent *Psoroptes communis var cuniculi* (Hendrix, 2002). *Psoroptes communis var cuniculi* was of public health interest because easily transmitted to human beings.

Polyacrylamide gel electrophoresis has become a standard tool in every laboratory in which proteins are analyzed and purified. Most frequently, the amount and location of the protein are of interest and

staining is then sufficient (Towbin *et al.*, 1979). SDS-PAGE of whole cell or surface cell proteins showed complete similarity among staph. isolates especially at higher molecular weight protein bands. Another associating gram negative bacterial isolates were differ in surface protein profile.

From our investigation, gram negative bacteria (*P. multocida* and *E. coli*) carrying single plasmid of the same molecular weight (3.5 Mda) as well as Hirsh *et al.*, 1981, reported on conjugative nature of the plasmid DNA from *P. multocida.* In addition, Lee and Wooly (1995) reported on presence of R-plasmids among *P. multocida* isolates.

There are four major mechanisms account for the evolution of bacterial resistance to antibiotics that correlates with the use of the drugs, one of them are plasmid borne and spread of known resistance genes into new bacterial hosts. The last mechanism has been known since the early finding that antibiotic resistance genes are often part of self-transferable plasmids or of transposable elements (Patrice Courvalin, 1994). In the present study the plasmids were detected in gram negative bacteria and not in gram positive bacteria. The observation of the same molecular plasmid (3.5 Mda) from different bacterial species (phylogenityically different) gave suggestion to plasmid transfer. Bacterial resistance was demonstrated against Ampicillin, spectinomycin and sulfadrugs may correlate with plasmid bearing.

On invitro testing, Flouroquinolanes (Ciprofloxacin, enrofloxacin and Flumequine) and Lincospectine showed higher effect on the causative bacteria than other antimicrobial agents. Moreover, the use of ciprofloxacin for treating the diseased rabbits resulted in complete recovery, the matter that supported by sequential negative reisolation of the identified causative agent. In addition, ivermectin was used for treatment. A finding that coinceded with that reported by Pandey (1989) reported that the infested rabbits with *Psoroptes cuniculi* given a good control when subjected to treatment with ivermectin by subcutaneous injection especially at 500 µg/kg body weight.

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Table 1. Aetiologic agents associating with otitis media in rabbits at Assiut and El-Menia Governorate

Colony No.	Breed	Locality	Total No.	Diseased (%)	Aetiologic agents ((Positive / Diseased)				
					Parasitic	Bacterial			
					Psoroptes cuniculi	*Staph. areus*	*P. multocida*	*B. bronchiseptica*	*E. coli*
1	New Zealand California Papion	Bani-Ghalib	40	6 (15.0)	5/6 (83.3%)	3/6 (50.0%)	0/6 (0.0%)	2/6 (33.3%)	0/6 (0.0%)
2	New Zealand California Papion Flemish	Assiut	34	9 (26.5)	7/9 (77.8%)	5/9 (55.6%)	2/9 (22.2%)	0/9 (0.0%)	3/9 (33.3%)
3	New Zealand California	Assiut	26	6 (23.0)	4/6 (66.7%)	5/6 (83.3%)	1/6 (16.7%)	0/6 (0.0%)	0/6 (0.0%)
4	New Zealand California Native	Dairut	50	8 (16.0)	5/8 (62.5%)	6/8 (75.0%)	0/8 (0.0%)	2/8 (25.0%)	1/8 (12.5)
5	California	Awlad-Morgan	20	5 (25.0)	3/5 (60.0%)	3/5 (60.0%)	2/5 (40.0%)	0/5 (0.0%)	0/5 (0.0%)
6	New Zealand	Glosna	36	6 (16.7)	4/6 (66.7%)	ND*	ND	ND	ND
Total			206	40 (19.4)	28/40 (70.0%)	22/34 (64.7%)	5/34(14.7%)	4/34 (11.8%)	4/34 (11.8%)

* ND. Not determined.

Table 2. Antimicrobial sensitivity testing

Antibiotic	*Staph. areus* (5)		*P. multocida* (5)		*B. bronchiseptica* (4)		*E. coli* (4)	
	S*	R*	S	R	S	R	S	R
Enrfloxacin	5	-	5	-	4	-	4	-
Ciprofloxacin	5	-	5	-	4	-	4	-
Flumequin	3	2	5	-	4	-	4	-
Lincospectin	4	1	5	-	3	1	4	-
Spectinomycin	-	5	3	2	1	3	-	4
Kitamox	4	1	4	1	2	2	-	4
Gentamicin	3	2	5	-	3	1	2	2
Exenel	3	2	5	-	4	-	3	1
Amoxacillin	-	5	5	-	-	4	-	4
Ampicillin	4	1	5	-	1	3	-	4
Sulphadimethoxine	-	5	2	3	-	4	-	4

* S. Susceptible R. Resistant

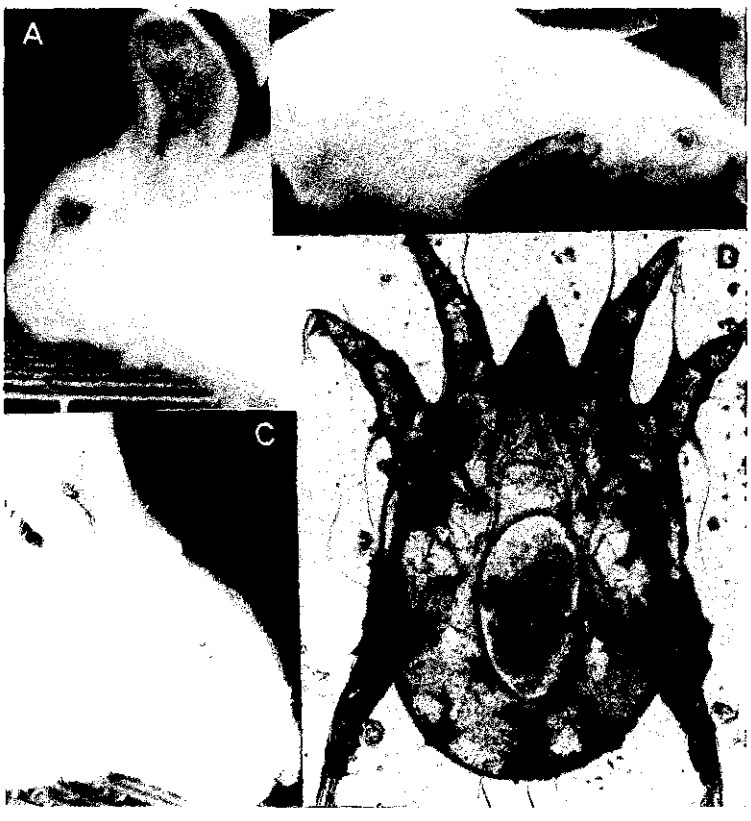

Fig. (1): A: Sever affection of the ear in New Zealand rabbit
B: A California rabbit with torticollis
C: Affection of ear and nose in New Zealand rabbit
D: *Psoroptes cuniculi* (Ear mange) (Microscopic picture)