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STAPHYLOCOCCAL MASTITIS IN DOMESTIC RABBITS

With 4 Tables & 2 Figs.)

By

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التهاب الثدى العنقودى في الأرانب الأليفة

طلبه يونس ، بخيت سالم

تم دراسة ثلاثة أوبئة حقلية لالتهاب الثدى في الارانب المتسببة بميكروب المكـــــــور العنقودى · وقد لوحظ احمرارورم في الثدى والجلد كأعراض اكلينيكية في احدى تلك المزارع (مزرعة نيوزلندى) مع قلة في الطعام وانتفاش للشعر مع نسبة نفوق ١٠٠٪ في الأرانب الرضيعة· أما في القطيعين الآخرين (كاليفورنيا، فلمش) فقد لوحظ وجود خراريج في الثدى والبطن وكانت نسبة النفوق في الأرانب الرضيعة ٣٠٪ ، ٥٠٪ على التوالي وذلك من بين ثمانية مزارع حكومية وأهلية تم فحصها· تم عزل ستة عترات من الأمهات المرضعة والأرانب الرضيعة المصابة،باجـــراء العدوى الصناعية باحد العترات المعزولة اتضح أنها ضاربة بالنسبة للأرانب القابلة للاصابـــــة· باجراء اختبار الحساسية في المعمل للعترات المعزولة اتضح أن جميعها حساسة لكل مـــــــن البنسلين والامبسيلين وأنها غير حساسة بالمرة لكل من الكلوكزاسيلين، سلفات الكولـــــستين، الاسبكتينومايسين أو السلفا المركبة· تم التحكم وعلاج الحالات المصابة حقليا بحقن البنســلين بنتائج مرضية·

SUMMARY

Out of 8 governmental and private rabbit farms, three natural outbreaks of rabbit mastitis caused by Staphylococcus aureus (S.aureus) coagulase - positive were reported. In one of these farms (Newzealand White) the disease was characterized by swelling and redness of skin and mammary gland together with reduction of food intake, ruffled fur and 100% losses in suckling rabbits. While abscesses of the subcutaneous (S/C) tissues of abdomen and mammary gland were observed in the other 2 farms (Californian and Flemish) with losses in suckling rabbits of 30% and 50%, respectively.

Six strains of B-hemolytic coagulase-positive S.aureus were isolated from infecting lactating does and their suckling rabbits.

Experimental infection with one isolate documented its pathogenic nature to susceptible rabbits.

In vitro, chemotherapeutic sensitivity tests showed that all of the 6 isolates examined were highly sensitive to each of Penicillin and Ampicillin but non was sensitive to each of Cloxacillin, Colistin sulphate, Spectinomycin or compound Sulphonamide. Trial for treatment of infected cases was conducted by injection of Penicillin which gave a satisfactory result.

INTRODUCTION

Staphylococcosis is a commonly occurring disease resulting in fatal septicemia or suppurative inflammation in nearly any organ or site (FLATT, 1974).

In a previous studies ADLAM, et al. (1976); VOROS (1982) and OKERMAN, et al. (1984) showed that rabbits were naturally susceptible to staphylococcal mastitis and when this condition was contracted early in lactation, litters were usually lost and unacceptable high losses from the disease were recorded so the rabbitry in some cases had been depopulated.

In some rabbitries the infection occurs sporadically and mortality varies according to the virulence of the organism. High mortality in young rabbits was first described by HAGEN (1963), a similar condition has also been reported by RENAULT (1980) and DEVRIESE, et al. (1981), who reported staphylococcosis with special rabbit virulent biotype of S.aureus.

The septicemic form is most common in young rabbits or rabbits stressed from various cases. The purpose of the present study was to describe some of the characteristic of naturally occurring staphylococcal mastitis in governmental and private rabbitries in Assiut province.

MATERIAL and METHODS

Isolation and identification of S.aureus:

Animals included in this investigation were collected from three private rabbit farms out of eight governmental and private examined rabbitries at different localities in Assiut Province. They were of different ages and breeds (NewZealand White, Californian and Flemish). A total of 24 adult does and 39 suckling rabbits were subjected to post-mortem examination for freshly dead animals and samples of milk or pus from mastitic does taken from the three infected farms as well as samples from heart blood, liver of suckling rabbits were cultured on nutrient broth, then on blood agar, suspected colonies were identified according to TOPLEY and WILSON'S (1984).

Pathogenicity test:

Twenty four, 6-week-old balady rabbits, obtained from private rabbit farms in Assiut Province were used in this experiment. The animals were kept in cages and observed for a period of a week. A random sample of 4 rabbits was slaughtered and exposed to post-mortem, parasitological and bacteriological examinations which proved their health status and freedom from known pathogens. Because S.aureus isolates differ in their pathogenicity to offsprings, so we selected one of the highly pathogenic isolates in our experimental work.

The rest of the animals (20) were divided into 3 groups as follows:

Group I. Comprised 8 animals was inoculated I/V with $30x10^{10}$ viable organisms/rabbit.
Group II. Of 8 animals was inoculated S/C with $30x10^{10}$ viable organisms/rabbit.
Group III. Consisted of 4 animals was inoculated with sterile broth and left as non-infected to serve as control. During an observation period, mortality rate, clinical signs and gross lesions were recorded and trials for reisolation were conducted.

Sensitivity of the isolates to antimicrobial agents:

The paper disc technique was carried out after FINEGOLD and BARON (1986) using 6 isolates of S.aureus and 21 chemotherapeutic discs produced by OXOID BASINGSTOKE, HAMPSHIRE, ENGLAND. The discs included Penicillin G (10 ug), Lincomycin (2 mg), Spectinomycin (10 ug), Cephalexin (30 ug), Streptomycin (10 ug), Ampicillin (10 ug), Neomycin (30 ug), Doxycycline hydrochloride (30 ug), compound Sulphonamide (300 ug), Furazolidone (50 ug),Oxytetracycline (30 ug), Colistin sulphate (10 ug), Flumequine (30 ug), Chlortetracycline (30 ug), Linco-spectin, Entroflocin (5 mcg), Framycetin (100 ug), Oxalinic acid (10 ug), Gentamycin (10 ug), Apramycin (15 ug) and Cloxacillin (5 ug). Interpretation of the results was recorded according to the recommendation of CASTLE and ELSTUB (1971).

RESULTS

Mastitis occurred at any time during lactation and can occur in different strains of rabbits at different times of littering clearly two kinds of natural staphylococcal mastitis with different pictures were observed in rabbits. The clinical disease which reported in three herds of commercial reared rabbits of different strains out of eight examined rabbits (New Zealand White, Californian and Flemish) appeared as swelling, redness of skin and mammary glands and the does refused to suckle their offsprings, together with anorexia and dull appearance with 100% mortality of their offsprings in the first strain of rabbit. Abscesses of the S/C tissue of the abdomen and mammary

glands were detected (Fig. 1). Also, diffuse swelling of the S/C tissue of abdominal region was noticed. The does in the later two strains of rabbits exhibited reduction in appetite and ruffling of fur with 30% and 50% losses of their offsprings, respectively. The high and rapid losses of suckling litters from infected does had occured specially if mastitis was contracted early during lactation period. The survival infected suckling rabbits manifested nasal discharge and diarrhea. The No. of does and their offsprings with active disease during the course of the study in the three infected rabbitries are illustrated in Table (1).

Bacteriological examination of samples yielded profuse growth of 6 isolants of staphylococci producing B-hemolysis on blood agar plates. These strains were recovered as follows: 2 from New Zealand White, one strain from Californian and 3 strains from Flemish breeds. Biochemical and enzymatic activities of the isolated organism are tabulated in Table (2).

In experimentally infected animals the inoculated strain documented its high virulence in both I/V and S/C inoculated groups as shown in Table (3). In group I, 100% mortality occured within 24 hours post infection. The experimental animals showing severe congestion of tracheal rings, and wide spreaded ecchymotic hemorrhages on lungs, myocardium and liver (Fig. 2). Severe congestion of small intestine with watery content, engorgement of heart blood vessels, and severe congestion of kidneys were noticed, only in one case fluid in pericardial sac and thoracic cavity was observed. In group II, 100% mortality occured within 5 days post infection. The experimental rabbit revealed nasal discharge and diarrhea with mild congestion of parenchymatous organs.

The results of in vitro sensitivity testing of 6 S.aureus isolates are given in Table (4).

DISCUSSION

In the rabbitries studied here the appearance of some highly pathogenic strain of S.aureus resulted in death of complete litter and a high frequency of staphylococcosis in adult rabbits. The results indicate that this strain of Staphylococci has serious consequences on the profitability of rabbit farms. Our results are supported by the finding of HOLLIMAN and GIRVAN (1986), who stated that 13 complete litters died due to staphylococcal infection within four days of birth. Our results of isolation agree to some extent with those reported by HAGEN (1963); McCOY and STEENBERGEN (1969); RENQUIST and SOAVE (1969); HAJEK and MARSALEK (1971); FARINHA, et al. (1982) and VOROS (1982). The results of biochemical and enzymatic activities are shown in Table (2). More or less similar results has been reported by TOPLEY and WILSON

(1984). Pathogenicity test of S.aureus conducted on healthy rabbits by I/V and S/C routes proved the pathogenic nature of the tested isolate with 100% mortality in both routes. The rapid death of experimentally infected animals suggesting the highly toxigenic nature of the organism, this is similar to that reported by ADLAM, et al. (1976).

The in vitro sensitivity testing of 6 isolates to each of 21 antimicrobial agents revealed that all isolates examined were sensitive to Penicillin and Ampicillin, only 5 isolates were sensitive to Linco-spectin, Chlortetracycline, Doxycycline hydrochloride, Flumequine, Entroflocin and Oxytetracycline; 3 isolates to Furazolidone, Neomycin, Cephalexin, Framycetin and Oxalinic acid; 2 isolates to Gentamycin, Apramycin and Lincomycin; one isolate to Streptomycin. None of the isolates proved to be sensitive to each of Cloxacillin, Colistin sulphate, Spectinomycin and compound Sulphonamide. Our results agree with those reported by OKAY (1986) about Colistin sulphate and disagree with other chemotherapeutic agent. Furthermore, our results are inagreement with those reported by NADER, et al. (1986) who maintained that no drug was effective against all S.aureus strains.

The field trial for controlling staphylococcal infection in adult does and minimizing mortality of their offsprings by parental injection of Penicillin 50000 IU/kg b.wt for three successive days gave a satisfactory result. This disagree with GROSINIC, et al. (1974) who reported that the treatment with antibiotic was unsatisfactory. Considering the heavy economic losses involved, it is important that improvement of hygiene and nutrition and early recognition of this severe form of staphylococcosis by practitioners are of great value for avoidance further spreading of rabbit pathogenic S.aureus strains and minimize losses from this disease in rabbits.

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Table (1): Number of does and their offspring with active disease during the course of the study in the three infected rabbitries

	Breed of Rabbit					
No.of does, offspring and % of infection.	New Zealand white	% of infec.	Californian	% of infec.	Flemish	% of infec.
Total No.of adult does	16		25		11	
No.of lactating does	7		9		8	
No.of infected does	2		3		4	
% of infected lactating does		28.6		33.3		50.0
Total No.of suckling rabbits	9		10		20	
No.of infected suckling rabbits	9		5		14	
% of infection		100.0		50.0		70.0

Table (2)
Biochemical and enzymatic activity of S.aureus isolates

Tests	Isolates number					
	1	2	3	4	5	6
Glucose	+	+	+	+	+	+
Fractose	+	+	-	+	+	+
Mannose	+	+	-	+	+	-
Galactose	-	-	-	-	-	-
Rhmnose	-	-	-	-	-	-
Maltose	+	+	+	+	+	+
Arabinose	-	-	-	-	-	-
Sucrose	+	+	+	+	+	+
Lactose	+	+	+	+	+	+
Trehalose	-	+	-	+	-	+
Mannitol	+	+	+	+	+	+
Dulcitol	+	+	-	-	-	-
Starch	-	-	-	-	-	-
Inulin	-	-	-	-	-	-
Dextrin	-	-	-	-	-	-
Salicin	-	-	-	-	-	-
Xylose	-	-	-	-	-	-
Other test:						
Indole	-	-	-	-	-	-
Urease	+	+	+	+	+	+
Nitrate	+	+	+	+	+	+
Bile solubility	-	-	-	-	-	-
Catalase	+	+	+	+	+	+
Motility	-	-	-	-	-	-
Hemolysis	+	+	+	+	+	+
H_2S	-	-	-	-	-	-
Litmus milk	+	+	+	+	+	+
Gelatin liquefaction	-	-	+	+	+	+
Citrate	+	+	-	+	+	+
Slide coagulase	+	+	-	+	+	+
Tube coagulase	+	+	+	+	+	+

Table (3)

Results of experimental infection of healthy rabbit with S.aureus by I/V and S/C routes

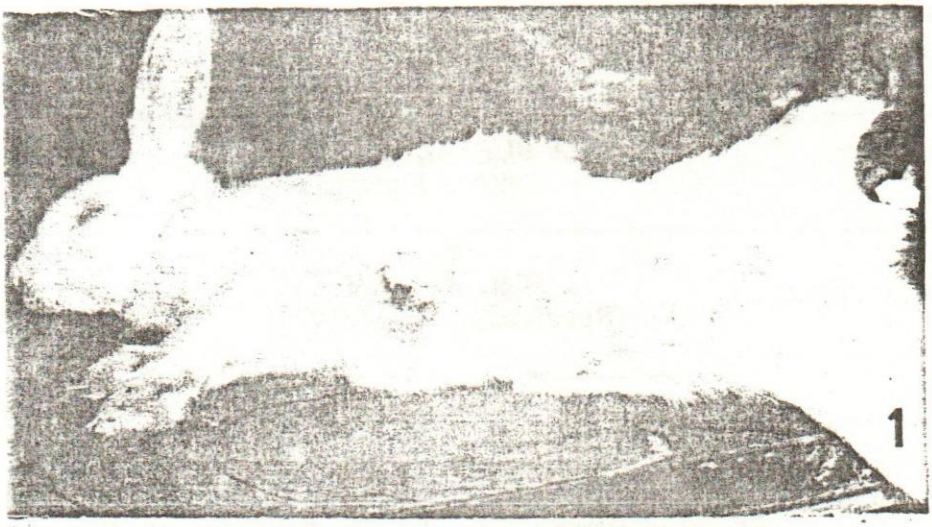
Group No.	1	2	3
No.of infected rabbits	8	8	4
Route of infection*	I/V	S/C	non-infected
Daily deaths post-infection			
1 st day.	8	4	-
2 nd day.	-	2	-
3 rd day.	-	-	-
4 th day.	-	1	-
5 th day.	-	1	-
Total No.of mortality	8	8	-
Mortality %	100	100	0

* A dose of $30x10^{10}$ viable organisms of 24 hours broth culture was used/animal.

Table (4)

Results of in vitro sensitivity tests

Antimicrobial agent	Sensitive No.of isolates	Sensitive %	Resistant No.of isolate	Resistant %
Penicillin G (10 ug)	6	100	0	0
Ampicillin (10 ug)	6	100	0	0
Linco-spectin (5 mcg)	5	83.3	1	16.7
Chlortetracycline (30 ug)	5	83.3	1	16.7
Doxycycline hydrochloride (30 ug)	5	83.3	1	16.7
Flumequine (30 ug)	5	83.3	1	16.7
Entroflocin (5 mcg)	5	83.3	1	16.7
Oxytetracycline (30 ug)	5	83.3	1	16.7
Furazolidone (50 ug)	3	50.0	3	50.0
Neomycin (30 ug)	3	50.0	3	50.0
Cephalexin (30 ug)	3	50.0	3	50.0
Oxalinic acid (10 ug)	3	50.0	3	50.0
Framycetin (100 ug)	3	50.0	3	50.0
Gentamycin (10 ug)	2	33.3	4	66.7
Apramycin (15 ug)	2	33.3	4	66.7
Lincomycin (2ug)	2	33.3	4	66.7
Streptomycin (10 ug)	1	16.7	5	83.3
Cloxacillin (5 ug)	0	0	6	100
Colistin sulphate (10 ug)	0	0	6	100
Spectinomycin (10 ug)	0	0	6	100
compound Sulphonamide (300 ug)	0	0	6	100



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