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STUDIES ON PASTEURELLA MICROORGANISMS PREVALENT IN RABBITS AT SHARKIA GOVERNORATE.

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ABSTRACT

Pasteurellosis is a debilitating disease occur in man and many animals due to either endogenous or exogenous infection with Pasteurella microorganisms especially under stress condition, so this study was done to detect the prevalence of such microorganism in the nasal swabs and blood samples to detect their antibodies. 105 samples were collected from apparently healthy rabbits and 154 samples from diseased ones.

- The obtained data revealed that the prevalence rate of *Past. multocida* was (4.8%) from apparently healthy rabbits and (12.3%) in diseased ones.
- Biochemical reactions of isolated *Past. microorganisms* revealed that all isolates (24) were belonged to *Past. multocida*.
- All isolates of *Past. multocida* were tested for their pathogenicity to mice with characteristic P.M.lesions, sever haemorrhage in subcutaneous blood vessels, septicaemia and congestion of the internal organs.
- Serodiagnosis by using ELISA test to detect the antibody titer of *Past. multocida* and the obtained data revealed that (31.4%) of tested apparently healthy rabbits were ELISA seropositive while it was (46.1%) in diseased ones.
- Concerning the antibiotic sensitivity test, it was found that all isolates were 100% sensitive to Ceftiofur sodium, Norflxacin, Gentamicin, highly sensitive to Enrofloxacin (95.8%) Sulphamethoxazole and Trimethoprim (87.5%) while complete resistance to chloramphenicol.

INTRODUCTION

Rabbits are considered to be one of the significant sources for establishing food security allover the world. like most creatures rabbits harbour pasteurella microorganisms in their upper respiratory tract. So it is of great value to study their incidence in such animals and the methods employed for their laboratory diagnosis. High morbidity and high mortality rate are considered to be main obstacles subjecting such industry. Pasteurella microorganisms are prevalent among rabbits population causing great losses in rabbits, Cheek, (1987). It playes an important role in producing the disease when the resistance of rabbits is lowered by concurrent disease or climatic stress.

Lu *et al.* (1978) stated that *Past. multocida* colonize the mucosal surfaces of the pharynges of rabbits in the carrier state with recurrent rhinitis., Digiacomo, *et al.* (1983) reported that number of clinical forms of infection can occur in pasteurellosis including upper respiratory infection (snuffles), otitis media, enzootic pneumonia, conjunctivitis, pyometra, orchitis, abscesses and septicaemia.

This work was planned as on attempt to throw spots lights on the following :

a- Isolation and biochemical identification of Pasteurella microorganisms isolated from both apparently healthy and diseased rabbits.

b- Pathogenicity of isolated microorganisms to laboratory animals.
c- Serodiagnosis of *Past. multocida* by using ELISA technique.
d- Studying their antimicrobial susceptibility pattern.

MATERIAL AND METHODS

I. Samples :

Atolal of 210 nasal swabs and blood samples were aseptically Collected from 105 apparently healthy rabbits and 308 nasal swabs and blood samples were collected from 154 diseased rabbits, from different Sharkia localities.

These samples were Examined bacteriologically to explore the possible existence of Past. microorganisms or thier Specific antibodies.

II- Bacteriological Examination

1- Isolation of Past microorganisms :-

The collected swabs were directly inoculated into N.broth and aerobically incubated at 37 C for 24 hours, subcultured on blood agar to detect haemolytic activity and MacConkey's ager to indicate thier growth ability on it, Cruickshank *et al.* (1975).

2- Morphological examination :

- Films were prepared from colonies and stained with Gram's stain and study their shape, size, arrangement, ...etc).

3- Motility test :- by stabing into semisolid media, according to the method of, Cruickshank *et al.*, (1975).

4- Biochemical identification :-

- pure colonies were biochemically tested as recommended by Kreig and Holt, (1984).

5- Pathogenicity test :

Two mice were used for each isolate, each mouse was given I/P 0.1 ml of (1.5×10^8 viable organism / ml), according to Stamp *et al.*, (1954) for the detection of pathogenicity and virulence, control was injected with 0.1 ml saline. Mortality rate and P.M.changes were recorded, also smears from blood were stained with leishman's stain for demonstration of bipolarity and reisolation of the organism on blood agar.

III- Serodiagnosis of *Past. multocida* using ELISA techniqus: it was performed according to the method adopted by Rhoads and Heddleston (1980) and Snyder *et al.*, (1984). IDEXX Company.

IV- Antibiotic sensitivity test :

- Pure culture from the tested isolates were inoculated in Mueller Hinton broth One ml of each bacterial suspension was inoculated on to the susface of Mueller Hinton plate by sterile pasteur pipette. The chosen antibiotic discs were applied as described by Bauer, *et al.*, (1966), Streptomycin 10 ug, Erythromycin 15 ug, Tetracycline 30 ug, Gentamycin 10 ug, Ampicillin 10 ug, Norfloxacin 10 ug, Chloramphenical 30 ug, Enrofloxacin 5 ug, Cefotaxim 30 ug, Ceftiofour sodium 50 ug, Sulphamethoxazole + Trimethoprim 25 ug, Kitamox 70 ug.

RESULTS AND DISCUSSION

Bacteriological examination of 259 nasal swabs collected from apparently healthy rabbits (105 samples) and diseased rabbits (154 samples). Five Pasteurella microorganisms were obtanied from apparently healthy rabbits with incidence of (4.8%), in addation 19 isolates of Pasteurella species were revealed from diseased rabbits with incidence of (12.3%) Table (1), so 24 isolates of Pasteurella were obtained with total incidence of (9.3%), these results simulated with those obtained by Nada (1994) who isolated Pasteurella from apparently healthy and diseased rabbits with incidence of (7.9%). In the present work biochemical identification of 24 Pasteurella isolates showed that all organisms were typed as *Past. multocida* Table (2).

Incidence of *Pasteurella multocida* among diseased rabbits was (12.3%), this was nearly in accordance with the finding of Percy *et al.* (1988) who isolated *Past. multocida* from diseased rabbits with incidence of (11.9%), and Nada (1994) who found that incidence of *Past. multocida* among 186 diseased rabbits was (9.1%). On the other hand these results were seemed to be disagreed with Fahmy *et al.* (1985) who reported that percentage of infection with *Past. multocida* in rabbits was (33.3%) and Mahmoud and Abdel-Baset (1991) who found that *Past multocida* infection was high and ranged from (70-78%) in rabbits.

Table (1): Prevalence of *Past. multocida* from nasal swabs of apparently healthy and diseased rabbits in different sharkia localities.

Locality	Apparently healthy rabbits			Diseased Rabbits		
	No.of examined samples	No. of positive casses	Percent-age %	No.of examined samples	No. of positive casses	Percent-age %
1- San-El-Hagar	57	3	5.3%	73	10	13.7%
2- Awlad-sakr	15	1	6.7%	15	2	13.3%
3- Abu-kabir	12	1	8.3%	24	3	12.5%
4- Al-Hussayneia	6	0	0%	16	1	6.3%
5- Fakous	5	0	0%	9	1	11.1%
6- Kafr-sakr	10	0	0%	17	2	11.8%
Total	105	5	4.8%	154	19	12.3%

Concerning the pathogenicity of isolated *Past. multocida* to mice it was noticed that 17 isolates were highly virulent to mice with a mean death time 24 hours with P.M finding of septicaemia and the microorganisms were recovered from heart blood. In addation 7 isolates were less virulent as they killed mice within 72 hours post – infection. These finding go hand in hand with those reported by Magda (1998) who observed that *Past. multocida* recovered from apparently healthy and diseased camels specially serotype B:2 were highly virulent to mice with mean death time 24 hours, while untypable strains were of less virulent, causing death of mice within 72 hours.

With regard to the results of serodiagnosis of *Past. multocida* by using ELISA test it was noticed that 33 serum samples (31.4%) from apparently healthy rabbits were ELISA positive. These reuslts agreed with those obtained by Holmes *et al.* (1986) who recorded that (32%) of examined apparently healty rabbits were ELISA positive. On the other hand these

results contradicted with those obtained by Kawamoto *et al.*, (1994) who tested 234 serum samples from nasal culture negative rabbits they found that 26 (11.1%) were ELISA positive. Also it was noticed that 71 serum samples from diseased rabbits (46.1%) were seropositive at different titers. These results showed high degree of agreement with those obtained by Zaoutis *et al.*, (1991) who found that (58.9%) of examined rabbits were seropositive using ELISA technique on the other hand these results disagreed with those reported by Rai *et al.*, (1987) who found that (96%) of examined diseased rabbits were ELISA positive. From the obtained data it was found that ELISA technique for testing serum antibodies against *Past. multocida* was a reliable diagnostic tool to screen rabbits colonies for *Past. multcida*.

With regard to the antibiotic sensitivty testing of *Past. multcida* to different antimicrobial agents. It was found that *Past. multcida* isolates were completely sensitive to Ceftiofur sodium, Norfloxacin and Gentamicin with activity percentage of (100%) for each. Table (3).

Table (2): Culture and biochemical characters of isolated Past. microorganisms (24 isolates).

Test	(+ ve) isolates		(- ve) Isolates	
	No.	%	No.	%
- Growth on macConkey's agar	0	0	24	100
- Haemolysis on blood agar	0	0	24	100
- Motility test	0	0	24	100
- Indole test	24	100	0	0
- Oxidase	24	100	0	0
- Catalase	24	100	0	0
- Nitrate reduction	24	100	0	0
- TSI	24	100	0	0
- Methyl red test	0	0	24	100
- Voges – proskauer	0	0	24	100
- Citrate utilization	0	0	24	100
- Urea hydrolgsis	0	0	24	100
- Glucose fermentation	24	100	0	0
- Sucrose	24	100	0	0
- Sorbitol	24	100	0	0
- Lactose	0	0	24	100
- Inositol	0	0	24	100
- Mannitol	18	75	6	25
- Maltose	16	66.7	8	33.3
- Xylose	14	58.3	10	41.7

Table (3): Results of the invitro chemotherapeutic sensitivity tests of *Past. multocida* isolated from rabbits (24 isolates).

Antibiotic discs	Disc conc.	No.of sensitive strains	Activity percentage
- Ceftiofur sod.	50 ug	24	100%
- Norfloxacin	10 ug	24	100%
- Gentamcin	10 ug	24	100%
- Enrofloxacin	5 ug	23	95.8%
- Sulphamethoxazole + Trimethoprim	25 ug	21	87.5%
- Streptomycin	10 ug	18	75%
- Cefotaxim	30 ug	17	70.8%
- Tetracycline	30 ug	16	66.7%
- Kitamox	70 ug	14	58.3%
- Ampicillin	10 ug	10	41.7%
- Erythromycin	15 ug	8	33.3%
- Chloramphenicol	30 ug	0	0

These finding agree with those obtained by Ahlam and Ibtisam (1998), Gaertner (1991), Saher (1994), Ibrahim *et al.* (1997), Amany (1998) and magda (1998) who found that *Past. multocida* were highly sensitive to : Ceftiofur sodium, Gentamicin, Neomycin, Enrofloxacin with activity perecntage of (100%) from the obtained data it was noticed that the isolated *Past. multocida* were resistant to chloramphenicol, these results agreed with Mercier (1992) who ststed that *Past. multocida* isolated from rabbits were highly resistant to chloramphenicol.

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دراسات على ميكروبات الباستريلا المنتشرة في الأرانب بمحافظة الشرقية.

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يعتبر مرض الباستريلوزيس من الأمراض التي تسبب هزال وضعف عام في الإنسان وكثير من الحيوانات وخصوصا تلك التي تكون تحت تأثير عوامل الإجهاد حيث يكون مصدر العدوى بميكروبات الباستريلا أما داخلية أو خارجية.

لذا فقد استهدفت الدراسة استبيان درجة الإصابة بالباستريلا بين الأرانب السليمة ظاهريا والتي تعاني من أعراض مرضية والكشف عن تواجد الأجسام المناعية لميكروب الباستريلا مالتوسيدا لعينات الأمصال من الأرانب جمعت مسحات قطنية من الأنف وكذلك عينات سيرم من ١٠٥ أرنب سليمة ظاهريا، ١٥٤ عينة من أرانب تعاني من أعراض مرض الباستريلوزيس. وبعد أجراء الاختبارات البكتريولوجية على العينات أسفرت النتائج عن عزل ميكروب الباستريلا بنسبة (٤,٨%) من الأرانب السليمة ظاهريا وبنسبة (١٢,٣%) من الأرانب المريضة.

وبأجراء التجارب البيوكيميائية أتضح أن كل العترات المعزولة (٢٤) تنتمي إلى صنف الباستريلا ملتوسيدا . تم تحديد ضراوة عترات الباستريلا ملتوسيدا المعزولة من الأرانب في الفئران البيضاء ونوقشت الأعراض الظاهرية والصفات التشريحية من حيث تواجد أنزفة في الأوعية الدموية تحت الجلد وحدوث تسمم دموي واحتقان في الأجهزة الداخلية كلها.

أسفرت نتائج التشخيص المصلي باستخدام اختبار تقدير المدمصة المناعية المرتبطة بالأنزيم (الأليزا) للكشف عن وجود أجسام مناعية لميكروب الباستريلا ملتوسيدا في الأرانب عن أن (٣١,٤%) من الأرانب السليمة ظاهريا وأن (٤٦,١%) من الأرانب التي تعاني من أعراض مرضية كانت إيجابية لاختبار المدمصة المناعية.

وباختبار حساسية المعزولات للمضادات الحيوية وجد أن جميع العترات كانت حساسة للسفيتوفور صوديوم، والنورفلوكساسين والجينتاميسين بنسبة (١٠٠%) لكل منهم، يليها الانروفلوكساسين بنسبة (٩٥,٨%) ثم التراي ميثيو بريم + السلفاميثوكسازول بنسبة (٨٧,٥%). بينما أظهرت جميع المعزولات مقاومة كاملة للكلورمفينكول .