Vet.Med.J.,Giza. Vol.42,No.3.(1994) 73-77

PASTEURELLA MULTOCIDA ISOLATED IN RABBITS: SEROLOGIC TYPES AND EXPERIMENTAL INFECTION

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Received 2/6/1994

SUMMARY

Out of 239 nasopharyngeal swabs obtained from apparently healthy and diseased rabbits, the prevalence rate of *P. multocida* was 7.9%. Such rate was lower among apparently normal rabbits (3.8%) and higher among diseased rabbits (9.1%).

Out of the 19 isolates of *P. multocida,* serotype (3:A) was the most predominant (42.1%), followed by serotype (12:A) with an incidence of 31.6%, then serotype (15:D) in an incidence of 10.5%.

An attempt was done to study the in vitro chemotherapeutic sensitivity of all isolates of *P. multocida.* Gentamicin and penicillin were the best drugs of choice against *P. multocida* infection in rabbits.

Experimental intranasal infection with serotype (3:A) showed that the mortality rate was 80%. Clinical signs and postmortem examination were described in details.

Gentamicin is the best drug for the initial therapy of infection with *P. multocida* with mortality rate not exceed 10.0% followed by penicillin with mortality rate reached 40.0%

INTRODUCTION

Infection of rabbits with *Pasteurella multocida* is widespread (Digiacomo et al., 1991) and is considered allover the world as one of the most dangerous diseases which affect rabbits (Mercier, 1992). The most common clinical manifestation of infection is disease of the proximal segment of the respiratory tract, principally rhinitis (Zimmerman et al., 1992).

P. multocida as a normal inhabitant in the upper respiratory tract of rabbits plays an important role in producing disease as the result of an interplay between infection with such microorganism and many factors other than the bacteriological agent (Kawamoto et al., 1990).

The capsular type of *P. multocida* most commonly isolated is type "A", whereas type "D" is recovered less frequently (Rimler and Brogden, 1986 and Frymus et al., 1990). The presence of carriers of *P. multocida* among apparently normal rabbits was recorded by some investigators such as Pestara de Castro et al., (1980) Rendondo et al., (1990)and Lu et al., (1991).

Various serological procedures have been used by utilizing extracted polysaccharides and protein antigens such as indirect haemagglutination and tube agglutination tests (Papp et al., 1992).

Many authors such as Kulkarni et al., (1990) ; Yuan and Fung (1990) and Gaertner (1991) had reported great variations in the sensitivity of the isolated strains to various chemotherapeutic agents.

The purpose of the study reported here was to isolate and identify *P. multocida* from rabbits in health and disease. Serotyping studies were carried out, to have an idea about the sensitivity of the isolated agaents, various drugs. Moreover, attempts for treatment of rabbits with pasteurellosis were done.

MATERIAL AND METHODS

Nasopharyngeal swabs were collected from 239 rabbits of different ages (4 weeks to 2 years) maintained at different commercial rabbit during the period from August, 1993 till May, 1994. The samples were obtained from 53 apparently healthy and 186 diseased rabbits with respiratory manifestations and rhinitis.

Within a few hours, the samples were inoculated into nutrient broth and incubated at 37°C for 24 hrs, then few loopfuls were plated onto DAS medium, 7% sheep blood agar, yeast proteose cystine agar and MacConkey agar medium. The plates were then incubated for 24-48 hrs at 37°C.

Pure colonies were subjected to clonial morphology and identification by biochemical reactions according to Balows et a l., (1991). Somatic type was determined by use of gel diffusion precipitin test (Chengappa et al., 1982). The capsular type of *P. multocida* was identified by use of indirect haemagglutination test (Carter and Rappy, 1962). Invitro-susceptibility of the isolated *P. multocida* strains to various chemotherapeutic agents using disk diffusion technique acording to Finegold and Martin (1982) were done.

Rabbits used for experimental infection were free of *P. multocida* infection (3:A) as determined by three negative nasal specimen culture results obtained at intervals before experimental infection. The inoculum consists of bacterial suspension prepared by growing on trypticase soy agar and incubated at 37°C in 5% Co_2 tension for 24 hrs. Bacteria were harvested in sterile saline solution and adjusted by MacFarland opacity tubes to give 10^8 viable organisms/ml.

Forty Boscat rabbits aged 3-4 months were divided into 4 groups each of 10 animals. They were kept undr optimun conditions of housing, food and water supply. All rabbits in the first three groups received 0.5 ml. of the inoculum intra-nasally, after 24 hrs they were re-infected again with the same dose (Gaertner, 1991). (1) The first infected group remained untreated. (2) the second infected group were assigned to receive daily infection of 100 i.u/kg of penicillin-G for 5 successive days. (3) the third infected group received daily 4 mg/kg gentamicin given S/C for 5 days. (4) The fourth uninfected group were injected with saline S/C for 5 days (Controls).

Rabbits were observed daily for clinical signs of proximal respiratory tract disease, culture was performed daily by inserting a swab deeply into both nasal cavities and examined bacteriologically. Survived rabbits were euthanatized at 4 weeks post-infection, post-mortem examination and bacterial re-isolation were done.

RESULTS AND DISCUSSION

Out of 239 samples collected from apparently healthy and diseased rabbits, 19 animals were positive for *P. multocida* with an overall incidence reached 7.9%. In diseased rabbits (186 cases) showed clinical respiratory disorders, such incidence reached 9.1 % with the recovery of 17 *P. multocida* isolates. Among apparently normal rabbits (53 cases), *P. multocida* rate was 3.8 % as shown in table (1). This agrees with the findings of Rendondo et al., (1990) who found that 3% of normal rabbits were carriers of *P. multocida*. Moreover, Mercier (1992) showed that the carrier rate of *P. multocida* among normal rabbits in the upper respiratory tract was in between 2-3.9%.

P. multocida is considered as a normal commensals of apparently normal rabbits (Kawamoto et al., 1990) which is capable of inducing severe rhinitis and respiratory disorders under stress environmental factors which lower the animal resistance such as cold, humidity, viral infection .....etc.

In the present work, incidence of *P. multocida* among diseased rabbits was 9.1%. This is nearly in accordance with the findings of Percy et al. (1988) and Frymus et al. (1990) who isolated *P. multocida* from dead rabbits showing various pathological lesions in an incidence of 11.9%.

Thus, care in management of rabbits, good protection from cold and humidity, moreover to

its vaccination may reduce the opportunity of infection or respiratory disease complication caused by *P. multocida*.

Data obtained in table (1) revealed that out of 19 strains of *P. multocida* isolated from rabbits, 4 strains (42.1 %) belonged to serotype 3:A, 6 strains (31.6 %) related to serotype 12:A and 3 strains (15.8%) belonging to serotype 15:D, while the remaining 2 strains (10.5%) were untyped serologically. These findings simulate with that of Rimler and Brogden (1986) and Frymus et al., (1990) who recommended that the capsular type (A) of *P. multocida* was the most common isolates, whereas serotype "D" was recovered less frequently.

These results are of importance because serotypes 3:A and 12:A of *P. multocida* are the causative agents of pasteurellosis in rabbits where these two serotypes are predominant in the nasopharynx of examined rabbits. Digiacomo et al., (1993) recorded that atrophic rhinitis was associated with capsular type-A isolates of *P. multocida* but the disease was rarely attributed to toxigenic capsular type-D isolates of *P. multocida*.

It is important to check the indiscreminatory advocation of antibiotics in treatment of pasteurellosis. Thus, an attempt was made to study the chemotherapeutic sensitivity of the 19 *P. multocida* isolates to evaluate their sensitivity

Table (1): Frequency and serotypes of *P. multocida* in healthy and diseased rabbits.

General Health Condition of animals	Total No. of rabbits examined	Positive for *P. multocida*		Capsular and somatic serotyping							
				3: A		12: A		15: D		Untypable	
		No.	%	No.	%	No.	%	No.	%	No.	%
Apparently healthy	53	2	3.8	1	5.3	--	--	--	--	1	5.3
Diseased	186	17	9.1	7	36.8	6	31.6	3	15.8	1	5.3
Total	239	19	7.9	8	42.1	6	31.6	3	15.8	2	10.6

* Incidence percentage according to the total *P. multocida* isolates.

Table (2): Antibiogram of the 19 *P. multocida* isolates.

Drug	Disk potency	Sensitive		Resistant	
		No.	%	No.	%
Chloramphenicol	30 ug	4	21.1	15	78.9
Colistin sulphate	10 ug	7	36.3	12	63.2
Erythromycin	15 ug	14	73.7	5	26.3
Penicillin-G	10 ug	15	78.9	4	21.1
Ampicillin	10 ug	10	52.6	9	47.4
Nitrofurantion	300 ug	4	21.1	15	78.9
Stereptomycin	10 ug	6	31.6	13	68.4
Gentamicin	30 ug	19	100.0	-	-
Sulphatriad	200 ug	10	52.6	9	47.4
Kanamycin	30 ug	8	42.1	11	57.9

spectrum to select the drug of choice. As shown in table (2), it was noticed that *P. multocida* isolates were compeltely sensitive to gentamicin (100.0%) and highly sensitive to penicillin-G (78.9%). On the contrary, most tested strains of *P. multocida* were highly resistant to nitrofurantoin and chloramphenicol (78.9% each), then streptomycin (68.4%), colistin sulphate (63.2%), kanamycin (57.9%) and sulphatriad (47.4%). These findings agreed with those described by Yuan and Fung (1990) and Gaertner (1991) who concluded that penicillin, and gentamicin followed by ampicillin and erythromycin were the best drugs of choice against *P. multocida* infection in rabbits. Moreover, Mercier (1992) stated that *P. multocida* of rabbit strains were highly resistant to chloramphenicol, nitrofurantoin and trimethoprim.

Therefore, gentamicin and penicillin-G affected with great tendency the rabbit strains of *P. multocida* and slight resistant strains were detected.

of 10 rabbits had no clinical signs nor were gross lesions seen at the end of the experiment. These obsrvations coincides with that of Rendodo et al., (1990) and Mercier, (1992).

In the penicillin treated group, one rabbit died on the second day, two rabbits on the seventh day and one on the 10th day and the remaining six rabbits surviving till the end of the experiment. There was an improvement in the respiratory clinical signs of the survivors and the mortality rate remained lower in the penicillin treated group reaching 40.0% . In the meantime, one rabbit of 10 that were given gentamicin S/C died on the second day and the remaining were in good health till the end of the experiment, and the mortality rate in gentamicin treated group was very low reaching 10.0%.

It is concluded that gentamicin is the drug of choice for initial therapy of *P. multocida* infection in rabbits. None of the control rabbits died during

Table (3): Comparative results and mortality rates between treated and untreated infected rabbits .

Group	No. of rabbits infected	No. of rabbits died	No. of died rabbits at intervals after infection							No./ died No./ exposed	Mortality rate (%)
			1 day	2	3	5	7	9	10		
Group I: Untreated infected group	10	8	2	4	1	1	-	-	-	8/10	80.0
Group II: Treated infected group with penicillin	10	4	-	1	-	-	2	-	1	4/10	40.0
Group III: Treated infected group with gentamicin	10	1	-	1	-	-	-	-	-	1/10	10.0
Group IV: Uninfected untreated group (control).	10	0	-	-	-	-	-	-	-	0.10	0.0

Eight out of 10 rabbits died during the experimental intranasal infection with *P. multocida* (3:A) and remained without treatment with antibiotics (group I). Death occurred between 24 hours and 5 days after inoculation. In the rabbits that died of *P. multocida* instillation, PM examination revealed severe pleuritis with the accumulation of purulent exudate in the thoracic cavity, serous rhinitis and pneumonia. A high fever was noticed before their death. The rabbits consumed almost no feed or water throughout the course of infection and lost weight rapidly. Two

the experimental period. The superiority of gentamicin for the in vivo action of *P. multocida* was also reported by some workers such as Kulkarni et al. (1990) and Gaertner (1991).

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