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INVESTIGATIONS OF THE KLEBSIELLAS OF THE GENITAL TRACT OF MARES AND STALLIONS.

BY

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INTRODUCTION

Klebsiella species are isolated with a relatively high frequency from equines and its environments. Incrimination of Klebsiella micro-organisms as an equine genital infective agent has been documented (Atherton, 1975). Several reports have attributed that Klebsiellas can produce an infection in the uterus of mares and Klebsiellosis in the equine species is a venereal disease as well as the organism can be transferred by mechanical means (Francis, 1975). In Japan, Kamada et al. (1984) isolated *K. pneumoniae* from 17.8% of 45 sampels from cervical swabs of mares suffered from metritis and infertility. Also, Weiss et al. (1976) isolated 712 Klebsiella strains from horses (252 from the nostrils, 289 from prepuce, 78 from the pre-ejaculate, 31 from semen samples and 62 from the cervix mare uteri) and 87.6% of the strains were *K. pneumoniae*. On the other hand, Brown et al., (1979); Eguchi et al., (1987) and Kikuchi et al. (1988) reported that the incidence of Klebsiellae in prepuce and semen of

stallions varied from 2% up to 43%. Furthermore, Greenwood and Ellis (1976) and Weiss et al. (1976) have shown that pathogenic types of the Klebsiella species were in the vestibula, urethera without necessarily infecting the cervix or uterus.

The main importance of genital tract infection with Klebsielleae in the equine species is the detrimental effect of infection has on the fertility of breeding stock (Kamada et al., 1984 and Kikuchi et al., 1988).

Antibiotics resistance has been encountered with various biotypes of klebsiellas, necessitating comprehensive sensitivity in vitro tests before a treatment is chosen. Marca et al., (1972) observed that most Klebsiella isolates were generally resistant to traditional antibiotics and chemotherapeutics but sensitive to neomycin, nalidixic acid, gentamicin, gabbromycin and kanamycin.

Some investigators reported that the pathogenicity of Klebsiellas for mice was not correlated with the presence or absence of the capsule (Marz, 1983 and Eguchi et al., 1987).

The objectives in the present study were: (i) to secure the isolation, identification and biotyping of Klebsiellas recovered from genital tract of both mares and stallions, (ii) to test in vitro sensitivity of isolates towards different chemotherapeutic agents and (iii) to compare their pathogenicity in experimentally induced lesions in the mice.

MATERIALS AND METHODS

A total of one hundred samples were collected from mares (thirty cervical samples were obtained from

apparently healthy animals and seventy both cervical and uterine samples were collected from mares suffering from endometritis.). Moreover forty five samples were collected from both prepuce and semen samples of stallions.

All samples were inoculated into sterile nutrient broth media and incubated overnight at 37°C. A loopful from inoculated fluid medium was streaked onto eosin methylene blue agar, MacConkey's lactose bile salt agar and blood agar plates and incubated at 37°C for 24 - 48 hrs. A single colony representing typical colonial and morphological appearance was picked up in semisolid nutrient agar for further identification. Five media were primarily used to differentiate Klebsiella from other Gram-negative rods. These were: TSI-agar, glucose fermentation broth, Christensen's urea hydrolysing, hydrogen sulfide-indole-motility agar and citrate agar slant media.

Isolated strains were furtherly divided into different Klebsiella biotypes in accordance with the instructions of Cowan and Steel (1974); Finegold and Martin (1982) and Kreig and Holt (1984).

Antibiotic sensitivities were determined by a disk assay on Mueller Hinton medium according to the methods of Stokes (1975). Ten chemotherapeutic disks used were (pre disk): ampicillin (10 ug), chloramphenicol (10 ug), dihydro-streptomycin (10 ug), nitofurantion (10 ug), nalidixic acid (30 ug), cephalothin (30 ug), gentamicin (10 ug), oleandomycin (15 ug), bacitracin (10 ug) and novobiocin (10 ug).

To study the clinico-pathological aspects of Klesiella infection in mice, S/C administration of four biovars of Klebsiellas i.e. *K. pneumoniae, K.ozaenae K. oxytoca* and *K. aerogenes* were carried out in an experimental study. 24 hrs pure culture of

the organism was suspended in sterile saline solution and matched with Mc Farland tube No. 7 (5×10^9 organisms per ml). 0.5 ml. of the prepared suspension was injected S/C into four groups of mice (each group contained 10 mice) and the fifth group was kept as control. All infected and control mice were observed for a period of 2 weeks to record any clinical signs, post-mortem lesions and bacteriological re-isolations.

RESULTS AND DISCUSSION

Klebsiellas are considered to be one of the most important causative agents which infect the genital system of horses and may have a variable clinical courses. Klebsiella is widely distributed in the nature but their main habitat is undoubted in the environmental conditions particullarly in the soil, hay, dust, air, water and feed stuffs.

In the present study, trials for the isolation of different biotypes from horses revealed the recognization of many biotypes of these organisms. The materials were cultivated onto a side variety of media inorder not to miss any Klebsiellae that might be present in the examined samples and the isolates were extensively studied and typed.

As shown in Table (1), out of 45 apparently healthy stallions, 6 exhibited Klebsiella organisms in an incidence of 13.3%. The higher frequency was obtained from prepuce washing but sharply decreased to 4.4% among semen samples. This nearly agrees with the findings of Merkt et al., (1974) who found Klebsiellas in 32 out of 176 stallions (18.18%) and higher incidence was found in the prepuce (48%). Moreover, Brown et al. (1979) and Kikuchi et al. (1988) observed that the incidence of Klebsiella among prepuce and semen of stallions varied from 2% up to 43%.

Table (1) : Prevalence of klebsiellae in different samples obtained from apparently healthy and diseased mares and stallions.

General Health condition.	Type of samples	No. of samples	No.of +ve samples	Prevalence rate
Apparently Healthy Stallions	Prepuce	45	6	13.3
	Semen	45	2	4.4
	Total No. of Stallions	45	6	13.3
Apparently Healthy mares	Cervical	30	2	6.7
Diseased mares	Cervical	70	8*	11.4
	uterine	70.	10@	14.3
	Overall total No. of examined mares	100	17	17

* Five cases showed cervical infection only three cases showed cervical uterine infection.

* @ Seven cases showed uterine infection only and three cases showed uterine and cervical infection.

Table (2) : Distribution and incidence of klebsiella biotypes obtained from the equine genital system.

Klebsiella biotype :	Total No. of isolates	%	Mares			Stallions		
			No.of isolates	%*	%**	No.of isola.	%*	%***
K.pneumoniae	13	56.6	9	39.2	53.0	4	17.4	66.6
K. ozaenae	5	21.7	4	17.4	23.5	1	4.3	16.7
K. oxytoca	2	8.7	2	8.7	11.7	0	-	-
K. aerogenes	2	8.7	1	4.3	5.9	1	4.3	16.7
Untyped	1	4.3	1	4.3	5.9	0	-	-
Total	23	100.0%	17	73.9	100.0	6	26.0	100.0

* On the basis of the total klebsiella isolates.

** On the basis of total examined mares.

*** On the basis of total examined stallions.

As shown in **Table (1)**, it was noticed that there was a marked variation between the incidence of Klebsiellae in mares as regards to their general health conditions as the frequency was higher among cervical and uterine samples collected from mares with endometritis (14.3%) as compared with cervical samples obtained from apparently healthy ones (6.7%). Generally speaking, the overall incidence of Klebsiella infection among mares (17%) was markedly higher as compared with those obtained from stallions (13.3%). These findings nearly simualtes those obtained by Weiss et al. (1976), Kamada et al.(1984) and Eguchi et al. (1987).

The presence of Klebsiella among apparently healthy genital system of mares and stallions may indicate that such organism may be harmless saprophytic, they may be also of opportunistic forms, only causing troubles under certain favourable circumstances. Horses susually live in a domestic life in close association with human beings and other animals. Therefore, such organisms may play a singnificant role in transmitting and spreading of Klebsileeosis to man, domestic animals or even to poultry.

As shown in **Table (2)**, *K. pneumoniae* was the most prevalent biotype recovered where its percentage was 56.6% and its incidence was higher than other species of Klebsiellae. This seems to agree with the results obtained by Weiss et al. (1976); Brown et al. (1979) and Kamada et al. (1984).

K. ozaenae, *K. oxytoca*, *K. aerogens* and untyped Klebsiella organism formulating 21.7%, 8.7%, 8.7% and 4.3% respectively of the total typed forms od isolates recovered from both mares and stallions. Similar biotypes of Klebsiella species had been recognized by Atherton (1975), Greenwood and Ellis (1976), Weiss et al. (1976) and Eguchi et al. (1987).

Table (3) : The results of the antibiogram of Klebsiella organisms isolated from the genital system of mares and stallions.

Chemotherapeutic agent	K. pneumoniae (13)			K. ozaenae (5)		K. oxytoca (2)		K. aerogenes (2)	
	R	I	S	R	S	R	S	R	S
Ampicillin	13 (100.0)	0	0	5 (100.0)	0	2 (100.0)	0	2 (100.0)	0
Chloramephenicol	6 (46.2)	3 (23.1)	4 (30.7)	3 (60.0)	2 (40.0)	2 (100.0)	0	1 (50.0)	1 (50.0)
Dihydrostreptomycin	8 (61.5)	0	5 (38.5)	2 (40.0)	3 (60.0)	1 (50.0)	1 (50.0)	1 (50.0)	1 (50.0)
Nitrofuration	11 (84.6)	0	2 (15.4)	5 (100.0)	0	2 (100.0)	0	2 (100.0)	0
Nalidixic acid	0	0	13 (100.0)	0	5 (100.0)	2 (100.0)	0	0	2 (100.0)
Cephalothin	13 (100.0)	0	0	5 (100.0)	0	2 (100.0)	0	2 (100.0)	0
Gentamicin	0	0	13 (100.0)	0	5 (100.0)	0	2 (100.0)	0	2 (100.0)
Oleandomycin	3 (23.1)	4 (36.7)	6 (46.2)	3 (60.0)	2 (40.0)	1 (50.0)	1 (50.0)	2 (100.0)	0
Bacitracin	12 (92.3)	1 (7.7)	0	5 (100.0)	0	2 (100.0)	0	1 (50.0)	1 (50.0)
Novobiocin	13 (100.0)	0	0	4 (80.0)	1 (20.0)	2 (100.0)	0 (100.0)	1 (50.0)	1 (50.0)

R= Resistant I = Intermediate S= Sensitive

The degree of susceptiability of Klebsiella isolates to the antibiotics tested is shown in Table (3). *K. pneumoniae* isolates were highly resistant to ampicillin, nitro furantion, cephalothin, bacitracin and novobiocin. The same strains were highly sensitive to nalidixic acid and gentamicin. These results are inagreement with that of Mdrca et al. (1972) who noticed that *K. pneumoniae* isolates were generally resistant to traditional antibiotics and chemotherapeutics but sensitive to neomycin, nalidixic acid, gentamicin, gabromycin and kanamycin. In addition, Weber et al. (1975) showed that *K. pneumoniae* strains were resistant to penicillin and sulphonamides.

K. ozaenae strains were highly resistant to ampicillin, nitrofurantion, cephalothin and bacitracin but all of them were sensitive to nalidixic acid and gentamicin. These results were nearly similar to that of Weiss et al. (1976) and Kamada et al. (1984).

In the present work, all strains of *K. oxytoca* and *K. aerogenes* were resistant to ampicillin, nitrofurantion and cephalothin but varied in their resistance to dihydro streptomycin and oleandomycin as reported also by Weber et al. (1975).

The present results emphasize the need of antibiotic sensitivity testing where Klebsiella inducing infection is suspected and act as a guide for selection of the most proper therapeutic agent to treat individual infection with Klebsiellosis.

Virulence tests were performed using representative biotypes listed in Table (2). This method is considered adequate for comparing the virulence of Klebsiella strains. Differences in virulence between different biovars were quite reproducible.

Table (4) : Virulence of Klebsiella biovars in experimentally induced lesions in the mice.

Bacterial biotype	Time of death in hrs.						No. of dead mice (D)	No. of surviving mice (S)	No. of dead / No.of exposed	Mortality Rate D + S x 100
	Less than 24hrs.	24 hr.	48 hr	72 hrs	More than 72 hrs	No death				
Kl. pneumoniae	2	3	4	1	0	0	10	0	10/10	100.0%
Kl. ozaenae	0	1	2	2	0	5	5	5	5 /10	50.0%
Kl. oxytoca	0	1	1	3	1	4	6	4	6 /10	60.0%
Kl. aerogenes	0	1	2	4	0	3	7	3	7 /10	70.0%
Control	0	0	0	0	0	10	0	10	0 /10	0.00%

* A total of 10 mice were inoculated with each biotype.

As shown in Table (4), *K. pneumoniae* was more virulent for mice than other serovars and have been reported to be highly virulent with a mortality rate reaching 100.0%. Death usually occured in between the first few hours after injection up to 72 hrs. post-injection. The macroscopical alterations in dead mice revealed congestion of all the blood vessels and most organs such as trachea, lungs, heart, liver, spleen, kidneys and intestinal tracts. Both lungs were dark red in colour, local to diffuse pneumonic and haemorrhagic areas could be noticed associated with congestion. Abnormal areas in the lungs were hard in consistency and many mucous exudate oozed from the cut surfaces. Petechial haemorrhages appears in the heart which was flabby and coronary fat showed pinkish in colour. The liver was frible, dark brown in colour and petechial haemorrhages could be demonstrated on its capsale. The spleen was dark brown, enlarged and petechial haemorrhages on the capsules were seen. The kidneys were swollen and dark red in colour. Hyperaemia along the intestinal tract and the mucous membrane covered by mucous mixed with the contents of the intestine. *K. pneumonia* could be reisolated from the internal organs of dead mcie. The present findings tend to agree with that of Weiss et al. (1976) and Brown et al. (1979).

In the present work, differences in the virulence could be detected between *K. ozaenae*, *K. oxytoca* and *K. aerogenes* and the mortality rate reached 50.0%, 60.0% and 70.0% respectively. Therefore, there is a correlation between biotype and virulence, and this was afforded by post-mortem examination and reisolation of the organism from the internal organs. Significantly lower virulence of such biotypes in virulence tests has not been reported previously as far as the authors know.

SUMMARY

Out of 45 apparently healthy stallions, Klebsiellas were found in an incidence of 13.3% from their genital tract. Prevalence rate was higher among prepuce washing (13.3%) than semen samples (4.4%).

Klebsiellas were observed in (6.7%) among cervical swabs of apparently healthy mares, on the contrary, such incidence reached 14.3% among mares with endometritis.

K. pneumoniae was the most prevalent biovar recovered (56.6%). Meanwhile, *K. ozaenae*, *K. oxytoca*, *K. aerogenes* and untyped Klebsiella organisms formulating 21.7%, 8.7%, 8.7%, 8.7% and 4.3% respectively.

The degree of susceptibility of Klebsiella biotypes to various chemotherapeutic agent is discussed in details.

Virulence tests were performed using representative biotypes. Differences in viulence between different biotypes were quite reproducibled.

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