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Klebsiella and *Enterobacter* organisms isolated from horses

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SUMMARY

An account is given of *K. pneumoniae* capsule types occurring in horses, with particular reference to strains originating from the genital tract in the mare and the external genitalia of the stallion.

A survey of the prevalence of *K. pneumoniae* and *E. aerogenes* strains in the preputial flora of healthy stallions is described. The majority of horses were found to be carriers of these organisms. The cultural characteristics of these preputial strains are described and compared with those of *K. pneumoniae* strains associated with epidemic metritis in mares. The epidemiological significance of certain *K. pneumoniae* capsule types is discussed.

INTRODUCTION

Klebsiella pneumoniae is an important cause of metritis in mares and the organism may occasionally be implicated in other disorders. Epidemics of metritis are usually associated with certain *K. pneumoniae* capsule types and the stallion plays an important role in their transmission (Dimock & Edwards, 1926–7; Crouch, Atherton & Platt, 1972). However, in many stallions strains of *K. pneumoniae* and *Enterobacter aerogenes* appear to be normal constituents of the preputial flora, and it is of practical importance to distinguish these from the strains associated with outbreaks of metritis.

In this article, the different *K. pneumoniae* capsule types isolated from horses are recorded, and their epidemiological significance is discussed. Evidence is also presented on the prevalence of *K. pneumoniae* and *Enterobacter* strains in healthy stallions, and the biochemical and other features of these resident strains have been compared with those of Klebsiella strains associated with outbreaks of metritis in mares (Dimock & Edwards, 1926–7; Crouch *et al.* 1972; Atherton, 1975).

MATERIALS AND METHODS

During the period 1967–75, 117 of the genital strains of *K. pneumoniae* isolated at the Equine Research Station were capsule typed. These originated from cervical swabs from mares with clinical or subclinical metritis or from preputial swabs from

stallions. Thirteen strains of non-genital origin, isolated from autopsy or other pathological material, were also capsule typed.

A detailed examination was made of the cultural and biochemical characteristics of 52 strains associated with outbreaks of transmissible metritis, comprising 49 strains from mares and 3 from stallions.

The survey of healthy stallions for the presence of *K. pneumoniae* and *E. aerogenes* in the preputial flora was carried out during the stud season (15 February to 15 July 1972) and was based on the weekly examination of preputial swabs, previously moistened in peptone water, from 16 thoroughbred stallions, aged 6–22 years, at six studs. An additional examination was made in October 1975, when single preputial swabs were obtained from 9 of these stallions.

Preputial and cervical swabs were plated on blood agar (diagnostic sensitivity test agar; Oxoid CM 261), and after incubation overnight at 37° C., representative colonies were subcultured into peptone broth. Indian ink preparations were examined for the presence of capsules, and motility was tested in motility gelatin infusion medium (Difco) incubated at 37° C. overnight. Strains were tested for ability to ferment glucose, lactose, inositol, adonitol, dulcitol and sorbose. Urease production was examined in Christensen's urea agar, and indole formation in 24 hr. peptone water cultures. Voges–Proskauer tests were done by Barritt's modification, and ability to utilize citrate was tested on Simmons' citrate agar. Strains unable to utilize citrate were further tested for growth on ammonium citrate and ammonium glucose media. Decarboxylase tests were performed with arginine, lysine and ornithine (Møller, 1955), and organic acid fermentation by the method of Ellis, Edwards & Fife (1957).

All strains received for serotyping at the Statens Seruminstitut were also examined biochemically. However, all reactions given in the tables are those carried out at the Equine Research Station according to the above-mentioned methods with the exception of liquefaction of gelatin which was tested at the Statens Seruminstitut only. The modified Kohn's method described by Lautrop (1956) was used. This test is a rapid one which shows liquefaction of slowly liquefying strains within about 5 days.

According to the characteristics of the strains, these have been classified as either *K. pneumoniae* or *E. aerogenes*. It should be emphasized that our nomenclature follows that of Ørskov (1974), according to whom the name *K. aerogenes* is non-existing, as all Klebsiella strains not being *K. rhinoscleromatis* or *K. ozaenae* are *K. pneumoniae*.

Capsular typing was carried out in the following way. A heavy suspension of the strains was first tested by slide agglutination in 72 antisera. A positive reaction was confirmed by the capsular swelling reaction under the microscope. In most cases cross-absorbed antisera had to be used finally. Some strains agglutinate to a varying extent on the slide in all antisera and can therefore only be examined by the capsular swelling method.

Mouse pathogenicity was tested by injecting pairs of adult male mice intraperitoneally with 0·1 ml. volumes of undiluted or serial tenfold dilutions (10^{-1} to 10^{-5}) of 24 hr. broth cultures of *K. pneumoniae* type 2 (NCTC 5055) or

K. pneumoniae type 7 isolated from the prepuce of a stallion. The culture of *K. pneumoniae* type 7 contained approximately 10^{10} organisms/0·1 ml.

RESULTS

Klebsiella pneumoniae *capsule types isolated from horses*

The capsule types of the strains of genital origin during the years 1967–75 are shown in Table 1. The results indicate the diversity of capsule types which have been isolated, but the numbers of each type do not necessarily reflect their relative prevalence in the horse population. This is particularly true of the stallions, which were usually only examined bacteriologically when Klebsiella infection had been diagnosed in mares that they had served.

The occurrence of Klebsiella metritis in more than one mare on a stud, after service by a single stallion, was regarded as an 'outbreak', and such cases were associated with *K. pneumoniae* types 1 or 5. Until 1975 *K. pneumoniae* capsule type 5 has been the predominant epidemic strain, but since 1973 type 1 has emerged, and appears to be superseding type 5. *K. pneumoniae* type 2 was isolated from one mare during the period under review but epidemiological details concerning this case were lacking. Sporadic cases of metritis involving single mares, with no tendency for spread by the stallion, have been attributed most frequently to capsule type 7, but a variety of other capsule types have occasionally been identified in such cases.

In males, *K. pneumoniae* capsule type 7 was the commonest capsule type isolated and was probably representative of the normal preputial flora (see below). Capsule types 1 and 5 were in stallions implicated in outbreaks of metritis, although capsule type 5 was unexpectedly isolated from the prepuce of one gelding sampled speculatively at post-mortem. The rare capsule type 14/68 was isolated from one stallion following an infection associated with this *K. pneumoniae* type in a mare which he had recently served. The organism was not found in other mares served by this horse. Capsule type 63 isolated from one particular stallion in 1969 and 1971 has not so far been recorded in mares.

The capsule types of 13 strains isolated from various lesions and sites unassociated with the genital tract are summarized in Table 2. *K. pneumoniae* was isolated from mammary secretion in 3 cases of mastitis in lactating mares, and in at least two of these cases the foal was also infected. *K. pneumoniae* capsule type 5 was associated with acute infections in at least five cases.

The occurrence of K. pneumoniae *and* E. aerogenes *in healthy stallions*

These organisms occurred, in varying numbers, in the prepuce of most of the stallions (Table 3). During the course of the 1972 survey, 91 strains of *K. pneumoniae* and 220 of *E. aerogenes* were obtained from 303 weekly swab samples (Table 4). *E. aerogenes* occurred in 15 out of the 16 stallions (94 %), and was present in most of the swabs examined. *K. pneumoniae* was isolated from 10 stallions (62 %) but was apparently absent from 6. One stallion in his first season at stud apparently carried neither organism.

Table 1. *Yearly incidence of* K. pneumoniae *capsule types isolated from the genitalia of mares and stallions*

	Mares (93 isolates)													Stallions (18)					Colts (1)	Geldings (5)			
Capsule types ...	1	2	5	5, 46	7	11	12	13	14, 68	33	35	39	47	1	5	7	63	14/68	73	5	7	8	69
1967	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2	—	—	—	—	—	—	—
1968	—	—	7	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—
1969	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	1	1	—	—	—	—	—	—
1970	—	—	14	—	3	3*	—	—	—	—	1	—	—	—	1	3	—	—	1	—	—	—	—
1971	—	—	24	1	1	—	—	—	—	—	—	—	1	—	1	3	1	—	—	1	1	2	1
1972	—	—	4	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—
1973	1	—	1	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
1974	5	1	3	—	2	—	1	1	1	1	—	1	—	—	1	—	—	1	—	—	—	—	—
1975	13	—	—	—	1	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—
Total	19	1	54	1	8	3	1	1	1	1	1	1	1	1	5	9	2	1	1	1	1	2	1

* Includes 1 case of *Klebsiella* chorionitis and necrosis in an aborted twin placenta.

Table 2. K. pneumoniae *capsule types isolated from extragenital sites in equines*

Site of origin	No. of isolates	*K. pneumoniae* capsule types					
		5	7	11	21	30	57
Mammary gland, with mastitis	3	2	—	—	—	—	1*
Blood and tissues (neonatal *Klebsiella* septicaemia)	2†	2†	—	—	—	—	—
Sacral abscess (donkey)	1	1	—	—	—	—	—
Alimentary tract	5	—	1	—	1	2	1*
Nasal cavity	2	1	—	1	—	—	—
Total	13	6	1	1	1	2	2

* Epidemiologically related isolates; from mammary gland of mare and rectal swab of foal respectively; the foal had severe diarrhoea.

† One case of neonatal *Klebsiella* septicaemia was known to be associated with *Klebsiella* mastitis in the dam.

Table 3. *Frequency of isolation of* Klebsiella pneumoniae *and* Enterobacter aerogenes *in the preputial flora of* 16 *stallions*

Frequency of isolation (% weekly swabs positive)	No. of stallions	
	K. pneumoniae	*E. aerogenes*
0	6	1
1–10%	3	0
11–50%	3	0
> 50%	4	15

In 15 of the stallions there was no history of association with transmissible *Klebsiella* metritis during the survey period but one horse, after serving a mare with subclinical *Klebsiella* metritis, became temporarily infected with *K. pneumoniae* capsule type 5 and transmitted infection to one other mare.

The single swabs from the 9 stallions examined in 1975 yielded *E. aerogenes* in 6 cases and *K. pneumoniae* in 2; from one horse neither organism was recovered. The two strains of *K. pneumoniae* were capsule type 7.

Cultural and other characteristics of preputial strains of K. pneumoniae *and* E. aerogenes

The cultural and other characteristics of the *E. aerogenes* strains and the preputial strains of *K. pneumoniae* from the 16 normal stallions are shown in Table 4. After overnight incubation on blood agar, *E. aerogenes* formed domed, glistening, white or cream, non-haemolytic, opaque colonies, 1–2 mm. in diameter, with entire edge. Cultures of the organism had a distinctive odour and colonies could be readily emulsified. Colonies of *K. pneumoniae* were similar but larger (2–3 mm. in diameter). Biochemically, most of these strains differed from typical *K. pneumoniae* strains by being urease-negative. Twenty-two strains, all urease-negative, were serologically typed; they all belonged to capsule type 7. The *E. aerogenes* strains usually reacted to *Klebsiella* capsule type 68 antiserum but one exceptional strain, which failed to decarboxylate ornithine, cross-reacted with *Klebsiella* capsule type 26. Two strains, otherwise typically *E. aerogenes* were non-motile.

In a mouse pathogenicity test carried out with capsule type 7, one of two mice inoculated intraperitoneally with undiluted broth culture died at 23 hr., and the remaining animal became temporarily sick; mice inoculated with 10^{-1} to 10^{-5} dilutions of broth culture showed no visible ill-effects. For comparison, a strain pathogenic for mice, *K. pneumoniae* capsule type 2 (NCTC 5055), was similarly tested, and in that case all the inoculated mice died. Those injected with undiluted culture died in 12 hr while those receiving 10^{-5} dilution died at 48–55 hr.

Strains of K. pneumoniae *associated with outbreaks of transmissible metritis*

The characteristics of 52 of the strains (included in Table 1) associated with transmissible metritis are shown in Table 5.

On blood agar plates they formed honey-coloured or brownish colonies, 3–4 mm. diameter, tending to coalesce. They were mucoid and often, although not

Table 4. *Cultural and other characteristics of preputial strains of* K. pneumoniae *and* E. aerogenes *isolated from* 16 *normal stallions*

	Percentage of isolates with specified cultural character			
	Enterobacter aerogenes		*Klebsiella pneumoniae*	
Character	No. of isolates examined	Isolates showing character (%)	No. of isolates examined	Isolates showing character (%)
Motility	220	99	90	0
Capsule	220	100 (small)	90	100
V-P reaction	220	100	90	95
Production of acid from				
Lactose	220	100*	90	100
Glucose	220	100	90	100
Inositol	220	100	90	100
Adonitol	220	100	90	100
Sorbose	57	0	22	100
Dulcitol	57	0	22	100
Production of gas from glucose	220	60	90	100
Citrate utilization	220	56	90	100
Production of				
Indole	220	0	90	0
Urease	220	8	90	15
Ornithine decarboxylase	57	99	22	0
Arginine decarboxylase	57	0	22	0
Lysine decarboxylase	57	100	22	100
Organic acid fermentation				
Mucate	14	78	10	90
Tartrate	14	100	10	20
Gelatin liquefaction	57	100	22	0
Klebsiella capsule types number examined	57		22	
	Capsule type 26: 1 strain		Capsule type 7	
	Capsule type 68: 55 strains		—	
	Non-capsulated: 1 strain		—	

* After 2 days.

invariably, were of sticky consistency. Of the two capsule types most frequently isolated in recent years from outbreaks of infection in mares in Britain, type 1 strains differed from type 5 strains in being dulcitol-positive, a characteristic which varies among different *Klebsiella* strains. A high percentage of type 5 strains were, in contrast to type 1 strains, ornithine decarboxylase-positive, a characteristic usually not found among *K. pneumoniae* strains.

The *K. pneumoniae* strain isolated from the stallion in the survey which had transmitted metritis between mares was capsule type 5, and its morphological, colonial and biochemical features were identical with those of other type 5 strains.

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Table 5. *Cultural and other characteristics of strains of* Klebsiella pneumoniae *isolated from the genitalia of horses in association with transmissible metritis*

	Klebsiella pneumoniae	
	Capsule type 1 (20 strains), % showing characteristic	Capsule type 5 (32 strains), % showing characteristic
Motility	0	0
Capsule	100	100
V-P reaction	100	100
Production of acid from		
Lactose	100	100
Glucose	100	100
Inositol	100	100
Adonitol	100	100
Sorbose	0	0
Dulcitol	100	0
Production of gas from glucose	100	100
Citrate utilization	100	100
Production of		
Indole	0	0
Urease	100	100
Arginine decarboxylase	0	0
Ornithine decarboxylase	0	87·5
Lysine decarboxylase	100	100

DISCUSSION

It is evident from the survey that many stallions are carriers of *K. pneumoniae* (capsule type 7) and the related organism, *E. aerogenes*, as part of the normal bacterial flora of the preputial skin. Introduction of these organisms into the genital tract of the mare at service would seem to be inevitable, and it is probable that under normal conditions they have little or no pathogenicity. Occasionally, other *K. pneumoniae* capsule types have been encountered in the external genitalia of male horses, but their epidemiological significance is uncertain. Metritis associated with *K. pneumoniae* type 7 and some of the rarer capsule types has been encountered as a sporadic, opportunist infection in isolated mares, presumably in association with some inadequacy in the normal defences of the genital tract. These cases show no tendency for spread by the stallion.

In contrast, certain capsule types have been found in association with outbreaks of metritis. During the period 1967–75 types 1 and 5 were those usually incriminated, but *K. pneumoniae* outbreaks associated with type 2 were recorded at the Equine Research Station in 1963 and 1964. The strains originally isolated in cases of metritis in mares in Kentucky and Virginia by Dimock & Edwards (1926–7) also belonged to capsule type 2 (Edwards, 1928). However, except for a single occurrence, *K. pneumoniae* capsule type 2 has not apparently been in evidence in Britain since 1964 and during the period under review type 5 predominated until

the recent appearance of type 1. It is evident that the predominant capsule type associated with outbreaks of metritis is subject to periodical change. Similar changes of serotype are well known in other diseases caused by Enterobacteriaceae both in man and animals.

Capsule types 1, 2 and 5 appeared to be definitely pathogenic compared with type 7 in their ability to cause uterine infection. It is of interest that *K. pneumoniae* type 5 may also be associated with acute infections of nongenital sites. Kauffmann (1949) working with strains of human origin, found that types 1 and 2 were usually highly virulent when tested intraperitoneally in mice, in contrast with urinary strains of *K. pneumoniae* types 8, 9 and 10 which as a rule were avirulent unless injected as undiluted broth cultures. In the present study, type 7 was also found to be avirulent in mice. The reason why some serotypes seem pathogenic and others not is unknown. It could be a particular trait of the bacteria, chromosomally or extrachromosomally linked, or it could depend upon immunological or other interactions within the host.

In most cases the role of the stallion in the transmission of the epidemic strains of *K. pneumoniae* is probably purely mechanical, resulting from transient contamination of the penis and prepuce. Occasionally, however, a pathogenic strain may become established in the prepuce as a resident constituent of the local flora. When this occurs the horse is likely to be a persistent carrier of infection, and may cause outbreaks of metritis over a long period (Crouch *et al.* 1972). This appears to be a relatively infrequent occurrence, and normally the indigenous flora may perhaps play some part in resisting the establishment of newly acquired Klebsiella strains.

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