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Klebsiella pneumonia: A commensal and cause of septicaemia in camel calves (*Camelus dromedarius)*

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Abstract

This paper highlights the aetiological involvement of *Klebsiella pneumoniae* in septicaemia of suckling camel calves in Kenya. Over a period of 22 months deaths and intestinal *K. pneumoniae* carrier rates were monitored in calves aged from birth to 12 weeks. The same number of calf deaths was caused by *K. pneumoniae* and by *Salmonella* sp.., while none could be attributed to *Escherichia coli*. *Klebsiella pneumoniae* was isolated from the intestines of healthy and diseased camel calves at frequencies of up to 100% (pastoralist herds) and 50% (ranch herds) respectively. The following *K. pneumoniae* capsular types were identified in camel calves: K2, K3, K5, K11, K13, K16, K26, K28, K31, K34, K36, K38, K54, K55, K60, K61, K64 and K81.

Keywords: Klebsiella, camel calf, septicaemia, diarrhoea, Salmonella, Escherichia coli

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Introduction

Klebsiella pneumoniae (identical with *Klebsiella pneumoniae ssp. pneumoniae* Ørskov 1984) occurs in the environment. It is an opportunistic pathogen and a saprophyte of the gastrointestinal and respiratory mucosa in humans (Bockemühl 1992, Podschun and Ullmann 1998) and in animals (Bisping and Amtsberg 1988, Carter and Chengappa 1990, Henton 1994, Songer and Post 2005). In humans, *K. pneumoniae* with multiple resistance to antibiotics are an important cause of nosocomial infections (Podschun and Ullmann 1998). Currently, 77 different capsule antigens can be differentiated by the Neufeld Quelling Reaction, with certain capsular types being particularly virulent for humans (Ørskov 1984, Podschun and Ullmann 1998).

In animals, *Klebsiella pneumoniae* is associated with: mastitis in cattle, venereal infections, endometritis, abortions, birth of weak infected foals and neonatal septicaemia in horses, neonatal

septicaemia in bovine calves and in kids (Henton 1994, Songer and Post 2005). *Klebsiella pneumoniae* was found in 23% of septicaemia cases in neonatal equine foals up to eight days old (Wilson and Madigan 1989) and has also been recorded from respiratory infections in foals (Boguta et al. 2002).

Klebsiella pneumoniae capsular type 11 was isolated from the lungs of two adult camels in India affected by 'Khurak', a condition characterised by respiratory distress, pyrexia, prolonged cough, high morbidity but low mortality (Arora and Kalra 1973). *Klebsiella sp*.. werecommonly found in lungs of slaughtered camels in Egypt (Thabet 1993), Jordan (Al-Tarazi 2001), Mauritania (Kane et al 2005), Pakistan (Zubair et al 2004) and Saudi Arabia (Abdulsalam and Alhendi 1999). From India and Saudi Arabia there are case reports of *Klebsiella sp*. mastitis in dromedary camels (Kapur et al. 1982, McGrane and Higgins 1986, Alhendi 2000). Involvement of *Klebsiella sp*. and of *K. pneumoniae* capsular types 6, 7, 21, and 68 in endometritis of camels has been documented (Wernery and Kaaden 2002, Yagoub 2005). In the internal organs of camels that died of endotoxicosis *K. pneumoniae* was present as part of a mixed flora (Wernery and Kaaden 2002).

In diarrhoeic camel calves in Mauritania *K. pneumoniae* was isolated in eight out of 29 cases (Dia et al., 2000) and in Niger *Klebsiella sp*. was suspected to cause diarrhoea in camel calves during their first year of age (Bada Alambedjir et al., 1992).

This paper describes the hitherto unrecognised role of *K. pneumoniae* as an intestinal saprophyte and as a cause of septicaemia in suckling camel calves.

Material and Methods

A point prevalence study was carried out in Kenyan camel calves aged from birth to twelve weeks of age to establish bacterial causes of death and diarrhoea. Sixty-five (55%) rectal swabs were collected from calves on seven ranches and 54 (45%) rectal swabs were collected from camel calves in various pastoralist herds. Parallel to this, a 22 months longitudinal study was carried out in 2002 and 2003in the same ranches on 86 camel calves of the same age group, 323 rectal swabs were collected at repeat visits and organ samples from nine dead ranch camel calves were examined. – Internal organ samples from post-mortems were cultured on McConkey (Oxoid CM0007B) and on blood agar (BA, Oxoid No. CM 0271B). Rectal swabs were examined for presence of *Klebsiella* sp.. on McConkey-agar (Oxoid CM0007B, 37ºC, 48h) and for presence of *Salmonella* sp.. through

selective culture: enrichment in Rappaport-Vassiliadis (Oxoid CM0669B), sub-culture on BPLS(Oxoid CM0329B) and XLD (Oxoid CM0469B). - Lactose-positive, H_2S-negative, mucoid colonies were sub-cultured and tested for indole and urease (Urea agar Oxoid No. CM 0053B, urea supplement Oxoid No. SR0020K). Urea-positive, indol-negative cultures were differentiated by API 20 E (BioMérieux). Capsular types of *K. pneumoniae* isolates were identified by Neufeld Quelling Reaction with monospecific rabbit anti-capsular sera at the Department of Infection Medicine, University Hospital SH, Kiel, Germany (for details see Podschun and Ullmann 1998). Lactose-negative cultures were differentiated on TSI (Oxoid CM0277B); isolates reacting positive in the Salmonella agglutination Latex-Test (Oxoid FT 0203A) were transferred to the German National Salmonella Reference Laboratory[1] for serotyping according to Kaufmann & White classification system. Lactose-positive coliform isolates from dead camel calves were tested for urease, indol, lysine reaction (LIM Agar), motility and presence of *E. coli* virulence associated genes. A boiling prep was prepared using colony material from blood agar plates and the DNA then extracted according to the QIAquick Gel Extraction Kit Protocol (QIAGEN, Hilden, Germany, Cat. No. 28706, QIAquick Spin Handbook 07/2002). DNA-DNA-Hybridization Method was used to detect virulence associated genes such as *est* Ia/Ib, *elt* I1/Ib, *eae*, *hly*EHEC, *stx*, *ast*. A control Polymerase Chain Reaction (PCR) was carried out to verify positive results from the hybridization (for further details see Glücks 2007).

Agar diffusion sensitivity tests for 41 *K. pneumoniae* isolates were conducted on Mueller Hinton Agar (Oxoid No. CM 0337B). A standard test procedure was followed (Stegemann and Beckmann, 1997). The antibiotic test discs used were: Penicillin G 10 I.U. (Oxoid code CT0043B), Amoxycillin 25µg (Oxoid code CT0061B), Sulphamethoxazole/Trimethoprim 25µg (Oxoid code CT0052B), Tetracycline 30µg (Oxoid code CT0054B) and Streptomycin 10µg (Oxoid code CT0047B).

Data were entered into ACCESS data base and analysed using SPSS Version 12.0 and 13.0. Descriptive statistics such as frequencies and cross tabs including calculations for Pearsons Chi-square were used.

[1] BfR, Nationales Salmonellenreferenzlabor, Diedersdorferweg 1, 12277 Marienfelde, Germany

Results

Sixty-two *Klebsiella pneumoniae* (species identity confirmed by API 20 E - BioMérieux) were isolated from 23 (37 %) healthy, 28 (45 %) diarrhoeic, two (3%) convalescent and nine (15 %) dead camel calves aged from birth to 12 weeks. The following capsular types were identified: K60 (13%); K2, K28, K54, K61 (9% each); K13, K34 (6% each); K5, K38 (4% each); K3, K11, K16, K26, K31, K36, K55, K64, K81 (2% each); untypeable (13%).- There was no significant correlation between health status and capsular type of *K. pneumoniae* present (p=0.18). There was also no statistically significant correlation between isolation frequency of *K. pneumoniae* and the management system (ranch or pastoralist herd, see Figure 1) or between *K. pneumoniae* prevalence and diarrhoea status of calves sampled. However, the majority of *K. pneumoniae* isolates (n=39, 62.9%) originated from calves that had suffered from diarrhoea or were found dead.

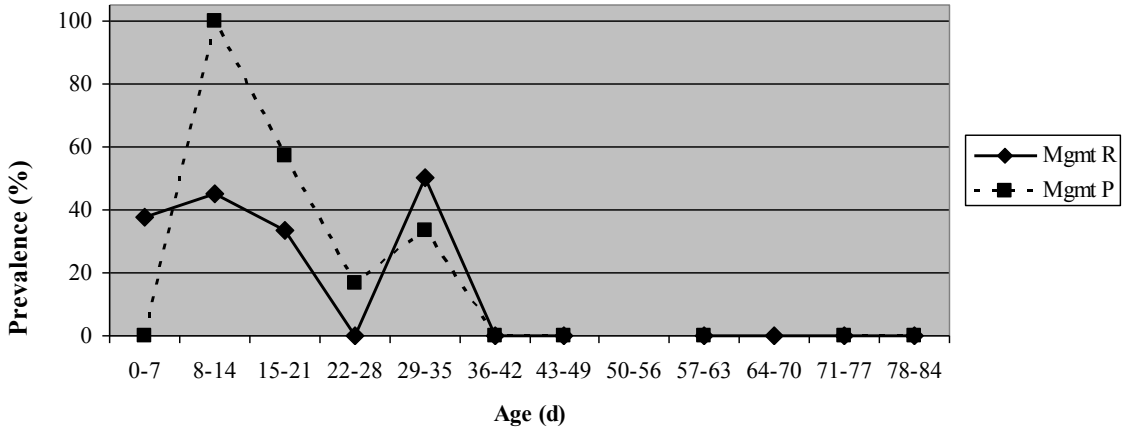

Figure 1. Point prevalences of *K. pneumoniae* in intestinal samples of camel calves; pastoralist (P) and ranch (R) management systems (Glücks 2007)

In the intestinal contents of both, healthy and diarrhoeic camel calves aged up to five weeks *K. pneumoniae* was present at point prevalences of up to 100% (pastoralist herds) and 50% (ranch herds) respectively. *Klebsiella pneumoniae* was less common in older camel calves. - Of the investigated nine deaths three were caused by *Salmonella sp*. (two *Salmonella bovismorbificans*, one *Salmonella* serovar not identified), three were attributed to *K.*

pneumoniae (capsular types K 2 and K28, one not typed), one death was due to bloat and in two cases the cause of death was not established. For the monitored ranch camel herds the pathogenic importance of *K. pneumoniae* in causing septicaemia in young camel calves equalled that of *Salmonella* sp.. Table 1 summarises case history and bacteriological results of the nine deaths.

Attributing relevance in causing death of the individual host to a particular isolate was based on:

1. The same serotype being present in more than one organ (for *Klebsiella* and *Salmonella*)
2. The possession of virulence factors (for *Escherichia coli*)
3. The isolation in pure culture from organs other than intestine

No virulence associated genes were found in *E. coli* isolated from organs of the dead calves.

Table 1. History and bacteriology results of nine dead Kenyan camel calves

Case No.	Age (d)	Clinical history	Specimen	Pathogen isolated	Capsular type /serovar/virulence gene identified	Pathogenic significance of isolate
1	6	Meconium retention	Liver	***K.pneumoniae*** in pure culture	Not done	**Relevant**
2	8	Diarrhoea for five days	*Proteus* contamination in all specimen			
3	17	Diarrhoea for three days, treatment with Amprolium	Spleen	***K.pneumoniae***	**Capsular type 28**	**Relevant**
			Blood	***K.pneumoniae***	**Capsular type 28**	**Relevant**
			Intestine	*K. pneumonia*	Untypeable	Doubtful
				K. pneumoniae	Capsular type 13	Doubtful
				E. coli	Not done	Doubtful
4	25	Diarrhoea since birth	*Proteus* contamination in all specimen			

5	46	No symptoms, found dead	Lung	*Salmonella sp*..	Not done	Relevant
			Liver	*Salmonella sp*..	Not done	Relevant
			Intestine	*Salmonella sp*..	Not done	Relevant
			Spleen	*Salmonella sp*..	Not done	Relevant
			Kidney	*Salmonella sp*..	Not done	Relevant
6	N/A	Tympany	Not done			
7	44	No history given	Liver	*S. bovis-morbificans*	Lysotype 6,8:r:1,5	Relevant
			Intestine	*K.pneumoniae*	Capsular type 38	Doubtful
8	70	Diarrhoea for five days, treated with rehydration solution	Lung	***K.pneumoniae***	**Capsular type 2**	**Relevant**
			Kidney	***K.pneumoniae***	**Capsular type 2**	**Relevant**
				E. coli	No virulence associated gene detected	Irrelevant
			Spleen	*E. coli*	No virulence associated gene detected	Irrelevant
			Intestine	*E. coli*	No virulence associated gene detected	Irrelevant
				S. butantan	Lysotype 3,10:b:1,5	Likely cause of diarrhoea
				S. bovis-morbificans	Lysotype 6,8:r:1,5	
9	10	Diarrhoea	Liver	*S. bovis-morbificans*	Lysotype 6,8:r:1,5	Relevant
			Intestine	*S. bovis-morbificans*	Lysotype 6,8:r:1,5	Relevant
				K.pneumoniae	Capsular type 26	Irrelevant
			Spleen	*S. irumu*	Lysotype 6,7:I,v:1,5	Doubtful
			Joints	*S. bovis-morbificans*	Lysotype 6,8:r:1,5	Relevant

Discussion

Dia et al. (2000) reported isolation of *K. pneumoniae* from eight out of 29 cases of calf diarrhoea in Mauritania. The presented 22 months longitudinal study in 86 camel calves aged from birth to 12 weeks describes the *Klebsiella sp.*. prevalence more in detail and is complemented by a point prevalence study in 119 camel calves of the same age group. *Klebsiella pneumoniae* was present at point prevalences of up to 100% in the intestines of camel calves during their first five weeks of life and was less common in older camel calves up to 10 weeks of age. There was no indication that *K. pneumoniae* played any aetiological role in diarrhoea of camel calves and its' very frequent presence in the intestine should be regarded as saprophytic, comparable to the situation in humans (Bockemühl 1992, Podschun and Ullmann 1998) and in other livestock (Bisping and Amtsberg 1988, Carter and Chengappa 1990, Henton 1994, Songer and Post 2005).

The number of camel calf deaths presented here is extremely low and is in no way representative of the district or region. Still the nine examined calves represent a miniature cross-section of deaths for the age group from birth up to 12 weeks. For one third of dead calves examined, *K. pneumoniae* septicaemia was rated as the most likely cause of death, the same number as for *Salmonella sp.*. In two calves, debilitation due to diarrhoea may have been a predisposing factor prior to the *K. pneumoniae* septicaemia.

Prior to this study only *Klebsiella pneumoniae* capsular type K11 had been described in respiratory disease of camels (Arora and Kalra, 1973) and capsular types 6, 7, 21 and 68 in conjunction with camel endometritis (Wernery and Kaaden, 2002). This study identified the following *K. pneumoniae* capsular types for the first time as being present in camels: K2, K3, K5, K13, K16, K26, K28, K31, K34, K36, K38, K54, K55, K60, K61, K64 and K81. The isolation of *K. pneumoniae* capsular type 2 from lungs and kidney of calf No. 8 is of particular interest as this capsular type is predominant in human clinical *Klebsiella* infections worldwide and is considered to be a highly virulent serotype; it is rarely encountered in the environment (Podschun and Ullmann, 1998). Calf No 1 had a history of meconium retention from birth and died on the sixth day. This case shows some resemblance with septicaemia in newborn foals due to *K. pneumoniae* (Wilson and Madigan 1989). Meconium retention is regularly seen in newborn camel calves (Köhler-Rollefson et al. 2001) and *Klebsiella* sp. are present in the reproductive

tract of camels (Wernery and Kaaden 2002, Yagoub 2005), which could be the potential site for intra-partum infection of camel calves.

The study confirms reports from other camel keeping regions on the major importance of *Salmonella sp*. in causing diarrhoea and septicaemia in camel calves (Berrada et al., 2000). According to Glücks (2007) the dominance of *Salmonella bovismorbificans* serovar 6,8:r:1,5 in ranch camel calves may reflect the fact that camels on ranches are kept in contact with cattle, a situation not typically found in all camel populations.

Twenty-five of the 41 *K. pneumoniae* isolates tested were resistant to tetracycline (61% resistance), the antibiotic most commonly used for livestock treatments in pastoralist regions in Kenya. Apart from tetracycline only a very limited choice of antibiotics is available to pastoralists in North Kenya, namely sulphamethoxazole-trimethoprim, amoxycillin and penicillin-streptomycin. The presented antibiotic sensitivity patterns for *K. pneumoniae* isolates from camel calves indicate that pastoralist camel owners in North Kenya (and in the Greater Horn of Africa in general) may often not have access to adequate means for treatment of septicaemic infections in young camel calves.

Conclusion

To the authors' best knowledge, this is the first report on the confirmed saprophytic presence of *Klebsiella pneumoniae* in the intestine of healthy camel calves and on the involvement of this pathogen in septicaemia of camel calves. *Klebsiella pneumoniae* should be considered for differential diagnosis when investigating septicaemic camel calf deaths and must be clearly differentiated from mucoid *E. coli*.

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