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"The Prevalence of Bacterial and Protozoal Intestinal Pathogens in Suckling Camel Calves in Northern Kenya"

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Ilona Viktoria Glücks

Veterinarian from Wesseling

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First Examiner:	Univ.-Prof. Dr. Wolfgang Müller
Second Examiner:	Univ.-Prof. Dr. Karl-Hans Zessin
Third Examiner:	Univ.-Prof. Dr. Lothar H. Wieler

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Für meine lieben Eltern

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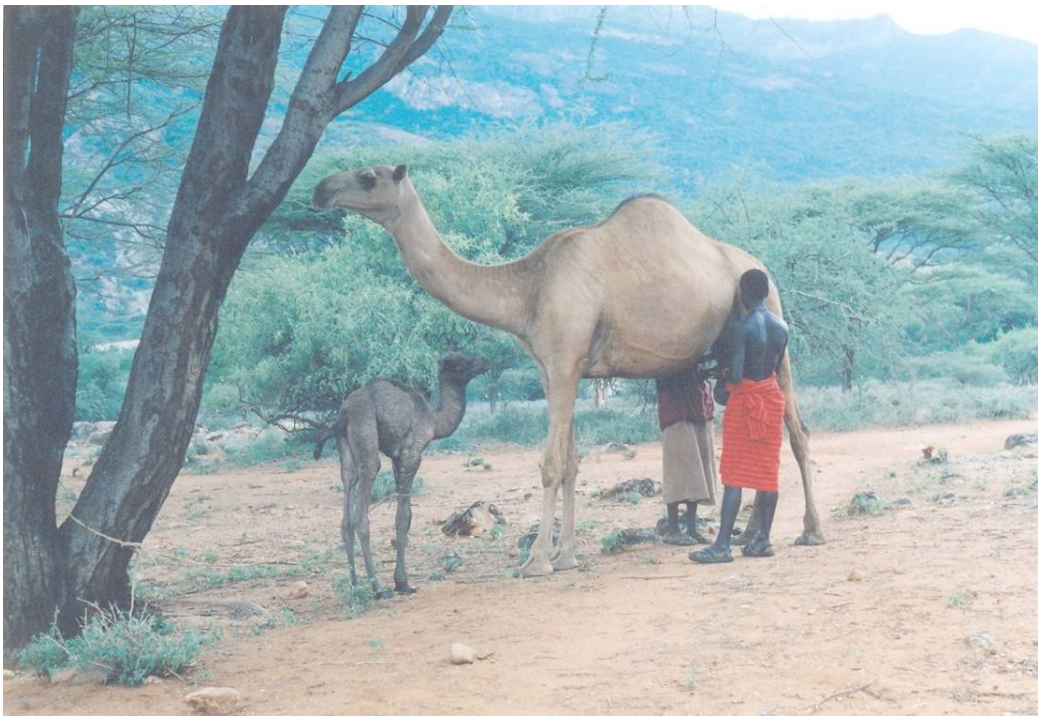
LIST OF ABBREVIATIONS

A/E lesions	Attaching and Effacing lesions
APEC	Avian pathogenic *E. coli*
ASAL	Arid and semi-arid land
DAEC	Diffusely adherent *E. coli*
E. coli	*Escherichia coli*
EAEC	Enteroaggregative *E. coli*
EHEC	Enterohemorrhagic *E. coli*
EIEC	Enteroinvasive *E. coli*
EPEC	Enteropathogenic *E. coli*
ETEC	Enterotoxigenic *E. coli*
FAO	Food and Agriculture Organization
GLiPHA	Global Livestock Production and Health Atlas
HC	Hemorrhagic Colitis
HUS	Hemorrhagic Uremic Syndrome
ITS 1	Internal Transcribed Spacer 1
LT	heat labile enterotoxin
Mgmt P	Camel herds kept under the pastoralist management system
Mgmt R	Camel herds kept under the ranch management system
MNEC	Meningitis associated *E. coli*
SSU rRNA	subunit RNA
ST	heat stabile enterotoxin
STEC	Shiga toxin producing *E. coli*
Stx	Shiga-like toxin
UAE	United Arab Emirates
UAEC	Uropathogenic *E. coli*
UTI	Urinary Tract Infection

Colour Plate 1: A “boma” at a ranch, camels, cattle, sheep and goats are kept over night

Colour Plate 2: Newborn camel calves remain in the “boma” during the day in a highly contaminated environment



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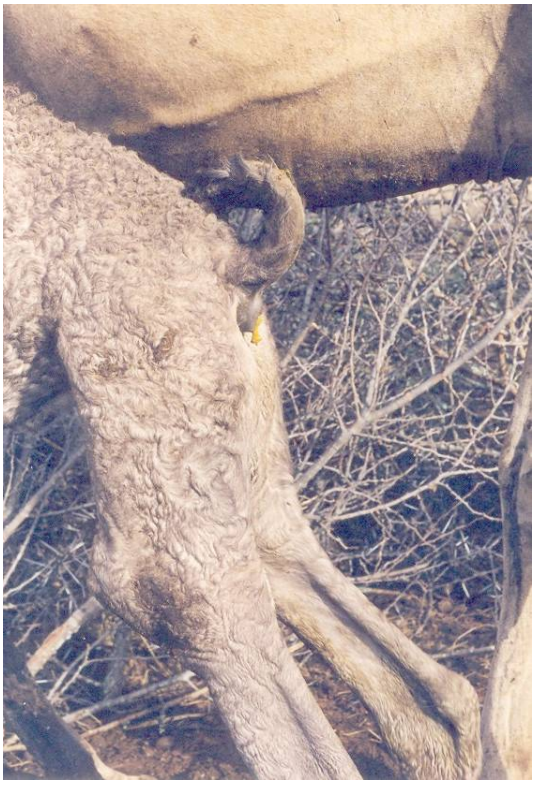

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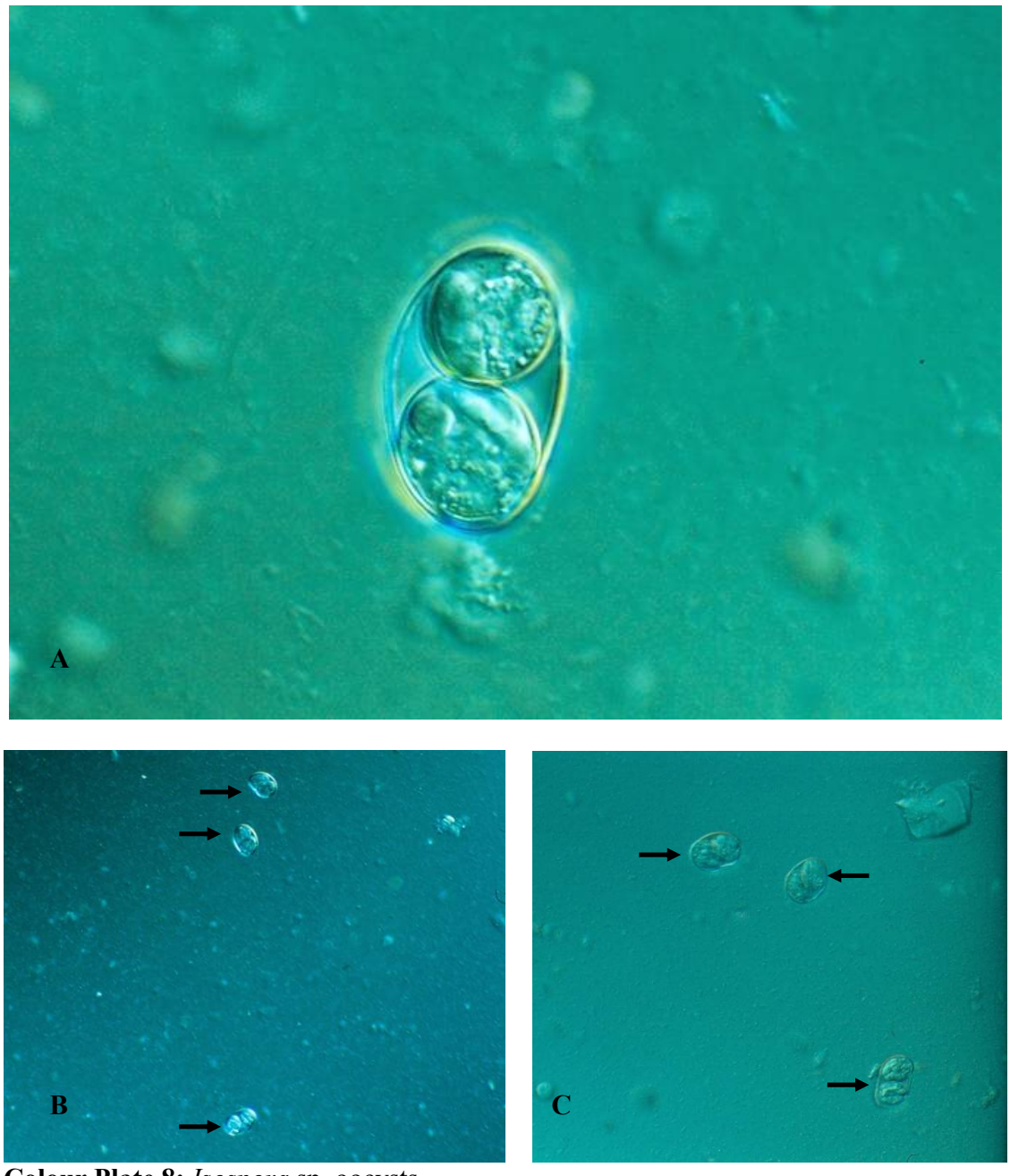

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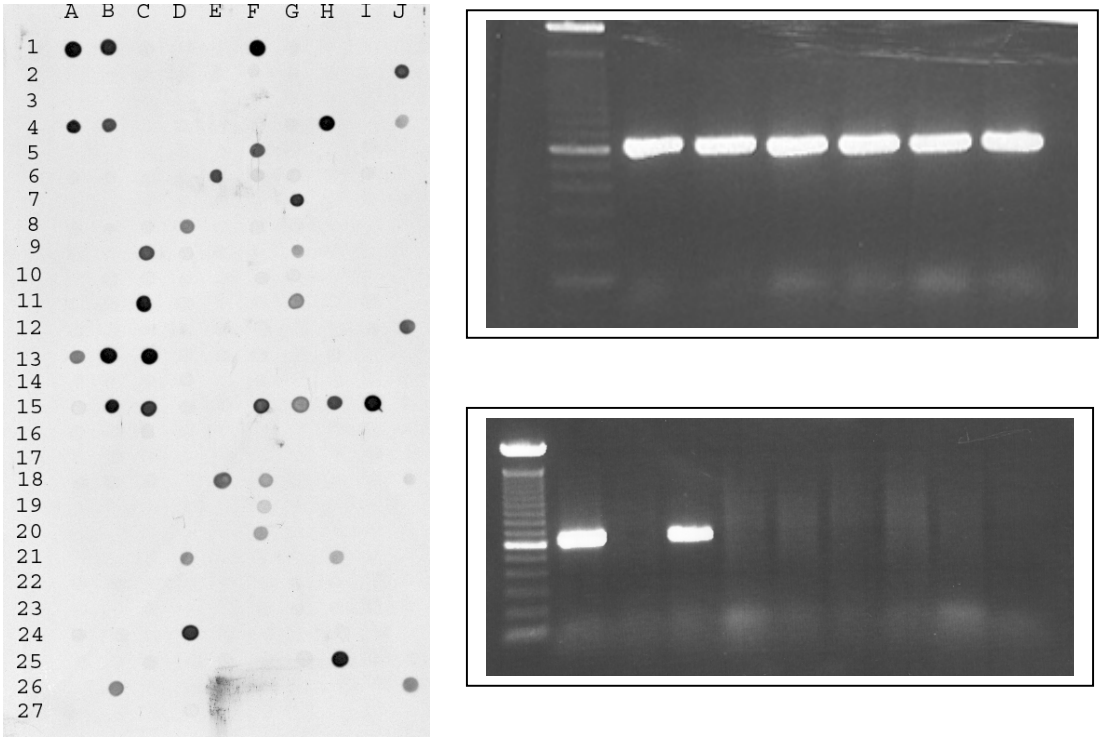

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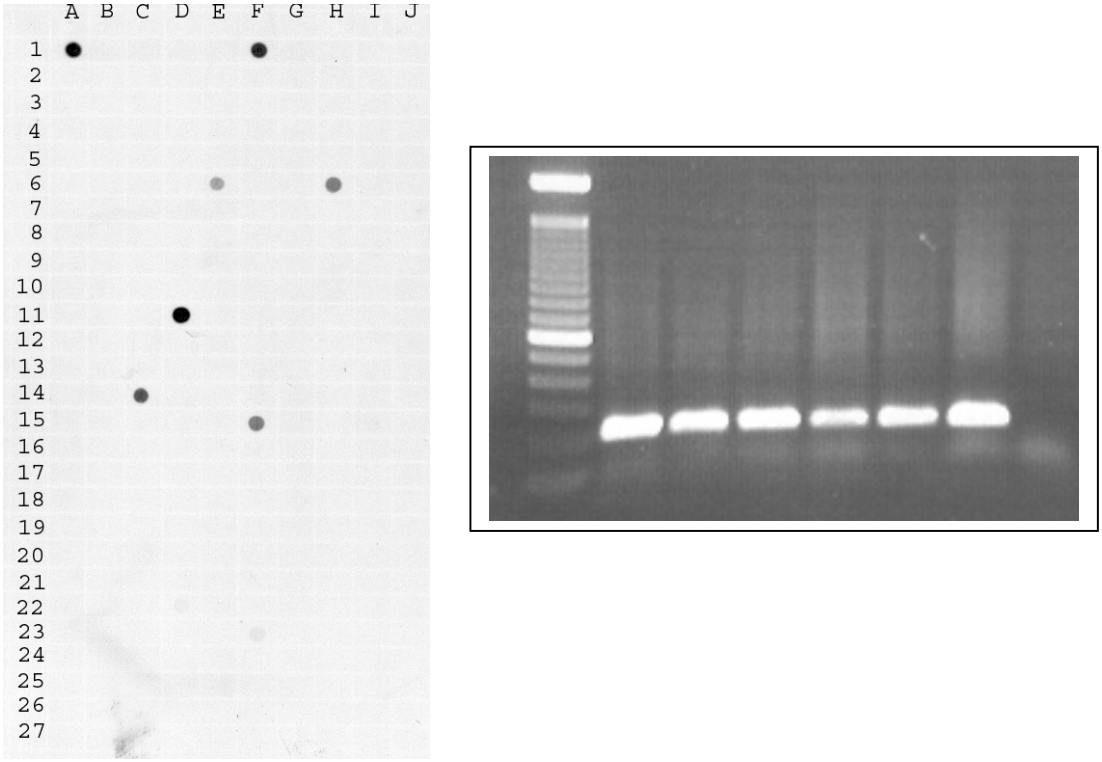

Colour Plate 10: Results of hybridization membrane with *E. coli stx*-probe and control PCR

1 INTRODUCTION

1.1 CAMELS IN GENERAL

1.1.1 Camel classification

Camels are in the taxonomic order Artiodactyla (even-toed ungulates), sub-order Tylopoda (pad-footed), and Family Camelidae. They are ruminants along with the giraffes, deer, cattle, sheep, goats and antelopes. Two different genuses are recognized: the Old World Camelids and the New World Camelids. The former is represented by two species:

> The dromedary or Arabian camel (*Camelus dromedarius*) is mainly found in Northern Africa and Middle East and the Bactrian camel (*Camelus bactrianus)*, which is found in Central Asia, China and Mongolia. They are overlapping in areas of Southwest Asia like Afghanistan and Pakistan.

The New World Camelids are represented by four species:

> The llama (*Lama glama*), alpaca (*Lama paco*), guanaco (*Lama guanacoe*) and vicuña (*Lama vicuna*). All species are living mainly in Southern America. However, their use as pets becomes more and more famous so that they can be found also in North America and Europe (Larson and Ho, 2004).

1.1.2 Camel population

According to The Statistic Division, Economic and Social Department, FAO (2004)[1] a total number of 18.9 million camels are found world wide, 95 % being dromedary camels. Of these 82 % (15.4 million) are living on the African continent in 18 different countries.
Somalia is the country with the highest camel population (7 million) followed by Sudan and Mauritania with 3.2 million and 1.292 million dromedaries, respectively. The camel population in Kenya is 830,000, representing five per cent of Africa's camel population. The camels are kept in at least 16 districts of the country, mostly in the arid and semi-arid lands (ASAL), covering over 80% of the total land surface of the country (Huelsebusch and Kaufmann, 2002). According to Mitaru (2002) 54% of the camels are kept in North Eastern Province, 29% in Eastern Province, 13% in Rift Valley Province and 4% in Coast Province.
The camels were present in Africa during pre-Roman times and first entered Africa through southern Arabia and the Horn of Africa (Farah et al., 2004).

[1] http://www.fao.org/es/ess/index_en.asp

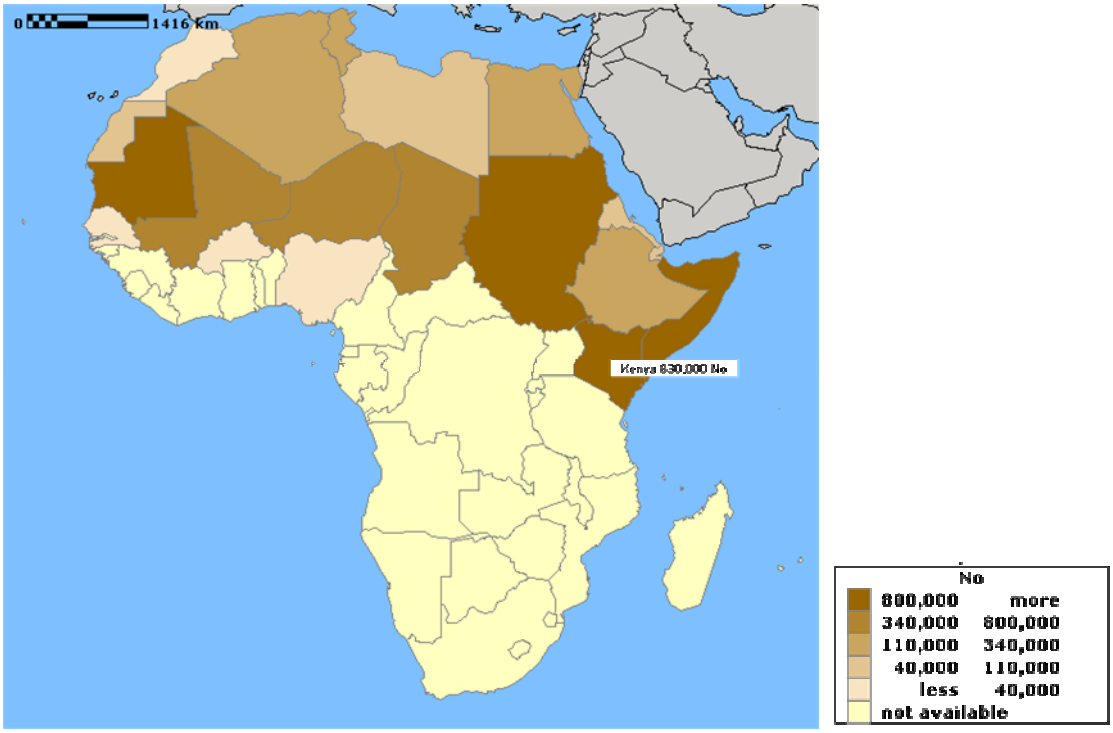

Figure 1: Camel population in Africa, Sources: FAO, GLiPHA, 2003

1.1.3 Physiological specifications

The last few decades have seen a gradual change from cattle to camels in the drylands of Eastern Africa. Traditional cattle cultures such as the Boran, Oromo, Samburu and Maasai, have gradually introduced camels into their herds. Regularly there are droughts hitting these vulnerable pastoralists, which for their survival depend on their livestock (Schwartz and Dioli, 1992). The camel is the livestock species least affected by drought due to its ability to adapt to extremely harsh conditions and has been quoted to be the most efficient domestic animal for converting vegetative matter into work, milk and meat (Ramet, 2001).

1.1.4 Camel husbandry

In Africa, the dromedary camel is generally kept by nomadic pastoralists and is an important part of their livelihood, essential to their subsistence economy (Farah et al., 2004). Many argue that dromedaries are the most important livestock species in terms of food security (Wilson, 1984, Schwartz and Dioli, 1992). The camel contributes to the household assets of the pastoralist communities as follows: Financial support is given to the household through the sales of milk, meat, hides, animals, transport services and the camel represents a saving instrument. Physically the camel is used for draught power for transport (e.g. in Africa, the dromedary camel is generally kept by nomadic pastoralists and is an important part of their livelihood, essential to their subsistence economy. transporting milk to the market, water from wells, household belongings when families move to a new area). Furthermore its milk and meat are available for household food consumption (Perry et al., 2002, Farah et al., 2004). Milk is the most important product of

the camel, and contributes between 50 – 60 % of the nutrient intake of some of the pastoralist communities of sub Saharan Africa (Perry et al., 2002, Kaufmann, 1998). The camel is often the main source of milk, especially during the dry season. Additionally the camel plays an important role for the socio-cultural set up of the community (e.g. payment of dowry, settlement of fines (e.g. in tribal feuds, recreational activities). The pastoralists, especially the Somali who represent the largest and oldest camel keeping tribe, see camels as a banking system or security against drought, disease, and other natural disasters that affect smaller stock more seriously (Farah et al., 2004).

The Somalis apply some management practices to their herds with selection and breeding being the most important husbandry techniques (Farah et al., 2004). As camel calf mortality is usually high, calf management is considered to be important and includes the attendance of the herdsman during parturition and the consideration that suckling within the first three hours post calving and sufficient milk supply is essential. However, they still believe that the intake of colostrum will result in ill-health to newborn calves, thus it is common practice to deny the newborn calf access to colostrum (Farah et al., 2004; Farah et al., 2007).
Apart from the traditional way of camel keeping, some ranches started keeping camels about 30 years ago. They mainly benefit from the camel milk, which can be sold to the markets and the usage of camels as transport animals for safaris in the tourist sector.

1.1.5 Camel breeds in Kenya

The classification of different camel breeds in Kenya is based on the pastoralist tribe: Somali, Rendille/Gabra, Turkana, Pokot and Samburu. The Somali breed is kept by most of the pastoralists in North Eastern Province of Kenya. They are known to be the largest animals with an adult female average weight of 500-600kg and an adult male average weight of 600-800kg. The average daily milk yield is between 5kg to 8kg during a lactation of 10 to 12 months (Farah and Fischer, 2004). A few imported camels from Pakistan and their crossbreeds with the above mentioned Kenyan camel breeds are also found on ranches.

1.2 DIARRHOEA COMPLEX IN CAMEL CALVES

In the literature high losses of camel calves have been described by several authors (Schwartz and Dioli, 1992; Kaufmann, 1998; Abbas and Omer, 2005; Farah et al., 2007). The reasons for the high mortality rate among camel calves is caused by different factors among which diarrhoea, predation and other diseases are mentioned as the main causes of death (Kaufmann, 1998). According to a questionnaire done by Kaufmann (1998), the mortality rates in pre-weaning camel calves are 22%, 27% and 31% in the Gabbra, Rendille and Somali tribes in Northern Kenya, respectively. Diarrhoea accounts for 21% of the calf mortality in the Gabbra tribes while it is the major cause of death in 33% and 23% in Rendille and Somali tribes, in that order. Kegode (1990) describes pre-weaning calf mortality with 22.4% on two commercial ranches in Kenya.
A survey carried out in Puntland, Somalia, attributes 73.2% of mortality in newborn camel calves to diarrhoea (Farah et al., 2007).

Dia et al. (2000) state that diarrhoea is the major cause of death in camel calves according to the herdsmen in Mauritania with more than 80% of calves being affected. The prevalence of diarrhoea in camel calves between birth and three months is described with 79.5%. The most affected age group observed is two month. Animals are severely dehydrated and death follows within four to five days. Dia et al. (2000) describe bacteria such as *E. coli* and *Salmonella* as the major pathogen for enteritis in camel calves up to three months of age. However, parasites such as *Strongyle* and coccidia as well as the nutritional status of the dam play a role, too. The authors conclude that many factors contribute to the aetiology of the diarrhoea complex in camel calves.
According to Faye et al. (1997) the reason for 68% of the camel calf losses in Niger is diarrhoea, while Bada Alambedji et al. (1992) states that the morbidity of diarrhoea can reach 80 to 90% with a minimum of 50% mortality. The authors conclude that diarrhoea in camel calves is caused by the synergetic effect of predisposing factors, nutritional factors, parasites (such as coccidia), bacteria (such as *Salmonella* and maybe *E. coli*) and virus (e.g. Rota- and Coronavirus). The absence of colostrum intake is described as a contributing factor to camel calf diarrhoea (Faye et al., 1997, Kaufmann, 1998, Agab and Abbas, 1999).
In Ethiopia the mortality rate of camel calves below on year is described with 20.4% (Zeleke and Bekeli, 2000). The authors observed difficulty in breathing as main symptom prior to death and attribute pneumonia as the major probable cause for calf mortality in the studied group.
Agab and Abbas (1999) state that diarrhoea is a significant cause of mortality and poor weight gain in camel calves and contributes to slow herd growth in Sudan. Diarrhoea was reported in 21.9% of the investigated calves, with a peak in the early summer. The authors attribute this peak to the calving period of the camels. To their knowledge the cause of diarrhoea is not sufficiently known, however *Salmonella* infections and bad management practices are mentioned.
In Morocco the mortality rate in camel calves up to six months of age reaches 20.2% resulting in a major threat for the development of camel husbandry (Bengoumi et al., 2000). Seventy two percent of mortality is attributed to diarrhoea and only 9%, 6% and 5% to malnutrition, stillbirth and abscess, respectively. Salmonellosis and colibacillosis are important causes of diarrhoea in new born camel calves up to ten weeks of age with a prevalence of 13.6% and 11.4%, respectively (Berrada et al., 2000).
Abass and Omer (2005) state that diarrhoea is a problem in “young camel calves”. They describe three clinical syndromes that can be observed in different age groups. The first one occurring in neonates is mainly caused by rotavirus and *E. coli*, the second syndrome occurs in calves between two weeks to two months of age with *Salmonella*, *Clostridium*, *Campylobacter* and possibly coccidia being the major cause of diarrhoea. The third syndrome is mainly found in older suckling calves up to one year of age and caused by the recurrence of an episode of acute or subacute gastroenteritis in this age group with *Salmonella* spp being most common. The authors stress on the importance of the investigation of risk factors, economic impact and control procedures for the diarrhoea complex in camel calves.

This study aims at investigating the prevalence of bacterial and protozoal intestinal pathogens in camel calves up to twelve weeks of age in Northern Kenya.

A point prevalence study is conducted to describe the existing intestinal pathogens according to age groups and health status and to compare their occurrence between different camel management systems (pastoralist and ranches).

A longitudinal study is carried out in the ranch management system in order to sustain the findings of the point prevalence study and to describe the age prevalence of the existing pathogens more comprehensively.

2 LITERATURE REVIEW

2.1 PARASITOLOGY

2.1.1 Protozoa

2.1.1.1 The genus Isospora

Isospora belongs to the family of Isosporidae, the suborder Eimeriina, the order of Eucoccidiida, the subclass of Coccidia, the class of Sporozoea, the phylum of Apicomplexa and the kingdom of Protozoa (Rommel et al., 2000).

Characteristically the genus Isospora form oocysts with two sporocysts, containing four sporozoites each. They are intracellular parasites, mainly of the gastrointestinal tract of the host. The lifecycle consists of three stages: Merogony (asexual cycle), Gametogony (sexual cycle) both taking place in the host and finally Sporogony, which takes place outside the host.

The host usually ingests sporulated oocysts. The sporozoites are released and invade epithelial or other cells of the intestines of the host, where they form meronts and multiply by merogony. The products are infective bodies called merozoites, which again invade neighbouring cells and multiply further by merogony. The last generation of merozoites become gamonts inside new host cells and develop either into macrogametocytes (female gamonts) or microgametocytes (male gamonts), the former producing a single macrogamete while the latter produce numerous microgametes. The microgamete penetrates the macrogametes forming finally zygotes (gametogony). With a wall formed around the zygote and finally released into the lumen of the intestines, the oocysts are formed and passed via faeces in an unsporulated stage. Outside the host, the oocyst sporulates depending on the environmental conditions (oxygen, temperature, humidity). With the sporulation, sporocysts containing the infective sporozoites are formed (Steward and Soll, 1994).

Coccidiosis is a disease usually affecting young hosts. The disease mainly occurs when factors such as stress (overcrowding, weaning or transportation) lead to a decline in the host's resistance. Most of the hosts are sub-clinically infected after ingesting sporulated oocysts. Hot, humid and unhygienic conditions favour the survival of the oocysts in the environment and lead to a higher infection rate. Oocysts can be shed by these sub-clinical infected hosts over a long period (Steward and Soll, 1994).

Factors playing an important role in the pathogenesis of the disease are:

the pathogenicity of the *Isospora sp.*, environmental conditions, the infective dose, the age of the host, the number of host cells destroyed, the location of the parasite and the presence or absence of acquired immunity (Steward and Soll, 1994). Infection is usually self-limiting due to the fact that the number of asexual generations is fixed for each species (Levine, 1985). The population of the organism grows to its maximum and finally fades away either to full extinction or to a very low level. Immunity develops after infection and is species-specific (Steward and Soll, 1994). Carriers can shed the oocysts for months, representing a constant threat and source of infection for the following generations (Bowman, 1999).

Invaded host cells are mainly destroyed or the usual function is impaired. This causes in severe cases acute inflammatory reactions leading to haemorrhages of the capillaries with hypoproteinaemia and anaemia as a result. Secondary bacterial infections usually aggravate the lesions. The result of these changes is an increased rate of peristalsis causing diarrhoea. Dehydration and acidosis follow in severe cases and are in combination with shock and secondary bacterial infections the main cause of death (Steward and Soll, 1994). Affected areas of the intestine are oedematous and thickened, and there may be focal or diffuse congestion or haemorrhage in the mucosa causing mal absorption (Steward and Soll, 1994).

Chronic diarrhoea is the cardinal sign of coccidiosis (Bowman, 1999). The passed faeces are soft, unpelleted and finally watery with blood being present occasionally. Anorexia and dehydration follow and with persisting infection weight loss, depression, recumbence and death may occur (Steward and Soll, 1994). In piglets for example the mortality is usually less than 20 %.

Several factors have to be taken into consideration when diagnosing coccidiosis:

the history of the case, clinical signs, necropsy findings and the demonstration of the parasite (Steward and Soll, 1994). The mere identification of the oocysts in the faeces does not justify the diagnosis of coccidiosis unless the history and clinical signs are in accord. It is possible to find large numbers of oocysts in the faeces of a healthy host or on the other hand find severe cases of coccidiosis without demonstrating the oocysts at all. The latter occurs in the early asexual stages of infection before oocysts had time to develop (Bowman, 1999).

Bowman (1999) and Steward and Soll (1994) both agree that prophylaxis is the most effective way of controlling the disease. Treatments with Sulphonamides or Thiamine Antagonists have not the expected effects, as it usually is administered too late. When oocysts are diagnosed in the faeces the damage has already been done in the small intestines. The most effective way to prevent a disease outbreak is the reduction of stress, improved management and hygiene. Sunlight for at least eight hours and desiccation are the most effective control methods for the destruction of oocysts in the environment (Steward and Soll, 1994). According to Rommel et al. (2000) disinfection of the surfaces with hot steam is currently more effective than the use of chemicals.

The prevention of the disease is based on a limited intake of sporulated oocysts to allow an infection to induce immunity but no clinical signs (The Merck Veterinary Manual, 2005).

Isospora cannot be found in all animal species and has not been described in domestic/wild ruminants but is a common pathogen in pigs, cats, dogs (now cytoisospora), wild birds, nonhuman primates and in humans.

2.1.1.2 Isospora infection in pigs

Levine and Ivens (1986) describe three *Isospora* species isolated from swine: *Isospora suis, I. almataensis and I. neyrai*. While *I. suis* is described as a pathogen in nursing piglets, *I. almataensis* and *I. neyrai* are only known from oocysts in the faeces (Lindsay et al., 1997).

Isospora suis may cause severe disease (coccidiosis) in piglets at an early age, in the first five to ten days according to Steward and Soll (1994), in the first two weeks according to Bowman (1999) and between five to 15 days according to Rommel et al. (2000).

Isospora suis occurs in the small intestines (Stuart et al., 1980, Lindsay et al., 1980), most numerous in the distal half of the small intestines (Hareleman and Meyer, 1984). Sometimes it is found in the colon (Steward and Soll, 1994). The oocysts are spherical to sub-spherical and 17-25 x 16-21 μm in size. They lack a micropyle, polar granule and residuum (Steward and Soll, 1994).

The parasite causes catarrhal, fibrineus and necrotic inflammation of the jejunum and/or ileum (Stuart et al., 1980, Rommel et al., 2000). The prepatent period is four to seven days (five days according to Bowman, 1999 and four to five days according to Lindsay et al., 1997) while the incubation period is three to four days. *Isospora suis* has three asexual and one sexual intra-intestinal conventional life cycle (Harleman and Meyer, 1984).

Oocyst excretion is cyclic with up to three peaks every two to three days according to Rommel et al., (2000) and every 5 days according to Christensen and Henriksen, (1994). The shedding of oocysts can last between one and three weeks (Bowman, 1999). The faeces are watery, whitish to yellowish with an unpleasant smell (Rommel et al., 2000, Lindsay et al., 1997). Apart from diarrhoea, dehydration and weight loss are described as the characteristic symptoms (Steward and Soll, 1994, Bowman, 1999, Rommel et al., 2000). Mortality can reach up to 20% (Steward and Soll, 1994) whereby morbidity is high and can reach up to 62% (Rommel et al., 2000). Survivors are immune to re-infection with this species and no clinical signs develop after re-infection, however a few or no oocysts are excreted in the faeces (Stuart et al., 1982).

The demonstration of *I. suis* oocysts in the faeces might be difficult as they do not occur until about two to three days after the onset of diarrhoea (Rommel et al., 2000, Stuart et al., 1980). Death may occur before this stage. In such cases histological examination is recommended (Steward and Soll, 1994).

According to Bowman (1999) piglets infected after three weeks of age develop only mild diarrhoea while Nilsson (1988) describes *Isospora suis* as the cause for post-weaning diarrhoea in five to six weeks old piglets. Diarrhoea occurs four to seven days after weaning. The morbidity is high with 80 to 90% but the mortality is very low.

Toltrazuril (1 x 20 mg 5% suspension) or Sulphonamide (e.g. 100mg/kg Sulfadimidin) administered orally may help as therapy. Furthermore Toltrazuril (20mg/kg BW) can be given to three to five day old piglets as prophylaxis (Rommel et al., 2000). It can reduce coccidiosis from 71 to 22% and lessens the severity of diarrhoea and oocysts excretion (from 4.9 to 2.5 days) in the treated piglets. Furthermore the detection of *I. suis* in piglets with diarrhoea can be reduced from 84% in the untreated piglets to 6% in the treated ones (Driesen et al., 1995). Lasalocid administered to early weaning pigs experimentally infected with *I. suis* prevents weight loss but not oocysts excretion, however the pigs develop a strong immunity. Halofuginone given to the same pigs inhibits oocyst production but reduces weight gains; developed immunity is weaker (Lindsay et al., 1997).

Good management practice including appropriate hygiene and reduced stress factors are the most effective ways of controlling *I. suis* infection in piglets (Steward and Soll, 1994). Available disinfectants are not efficient enough, as sporulated oocysts are highly resistant to them. The usage of steam is effective in killing sporulated and unsporulated oocysts. Crate to crate spread due to the farmer himself should be avoided (Lindsay et al., 1997).

According to Lindsay et al. (1997) the epidemiology of neonatal isosporiasis in piglets is not clear. The role of the sow as the logical source of infection could not be confirmed. Sows are mainly infected with *Eimeria* species and only with a prevalence of 5% with *I. suis*. However, piglets suckling from sows infected with *Eimeria* species develop coccidiosis and excrete oocysts of *I. suis* after four to eight days of age. Milk and placenta sample are negative for parasites. Lindsay et al. (1997) suggest that once *I. suis* is set up on a farm, it is maintained by infection of piglets from the contaminated farrowing crate.

2.1.1.3 Isospora infection in dogs and cats

Rommel et al. (2000) have introduced the genus name *Cytoisospora* for *Isospora* in dogs and cats. This genus name is used throughout this thesis.

Three *Cytoisospora* species are described in dogs: *C. canis*, *C. ohioensis*, *C. burrowsi* and two species in cats: *C. felis*, *C. rivolta* (Rommel et al., 2000, Bowman, 1999). Apart from *C. felis*, being egg shaped, most of the other oocysts species are round to oval with different sizes (Rommel et al., 2000).

The prepatent period varies among the different species and has a minimum of four days (*C. ohiohensis* and *C. felis*) and a maximum of ten to eleven days (*C. canis* and *C. burrowsi*). For more details see Table 1 below:

Species	Prepatent period	Patent period
C. canis	10 days	12 to 28 days
C. ohioensis	4 days	
C. burrowsi	11 days	4 to 12 days
C. felis	4 days	13 to 23 days
C. rivolta		13 to 23 days

Table 1: Prepatent and patent period of *Cytoisospora sp.* infections in dogs and cats

The patent period varies as well, with the shortest period with *C. burrowsi* (4 to 12 days) and the longest period with *C. canis* (12 to 28 days), *C. felis* and *C. rivolta* (13 to 23 days). The sporulation time has been described with four days under an optimum temperature of 21 °C (Rommel et al., 2000).

Cytoisospora sp. are facultative heteroxenous and multiply in the cells of the epithelium of the lamina propria of the small intestines, caecum or colon.

Paratenic hosts are mainly rodents, where multiplication usually does not take place. The sporozoites invade the tissue of the paratenic host (mainly mesenterical lymphnodes, Payer's plaques, spleen, liver, heart, lung, skeletal muscles) and remain there as

dormocoites (Hypnosoites). They can remain for at least two years and only multiply if digested by the primary hosts through meat consumption (Rommel et al., 2000).

Vegetative stages of *C. felis* and *C. rivolta* were found in camels, cattle, sheep and rabbits, of *C. canis* in pigs, buffalo, and camels, of *C. ohiohensis* in pigs, buffalo and donkeys and of *C. burrowsi* in buffalos (Zayed and El-Ghaysh, 1998). Furthermore, dogs feeding on camel meat started shedding *C. (I.) canis* while dogs fed with sheep meat were excreting *C. (I.) canis* and *C. (I.) ohiohensis*, among others (Hilali et al., 1992).

Cytoisospora sp. are stenoxenous and affect mainly young animals. Almost every puppy/kitten have experienced infection in early months (Bowman, 1999), 3 to 38% of dogs and 3 to 36% of cats have been positive for coccidial oocysts (Kirkpatrick and Dubey, 1987; Lindsay et al., 1997). The infection can be a major problem in kennels and catteries where it can spread explosively. Non-immune adults can be affected when eating raw infected meat. Outside the hosts the oocysts stay infectious for several months (Rommel et al., 2000). Stray dogs and cats are more affected as they need to hunt for food and are therefore more exposed to paratenic hosts (Lindsay et al., 1997).

Severe infection with *Cytoisospora* sp. leads to hemorrhagic enteritis of the jejunum and ileum. While light infections usually pass without symptoms, severe cases are accompanied by copious watery diarrhoea, fever, anorexia, emaciation and death. The symptoms persist for one week according to Rommel et al. (2000) and several weeks according to Bowman (1999). In kittens the infection occurs primarily during weaning stress (The Merck Veterinary Manual, 2005). Immunity usually develops, however, it is not known how strong the immunity is and for how long it can last. *C. canis* has been described as the most pathogenic species (Rommel et al., 2000).

Therapeutically the administration of Sulfonamides, such as Sulfadimethoxin (25mg/kgBW daily for 2-3 weeks) alone (The Merck Veterinary Manual, 2005) or in combination with Trimethoprim (Sulfadiazin plus Trimethoprim twice daily, 15mg/kgBW orally for five days; Sulfadoxin plus Trimethoprim daily, 15 – 20mg/kgBW orally for six days; Sulfadimethoxin plus Trimethoprim dogs 24mg/kgBW, cats 32mg/kgBW orally for six days; Rommel et al., 2000) is recommended.

Furthermore Toltrazuril (daily 10mg/kgBW orally for four to five days) or Clazuril (daily 2.5 – 5mg/kgBW orally) can be used. Supportive fluid therapy should accompany the treatment.

Extinction of the infection is usually impossible, but with normal hygienic measures (e.g. clearance of faeces) and no feeding of raw meat, the infection rate can be limited (Rommel et al., 2000).

The cages in catteries and cannels should be disinfected on a daily base (The Merck Veterinary Manual, 2005). The extinction of the oocyst is best with hot steam. So far chemical disinfection does not fully extinguish the oocysts (Rommel et al., 2000). The treatment of the pregnant and lactating cats prevents the infection of the kittens.

2.1.1.4 Isospora in wild birds

Isospora is a common pathogen in sing birds such as finches, canaries and sparrows. However, the pathogenicity is rather low but in young or stressed birds the infection with *Isospora* sp. can lead to high flock mortality.

Isospora lacazei is a common pathogen in the domestic sparrow first described by Labbé 1893 (according to Grulet et al., 1982). The excretion pattern of the oocysts has been investigated and Wild (2003) found out that it is rhythmic at a specific time of the day, mainly towards the evening. This way, oocysts are excreted while the birds have re-joined. Therefore, the possibility of *Isospora* infections in a new host is increased. Before sleeping, the sparrow tends to take a bath in oocyst-contaminated sand, after which it cleans its feathers and ingests the oocysts.

Oocysts are passed with the faeces unsporulated and sporulate within 48 hours at room temperature.

2.1.1.5 Isospora infection in humans

Isospora natalensis and *I. belli* have been described as pathogenic parasites of humans. However, little is known of *I. natalensis* which was found in 1953 in a 21 year old patient suffering from dysentery. The infection was self-limiting and has never been described since (Lindsay et al., 1997a).

Isospora belli infection is asymptomatic or causes self-limiting diarrhoea in immuno-competent individuals while it leads to a severe chronic disease in immunocompromised patients (Meyer, 1996). However Lindsay et al. (1997) and Raymondo (http://www.practicalscience.com/isospora.html) indicate that *I. belli* could sometimes cause serious, even fatal infections in immunocompetent people as well. Children are more susceptible to infection with *I. belli*. The infection rate decreases with increasing age (Sorvillo et al., 1995).

Infection with *I. belli* is cosmopolitan in distribution but more common in tropical and subtropical regions (Lindsay et al., 1997a). Humans acquire the infection by ingesting infective oocysts from symptomatic or asymptomatic cases (Bijay et al., 2002).

The prepatent period has been described with 10 to 11 days and oocysts excretion can last up to 38 days. Symptoms include diarrhoea, steatorrhoea, headache, fever, malaise, abdominal pain, vomiting, dehydration and weight loss (Lindsay et al., 1997a, DeHovitz et al., 1986). The disease is often chronic and oocysts can be found for several months up to years in faeces or biopsy specimen. Recurrences are common (Lindsay et al., 1997a and 1997b). In immunodeficient people, e.g. with Aquired ImmunoDeficiency Syndrome (AIDS) or Hodgkin's disease[2] symptoms of *I. belli* infection are similar but more severe (Bijay et al., 2002) and more common (Dehovitz et al., 1986; Pape et al., 1989). Furthermore, extra intestinal infections in AIDS patients have been described by Restrepo et al. (1984) and Michiels et al. (1994), whereby the oocysts of *I. belli* were found in the mucosa and lamina propria of small and large intestines and in the mesenteric and tracheobronchial lymph nodes in the former and in the mesenteric and mediastinal lymph nodes, liver and spleen in the latter case.

In children *I. belli* infection can cause diarrhoea, vomiting, abdominal pain, nausea and flatulence (Bijay et al., 2002) and may be fatal as described by Liebman et al. (1980), of

[2] Hodgkin's disease is a malignant disease of lymphatic tissue, characterized by painless enlargement of the lymph nodes in the neck, axillae, groin, chest or abdomen. Even liver, spleen, bone marrow and bones can be affected. Typical Sternberg-Reed cells (large binucleate cells) are found in the affected lymph nodes.

one case in a six months old infant that died after 30 weeks of continuous parenteral nutrition. The infant suffered from severe diarrhoea due to cholera – like hypersecretion of intra-luminal fluid.

Relapse of infections with *I. belli* is common, especially in patients with AIDS (Lindsay et al., 1997a and 1997b, DeHovitz et al., 1986) and are associated with the presence of extraintestinal stages (Lindsay et al., 1997b).

Treatment is most commonly done with Timethoprim-Sulfamethoxazole and less frequently with Sulfadoxine-pyrimethamine, both having a positive effect. Furthermore they can be used as a prophylactic measure to prevent recurrences, especially in patients with AIDS (Pape et al., 1989). Lindsay et al. (1997) stated that other agents such as metronidazole, tinidazole, quinacrine and furazolidone are probably of little value in the treatment of *I. belli* infections. The authors furthermore stated that trials with veterinary anticoccidial drugs like Amprolium and Diclazuril were partially useful.

Whether dormocoites of sheep and cattle can be infectious for humans is not known. So far these vegetative stages have not been found in humans.

2.1.1.6 Isospora in camel calves

There are only few reported cases of *Isospora sp.* infections in camels. Two species of *Isospora* (*I. orlovi* and *I. cameli*) have been found in dromedary camels according to Kaufmann (1996). Apart from Kaufmann (1996) there are no other references to *Isospora cameli*.

Tsygankov (1950) was the first to describe *I. orlovi* in faeces of 10 out of 19 sampled 10 to 35 day old camels. However, Pellérdy (1974) questioned that *I. orlovi* was a true parasite of the camels. He hypothesised that the detected oocysts found by Tsygankov were *Isospora sp.* from wild birds. This hypothesis is supported by Daruish and Golemansky (1993), who found *Isospora sp.* in three out of 112 samples (no age indicated) that rather resembled *I. lacazei* (found in the English sparrow) than *I. orlovi*.

Raisinghani et al. (1987) describe *Isospora sp*. found in the faeces of a six month old camel calf in India showing signs of diarrhoea and abdominal pain. Faeces contained a lot of mucus, but no blood. The detected sporulated oocysts (29.5 x 18.4µm in size) were described oval to sub-spherical with two sporocysts (13.8 x 9.6µm in size) containing four sporozoites (6-8 x 4-5 µm in size) each. The oocysts had no micropyle, no polar granule and no oocystic residium. They resembled the measurements reported for *I. orlovi*. The authors stressed on the importance of clinical coccidiosis caused by *Isospora sp.* in Indian camels.

In a limited field study in Northern Kenya (Younan et al., 2002) evidence has been found, that *Isospora sp*. can cause diarrhoea and death in camel calves. Five camel calves aged between 18 and 32 days were found excreting *Isospora sp.* in three different herds. Four of them were diarrhoeic for about ten days. One of the calves died and on gross pathology coccidiosis-like lesions were found in the colon. The authors concluded that *Isospora sp.* is a causative agent for diarrhoea in camel calves in Kenya.

Kinne et al. (2002) found coccidia oocysts resembling *I. orlovi* in eight camel calves aged between four to eight weeks in Dubai, UAE. The calves were suffering from severe diphteroid colitis. In their study small coccidian stages were found in the lamina propria of the colon. According to histological examination, sections of the mucosa were destroyed, disorganised and showed haemorrhages. The mucosa was infiltrated with eosinophilic granulocytes and macrophages. Furthermore oocysts were found in colon smears and faeces. Most of the oocysts were sporulated, ellipsoidal, 30-33 x 18-21µm in size with a two layered wall. The oocysts contained two sporocysts (10-15 x 17-19µm in size) including four sporozoites (elongated, ellipsoidal, 4-6 x 11-13µm in size). As in the findings of Raisinghani et al. (1987), no micopyles, residiums or polar granules could be detected. The authors clearly indicate that due to the multiplication of *Isospora sp.* in the colon mucosa leading to diphteroid colitis, *Isospora sp*. can be considered a true camel pathogen.

Morrison et al. (2004) confirmed the suggestions of the previous authors by sequence analysis of the small subunit rRNA gene (SSU rRNA) and the internal transcribed spacer (ITS1) that the previous described oocysts isolated by Kinne et al. (2002) belong to *Isospora* species. Furthermore, they found out that the sequenced *Isospora* formed part of a clade with the other mammal host *Isospora* species making it a true camel parasite. The lack of stieda bodies in the sporocysts of *I. orlovi* is a typical feature of a mammal host species, not a bird host species. Based on the ssu rRNA data, the phylogenectic placement of *I. orlovi* showed close genetic relationship to *I. belli* as does *I. suis. Isospora suis* and *I. orlovi,* on the other hand, were less closely related to each other (Bornstein et al., 2006).

2.2 BACTERIOLOGY

2.2.1 Salmonella

2.2.1.1 The genus Salmonella

The genus *Salmonella* belongs to the family of *Enterobacteriaceae* and consists of a single species *Salmonella (S.) enterica* which comprises seven subspecies (*enterica, salamae, arizonae, diarizonae, houtenae, bongori, indica*). All serovars found in warm blooded animals are contained by *S. enterica subsp. enterica* (Coetzer et al., 1994, Bisping and Amtsberg, 1988). Serovars are identified mainly on the basis of specific combinations of somatic and flagellar antigens and to a lesser extend by their biochemical reactions (Coetzer et al., 1994).

Salmonella are gram-negative rods which are motile due to peritrichous flagella except for *S. gallinarumpullorum* (Burckhardt, 1992).

2.2.1.2 Salmonella in bovines

Septicaemia, acute or chronic enteritis or abortion are the usual syndromes associated with salmonellosis in bovines, especially affecting calves below three months of age or animals exposed to stressful situations (Venter et al., 1994). *Salmonella dublin*, host-

specific, and *S. typhimurium*, not host-specific, are quoted to be the most common servovars in bovines (Venter et al., 1994), both affecting calves severely between six and twelve weeks of age and in the first three weeks of age, respectively (Bisping and Amtsberg, 1988, Venter et al., 1994). Due to management practices the disease is more commonly found in dairy than in beef cattle (Venter et al., 1994).

Source for infection are mainly latent carriers or contaminated environment, where *Salmonella* can persist for a long period of time. The infective dose, predisposing factors and the immunity status of the hosts determine the outcome of the infection (Venter et al., 1994).

Affected calves suffer from peracute, acute or chronic salmonellosis. The peracute form is often fatal with signs of diarrhoea and septicaemia. The acute form goes along with fever, inappetence, diarrhoea and polypnoea. In chronic cases of salmonellosis, calves are unthrifty, have long and scruffy hair and are stunted (Venter et al., 1994). Survivors might develop pneumonia, meningoencephalitis, purulent polyarthritis and osteomyelitis.

Isolation of the causative agent, supported by the case history, the clinical signs and lesions seen during post mortem make the diagnosis. The use of antimicrobial drugs like ampicillin, amoxicillin, gentamycin, timethoprim-sulphonamide and fluoroquinolones is controversial but might help when administered at an early stage of the disease (Venter et al., 1994). Of utmost importance is supportive therapy like oral rehydration and good nursing.

2.2.1.3 Salmonella in equine

Salmonellosis in horses is affecting all age groups but foals and stressed or aged horses are specifically susceptible (Wilkens, 1994). *Salmonella typhimurium* and *S. typhimurium* var. *copenhagen* are the most pathogenic, however, other serovars like *S. enteritidis, S. anatum, S. newport, S. heidelberg, S. cottbus* and *S. saint-paul* have been isolated (Wilkens, 1994).

Infection occurs via the oral route through contaminated water or feed with asymptomatic carriers being the source for the *Salmonella* contamination as they are active shedders (Wilkens, 1994).

Infection with *Salmonella* in foals leads to acute colitis, profuse diarrhoea and septicaemia with or without diarrhoea. The peracute form with septicaemia is often fatal and affects foals at one to six months of age. Symptoms shown by the affected animal are fever, anorexia, depression, weakness and they die within 24-72 hours. The acute form can go over a period of three weeks with anorexia, severe enteritis and dehydration but foals usually recover. In chronic cases which can last for several month the animals have loose faeces, intermittent fever and weight loss (Wilkens, 1994).

Isolation of *Salmonella* as a pathogen confirms the diagnosis. Again treatment with antimicrobial drugs is controversial but trimethoprim alone or in combination with sulphadiazine seems to have some success. The treatment with antimicrobial drugs is most effective when administered prior to the development of diarrhoea. Symptomatic and supportive therapy in addition remains important, too (Wilkens, 1994).

2.2.1.4 Salmonella in camels

Infection with *Salmonella* sp. in camels has been described by various authors since 1912. Wernery and Kaaden (2002) give a comprehensive overview on the existing literature of *Salmonella* sp. in camels: The pathogen can cause enteritis, septicaemia and abortion. Acute enteritis is common both in camel calves and adults with diarrhoea, which may develop into severe haemorrhagic enteritis, while the chronic form is more prevalent in adult camels with symptoms of persistent diarrhoea, fever, emaciation and poor response to treatment. Septicaemia is a syndrome seen in newborn calves up to six months of age. Calves develop fever and depression and die within 48 hours.

For treatment, supportive therapy is most important. Treatment with antibiotics is controversial, however parenteral use of antimicrobials (e.g. ampicillin, amoxicillin, and timethoprim-sulfonamide) is recommended by the authors. Furthermore, where possible autogenous *Salmonella* vaccines, administered twice before parturition, might provide protection against salmonellosis in newborns (Wernery and Kaaden, 2002).

The camel has been reported to be an important reservoir for *Salmonella* sp. and possible source of infection and therefore a health hazard for humans (Malik et al., 1967, Cheyne et al., 1977, Kwaga, 1985, Molla et al., 2004, Abbas and Omer, 2005). Wernery and Makarem (1996) however found it unlikely that camel products are an important reservoir for salmonellosis in humans in UAE. They conducted a study comparing *Salmonella* serovars isolated both from humans and camels and found out that *Salmonella typhimurium* and *S. enteritidis* were the main serovars isolated from humans suffering from salmonellosis, two serovars that were rarely (*S. typhimurium*) or not at all (*S. enteritidis*) isolated from camels in UAE.

Abbas and Omer (2005) stress on the importance of the role of *Salmonella* sp. in the complex of camel calf diarrhoea. In the Moroccan Sahara calves with diarrhoea (n=44) in the age between one and ten weeks were examined and four *Salmonella* serotypes (*S. tennessee, S. tananarive, S. tallahassee, S. enteritidis*) were identified from 13.6% of the examined calves (Berrada et al., 2000). Bada Alambedjir et al. (1992) analysed 42 camel calves (22 with diarrhoea, 20 healthy), age 0 to one year, in Niger and found five calves positive for five *Salmonella* sp.. Four different *Salmonella* serotypes (*S. brandenburg, S. johannesburg, S. nottingham, S. havana*) were identified. The authors explain that the found serotypes are rare and usually not associated with salmonellosis in animals. However, as they were all isolated from diarrhoeic camel calves the authors suggest that the bacteria intervene in the aetiology of camel calf diarrhoea. Salih et al. (1997) isolated *Salmonella* sp. (13%) from 106 diarrhoeic camel calves in Sudan with *S. typhi* being the most prominent serotype. Moore et al. (2002) examined 67 camel calves of racing camels in the age group between 11 and 45 days for intestinal pathogens. *Salmonella hindmarsh* was isolated as the most common serovar (n=9). However, only two of the camel calves with *Salmonella* sp. showed signs of diarrhoea. The authors conclud that *Salmonella* sp. might not be the major cause of diarrhoea in camel calves. As they were found in asymptomatic animals, the camel is a sub-clinical carrier and excretes the organism.

2.2.2 Escherichia coli

2.2.2.1 The genus Escherichia

Bacteria of the genus *Escherichia* belong to the family of *Enterobacteriacae* and are mostly motile gram negative rods.

The organism is known to colonize the infant intestines within the first hours of life and both host and *E. coli* derive mutual benefit (Nataro and Kaper, 1998). It is referred to as a mucosal pathogen and follows a strategy of infection, starting with the colonization of the mucosal site, followed by the evasion of the host defences and multiplication leading to host damage (Nataro and Kaper, 1998, Kaper et al., 2004). Colonization is done with the help of surface adherence fimbriae known to all *E.coli* strains, whereby the pathogenic ones possess specific fimbrial antigens leading to enhanced intestinal colonization. Except for EIEC (entero invasive *E. coli*), all *E. coli* strains are extra-cellular (Kaper et al. 2004). After colonization the pathogenic strategies of diarrheagenic *E. coli* strains vary and they are highly adapted and have acquired specific virulence attributes. The most successful combinations of specific virulence factors have persisted to become specific „pathotypes“ that can cause disease in healthy individuals. There are three clinical syndromes caused by these pathotypes, namely enteritis/diarrhoeal disease, urinary tract infections (UTI) and sepsis/meningitis. Among those pathotypes, there are six well known categories: enteropathogenic *E. coli* (EPEC), enterohemorrhagic *E. coli* (EHEC), entertoxigenic *E. coli* (ETEC), enteroaggregative *E. coli* (EAEC), enteroinvasive *E. coli* (EIEC) and diffusely adherent *E. coli* (DAEC). Furthermore uropathogenic *E. coli* (UAEC) are known to cause UTIs in humans, while meningitis-associated *E. coli* (MNEC) cause meningitis and sepsis. Avian pathogenic *E. coli* (APEC) cause extraintestinal infections in poultry (Kaper et al., 2004).

In the following review only *E. coli* strains causing enteric disease are reviewed.

Enterotoxigenic *E. coli* (ETEC)

ETEC strains produce either heat stabile (ST) and/or heat labile (LT) enterotoxins. After the colonization of the mucosa, these enterotoxins (either alone or together) cause diarrhoea through a rise in the net secretory rate in the intestines.

The heat labile enterotoxin (LT), a big oligomeric toxin, has two major serogroups of LT-I and LT-II, the former being known to be pathogenic to both animals and humans while the latter is mainly found in animals but not associated to disease.

The heat stabile enterotoxin (ST) is a small monomeric toxin and two classes can be differentiated: STa and STb. STa toxin is produced by ETEC and other bacteria such as *Yersinia entercolitica* and *Vibrio cholerae*. STa can be found in human *E. coli* strains while STb is mainly associated with ETEC isolated from pigs.

Enteropathogenic *E.coli* (EPEC)

Enteropathogenic *E. coli* cause characteristic histopathological lesions due to its attaching-and-effacing (A/E) factors, leading to the effacement of the microvillis and an intimate attachment between the bacterium and the epithelial cell membrane (Kaper et al., 2004). Donnenberg and Kaper (1992) proposed a three stage model of EPEC

pathogenesis: a) localized adherence, b) signal transduction and c) intimate adherence. Multiple mechanisms finally lead to diarrhoea: EPEC stimulates an active chloride secretion which might lead to the rapid onset of diarrhoea. The destruction of the microvilli could account for the prolonged diarrhoea due to malabsorption. Furthermore local inflammatory response and increased intestinal permeability might contribute to the diarrhoea as well (Nataro and Kaper, 1998).

Enterohemorrhagic *E. coli* (EHEC)

All EHEC strains are considered to be pathogens, having either „typical EHEC" that denote STEC strains such as O157:H7 that produce shiga-like Toxin (Stx), cause A/E lesions and possess the 60-MDa plasmid or „atypical EHEC" that denote STEC strains which do not produce A/E lesions and/or do not possess the 60-MDa plasmid.

The major virulence factor of EHEC is the production of Shiga-like toxin (Stx), a potent cytotoxin that can lead to death (Nataro and Kaper, 1998). Two major toxin groups can be differentiated: Stx1 and Stx2. Shiga-like toxin play an important role in intestinal diseases such as diarrhoea and enterocolitis. Diarrhoea might be the result of the death of the absorptive cells due to the toxin that leads to an imbalance of intestinal absorption and secretion. A/E lesion may lead to non-bloody diarrhoea whereas Stx is essential for the development of hemorrhagic colitis and bloody diarrhoea. EHEC is known to cause Hemorrhagic Uremic Syndrome (HUS) and Hemorrhagic Colitis (HC) (see below).

Enteroaggregative *E. coli* (EAEC)

Kaper et al. (2004) describe EAEC as *E. coli* that do not secrete LT or ST and that adhere to Hep-2 cells in a pattern known as auto-aggregative. There are a few EAEC known to be human pathogens. They colonize the intestinal mucosa mainly of the colon forming a thick layer of auto-aggregated bacteria on the surface of the mucosal membrane. This is followed by the secretion of enterotoxins and cytotoxins. The result is mild diarrhoea but with significant mucosal damage. In the developing and developed world EAEA plays more and more an important role as cause of often persistent diarrhoea in children and adults (Kaper et al., 2004).

Enteroinvasive *E. coli* (EIEC)

EIEC is closely related to *Shigella* sp. but have – unlike the other *E. coli* strains that remain extra-cellular – the capability to invade and disseminate within the epithelial cells. It penetrates the epithelial cell, followed by the lysis of the endocytic vacuole. Multiplication takes place inside the epithelial cell and the bacteria moves through the cytoplasm (directional movement) and invades adjacent epithelial cells. This results in invasive inflammatory colitis with occasional dysentery leading mainly to watery diarrhoea (Kaper et al., 2004).

Diffusely adherent *E. coli* (DAEC)

DAEC are known to cause diarrhoea especially in children older than 12 months. Their characteristic is the diffuse pattern of adherence to Hep-2 cells inducing a cytopathic effect characterised by the development of long cellular extensions, which wrap around the bacteria.

2.2.2.2 E. coli in humans

Weanling diarrhoea among children in the developing world and traveller's diarrhoea are caused by ETEC. Three factors are known to be leading to disease: one being the mucosal immunity to ETEC that develops in exposed individuals. Another factor is asymptomatic individuals that shed a large number of virulent ETEC organisms and the last factor represents the high infectious dose that is required to cause disease (Nataro and Kaper, 1998). Contaminated food and water are the most common vehicles for ETEC infections. In an endemic area infants encounter ETEC mainly upon weaning and infection tends to occur during wet and warm months. Traveller's disease is caused by ETEC infection of adults from the developed world visiting endemic areas (20 to 60% of such travellers experience diarrhoea and 20-40% are due to ETEC). Again the source of infection is mainly contaminated food and water and occurs mainly during the warm and wet months of the year (Nataro and Kaper, 1998). Both infants and adults infected by ETEC develop after short incubation period mainly mild watery diarrhoea which is self-limiting. In severe cases the symptoms are similar to *Vibrio cholerae* infections. Antibiotics such as fluoroquinolones are recommended for treatment; however the cornerstone of the therapy is oral rehydration to maintain a normal hydration status, especially in children (Nataro and Kaper, 1998; Kaper et al., 2004).

Enteropathogenic E. coli (EPEC) mainly cause diarrhoea in infants up to six months of age. Especially in the developing world EPEC is a major pathogen contributing to 30-40% of diarrhoea in infants (Nataro and Kaper, 1998, Kaper et al., 2004). The infectious route is faecal-oral and the reservoir of EPEC infection are symptomatic or asymptomatic children and asymptomatic adults. EPEC causes acute or protracted diarrhoea, often severe with a mortality rate of 30% in children of the developing countries. The symptoms combine watery faeces with vomiting and low grade fever. Again the main pillar of the treatment is to prevent dehydration. In addition antibiotic therapy has been reported to be successful, however, multiple antibiotic resistance is common (Nataro and Kaper, 1998).

Hemorrhagic Uremic Syndrome (HUS) and/or Hemorrhagic Colitis (HC) are known to be the most common syndromes of EHEC infections in humans. Nataro and Kaper (1998) describe the frequencies of the various syndromes, especially related to infection with *E. coli* O157:H7 with ca. 10% non-bloody diarrhoea, ca. 90% HC and ca. 10% HUS in children younger than 10 years. Symptoms related to HC are diarrhoea (first non-bloody progressing into bloody diarrhoea), abdominal pain and short-lived fever. In most infected persons the diarrhoea resolves on its own, only children younger than 10 years can develop HUS with mortality up to 3 – 5%. HUS classically goes along with haemolytic anaemia, thrombocytopenia, and renal failure. The intestinal tract of cattle and other animals provide a reservoir of EHEC; the transmission is done through a variety of food items, beef being the major vehicle. Furthermore person to person transmission, water and even potential airborne transmission has been reported. Low infectious dose are sufficient for high rates of infection (Kaper et al., 2004). Supportive care is the main treatment to consider, while the use of antibiotic therapy is rather controversial.

2.2.2.3 E. coli in camels

Very little is known about the pathotypes and pathogenic strains of *E. coli* in camels. Published papers refer to isolation of different *E. coli* from faecal samples of camels with or without diarrhoea.

Chauhan and Kaushik (1991) describe the isolation of *E. coli* serogroup O2, O8, O83, O103 and O120 from seven camels (five adults and 2 calves aged one month) with diarrhoea. Out of the identified serogroup O2, O8 and O83 were found to be enterotoxigenic. Schwartz and Dioli (1992) suspect *E. coli* as a major cause of neonatal diarrhoea in East Africa with a morbidity of 30% and a mortality of up to 100% if treatment is not appropriate and timely. According to the authors, poor management and inadequate colostrum intake facilitate the disease. Calves show signs of diarrhoea with abdominal pain, fever and might become debilitated, depressed and anorectic. As treatment therapy long acting antibiotics, oral re-hydration and good sanitation have been recommended.

The description of colibacillosis by Manefield and Tinson (1996) is similar to that of other authors. They observed a morbidity of 10% in around 700 calves in a calving season on larger farms in the United Arab Emirates. In their opinion mostly calves of three weeks of age are affected, as it is around that time that calves become interested to lick, taste and eat objects of the surrounding. Infected calves are weak and feverish, with watery yellowish diarrhoea and they die within two to three days. Re-hydration therapy combined with administration of antibiotics (e.g. Enrofloxacin) and antipyretics are recommended for treatment.

Dia et al. (2000) found *E. coli* with 60.8% as a major pathogen in diarrhoeic camel calves in the age between one and three months in Mauritania. Bala Alambedjir et al. (1992) isolated *E.coli* from diarrhoeic camel calves in Niger, however the authors stress on the importance of the identification of enterotoxins, which can give an indication on the pathogenicity of *E. coli*. Berrada et al. (2000) isolated *E. coli* $K99^+$ from 11.4% of 44 camel calves suffering from diarrhoea.

Wernery and Kaaden (2002) stress on the economic impact colibacillosis and colisepticaemia have on the camel industry with losses reaching up to 40%. In the United Arab Emirates (UAE) *E. coli* infections occur regularly and mainly in two to four weeks old camel calves. Affected animals develop fever, yellowish watery diarrhoea resulting in dehydration and death within two to three days. At necropsy inflammation of the intestinal mucosa, varying amounts of sand and extreme pallor of the whole corps have been observed. The treatment regime mentioned by the authors resembles the one already described however they recommend maternal vaccination with herd-specific *E. coli* strains annually or oral vaccination in young camelids. Bornstein et al. (2000) describe a case study of coli septicaemia in a one week old camel calf in Kenya. The calf was unthrifty for two to three days and died without any signs of diarrhoea. *E. coli* were isolated from the body lymph nodes, tonsils, spleen, lungs, bone marrow, heart blood and pericardial fluid.

Mohamed et al. (1998) found 40 % of 42 examined 1-3 month old calves infected with *E. coli* in Sudan. They identified EIEC, EPEC and VT2 pathotypes.

In a study conducted by Moore et al. (2002) no *E. coli* O157:H7 was isolated out of 67 faecal samples from camel calves of racing camels in the UAE.

2.2.3 Klebsiella

2.2.3.1 The genus Klebsiella

Bacteria of the genus *Klebsiella* belong to the family *Enterobacteriaceae* and are named after the German pathologist Edwin Klebs. They are opportunistic pathogens found in the intestines of humans and animals and in the environment, where they can multiply in the water or on the surface of leaves. The forming of a capsule is characteristic (Bockemuehl, 1992, Selbitz, 2002).

In culture they appear as mucoid colonies. *Klebsiella* are coccoid, non-motile, gram-negative rods, which form a capsule (Henton, 1994).

The classification of capsular types (done with the quelling reaction according to Neufeld) gives a much clearer indication of pathogenicity than biochemical typing (Henton, 1994).

Currently 82 defined polysaccharide-capsule-antigens can be differentiated, which can be expressed by any of the capsule producing *Klebsiella* species (Bockemuehl, 1992). In respiratory tract infections capsule type 1 to 6 were found while in Pneumonia cases type 1 is more prominent than type 2 and type 3. Infections of the urinary tract are associated with type 2 and above type 6.

Capsular type 1 and 2 followed by the less frequent type 3 and 4 are the most pathogenic capsular types for humans and animals (Podschun and Ullmann, 1998, Euzéby, 2004). Apart from the capsule, also endo- and enterotoxins and adhesion antigens are virulence factors. However, the virulence is rather low, as *Klebsiella* is a typical pathogen of multifactorial diseases (Selbitz, 2002).

According to the classification of Ørskov (1984) there are five major Klebsiella species:

Klebsiella pneumoniae
  subsp. pneumoniae
  subsp. ozaenae
  subsp. rhinoscleromatis
Klebsiella oxytoca
Klebsiella terrigena
Klebsiella planticola (syn. K. trevisanii)
Klebsiella ornithinolytica

K. pneumoniae subsp. pneumoniae is present in the intestines and the respiratory tract of humans and animals and is ubiquitous in the environment. Bacteria of this species cause respiratory diseases, nosocomial infections of the urinary tract, wounds and sepsis (Bockemuehl, 1992, Podschun and Ullmann, 1998). Furthermore *K. pneumoniae ssp. pneumoniae* has been described as a causative agent of mastitis in cows, cervicitis and metritis in mares and pneumonia in dogs (Carter and Chengappa, 1990). It has also been

associated with respiratory tract infection in horses in Poland (Boguta et al., 2002).

Klebsiella pneumoniae subsp. rhinoscleromatis contributes to rhinoscleroma in humans (Bockemuehl, 1992).

Klebsiella pneumoniae ssp. ozaenae can cause nasal infections in human producing glutinous, fetid mucus.

In animals and human *Klebsiella oxytoca* causes similar diseases like *K. pneumoniae* (Bockemuehl, 1992). It can be recovered from the intestinal tract of healthy animals, mastitic bovine milk samples and from the environment (Carter and Chengappa, 1990).

K. terrigena has been found only in the environment so far. The clinical importance of *K. terrigena* is not known (Bockemuehl, 1992).

K. planticola has been found mainly on plants but isolations from human infections are becoming more frequent. So far the possible pathogenicity factors are unknown; however, *K. planticola* is considered to be of clinical significance (Podschun and Ullmann, 1998).

K. ornithinolytica has been described in humans and animals as a biovar of *K. planticola* (Stock and Wiedemann, 2001).

2.2.3.2 Klebsiella in humans

In humans, *Klebsiella* is a saprophyte in the nasopharynx and in the intestinal tract. It is said to be among the eight most important infectious pathogens in hospitals in the United States and is of similar importance in the United Kingdom and in Germany. It is a major cause of nosocomial infections with medical equipment, blood products, the gastro-intestinal tract of patients and the hands of hospital personnel being the principal reservoirs. The most common site for nosocomial infections is the urinary tract and *Klebsiella* accounts for 6 to 17 % of these infections, which cause significant renal scarring and may lead to death (Podschun and Ullmann, 1998).

In paediatric wards *Klebsiella* infections are involved in neonatal sepsis and meningitis. In Bangladesh and neighbouring countries *K. pneumoniae* has been quoted to be the leading pathogen causing neonatal sepsis (Afroza, 2006). Multi-drug resistant strains (mainly serotype K55) cause severe problems especially in premature infants and in those kept in the intensive care units and are becoming a major concern for paediatricians (Podschun and Ullmann, 1998). Furthermore, the isolation of *Klebsiella planticola* from infants has been found with surprisingly high frequency. Out of 131 infants sampled for *Klebsiella*, 8.7% were positive for *K. planticola* confirming its high frequency in human specimens (Podschun et al., 1998).

2.2.3.3 Klebsiella in horses

Klebsiella pneumoniae ssp. pneumoniae causes mainly genital infections in horses which lead to economic losses. Mares are commonly infected venereally (mainly capsular types 1, 2 and 5), (Henton, 1994). Immunocompromised uteri (with any malfunction, either structural or hormonal) are easily infected by capsular types 6, 7, 20, 21 and 30. The transmission of theses types is usually mechanically by stallions (Henton, 1994). The infected mare develops endometritis characterized by vaginal discharge (greyish, viscid with possible floccules of pus) within 3 to 5 days of being served by a carrier stallion.

The mucus membranes are inflamed and in advanced cases, pyometra may result. Systemic symptoms are usually not present (Henton, 1994).

If a foetus becomes infected, it may lead to abortion or a septicaemic, weak foal at the time of birth or soon afterwards. Septicaemic foals show signs of anorexia, depression, high fever. They usually develop severe polyarthritis, pneumonia and/or meningo-encephalitis. They die within a few days if left untreated (Henton, 1994). *Klebsiella pneumoniae* was found in 23 % septicaemic foals (n=47) up to eight days old (Wilson and Madigan, 1989). Main source of the infection is the latent infected mare. Prophylactic control of the mare and strict hygienic measures can prevent the infection of the foal. Antibiotics such as Tetracycline, Chloramphenicol, Polymycin B and Sulmycin can be used for treatment (Dietz and Wiesner, 1982).

2.2.3.4 Klebsiella in pigs

It has been reported that infection of neonatal piglets with *K. pneumoniae* leads to severe diarrhoea 12 hours post inoculation. The piglets became dehydrated and weak but continued to drink. The bacteria could be isolated from the intestine and faeces but not from the liver or spleen. No morphological changes could be found in the intestinal mucosa (Wilcock, 1979).

In sows, *K. pneumoniae ssp. pneumoniae* is known to contribute to the Mammitis-Metritis-Agalactiae-Syndrome (MMA) and to diarrhoea and respiratory diseases (Rolle and Mayr, 2002).

2.2.3.5 Klebsiella in cattle

In cattle *Klebsiella pneumoniae sp. pneumoniae* has been associated with Mastitis and as a cause of calf diarrhoea (Rolle and Mayr, 2002).

2.2.3.6 Klebsiella in camels

Very little is known about the involvement of *Klebsiella* spp. in infections of camels in general and of camel calves in particular.

Klebsiella pneumoniae (capsular type 11) has been isolated together with Diplococci from two dead adult camels, which suffered respiratory distress, pyrexia and prolonged cough in Haryana, India. The disease called ‘khurak’ had a low mortality but high morbidity (20 to 30%) rate and affected mainly adult camels. Histological examination showed microbial broncho-pneumonia, characterized by acute capillary hyperaemia and the presence of serofibrinous exudates in the alveoli. The authors suggest that *K. pneumonia* and/or the isolated Diplococci were involved in the development of the disease in camels (Arora and Kalra, 1973).

Klebsiella pneumoniae has been isolated from diarrhoeic calves in eight out of 29 cases in Mauritania (Dia et al., 2000).

Klebsiella ozaenae, among others, has been found in camel calves in their first year of age in Niger. The authors suggest that the involvement of *Klebsiella* in diarrhoea is linked to its ability to inhibit the absorption of fluids in the jejunum (Bada Alambedjir et al., 1992).

3 MATERIAL AND METHODS

3.1 STUDY HERDS

The field work was carried out in eight districts of North Eastern, Eastern and Rift Valley Provinces of Kenya.

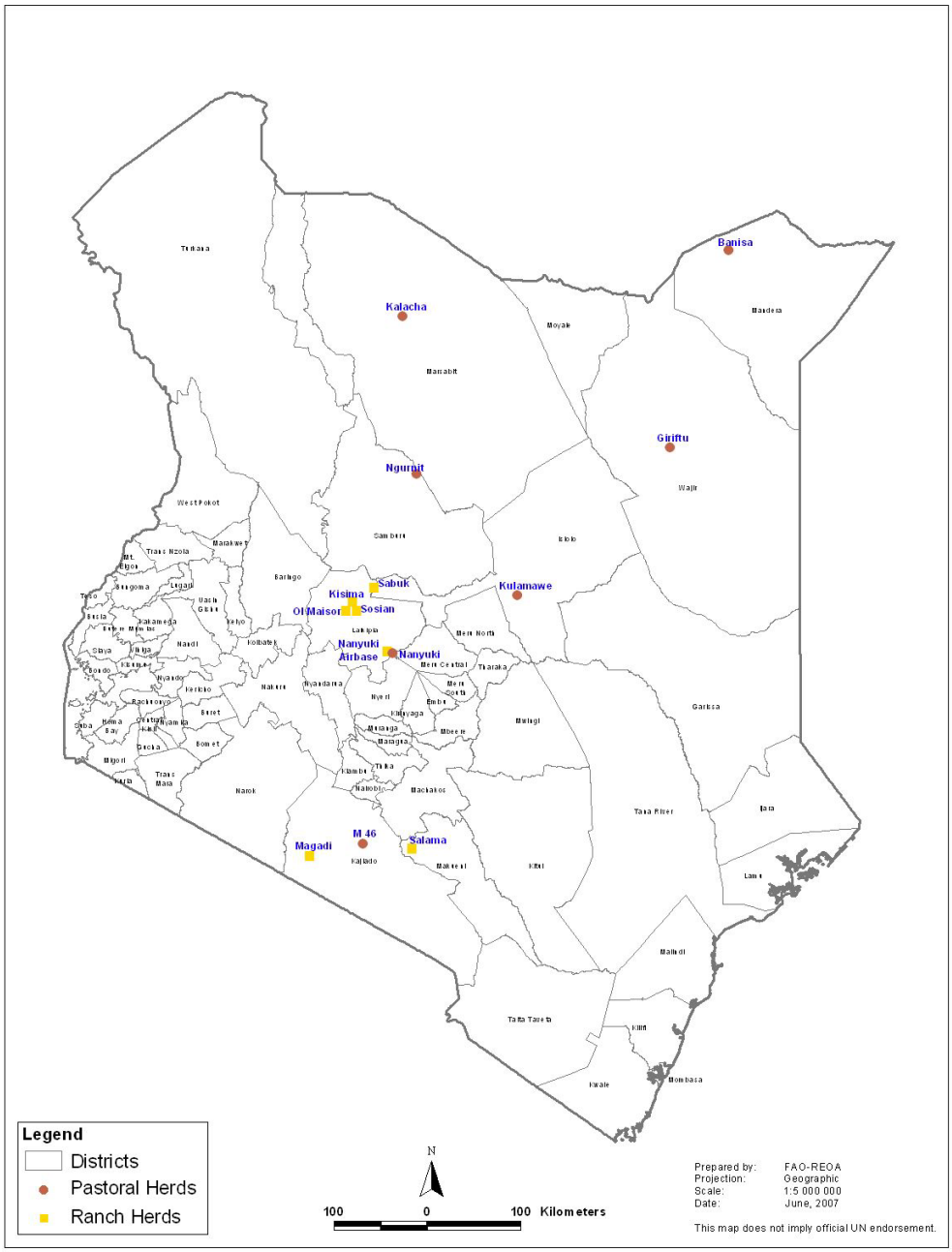

Figure 2: Map of Kenya indicating locations visited during the study period 2002-2004

Table 2: Ranch and pastoralists study herds visited in 2002

Ranch herds (Mgmt R)			Pastoralists Herds (Mgmt P)		
Location	**District**	**Province**	**Location**	**District**	**Province**
Ol Maisor, Kisima, Sosian, Nanyuki Airbase, Sabuk	Laikipia	Rift Valley	M 46	Kajiado	Rift Valley
Magadi	Kajiado	Rift Valley	Nanyuki surroundings	Laikipia	Rift Valley
			Kulaley, Giriftu, Dela	Wajir	North Eastern
			Ngurnit, Korr	Marsabit	Eastern
			Bore, Nana	Moyale	Eastern

Table 3: Ranch and pastoralists study herds visited in 2003

Ranch herds (Mgmt R)			Pastoralists Herds (Mgmt P)		
Location	**District**	**Province**	**Location**	**District**	**Province**
Ol Maisor, Kisima, Sosian, Nanyuki Airbase and Mugambe Ranch	Laikipia	Rift Valley	Kulamawe	Isiolo	Eastern
Magadi	Kajiado	Rift Valley	Kalacha	Marsabit	Eastern
Salama	Machakos	Eastern			

Table 4: Ranch and pastoralists study herds visited in 2004

Ranch herds (Mgmt R)			Pastoralists Herds (Mgmt P)		
Location	**District**	**Province**	**Location**	**District**	**Province**
			Banisa, Eymole, Gither	Mandera	North Eastern
			Kalacha	Marsabit	Eastern

The field visits carried out in the period between January 2002 and February 2004 are reflected in the tables above.

The herds could be allocated to two management systems:

Ranch Herds (Mgmt R) were represented by stationary herds. The animals were herded in a restricted area and were rotated depending on pasture availability.

According to Herlocker (1999) these ranches and herds are all located in the same agro-ecological zone, called the Themeda Mid-Grass Region. This region is in a sub-humid to semi-arid climate, where the rainfall varies between below 500 to 1500 mm a year. The altitude lies between 1170 and 2330 m.

Pastoralists herds (Mgmt P) stands for camels kept by pastoralists in Northern Kenya. The herds were mobile and followed pasture and water availability. In the study locations different tribal groups were present: the Somali, Rendille, Gabra and Samburu.

For the **point prevalence study** camel calves between birth and 12 weeks of age were sampled belonging either to Ranch Herds (Mgmt R) or Pastoralists Herds (Mgmt P). Each calf in the investigated age group was sampled in herds belonging to Mgmt R while in Mgmt P, due to logistic issues and the reluctance of the herd owner, not every camel calf in the pastoralist herds could be sampled. Mainly calves showing signs of diarrhoea were presented by the owner and could be included in the sampling procedure. Where possible, one healthy cohort was sampled for each diseased calf in Mgmt P.

For the **longitudinal study** only calves kept under Mgmt R aged from birth till three months of age were included. Regular (interval between one and three weeks) visits were made to herds of Ol Maisor and Kisima Ranch. Less regular visits were made to herds in and around Nanyuki, in Magadi, in Salama and of Sosian Ranch. All calves belonging to the age group between birth and three months of age were sampled at each visit.

3.2 DATA COLLECTION AND ANIMALS SAMPLED

During every field visit, the following data were collected either through interviews with the owner or with the herdsman:

3.2.1.1 *General data (herd)*

Name of owner

Location of the herd

Management system

Herd-size

Camel breeds kept

3.2.1.2 *Specific data (calf)*

Date of birth/age

Name/number (if not available, name/number of the dam)

Sex

Health status at the time of sampling

1. *Healthy* → Animals showing no signs of diarrhoea
2. *Diseased* → Animals exhibiting diarrhoea
3. *Convalescent* → Animals that had suffered from diarrhoea previously but have recovered
4. *Dead*

The data were noted down and later entered into an ACCESS data base and in SPSS (VERSION 12.0 and 13.0) for statistical analysis.

3.2.1.3 General condition of the calf:

Before the samples were taken, every calf was examined clinically and its general physical condition was assessed. Special emphasis was put on

- ✓ mucous membranes (conjunctiva, gum)
- ✓ skin turgor to check on the hydration status
- ✓ the adspection of the perianal area for any signs of diarrhoea
- ✓ the adspection and palpation of the umbilicus to confirm the age and check for infections

3.3 PARASITOLOGY

3.3.1 Solutions

Flotation Solution (Saturated Saline/Salt Solution)

NaCl	340	g/l tap water

Specific gravity 1.18 – 1.2

Normal Saline (0.9%)

NaCl	0.9	g/l distilled water

Potassium Bichromate Solution (2.5 %)

$K_2Cr_2O_7$	2.5	g/l distilled water

3.3.2 Equipment

Beaker

Spoon

Sieve (200µm)

Glassware

Microscope

Slides

Cover slip (size)

3.3.3 Sampling methods for Parasitology

A faecal sample was taken directly from the rectal ampulla of camel calves using a clean plastic bag. The sample was then stored in a portable fridge at 4ºC until it reached the laboratory.

3.3.4 Laboratory Methods

3.3.4.1 Native Smear

In case of very little sample material available for the parasitological analysis, a loop full (Copan Disposable loops) was used to transfer material onto a slide and mixed with an equal amount of normal saline. Covered with a cover slip, it was then examined under the microscope (magnification 10 x 10 and 10 x 40).

3.3.4.2 Flotation (according to Fülleborn)

In order to detect parasitic stages in the faeces, the flotation method according to Fülleborn was used.

A teaspoon full of faecal material was mixed with the flotation solution, and poured into a glass beaker through a sieve. The beakers were filled to the top with the flotation solution and kept standing for 30 minutes. Three loops taken from the surface were transferred onto a slide and examined under a cover slip under the microscope at magnification 10 x 10 and 10 x 40. Protozoa oocysts and/or helminth eggs found were described and measured.

Measurements were taken with a calibrated measuring slide (µm).

Oocysts were identified according to size and morphology:

Isospora sp. oocyst with two sporocysts containing four sporozoites each.

Eimeria sp. oocyst with four sporocysts and two sporozoites each.

Helminth ova morphology:

Strongyloides sp. ova containing a larva

Any other *Strongyle* ova

3.3.4.3 Sporulation Culture

Faecal material containing *Isospora sp.* oocysts was mixed with 2.5 % Potassium Bichromate solution (1:1) in a covered petri dish and kept in the dark at room temperature. The mixture was aerated once a day for one week, than stored in 4 ml cryo vials at 4ºC in the fridge and finally send to the National Veterinary Institute, Department of Parasitology in Uppsala for sequence analysis of the small subunit rRNA gene (SSU rRNA) and the internal transcribed spacer (ITS1) according to Morrison et al. (2004).

3.4 BACTERIOLOGY

3.4.1 Sampling methods for Bacteriology

A faecal sample was taken by swabbing the rectal ampulla of the camel calf with a sterile cotton swab. The swab was stored in a screw-cap Bijoux bottle with Stuart Transport

Medium and kept at 4ºC in a portable fridge until it reached the laboratory.

In addition, the faecal sample collected as described under 3.1.3. was used for enrichment culture.

3.4.2 Isolation and Differentiation Procedure

The culture media used are listed in Annex 9.1.

3.4.2.1 Salmonella sp.

A loop full of faeces was suspended in Rappaport Vassiliadis Enrichment Broth and incubated for 18 h in a 42ºC water-bath. One loop was than streaked onto two selective media (XLD and BGA Agar) and incubated for 18h at 37ºC and examined for:

Lactose-fermentation (L+, L-), and

Hydrogen-sulphide-production (H_2S+, H_2S-).

Any colony with *Salmonella*-like morphology (L-, H_2S+/-) was sub-cultured on MacConkey Agar and incubated for 18h at 37ºC. A gram stain was carried out and gram negative rods were tested for cytochrome oxidase and catalase reaction and differentiated further:

One loop full was streaked out on Urea Agar and incubated for 18h at 37ºC. Urease reaction was:

Positive = (red/pink coloration)

Negative = (yellow coloration).

One loop full was streaked out on the surface of Triple Sugar Iron Agar, the butt was stabbed and the agar incubated for 18h at 37ºC. The following readings were made:

Carbohydrate utilisation:

	Slant reaction	**Butt reaction**
Positive	Yellow colour (=acid)	Yellow colour (=acid)
Negative	Red colour (=alkaline)	Red colour (=alkaline)

Gas production:

Positive	**Negative**
Bubbles splitting of the agar	No gas production

H_2S production:

Blackening in whole or part of butt

Cultures with the following reaction pattern:

Urea negative,

TSI acid butt, acid/alkaline slant, Gas +/-, H_2S +/-

were used for serotyping with Poly A-67 and Poly A-E (Reagensia AB):

One colony was mixed with a drop of the agglutination solution and the reaction noted:

Positive	**Negative**
visible agglutination (floccules, clouds)	mixture remains homogenous

Agglutination positive cultures were stored in a stab culture in CTA Agar at room temperature. A duplicate was stored in Succhrose Solution (8.6g / 100mlH_2O) at -20ºC.

The stab cultures were sent to Germany to the National Salmonella Reference Laboratory in Marienfelde[3] for further serotyping.

3.4.2.2 Klebsiella sp.

Following the enrichment procedures described under 3.4.2.1 colonies with Klebsiella-like morphology (L+, H_2S-, mucoid) were sub-cultured on MacConkey Agar and incubated for 18h at 37ºC. After gram staining, gram negative rods were tested for cytochrome oxidase and catalase reaction and subcultured into Tryptone Water and Urea Agar and incubated for 18h at 37ºC. Kovacz reagent was added to the Tryptone Water and the reaction noted:

Indole Positive	**Indole Negative**
Formation of a pink layer	Transparent layer

Mucoid, Urea positive and Indol negative cultures were differentiated further by API 20 E (Biomérieux, Marcy l'Etoile, France). Isolates identified as *Klebsiella pneumoniae ssp. pneumoniae* were stored in Nutrient Agar and sent to the Department of Infection Medicine, University Hospital SH, Kiel, Germany[4] for capsule typing using the Neufeld quelling reaction (Burckhardt, 1992).

3.4.2.3 Escherichia coli

The rectal swabs were plated onto MacConkey Agar and incubated at 37ºC for 18h. Lactose non-fermenting cultures (Salmonella-like) and mucoid Lactose-fermenting cultures (Klebsiella-like) were differentiated as described under 3.4.2.1. and 3.4.2.2. Lactose-fermenting colonies were gram stained. Gram negative rods were tested for cytochrome oxidase and catalase reaction and differentiated further by:

Indol test	see 3.4.2.2
Urease	see 3.4.2.1
TSI	see 3.4.2.1

Urease negative, Indol positive coliform isolates were then stored as stab cultures in CTA Agar at room temperature and in Succhrose solution at -20ºC. CTA Agar cultures were transferred to the Institute of Microbiology and Zooepidemiology of the Freie Universität

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

[4] Institute of Infection Medicine, Reference Laboratory for Klebsiella species, University Hospitals SH - Campus Kiel-, Brunswiker Str. 4, 24105 Kiel, Germany

(FU) Berlin in Germany[5].

At the FU Berlin, CTA stab cultures were checked for purity and lactose reaction (incubated on Gassner Agar for 18h at 37ºC). Pure lactose fermenting cultures were subcultured on Blood Agar, incubated for 18h at 37ºC and the haemolysis was noted (α-, β-, γ-haemolysis).

A single colony was put into Lysine Indol Mobility (LIM) Agar and incubated for 18h at 37ºC. The following reactions were noted:

	Positive	**Negative**
Indol reaction	formation of a pink layer	layer remains transparent
Mobility	growths inside and outside stab line	growths only along stab line

3.4.3 Molecular Biology

3.4.3.1 Escherichia coli and other Reference and Control Strains

Table 5: List of *Escherichia coli* and other Reference and Control Strains used

Strain	**Species**	**Serovar**	**Virulence-associated factor /-gene**	**Origin**	**Reference**
TTP-1	Escherichia coli	O157:H-	stx1, stx2	Human	Wieler et al., 1992
E2348/69	Escherichia coli	O127:H6	eae, espB, bfpA	Human	Levine et al., 1978
H10407	Escherichia coli	O78:K80:H11	cfa, est-Ia, est-Ib, elt-Ib	Human	Evans et al., 1975
B41	Escherichia coli	O101:H$^-$	F5, F41, fan, elt-Ia	Calf	So and McCarthy, 1980
EDL933	Escherichia coli	O157:H7	Stx1, stx2, eae, hlyEHEC, LEE+, pssA/EspP+	Human	O'Brien et al., 1983
MG1665	Escherichia coli	K12			

[5] Institute of Microbiology and Zooepidemiology, Freie Universität Berlin, Philippstrasse 13, 10115 Berlin, Germany

Strain	Species	Serovar	Virulence-associated factor /-gene	Origin	Reference
K1068/99	Escherichia coli	O2:K1		Chicken	
293/Rom	Yersinia. enterocolitica	O:3		Dog	
SL1344	Salmonella typhimurium				

3.4.3.2 Solutions and buffer

Polymerase Chain Reaction (PCR)

AmpliTaq® DNA Polymerase — Perkin Elmer Cetus, Überlingen
Stoffel Fragment (10U/µl)

Magnesium Chloride Solution — Perkin Elmer Cetus, Überlingen
25 mM $MgCl_2$

10 x Stoffel Buffer — Perkin Elmer Cetus, Überlingen
100 mM KCl
100 mM Tris/HCl

dNTP

Milipor

Table 6: PCR conditions applied for the detection of virulence-associated genes in *E. coli* strains

Primer	Detected gene	Location within gene	PCR conditions (°C / sec)[1]			Number of cycles
			Denaturation	Annealing	Elongation	
EAST-1s	astA	135-155	94 / 40“	50 / 80“	72 / 60“	25
EAST-1as		219-245				
ECW1 s	eae	563-582	94 / 60“	68 / 90“	72 /90“	25
ECW2as		1191-1172				
EHLY1s	hly_{EHEC}	972-991	94 / 60“	63 /90“	72 / 90“	25
EHLY5		1322-1302				

Primer	Detected gene	Location within gene	PCR conditions (°C / sec)[1]			Number of cycles
			Denaturation	Annealing	Elongation	
as						
MK1 a MK2 as	stx	115-134 300-280	94 / 40“	50 / 80“	72 / 60“	25
ST-IB s ST-IC as	est-Ia / est-Ib	360-383 481-458	94 / 40“	50 / 80“	72 / 60“	25
LT-I1 s LT-I2 as	elt-Ia / elt-Ib	250-269 571-553	94 / 40“	55 / 80“	72 / 60“	25

[1] Initial denaturation step for all protocols: 94°C, 3min, final elongation: 72°C, 10min.

Agarose Gel Electrophoresis

Agarose — Roth, Karlsruhe

Rotigarose for Gel Electrophoresis, NEEO Ultra Quality

Ethidium Bromide Solution — Serva, Heidelberg

Ready Made Solution 10 mg/ml

25 x TBE Buffer

2,5 M Tris

2,5 M Boric Acid

50 mM EDTA

ad 1000 ml H_2O

Stop-Mix

0,25 % (w/v) Bromphenol Blue

0,25 % (w/v) Xylenzyanol

30,00 % (w/v) Glycerol

ad 10 ml H_2O

Marker (Counterselective marker)

3.4.4 Molecular biological methods

3.4.4.1 DNA Extraction

Boiling prep

All colony material, taken from a blood agar plate, was diluted in 500 µl Milipore and frozen for 10 min at -20°C. The sample was then defrosted and centrifuged for 10 min at 16.000 x g at room temperature. The supernatant was discarded; the remaining

pellet re-suspended in 100 µl dH_2O and boiled for 15 min at 100°C in a water bath (Julabo 5). The sample was again centrifuged for 10 min at 16.000 x g at room temperature and finally the supernatant transferred into a sterile tube.

DNA Extraction

The DNA was extracted according to the QIAquick Gel Extraction Kit Protocol (QIAGEN, Hilden, Germany, Cat. No. 28706, QIAquick Spin Handbook 07/2002).

3.4.4.2 DNA Hybridization

DNA-DNA Hybridization

The DNA-DNA Hybridization helps to detect sequences of the sample nucleine acid that are akin to or like the one of the probes. Through the hybridization process a contact between the denaturated DNA bound to the membrane and the DNA probe can be established. The DNA probe will then be able to bind on the corresponding areas during the incubation time. So called "hybrid-molecules" are formed which are made visible after the removal of the DNA probe.

Dot Blot

Samples were heated for 10 min at 100 °C in a water bath and put on ice directly afterwards to avoid the renaturation of the DNA. The denaturated DNA (2.5 µl) were transferred on to a positively charged Nylon-membrane (Boehringer Mannheim GmbH, Mannheim) in form of a dot. After air drying, the DNA was fixed onto the membrane through incubation at 120 °C for 30 minutes. Until further usage, the membrane was stored at +4 °C.

Generation of Oligo-nucleotide Probes

All used DNA-Probes for the detection of virulence associate Genes (*est* Ia/Ib, *elt* I1/Ib, *eae*, *hly*EHEC, *stx*, *astA*) were labelled with Digoxigenin-11-2'-desoxyuridin-5'-triphospate (DIG-11-dUTP). The labelling reaction was carried out via Polymerase Chain Reaction (PCR), according to the protocol of the manufacturer (PCR DIG Probe Synthesis Kit, Roche Diagnostic GmbH, Mannheim, Cat. No. 1 636 090, Version 4, Dec. 2002). The success of the labelling-reaction was verified through the slower migration of the product in the Agarose Gel Electrophoresis as compared against a positive control.

Hybridization with DIG labelled Oligo-nucleotide Probes

The Nylon membrane with the fixed DNA samples was incubated rotating for 2-3 h in prehybridization solution (20 ml/100 cm^2) and overnight in 2-4 ml hybridization solution at 68 °C. The unbound parts of the probes were then washed away at room temperature and 68 °C, respectively. Solutions and buffers used are detailed in Annex 9.2.

Detection of bound labelled probes

For the detection of the bound labelled probes the membrane was blocked with "blocking reagent", incubated with an antibody conjugate (Anti-DIG, Roche) and washed again. Finally the DIG-labelled probe could be made visible through the adding of substrate solution (chemiluminiscent substrate).

Chemoluminescence

Substrate solution (0.5 ml / 100 cm^2) was evenly distributed on the nylon membrane. The nylon membrane was then wrapped in cling film and placed on an x-ray film which was put into a light proof x-ray cassette. The film was exposed over night. The chemiluminiscent reaction could be detected according to the blackening of the areas of the x-ray film were the DNA had been put and was compared to the intensity of the control samples.

3.4.4.3 Control Polymerase Chain Reaction (PCR)

A control PCR was carried out to verify positive results from the hybridization.

The following preparation was used (pipetted on ice):

1 µl	$MgCl_2$
2 µl	10xPCR Buffer
2 µl	DNA (boiling prep)
0.1 µl	Primer forward (100pmol)
0.1 µl	Primer reverse (100pmol)
0.5 µl	dNTP
0.1 µl	*Taq* DNA Polymerase
Ad 25 µl	Aqua bidest

The PCR-preparation was transferred to the thermocycler and run according the specific program (see table under *3.4.3.2*).

3.4.4.4 Agarose-Gel-Electrophoresis

Agarose Gel electrophoresis is used to separate, identify and purify DNA fragments sized from 0.1 to 25 kb length. Gen-specific PCR products can be detected in 1% Agarose Gel. Therefore 1g of Agarose was suspended through boiling into 100 ml of 1 x TBE. The suspension was cooled down to 55 °C and 0.5 µl of Ethidiumbromide was added in order to visualise the DNA-wells in a UV light box. The liquid Agarose-suspension was poured into a gel rack and the slots were prepared with the help of a comb. The solid gel was put into an electrophoresis chamber and covered with 1 x TBE buffer. The DNA samples were stained with 1/10 volume of Bromphenolblue (BPB) before injected into the slots. The electrophoretic separation resulted from the applied current (80 – 100V run for 60 min) to the electrophoresis chamber. The negatively charged DNS fragments migrated to the anode. The colored dye in the DNA ladder and DNA samples acted as a "front wave" that runs faster than the DNA itself. When the "front wave" approaches the end of the gel, the current is stopped. The DNA was visualized with ultraviolet light.

3.5 STATISTICAL METHODS

The 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. For visualisation of the findings graphs such as boxplots and line charts were included.

4 RESULTS

4.1 POINT PREVALENCE STUDY

4.1.1 Clinical Observation

For the **point prevalence study** camel calves between birth and twelve weeks of age were sampled belonging either to Ranch Herds (Mgmt R) or Pastoralists Herds (Mgmt P). Each calf in the investigated age group was sampled in herds belonging to Mgmt R during the observation period while only camel calves presented by the herdsman could be sampled from Mgmt P.

4.1.1.1 Sample size

A total of 229 individual camel calves in their first twelve weeks of age were sampled during the period from January 2002 to February 2004.

4.1.1.2 Management systems

157 samples (68.6%) were taken from calves kept in Mgmt R while 72 samples (31.4%) were taken from calves kept in Mgmt P.

4.1.1.3 Management system / Age:

The animals sampled in Mgmt R were in general younger than those of Mgmt P with 14 days and 39 days being the median, respectively.

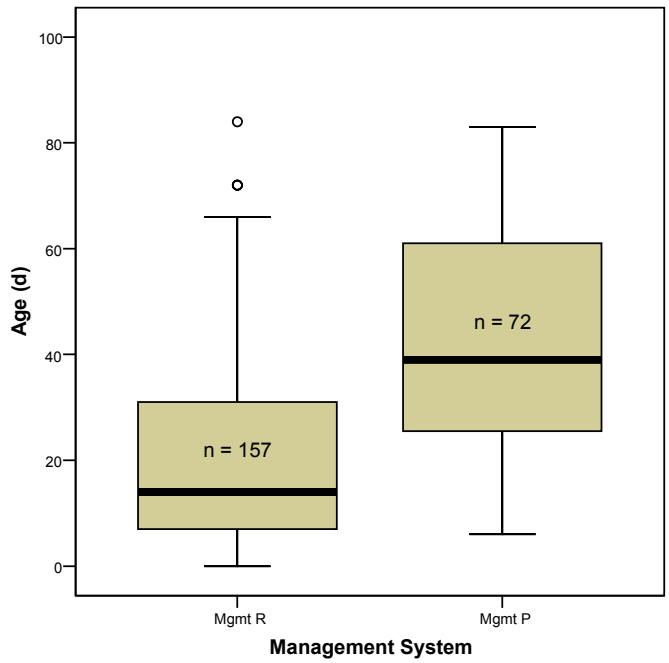

Figure 3: Age distribution of camel calves compared between the two management systems

For Mgmt R the 25.percentile was seven days and the 75.percentile 31 days while it was 25 days and 61 days for Mgmt P respectively (see Figure 3).

4.1.1.4 Health status of camel calves sampled

Out of the 229 individual camel calves sampled in both management systems, 155 (67.7%) were healthy, 53 (23.1%) were diseased, exhibiting diarrhoea, 15 (6.6%) were convalescent and five (2.2%) were dead. One animal (0.4%) was not categorised at the time of sampling.

4.1.1.5 Age of camel calves sampled:

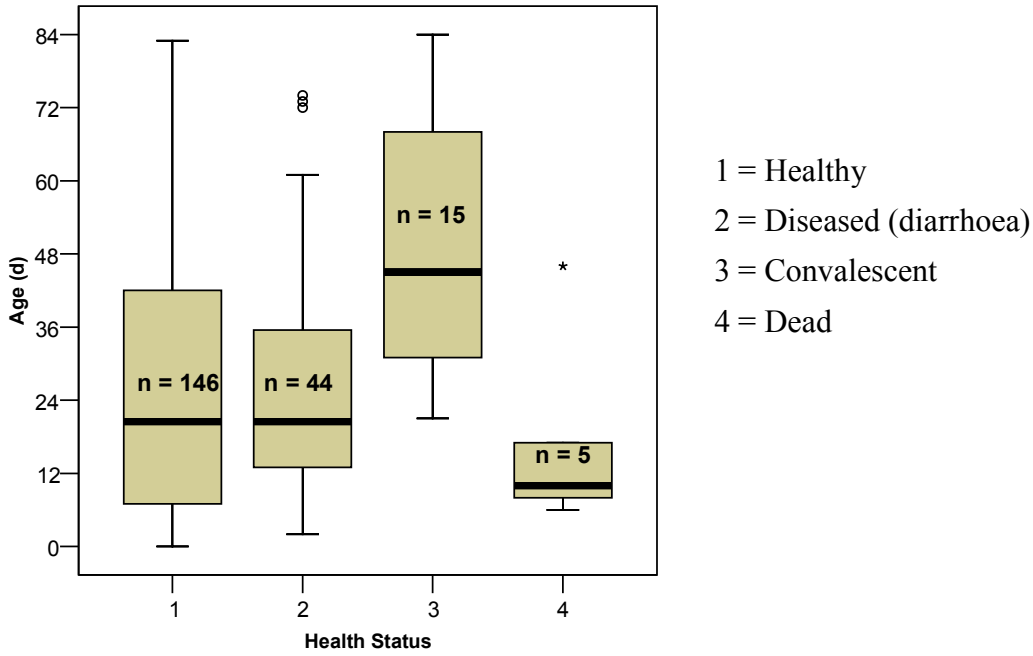

Figure 4: Age distribution of camel calves (n=210)[6] according to the different health status categories

Healthy calves were found in the whole age range between just born to 84 days old. The median was 21 days (seven days being the 25.percentile and 42 days the 75.percentile).

Among individuals that fell into the category of calves, diseased with diarrhoea, the youngest was two and the oldest 74 days old. The median was calculated with 21 days, seven days being the 25.percentile and 42 days being the 75.percentile.

Those categorised as convalescent calves were found with a median age of 45 days, 31 days being the 25.percentile and 75 days the 75.percentile.

The dead calves were in the age range of six to 46 days, with a median of ten, the 25.percentile of seven and the 75.percentile of 32 days.

[6] The age of 18 individual camel calves was not recorded, as the data were not available at sampling time

4.1.1.6 Management system / health status:

The table below describes the number of camel calves categorised by their health status according to management systems.

Table 7: Comparison of the health status of the sampled camels according to management systems

			Management System		Total
			Mgmt R	Mgmt P	
Health status	**Healthy**	No.	117	38	155
		% of Mgmt	75.0%	52.8%	68.0%
	Diseased (diarrhoea)	No.	30	23	53
		% of Mgmt	19.2%	31.9%	23.2%
	Convalescent	No.	4	11	15
		% of Mgmt	2.6%	15.3%	6.6%
	Dead	No.	5	0	5
		% of Mgmt	3.2%	0%	2.2%
Total		No.	156	72	228
		% of Mgmt	100.0%	100.0%	100.0%

75% (117) of the camel calves sampled in Mgmt R were healthy, while 19.2% (30) suffered from diarrhoea at time of sampling. 2.6% (4) and 3.2% (5) of the calves were convalescent and dead, respectively.

In Mgmt P, 52.8% (38) were healthy, 31.9% (23) were diseased with diarrhoea and 15.3% (11) were convalescent.

All dead camel calves belonged to Mgmt R. The only post mortems carried out during the observed study were performed on camel calves kept on ranches, because only here owners were able to inform the researcher immediately after a death had occurred. This was not possible in herds kept under Mgmt P.

4.1.1.7 Health status and age compared between the two management systems:

Diseased (diarrhoea)

As shown in Figure 5, in both management systems the occurrence of diarrhoea started in the first week of age. The first peak in Mgmt R was seen in the second week of age with 37.5 % (12 calves). Afterwards the prevalence of calves with diarrhoea was decreasing until the sixth week with 12.5 % (one calf). A second peak could be found in the tenth week of age with 50 %.

In Mgmt P the graph shows an almost similar age distribution, with a peak in the third week of age with 42.9 % (3); however the decrease of diseased calves was not so prominent, it rather stagnated between 16.7 % to 28.7 % from the sixth week until the twelfth week.

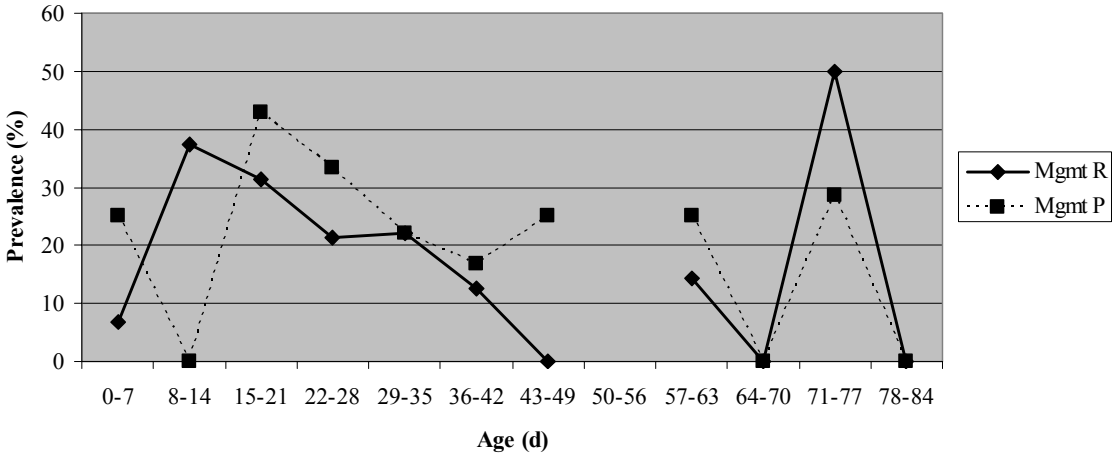

Figure 5: Age prevalence of diseased camel calves compared between the two management systems

In the second week of age, no calf suffering from diarrhoea was sampled in Mgmt P. However sampling was done sporadically and the sample size was too small, to be significant (see Annex 9.3).

4.1.2 Parasitological Findings

4.1.2.1 Sample size

Out of the 229 samples taken from individual camel calves in both management systems, 197 were analysed for protozoa oocysts and helminth eggs.

4.1.2.2 Parasitic pathogens

Isospora sp. oocysts were excreted by 6.6 % (13) of the sampled calves while 6.6 % (13) and 4.6 % (9) were shedding *Strongyloides* eggs or other *Strongyle* eggs, respectively (see Table 8).

Table 8: No of sampled camel calves excreting *Isospora sp.* oocysts, *Strongyloides sp., Strongyle* eggs

	Isospora sp.		***Strongyloides sp.***		***Strongyle sp.***	
	No.	**%**	**No.**	**%**	**No.**	**%**
Negative	184	93.4	184	93.4	188	95.4
Positive	**13**	**6.6**	**13**	**6.6**	**9**	**4.6**
Total	**197**	**100**	**197**	**100**	**197**	**100**

4.1.2.3 Management systems:

A total number of 135 (68.5%) samples were collected from camel calves kept under Mgmt R while 62 (31.5%) were collected from calves that belonged to Mgmt P.

In Mgmt R *Isospora* sp. was isolated in five camel calves (3.7 %) while they were more frequently found in Mgmt P with eight camel calves (12.9 %).

Strongyloides sp. were isolated from six calves in Mgmt R (4.4%) and Mgmt P (9.7%) while *Strongyle* sp. eggs were isolated in five (3.7%) and four (6.5%) camel calves in Mgmt R and Mgmt P, respectively. (See also Table 9)

Table 9: Distribution of *Isospora sp., Strongyloides sp. and Strongyle* according to the management system

	Isospora sp.				***Strongyloides sp.***				***Any other Strongyle eggs***			
	Mgmt R (n=135)		**Mgmt P (n=62)**		**Mgmt R (n=135)**		**Mgmt P (n=62)**		**Mgmt R (n=135)**		**Mgmt P (n=62)**	
	No.	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**
Positive	**5**	**3.7**	**8**	**12.9**	**6**	**4.4**	**6**	**9.7**	**5**	**3.7**	**4**	**6.5**
Negative	130	96.3	54	87.1	129	95.6	56	90.3	130	96.3	58	93.5

4.1.2.4 Health status compared to parasitic infection

Table 10: Point prevalence of *Isospora sp., Strongyloides sp*. and any other *Strongyle sp.* according to the health status of the calves sampled

Health status		*Isospora sp.*		*Strongyloides sp.*		*Strongyle sp.*	
		Negative	**Positive**	Negative	**Positive**	Negative	**Positive**
Healthy	**No.**	128	**0**	120	**8**	121	**7**
	%	69.6	**0**	64.9	**66.7**	64.4	**77.8**
Diseased	**No.**	40	**12**	48	**4**	50	**2**
	%	21.7	**92.3**	25.9	**33.3**	26.6	**22.2**
Convalescent	**No.**	13	**1**	14	**0**	14	**0**
	%	7.1	**7.7**	7.6	**0**	7.4	**0**
Dead	**No.**	3	**0**	3	**0**	3	**0**
	%	1.6	**0**	1.6	**0**	1.6	**0**
Total	**No.**	184	**13**	185	**12**	188	**9**
	%	100	**100**	100	**100**	100	**100**

Of the 13 camel calves diagnosed with *Isospora sp.* oocysts excretion, twelve (92.3%) were diseased and had diarrhoea. Only one calf (7.7%) was in a convalescent state. Hence, there was a significant relation (according to Chi-Square, Pearson) between the infection with *Isospora sp.* and the health status ($p < 0.05$, $p = 0.000$).

Four (33.3%) of the twelve calves infected with *Strongyloides* had diarrhoea, eight (66.7%) were healthy.

Only two of the calves shedding *Strongyle* eggs (22.2%) showed signs of diarrhoea. Seven *Strongyle* egg positive calves (77.8%) were asymptomatic and showed no signs of diarrhoea.

Table 11: Number of positive cases with *Isospora sp., Strongyloides sp*. and *Strongyle sp*. in camel calves suffering from diarrhoea

Species found	No of positive cases with diarrhoea (n=52)	% of all diarrhoea positive cases
Isospora sp.	12	23%
Strongyloides sp.	4	8%
Strongyle sp.	2	4%

The table above shows that with 23% (12 calves) *Isospora sp.* was commonly found in camel calves suffering from diarrhoea at the time of sampling.

4.1.2.5 Point prevalences of parasitic infection according to age groups (first to twelfth week of age)

Figure 6 shows the age distribution in camel calves shedding *Isospora* sp. oocysts, *Strongyloides* sp. and *Strongyle* sp. eggs in their first twelve weeks of age.

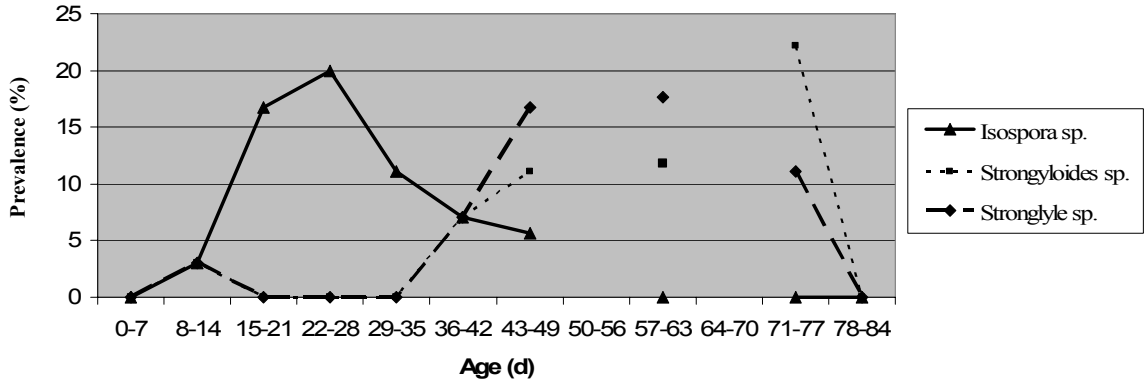

Figure 6: Point prevalences of *Isospora sp., Strongyloides sp.* and other *Strongyle sp.* in camel calves from birth up to 12 weeks of age

The excretion of *Isospora sp*. oocysts in camel calves up to twelve weeks of age gave a clear picture: There was a prevalence of 3% during the second week of age, continuously increasing to 16.7 % in the third week and reaching a peak in the fourth week of age with a prevalence rate of 20.0%. Afterwards the excretion rate decreased to 11.1%, 7.1% and 5.6% in the fifth, sixth and seventh week, respectively. No shedding of *Isospora sp.* oocysts was diagnosed at an older age.

Both the excretion of *Strongyle sp.* eggs and *Strongyloides sp.* eggs from camel calves started during the sixth week of age with 7.1% and 5.6%, respectively. The excretion rate increased for both parasites towards older age with 16.7% (seventh week) and 17.6% (eighth week) for *Strongyle sp.* eggs and 7.1% (sixth week), 11.1% (seventh week) and 11.8% (eighth week) for *Strongyloides sp.* eggs. There was a decrease in the excretion of *Strongyle* eggs in the eleventh week of age (down to 11.1%), while the shedding of *Strongyloides sp*. eggs still increased in this age group (up to 22.2%). For details refer to Annex 9.4

No samples for parasitological analysis were taken from camel calves in the eighth week of age (days 50 to 56). Hence this age group does not appear in the graph above. No parasitic stages could be found in samples taken in the age group between 64 to 70 days (tenth week), therefore the graph is interrupted.

4.1.2.6 Prevalences of parasitic infection according to age group, comparison of the two management systems:

Infection with *Isospora sp.*

As seen in Figure 7, *Isospora sp.* infection occurred in calves belonging to Mgmt R during the second week of age (3.3%), increasing to 7.1% in the third week of age and reaching a peak of 16.7% in the fourth week of age. No *Isospora sp.* oocysts were detected in calves older than four weeks.

Calves kept in Mgmt P showed a high prevalence of 50%, within the third week of age decreasing slowly over the following weeks to 33.3 %, 22.2%, 16.7% and 12.5% in the fourth, fifth, sixth and seventh week, respectively. Calves older than eight weeks were not diagnosed positive for excretion of *Isospora sp.* oocysts.

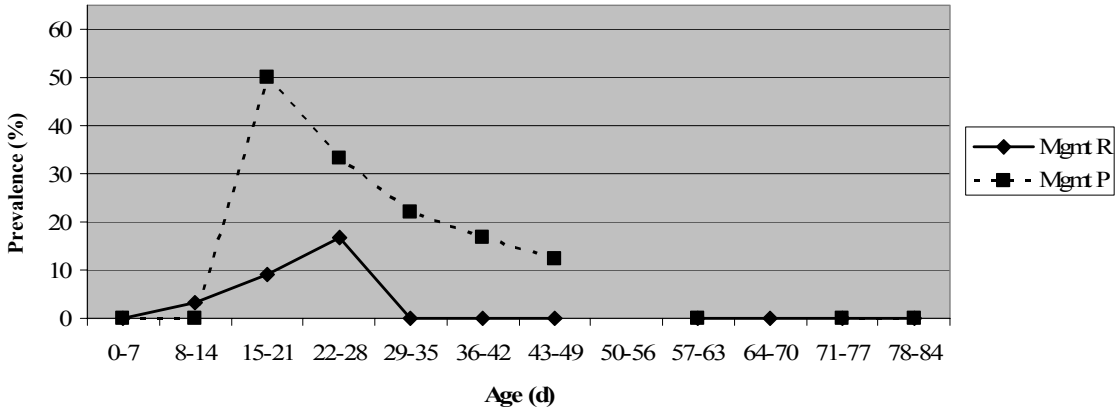

Figure 7: Point prevalences of infection with *Isospora* sp. according to age, compared between the two management systems

Infection with *Strongyloides sp.*

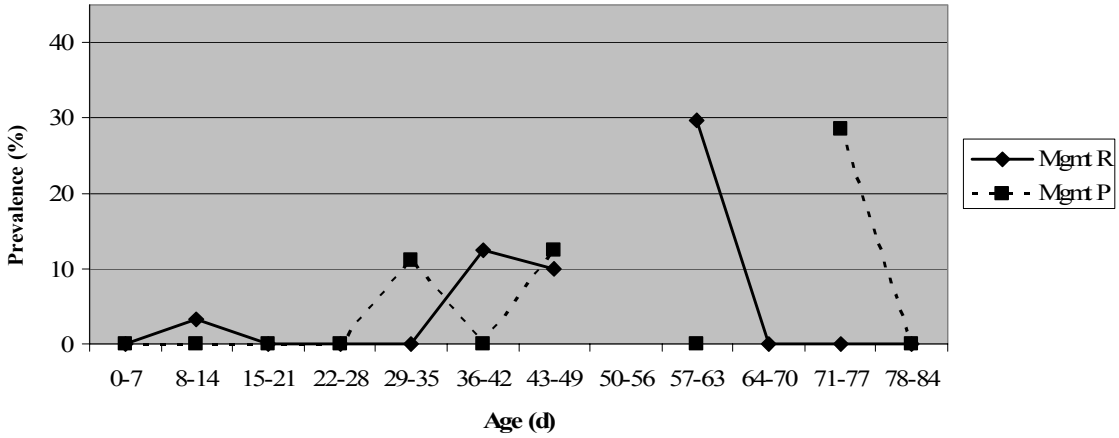

Figure 8: Point prevalences of infection with *Strongyloides sp.* according to age compared between the two management systems

The graph in Figure 8 above shows that *Strongyloides sp.* infection was diagnosed in calves kept in Mgmt R within the sixth week of age (12.5%), increasing steadily to reach a peak within the eighth week of age (28.6%). No *Strongyloides sp.* eggs were found at a later age. Infection with *Strongyloides sp.* eggs occurred in Mgmt P at an earlier stage, within the fifth week of age (11.1%). Although no *Strongyloides* eggs were diagnosed in the sixth and eighth week of age, there is a clear increase (12.5% in the seventh week), reaching a peak in the ninth week of age (28.6%). No *Strongyloides sp.* eggs were diagnosed at an older age.

Infection with *Strongyle sp.*

Figure 9 shows the age prevalences of infections with *Strongyle sp.* eggs compared between the two management systems.

Calves kept in Mgmt R started to show infections within the sixth week of age (12.5%), with a peak in the tenth week of age (50%). There seemed to be an increasing prevalence towards older age. However in the eighth week no samples were collected while in the tenth week of age, no *Strongyle sp.* eggs were diagnosed.

Faecal samples from calves kept in Mgmt P were diagnosed positive with *Strongyle* eggs in the seventh week of age (12.5%), having a peak in the eighth week (30%). No *Strongyle* eggs were diagnosed after the tenth week of age.

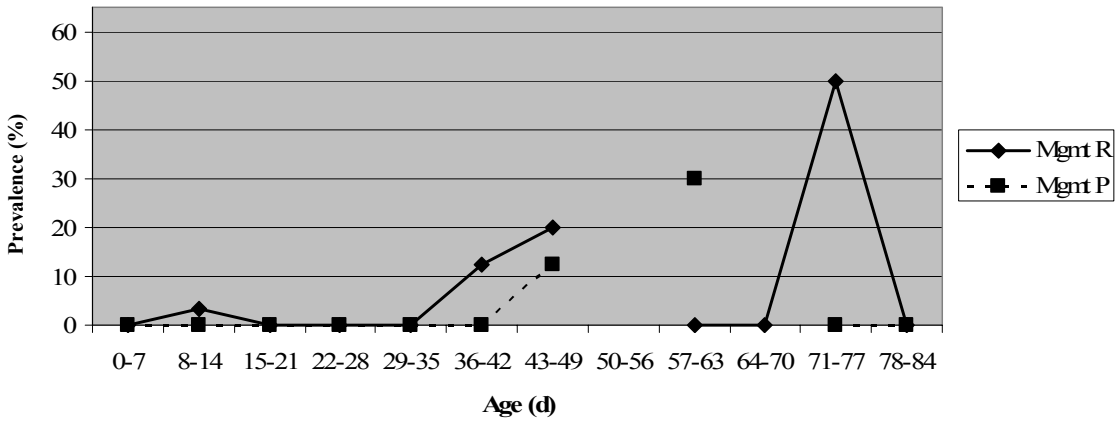

Figure 9: Point prevalences of infection with other *Strongyle sp.* according to age compared between the two management systems

For details refer to Annex 9.5.

4.1.3 Bacteriological Findings

4.1.3.1 Sample size

Out of the 229 samples taken from individual camel calves in both management systems, 119 were screened for *Klebsiella* sp., 226 for *Salmonella* sp. and 200 for *E. coli*.

Table 12: Number of valid samples analysed for *Klebsiella pneumoniae, Salmonella sp.* and *E. coli*

		Klebsiella pneumoniae	*Salmonella sp.*	*E. coli*
N	**Valid**	**119**	**226**	**200**
	Missing	**110**	**3**	**29**

4.1.3.2 Bacterial pathogens

The following bacterial pathogens were isolated from faecal (and post mortem) samples:

Klebsiella pneumoniae (ssp. pneumoniae)

Salmonella sp.

Escherichia coli

Table 13: No of examined camel calves positive for *Klebsiella pneumoniae, Salmonella sp.* and *E. coli*

	Klebsiella pneumoniae		***Salmonella sp.***		***E. coli***	
	No.	**%**	**No.**	**%**	**No.**	**%**
Negative	87	73.1	181	80.1	5	2.5
Positive	**32**	**26.9**	**45**	**19.1**	**195**	**97.5**
Total	119	100	226	100	200	100

Klebsiella pneumoniae was isolated in 32 (26.9 %) out of 119 camel calves sampled. In 45 (19.1 %) out of 226 sampled camel calves, *Salmonella sp.* was isolated, while in 195 (97.5 %) out of the 200 samples analysed *E. coli* was present.

4.1.3.3 Management systems

Out of the 119 samples screened for *Klebsiella (K.) pneumoniae*, 65 samples (54.6 %) were taken from calves belonging to Mgmt R and 54 (45.4 %) were taken from camel calves belonging to Mgmt P.

In Mgmt R *K. pneumoniae* was isolated in 21 camel calves (32 %) while in Mgmt P 11 (20 %) camel calves were found to be *K. pneumoniae* positive.

155 samples (68.6 %) out of the 226 samples analysed for presence of *Salmonella sp.* were taken from calves belonging to Mgmt R while 71 (31.4 %) samples originated from Mgmt P. 28 (18 %) and 17 (24 %) *Salmonella sp.* positive animals were detected in Mgmt R and P, respectively.

Of the samples screened for *E. coli*, 141 (70.5 %) were from Mgmt R and 59 (29.5 %) from Mgmt P. *E. coli* was present in almost all samples screened. In 137 (97 %) out of 141 samples from Mgmt R and in 58 (98 %) out of 59 samples in Mgmt P *E. coli* was isolated. (See also Table 14)

Table 14: Distribution of *K. pneumoniae, Salmonella sp*. and *E. coli* according to the management system

	Klebsiella pneumoniae				***Salmonella sp.***				***E. coli***			
	Mgmt R (n=65)		**Mgmt P (n=54)**		**Mgmt R (n=155)**		**Mgmt P (n=71)**		**Mgmt R (n=141)**		**Mgmt P (n=59)**	
	No.	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**
Positive	**21**	**32%**	**11**	**20%**	**28**	**18%**	**17**	**24%**	**137**	**97%**	**58**	**98%**
Negative	44	68%	43	80%	127	82%	54	76%	4	3%	1	2%

No significant relation between the isolation frequency of *K. pneumoniae, Salmonella sp.* and *E. coli* and the management system was found.

4.1.3.4 Health status in relation to bacterial infection

In total *K. pneumoniae* was isolated 32 times. In 17 cases (53.1%) *K. pneumoniae* was isolated from healthy calves. *Klebsiella pneumoniae* was found in calves showing signs of diarrhoea in 12 cases (37.5 %). No *K. pneumoniae* was isolated from convalescent calves. However three isolates (9.4 %) originated from dead calves.

Salmonella sp. was isolated from all health status categories: 24 (53.3 %), 17 (37.8 %), 3 (6.7 %) and 1 (2.2 %) from healthy, diseased, convalescent and dead calves, respectively.

Table 15: Prevalences of *K. pneumoniae, Salmonella sp*. and *E. coli* according to the health status of the calves sampled

Health status		***Klebsiella pneumoniae***		***Salmonella sp.***		***E. coli***	
		Negative	**Positive**	Negative	**Positive**	Negative	**Positive**
Healthy	**No.**	56	**17**	130	**24**	4	**125**
	%	64.4	**53.1**	72.2	**53.3**	80	**64.4**
Diseased	**No.**	21	**12**	36	**17**	0	**52**
	%	24.1	**37.5**	20	**37.8**	0	**26.8**
Convalescent	**No.**	8	**0**	12	**3**	0	**13**
	%	9.2	**0**	6.7	**6.7**	0	**6.7**
Dead	**No.**	2	**3**	2	**1**	1	**4**
	%	2.3	**9.4**	1.1	**2.2**	20	**2.1**
Total	**No.**	87	**32**	180	**45**	5	**194**
	%	100	**100**	100	**100**	100	**100**

E. coli isolates originated from calves in all the four categories: 125 (64.4 %) isolates were found in healthy, 52 (26.8 %) in diseased, 13 (6.7 %) in convalescent and 4 (2.1 %) in dead calves.

9.4% of *K. pneumoniae* isolations were made from dead camel calves compared to only 2.2% for *Salmonella sp.* and 2.1% for *E. coli.*

4.1.3.5 Point prevalences of bacterial infections according to age groups (first to 12th week of age)

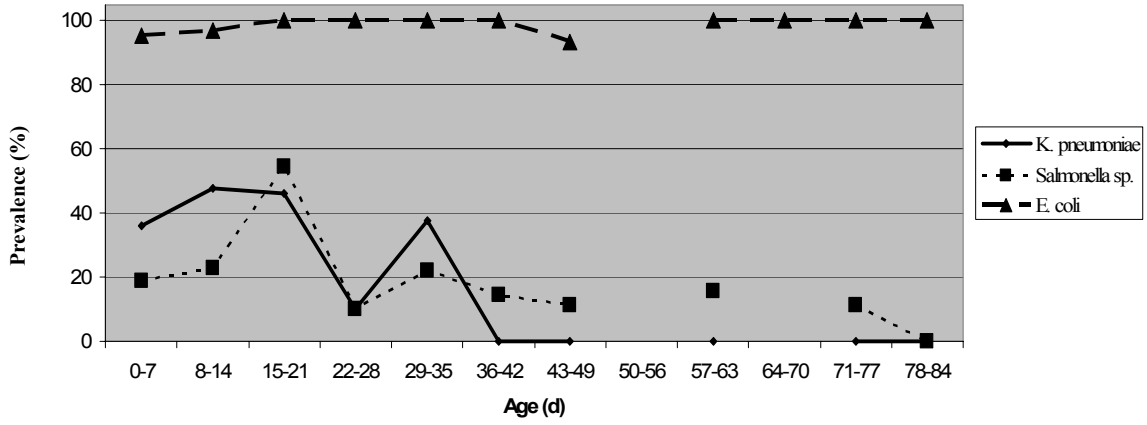

Figure 10: Point prevalences of K. *pneumoniae, Salmonella sp.* and *E. coli* in camel calves up to twelve weeks of age

The graph (Figure 10) reflects the point prevalences of *K. pneumoniae*, *Salmonella sp.* and *E. coli* in camel calves within their first 12 weeks of age.

With 36 % (nine positive cases) *K. pneumoniae* point prevalence was particularly high in one week old camel calves and increased further to 47.6 % (10) and 46.2 % (6) in the second and third week, respectively. In the fourth week the point prevalence decreased to only 10 % (1) and was at 37.5 % (3) in the fifth week of age. No *K. pneumoniae* was isolated from camel calves older than six weeks.

The point prevalence of *Salmonella sp*. infections started with 18.7 % (9) in the first week of age rising to 22.9 % (8) in the second week and 54.5 % (12) in the third week of age. In the following weeks *Salmonella* sp. was constantly present with a prevalence of 10 % (2), 22.2 % (4), 14.3 % (2), 11.1 % (2), 15.8 % (3) and 11.1 % (1) in week 4, 5, 6, 7, 9 and 11 in that order. No *Salmonella* sp. was isolated in camel calves in their tenth week of age, while no samples were taken from camels calves aged eight weeks.

E. coli was always present in the camel calves. Only in 4 calves (2 in the first, 1 in the second and 1 in the seventh week of age) *E. coli* was not isolated.

No samples were taken from camel calves in the eighth week of age (days 50 to 56). Hence this age group does not appear in the graph above. No *K. pneumoniae* or *Salmonella sp.* was found in samples taken in the age group between 64 to 70 days (tenth week); therefore the graph is interrupted at that stage for the two pathogens.

For further details refer to Annex 9.12

4.1.3.6 Point prevalences of bacterial infections according to age group, comparison of the two management systems

Infection with *Klebsiella pneumoniae*

Comparison of *K. pneumoniae* point prevalences between Mgmt R and P revealed no significant differences. There was a major decrease in *K. pneumoniae* point prevalence after day 42 in both systems.

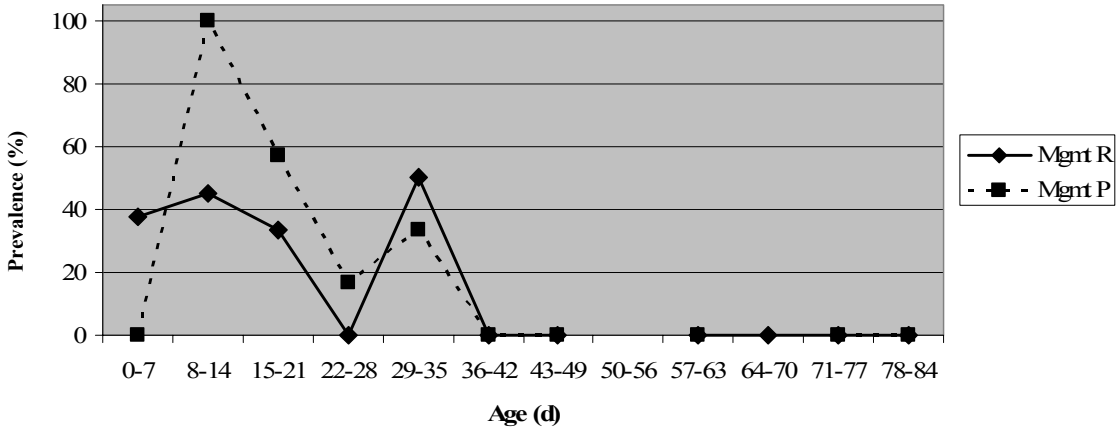

Figure 11: Point prevalences of *K. pneumoniae* infection in camel calves compared between the two management systems

The point prevalence was high in the first three weeks of age with 37.5 % (9), 45 % (9) and 33.3 % (2) in Mgmt R and 100% (1) and 57.1 % (4) in week two and three in Mgmt P. Both showed a second peak in week five with 50 % (1) and 33.3 % (2) in Mgmt R and P, respectively. From the sixth week onwards no *Klebsiella pneumoniae* were isolated in both management systems.

Infection with *Salmonella sp.*

For *Salmonella sp.* in calves in the two different management systems, higher point prevalence was seen in Mgmt P (see Figure 12).

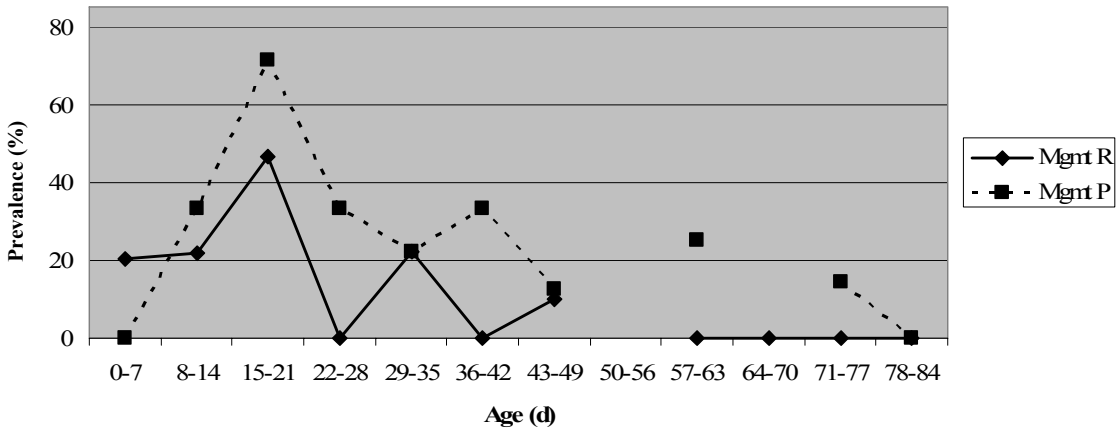

Figure 12: Prevalences of *Salmonella sp.* infections in camel calves compared between the two management systems

In Mgmt R the point prevalence of *Salmonella sp.* in camel calves increased from 20.5 % (9) to 21.9 % (7) in week one and two, respectively and was at 46.7% (7) in week three. In Mgmt P the increase was from 33.3 % (1) in week two to a peak of 71.4% (5) in the third week of age. After the forth week of age, the prevalence of *Salmonella sp.* in Mgmt R decreased to 22.2 % (2) in week five and 10% (1) in week seven. No *Salmonella sp.* was isolated in older calves in Mgmt R.

In Mgmt P *Salmonella sp.* was isolated until the twelfth week of age at a relatively constant level with a prevalence of 33.3 % (2), 22.2 % (2), 33.3 % (2), 12.5 % (1), 25 % (3) and 14.3 % (1) in week 4, 5, 6, 7, 9 and 11 in that order.

Infection with *E. coli*

Escherichia coli was present throughout the different age groups in both management systems.

4.2 LONGITUDINAL STUDY

4.2.1 Clinical Observation

4.2.1.1 Time frame and sample size

A total of 323 samples were taken from 86 individual camel calves aged between birth and three months. The camels were all kept on ranches in Mgmt R. The samples were taken during the period from February 2002 till November 2003.

4.2.1.2 Health status

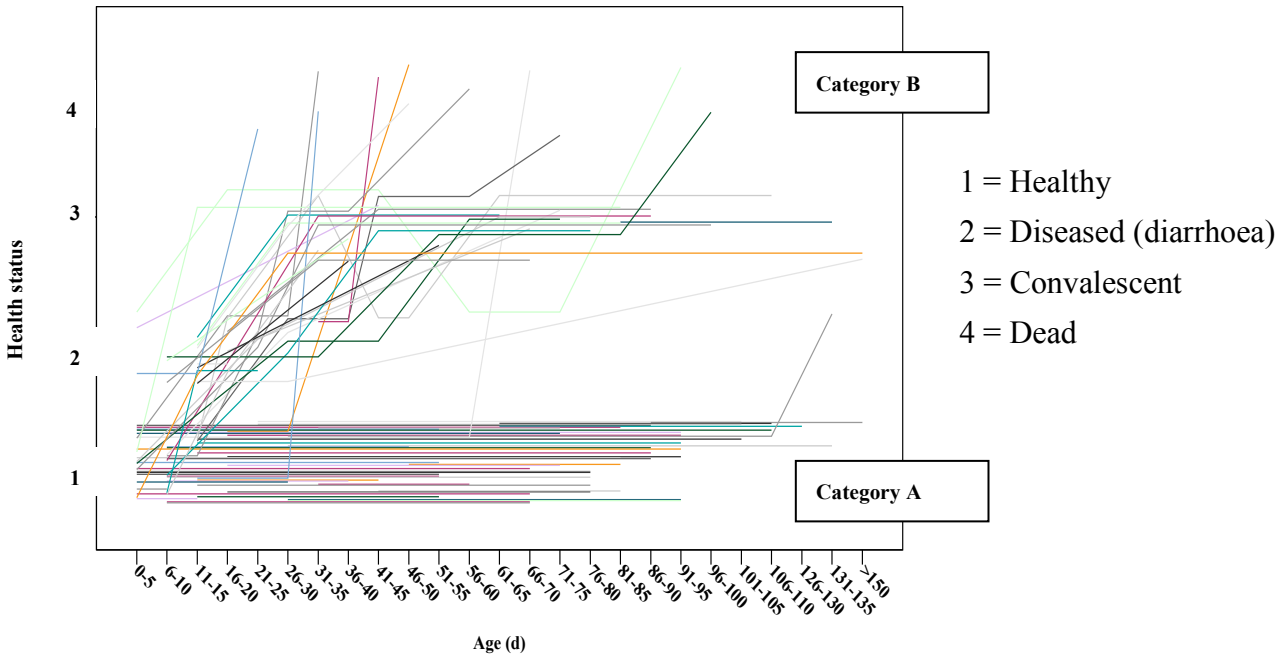

Figure 13: Health status of individual camel calves

The health status of 86 individual camel calves is illustrated in the graph above. Each line is representing an individual calf and its health status at the sampling time.

According to this the individual calves could be divided into two categories:

Category A: Representing camel calves never recorded with diarrhoea throughout the observation period.

Category B: Representing camel calves, which were either diseased with diarrhoea or convalescent from diarrhoea, meaning they suffered from diarrhoea at least once during the observation period but not necessarily at the time of sampling.

Fifty two and 34 individuals fell into Category A and B, respectively (see Table 16).

Table 16: Distribution of camel calves according to Category A and B

Category	N# of camel calves	Percentage %
Camel calves healthy throughout the observation period (Category A)	52	60,5
Camel calves recorded with diarrhoea at least once during the observation period (Category B)	34	39,5
Total	**86**	**100**

Category A:

A total of 181 samples from 52 individuals fell in Category A.

177 samples were from healthy calves while four samples were taken from calves that had died of non-diarrhoea related causes.

Category B:

Out of the 142 samples analysed from 34 individuals falling into Category B, 22 (15.5%) were taken from healthy calves on the day of sampling, while 49 (34.5%) and 61 (43%) were from calves being diseased with diarrhoea and convalescent, respectively. Ten samples (7%) were taken from dead calves with a previous diarrhoea history.

In the following, obtained data were compared according to the two categories A and B.

4.2.1.3 Health status according to age in calves falling under Category B

The interval of five days was chosen, because no calf was sampled twice during this time period.

Health status: Healthy

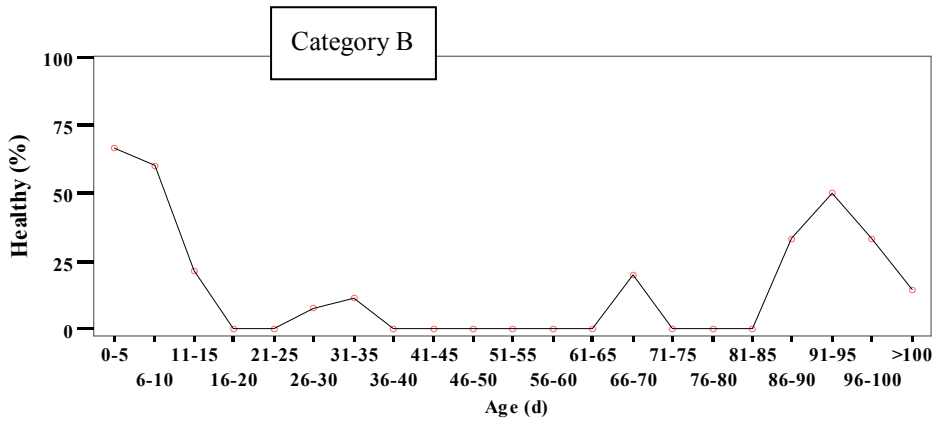

Figure 14: Age distribution of samples (n=22) taken from healthy calves falling into Category B

Health status: Diseased/diarrhoea

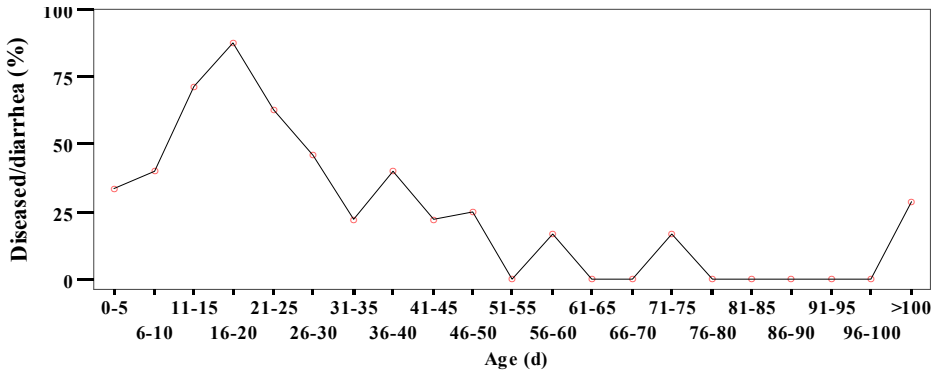

Figure 15: Age distribution of samples (n=49) taken from calves with diarrhoea falling into Category B

Figure 15 shows the presence of diarrhoea according to age within Category B. The highest prevalence of calves recorded with diarrhoea was during days six and 75.

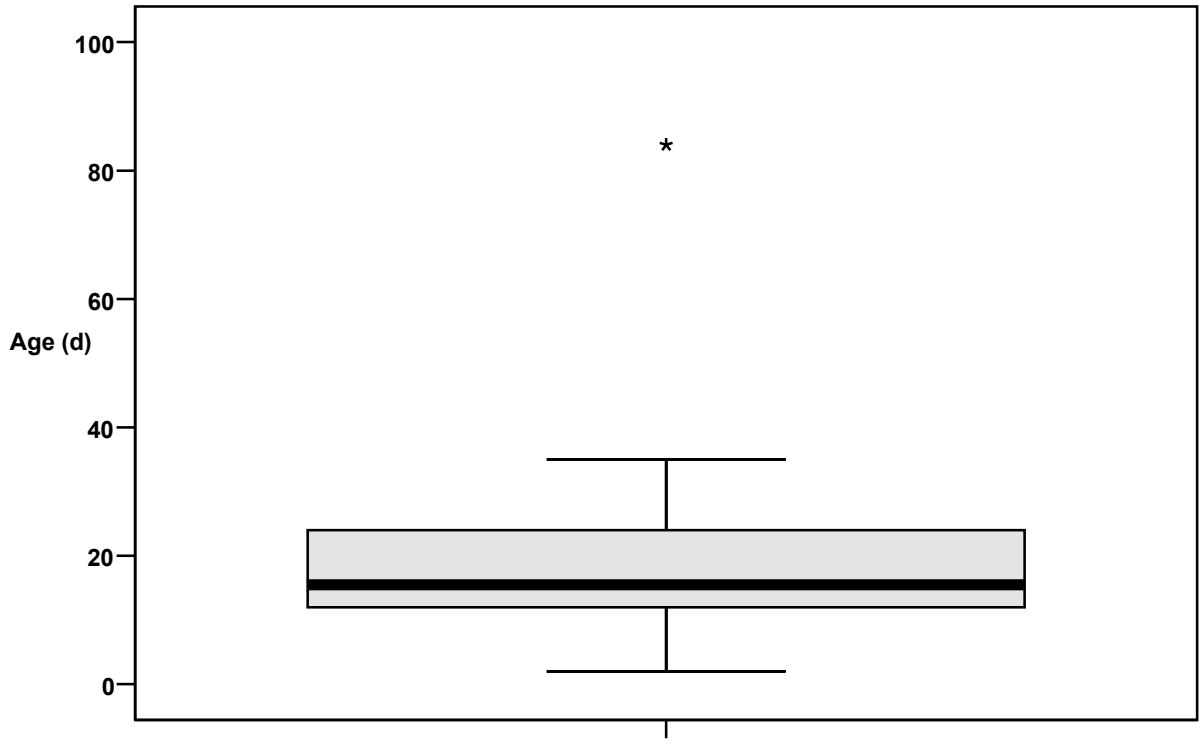

Figure 16: Earliest age at which diarrhoea was recorded in Category B calves

Figure 16 shows the first appearance of diarrhoea according to the age group. First symptoms of diarrhoea occurred at an early age, mainly within the first three weeks with a peak between day 16 and 20. The youngest calf sampled showing signs of diarrhoea was two days old.

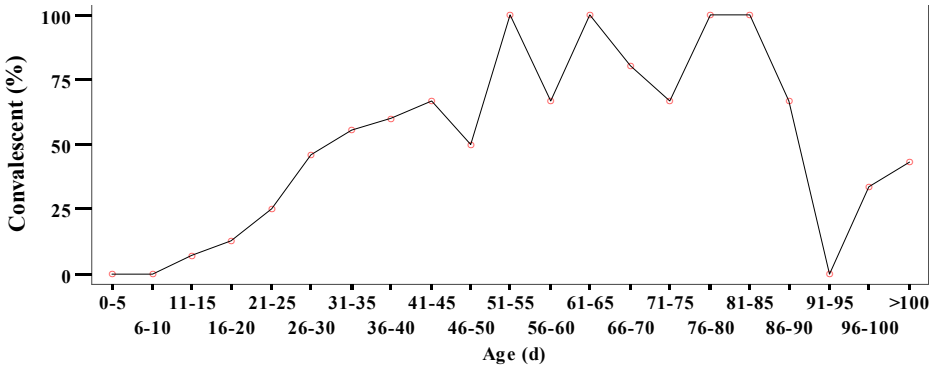

Figure 17: Age distribution of samples (n=61) taken from convalescent calves falling into Category B

Figure 17 shows that the majority of diarrhoea episodes occur before 51 to 55 days of age.

Dead calves found belonged to both categories. Death occurred at any age between birth and the end of observation period, day 96 to 100.

4.2.2 Parasitological Findings

4.2.2.1 Prevalence of parasitic infection according to age group

The following graphs (Figure 18, 19, 20) show the prevalence of the different parasitic infections according to age groups in Mgmt R.

Infection with *Isospora sp.*

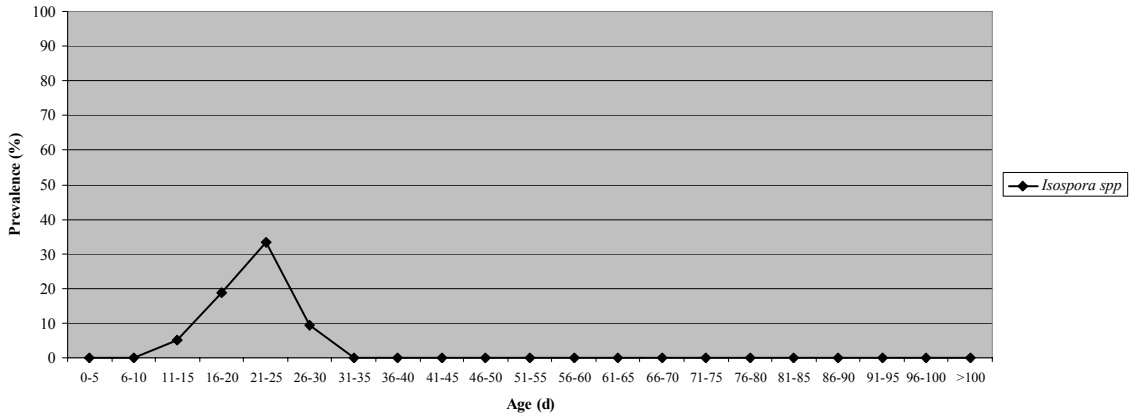

Figure 18: Age distribution of infection with *Isospora sp*. in camel calves kept in Mgmt R

Infection with *Isospora sp.* was detected in camel calves from day 11 to 15 (second week of age) with 5% (1). From days 16 to 20 (third week of age) it increased to 18.8% (3). The prevalence increased constantly towards a peak (33.3% (4)) between day 21 and 25 (fourth week of age). The prevalence rate then declined to 9.5% (2) between day 26 and 30. No *Isospora sp.* shedding was diagnosed in the monitored population after 31 days of age.

Refer to Annex 9.6 for further details.

Infection with *Strongyloides sp.*

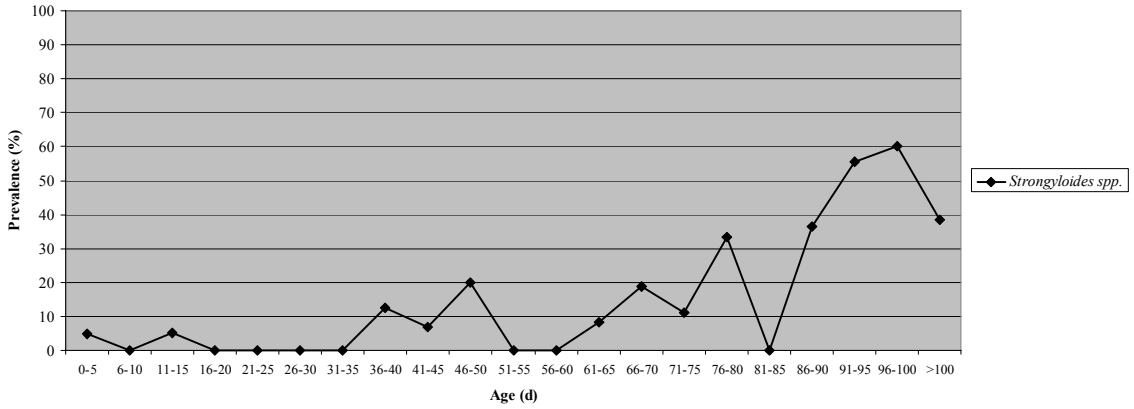

Figure 19: Age prevalence of infection with *Strongyloides sp.* in camel calves kept in Mgmt R

Strongyloides sp. eggs were shed already between day zero and five (first week of age) and day 11 and 15 (second week of age) with 4.8% (1) and 5% (1), respectively. An increase in the excretion of the eggs started with 12.5% (1) between day 36 and 40 (sixth week of age), peaking between day 96 and 100 (60% (3)). Similar to the infection with *Strongyle sp*., there is a tendency of a higher *Strongyloides sp.* prevalence with age in the camel calves.

See also Annex 9.7 for more details.

Infection with *Strongyle sp.*

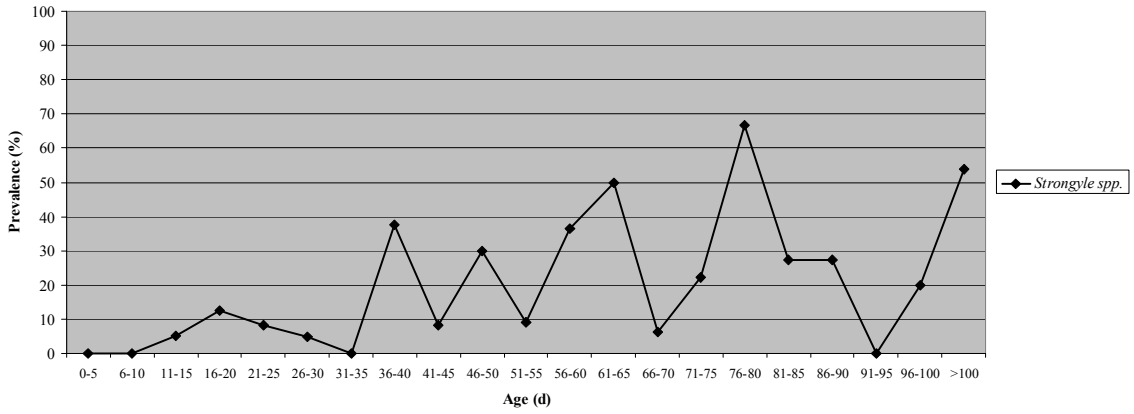

Figure 20: Age prevalence of infection with other *Strongyle sp.* in camel calves kept in Mgmt R

Strongyle sp. infections were diagnosed first between day 11 and 15 (second week of age) with a prevalence of 5% (1). Showing various small peaks, the main peak occurred between day 76 and 80 (twelfth week of age) with 66.7% (6). The general tendency was that of an increase towards older age.

Refer to Annex 9.8 for further details.

4.2.2.2 Comparison of the age prevalence of Isospora sp., Strongyloides sp. *and other* Strongyle sp. *according to different health categories (A and B)*

A total of 157 out of 181 samples were analysed for helminth eggs and/or coccidian oocysts from calves belonging to Category A, while 120 out of 142 samples were analysed for parasitic stages from calves belonging to Category B.

Table 17: Number and percentage of *Isospora sp., Strongyloides sp.* and other *Strongyle sp.* excretion rates compared between the two categories

	Category A n = 157				**Category B N = 120**			
	Negative	%	**Positive**	**%**	Negative	%	**Positive**	**%**
Isospora sp.	157	100	**0**	**0**	108	90	**12**	**10**
Strongyloides sp.	139	88.5	**18**	**11.5**	105	87.5	**15**	**12.5**
Other *Strongyle sp.*	126	86.6	**21**	**13.4**	94	78.3	**26**	**21.7**

Infection with *Isospora sp.*

No *Isospora sp.* or any other coccidian oocysts could be detected in any of the samples from calves belonging to Category A, while in twelve cases (10%) *Isospora sp*. oocysts could be demonstrated in the faeces of camel calves falling under Category B.

In calves belonging to Category B the occurrence of *Isospora sp*. was limited, starting from day eleven (7.7% (1)), increasing to 37.5% (3) between day 16 and 20, to a peak of 57.1% (4) between day 21 and 25. The prevalence of the infection decreased to 18.2% (2) between days 26 to 30. No *Isospora sp.* excretion was diagnosed in camel calves older than 30 days in Mgmt R.

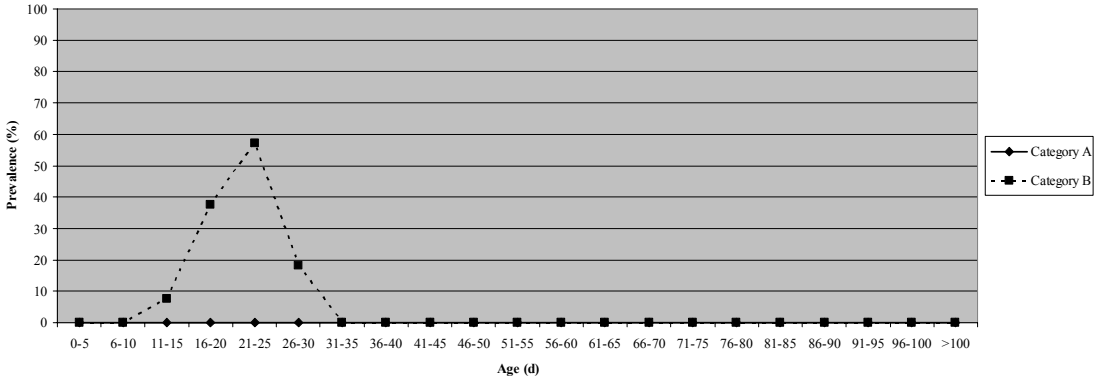

Figure 21: Age prevalence of *Isospora sp.* infection in camel calves falling under Category A and B

Refer to Annex 9.9 for details.

Infection with *Strongyloides sp.*

Infection with *Stronyloides sp.* was found in camel calves belonging to both categories. In 18 samples (11.5%) and in 15 samples (12.5%) *Strongyloides sp.* eggs were found in Category A and Category B, respectively.

The prevalence of the infection tended to increase with age. However, the prevalence was higher in camel calves belonging to Category B. In Category A, *Strongyloides sp*. infection were diagnosed within the first and third week of age (8.3% (1) between day one and five and 14.3% (1) between day eleven and 15 while it occurred in calves belonging to Category B only after the sixth week of age between day 36 and 40 with 25% (1). The prevalence of *Strongyloides sp.* infection increased slowly in Category A and showed a mild peak between days 41 and 45 (16.7% (1)), day 76 and 80 (28.6% (2)) and a major one between day 91 and 95 with 62.5% (5).

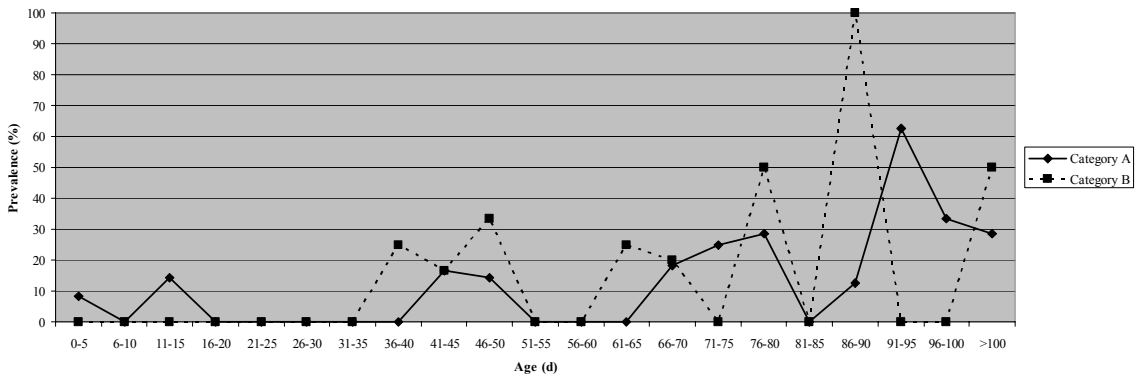

Figure 22: Age prevalence of *Strongyloides sp.* infection in camel calves compared between Category A and B

The increase of *Strongyloides sp.* infection in calves belonging to Category B was slow in the beginning (33.3% (1) between day 46 and 50, 25% (1) between day 61 and 65) but exploded after day 76 to 80 from 50% (1) to a prevalence of 100% (3) at an age from day 86 and above.

Infection with *Strongyloides sp*. clearly affected camel calves at an older age, beginning slowly (after day 36) and increasing impressively after the eleventh week. The infection could occur in any camel calf no matter in which health condition it was found. However the prevalence was higher in calves being either diseased with diarrhoea or convalescent.

Refer to Annex 9.10 for details.

Infection with *Strongyle sp.*

Infection with *Strongyle sp.* eggs were diagnosed from camel calves belonging to both categories with 21 (13.4%) out of 157 and 26 (21.7%) out of 120 analysed samples in Category A and B, respectively.

Strongyle sp. eggs were found in the faeces of healthy calves with no symptoms of diarrhoea at an early age of eleven to 15 days (14.3% (1)). However, the prevalence of this infection remained low throughout the first five weeks of age. A high prevalence was seen between day 36 and 40 (75% (3)) and from day 76 to 80 (57.1% (4)). Hence, infection with *Strongyle sp.* occurred constantly after day 36 and 40 with a changing prevalence throughout.

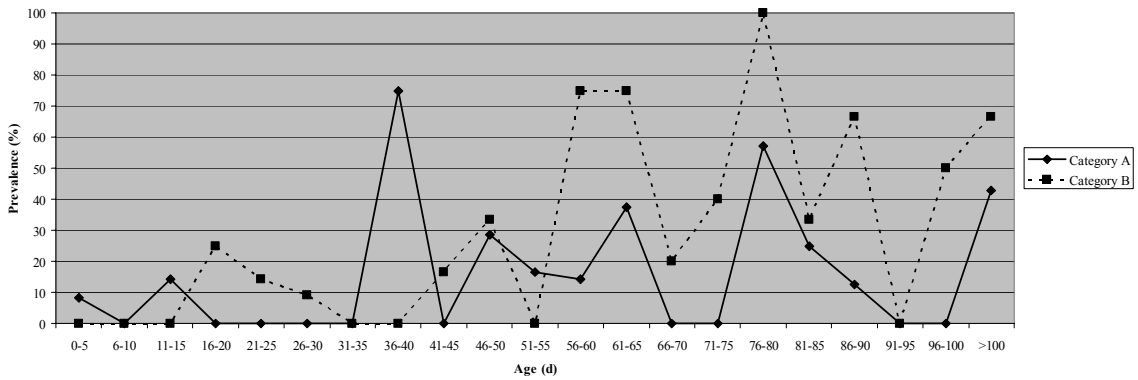

Figure 23: Age prevalence of *Strongyle sp.* infection in camel calves compared between Category A and B

Camel calves belonging to Category B started shedding *Strongyle sp.* eggs within the third week of age (day 16 to 20, 25% (2)). A clear increase of the prevalence with *Strongyle sp.* infection could be seen from day 41 to 45 (16.7% (1)) onwards, with peaks between day 56 and 65 (both 75% (both 3)) and a peak of 100% (2) between day 76 and 80. The prevalence of *Strongyle sp.* infection was clearly higher in camel calves represented in Category B than in Category A.

Refer to Annex 9.11 for details.

4.2.3 Bacteriological Findings

4.2.3.1 Bacterial pathogens

The following bacterial pathogens were isolated from calves belonging to Mgmt R:

Klebsiella pneumoniae sp. pneumoniae

Salmonella sp.

Escherichia coli

4.2.3.2 Prevalence of bacterial infection according to age group

Infection with *Klebsiella pneumoniae*

In the longitudinal study the prevalence of intestinal infection with *K. pneumoniae* was high in the first three weeks of age (37.5 % (6) between day 0 to 5, 50 % (5) day 6 to 10, 36.4 % (4) days 11-15 and 50 % (4) day 16 to 20). It declined until the tenth week of age (day 66 to 70). The prevalence varied from 13.3 % (2) day 26 to 30, 22.2 % (2) day 31 to 40, 20 % (2) day 41 to 45, 12.5 % (1) day 46 to 50 and 14.3 % (1) day 51 to 55. A *K. pneumoniae* positive calf was found at an age between 66 to 70 days representing 20 % (1) prevalence. No *K. pneumoniae* positive camel calves were found in the age group between 21 to 25 days, 56 to 65 days and particularly from day 71 onwards. This indicates that *K. pneumoniae* was more prevalent in very young calves during their first ten weeks of age and less common in older camel calves (see Figure 24 below).

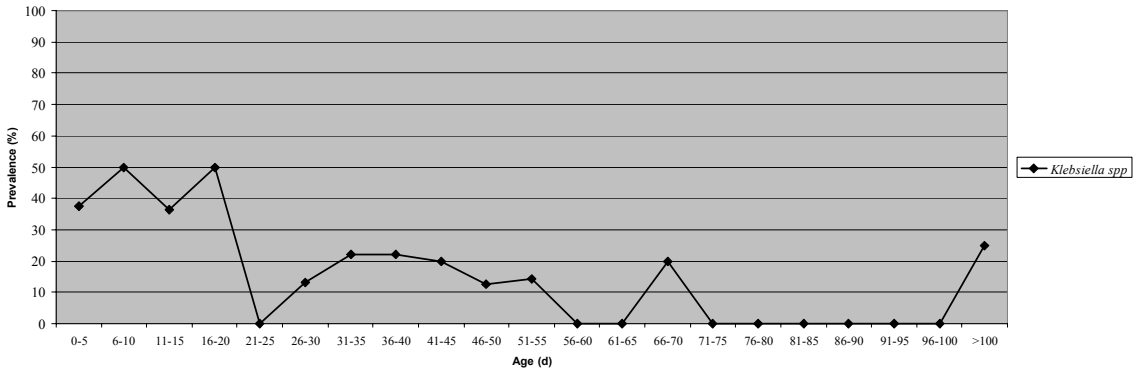

Figure 24: Prevalence of *K. pneumoniae* in camel calves according to their age

Refer to Annex 9.14 for details.

Infection with *Salmonella sp.*

Salmonella sp. prevalence in camel calves was high with the beginning of the second week of age. Starting with a prevalence of 26.9 % (7) in the first five days, it increased continuously to 28 % (7), 43.5 % (10), 64.7 % (11) and 66.7 % (8) between day 6 to 10, 11 to 15, 16 to 20 and 21 to 25, respectively. Even though another two peaks were seen at an age between 41 to 45 days (60 % (9)) and 71 to 75 days (40 % (4)), the prevalence of *Salmonella* sp. infection declined till day 86 to 90 with 50 % (8) day 31 to 35, 30 % (3) day 46 to 50, 16.7 % (2) day 56 to 60, 17.6 % (3) day 66 to 70, 10 % (1) day 76 to 80 and 8.3 % (1) day 81 to 85. A final increase was seen after day 100 with 30.8% (4).

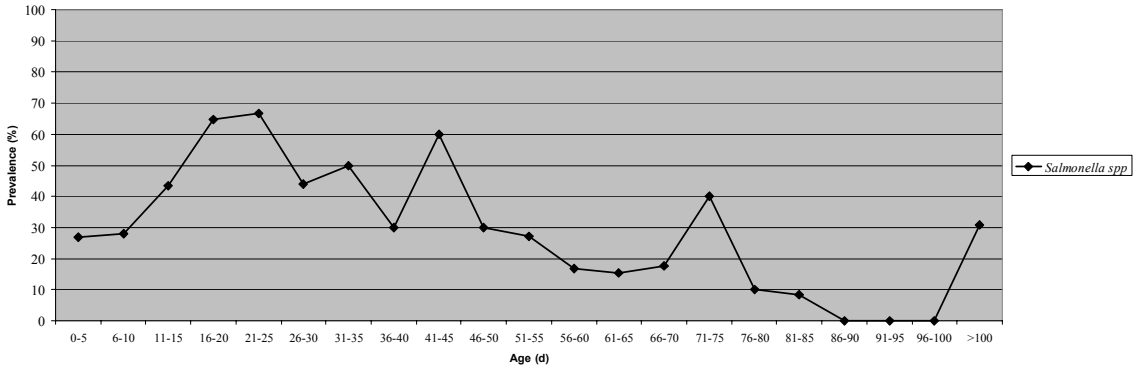

Figure 25: Prevalence of *Salmonella sp.* in camel calves according to their age group

Refer to Annex 9.15 for details.

Infection with *E. coli*

Escherichia coli infection was present in almost all camel calves throughout all age groups without variation in the prevalence.

4.2.3.3 *Comparison of the age prevalence of* Klebsiella pneumoniae, Salmonella sp. *and* E. coli. *according to Category A and B*

In category A, a total of 88 out of 181 samples were analysed for *Klebsiella pneumoniae*, 176 for *Salmonella sp.* and 162 for *E. coli*.

In category B, 79 out of 142 samples were analysed for *Klebsiella pneumoniae*, 133 for *Salmonella sp.* and 126 for *E. coli*.

Table 18: Number and percentage of *Klebsiella pneumoniae., Salmonella sp.* and *E. coli* excretion rate compared between Category A and B

	Category A				Category B			
	Negative	%	**Positive**	**%**	Negative	%	**Positive**	**%**
Klebsiella pneumoniae	77	87.5	**11**	**12.5**	59	74.7	**20**	**25.3**
Salmonella sp.	136	77.3	**40**	**22.7**	75	56.4	**58**	**43.6**
E. coli	2	1.2	**161**	**98.8**	4	3.2	**122**	**96.8**

Infection with *Klebsiella pneumoniae*

Comparing the two categories, there was a higher prevalence of *K. pneumoniae* infection in camel calves falling into Category B (25.3 % positive) than into Category A (12.5% positive).

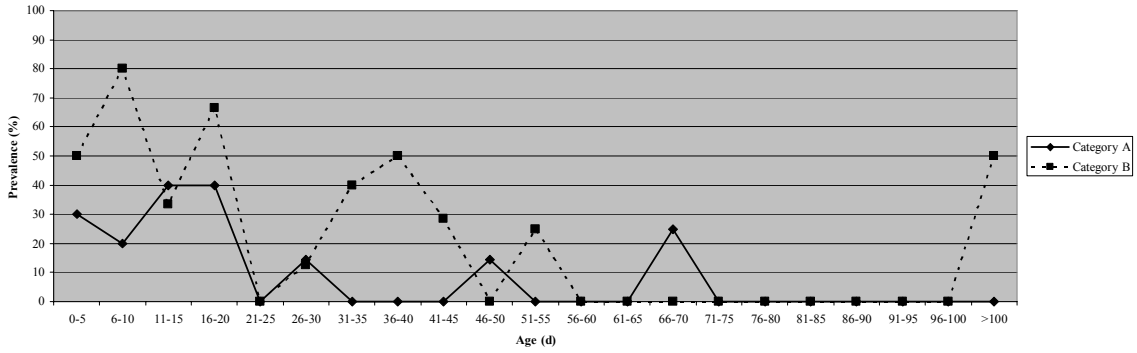

Figure 26: Age prevalence of *Klebsiella pneumoniae* infection in camel calves compared between Category A and B

In Category B, in the first five days of age the prevalence was at 50 % (3) and increased to 80 % (4) between days six and ten. From that age on the prevalence decreased constantly with peaks at day 16 to 20 with 66.7 % (2), day 36 to 40 with 50 % (2) and day 51 to 55 with 25 % (1).

In Category A, the prevalence was higher in the first 20 days with 30 % (3), 20 % (1), 40 % (2) and 40 % (2) between day 0 to 5, day 6 to 10, day 11 to 15 and day 16 to 20, respectively. The prevalence decreased in older camel calves to 14.3 % (1) at day 26 to 30 and day 46 to 50 was at 25 % (1) at day 66 to 70. See Annex 9.17 for more details.

Infection with *Salmonella sp.*

A total of 22.7 % (40 out of 176 samples) of the screened camel calf samples from Category A were positive for *Salmonella sp.* In Category B 43.6 % (58 out of 133 samples) of the faecal samples screened for *Salmonella sp.*, isolates were produced.

Similar to *K. pneumoniae*, the prevalence of *Salmonella* sp. was more prominent in calves belonging to Category B.

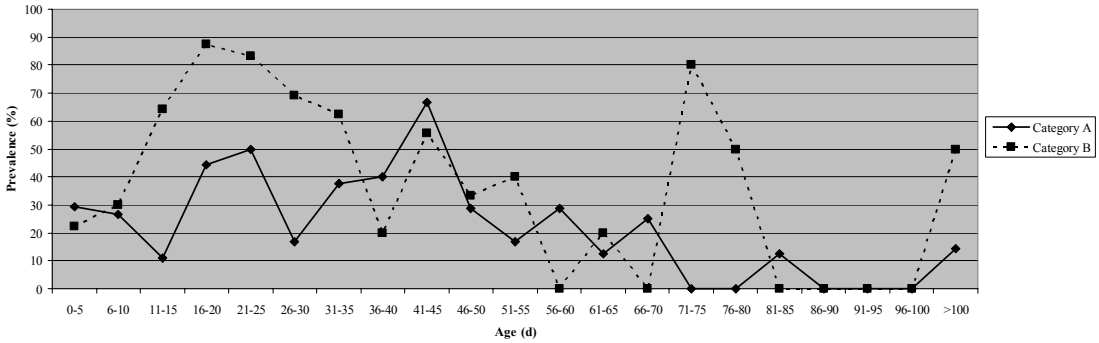

Figure 27: Age prevalence of *Salmonella sp.* infection in camel calves compared between Category A and B

There was a high prevalence of *Salmonella sp.* in Category B between day 11 to 15, 16 to 20, 21 to 25, 26 to 30 and 31 to 35, with 64.3 % (9), 87.5 % (7), 83.3 % (5), 69.2 % (9) and 62.5 % (5), respectively. After day 31 to 35 the prevalence pattern was undulating, decreased to 20 % (1) between day 61 and 65 and increased towards a peak between days 71 to 75 with 80 % (4).

The prevalence of *Salmonella* sp. in calves belonging to Category A started with 29.4 % (5) at day 0 to 5 and increased to a peak between day 41 and 45 with 66.7% (4). The prevalence in older camel calves decreased constantly with 16.7 % (1) between day 51 and 55, 12.5 % (1) between day 61 to 65 and day 81 to 85. Refer to Annex 9.18 for more details.

Infection with *E. coli*

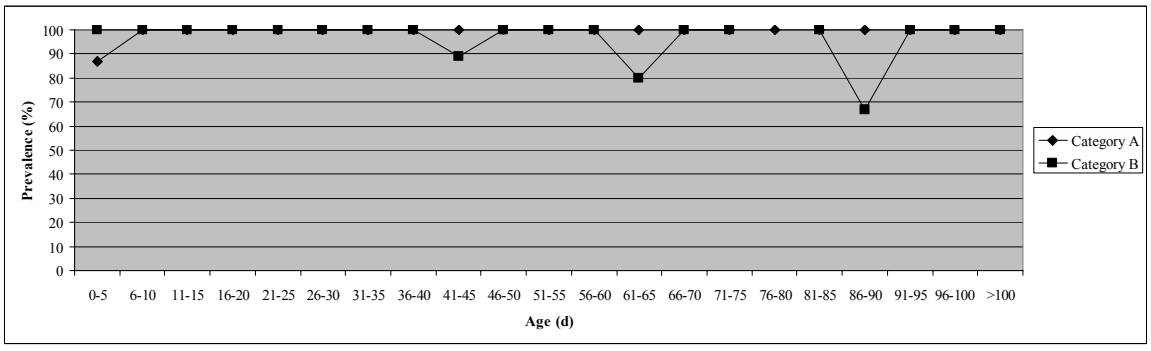

Figure 28: Age prevalence of *Escherichia coli* infection in camel calves compared between Category A and B

Out of the 163 samples from Category A screened for *E. c*oli only two samples were negative, while 98.8 % were positive. Only four (3.2%) of the samples taken in Category

B were *E. coli* negative. Refer to Annex 9.19 for more details.

As seen in the graph (Figure 28) above, there were no major differences in the prevalence of *E. coli* infection between the two categories. *E. coli* infection was present throughout the different age groups.

4.3 ADDITIONAL DIFFERENTIATION OF PATHOGENS

4.3.1 Isospora orlovi

Sequence analysis of the SSU rRNA gene and ITS 1 confirmed that the *Isospora* isolates from this study belonged to the species *I. orlovi*.

4.3.2 Klebsiella pneumoniae

4.3.2.1 Capsular typing of Klebsiella pneumoniae *isolations:*

Klebsiella pneumoniae infection was present in 32 camel calves. Out of these 32 camel calves a total of 62 *K. pneumoniae* were isolated, including multiple and repeat isolations. Of the 62 isolates, 23 (37.1 %) were from healthy, 28 (45.2 %) from diseased, two (3.2 %) from convalescent and nine (14.5 %) from autopsied camel calves aged from birth to twelve weeks.

47 of the 62 isolates were typed and the following 18 capsular antigens types identified:

K2 (n=4, 8.5%), K3 (n=1, 2.1%), K5 (n=2, 4.3%), K11 (n=1, 2.1%), K13 (n=3, 6.4%), K16 (n=1, 2.1%), K26 (n=1, 2.1%), K28 (n=4, 8.5%), K31 (n=1, 2.1%), K34 (n=3, 6.4%), K36 (n=1, 2.1%), K38 (n=2, 4.3%), K54 (n=4, 8.5%), K55 (n=1, 2.1%), K60 (n=6, 12.8%), K61 (n=4, 8.5%), K64 (n=1, 2.1%), K81 (n=1, 2.1%), six isolates were untypable (12.8%).

According to Chi-Square (Pearson), there was no significant relation ($p<0.05$, $p=0.18$) between health status and capsular type of *K. pneumoniae* present. However, the majority of *K. pneumoniae* isolates (n=39, 62.9%) originated from calves that had suffered from diarrhoea or were found dead (see Table 19).

Table 19: Spectrum of capsular types of *K. pneumoniae* in Kenyan camel calves up to twelve weeks of age in relation to their health status*

Capsular Type	No of isolates from calves being:				Total
	Healthy	Diseased	Convalescent	Dead	
2	1	**1**		**2**	**4**
3		**1**			**1**
5	2				**2**
11		**1**			**1**
13	2			**1**	**3**
16		**1**			**1**
26				**1**	**1**
28		**2**		**2**	**4**
31		**1**			**1**
34	1		2		**3**
36	1				**1**
38		**1**		**1**	**2**
54	2	**2**			**4**
55		**1**			**1**
60	3	**3**			**6**
61	3	**1**			**4**
64	1				**1**
81		**1**			**1**
Untypable	1	**4**		**1**	**6**
Not typed	6	**8**		**1**	**15**
Total	23 (37.1%)	**28 (45.2%)**	2 (3.2%)	**9 (14.5%)**	**62 (100%)**

* including multiple and repeat isolations

Table 20 below shows *K. pneumoniae* capsular types identified in the same individual camel calf during repeat sampling and isolations.

Table 20: Presence of *K. pneumoniae* capsular types in Kenyan camel calves up to twelve weeks of age at repeat sampling

Calf No	Location	Age (d) at sampling	No of *K. pneumoniae* isolated	Capsular Type	API 20E	Health status
c. 555	Ol Maisor	8	1	**54**	5215773	Healthy
		70	2	**2**	5215773	Dead
				2	5215773	
c. 576	Ol Maisor	11	1	-	5737773	Healthy
		16	2	**38**	1215773	Diseased
				-	5215773	
		42	1	**16**	5215773	Diseased
c. 588	Ol Maisor	35	1	-	5215773	Diseased
		40	2	-	1215773	Diseased
				-	5215773	
		44	1	**38**	5215773	Dead
c. 701	Ol Maisor	6	2	**5**	5215773	Healthy
				-	5215773	
		30	1	**60**	5215773	Diseased
c. no number	Kisima	8	1	**11**	5215773	Diseased
		33	1	-	5214773	Diseased
		51	2	**34**	5215773	Convalescent
				34	5215773	

4.3.2.2 Isolation of Klebsiella pneumoniae *in post mortem samples:*

A post mortem with subsequent bacteriological and parasitological analysis of autopsy specimen was possible in nine cases. In one third of the dead camel calves that had died *K. pneumoniae* was the only noteworthy pathogen present. Of the three *K. pneumoniae* positive camel calves case No 3 and 8 (see Table 22 below) had suffered from diarrhoea for five days prior to death. Case No 1 had a history of meconium retention and respiratory distress.

Capsular type 28 and type 2 were involved in cases No 3 and No 8, while in case No 1, no capsular typing was carried out. The camel calves were 17 days (No 3), 70 days (No 8) and six days (No 1) old.

In autopsy case No 7 *K. pneumoniae* capsular type 38 was isolated from the faeces of a 44 day old camel calf and *S. bovismorbificans* lysotype 6,8:r:1,5 was isolated from the liver. However, the clinical history of this calf was not known.

Bacteriological results from post mortem samples are summarised in Table 21.

Table 21: Pathological findings of the nine post mortem cases in Kenyan camel calves up to twelve weeks of age

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

Case No.	Age (d)	Clinical history	Specimen	Pathogen isolated	Capsular type /serovar/virulence gene identified	Pathogenic significance of isolate
		with Stop Bloat				
7	44	No history given	Liver	*S. bovismorbificans*	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
			Spleen	*S. irumu*	Lysotype 6,7:I,v:1,5	Unclear
			Joints	*S. bovis-morbificans*	Lysotype 6,8:r:1,5	Relevant

4.3.2.3 Antibiotic sensitivity of Klebsiella pneumoniae *isolated:*

As shown in Table 22, *K. pneumoniae* isolates were highly resistant to Amoxicillin (100%), Sulphonamide-TMP (78%) and Tetracycline (61%). However, they showed sensitivity towards Streptomycin.

Table 22: Antibiotic sensitivity of *K. pneumoniae* isolated from Kenyan camel calves up to twelve weeks of age

Antibiotic tested (n=41 isolates)	Amoxycillin	Sulphonamide-TMP	Tetracycline	Streptomycin
Isolates showing resistance (n = 41)	41 (100%)	32 (78.0%)	25 (61.0%)*	4 (9.8%)

- Of four autopsy isolates only one was sensitive to Tetracycline.

4.3.3 *Salmonella* sp.

4.3.3.1 Sample size

A total of 144 *Salmonella sp.* were isolated during the study period and sent to the German National Salmonella Reference Laboratory in Marienfelde[7] for sero-typing.

The following *Salmonella sp*. were identified:

Table 23: *Salmonella* serotypes found in Kenyan camel calves

Salmonella sp.	Number of Isolates	Percentage (%)
S. bovismorbificans	47	32.6 %
S. butantan	31	21.5 %
S. typhimurium	16	11.1 %
S. kiambu	13	9.0 %
S. muenchen	11	7.6 %
S. fayed	4	2.8 %
S. subspecies I	4	2.8 %
S. subspecies IIIb	4	2.8 %
S. Poona	3	2.1 %
S. adelaide	2	1.4 %
S. milwaukee	2	1.4 %
S. derby	1	0.7 %
S. enteritidis	1	0.7 %
S. Havana	1	0.7 %
S. irumu	1	0.7 %
S. montevideo	1	0.7 %
S. schwarzengrund	1	0.7 %
S. of group E1	1	0.7 %
TOTAL	**144**	**100 %**

[7] BfR, Nationales Salmonellenreferenzlabor, Diedersdorferweg 1, 12277 Berlin-Marienfelde, Germany

4.3.3.2 Management system

Out of the 144 *Salmonella sp.* isolated 124 (86.11 %) were from calves belonging to Mgmt R while 20 (13.89 %) were isolated from camel calves kept in Mgmt P. ***Salmonella typhimurium*** and ***S. adelaide*** were the only two serotypes found in both management systems. *Salmonella bovismorbificans, S. butantan, S. kiambu, S. muenchen, S. subspecies IIIb, S. derby, S. enteritidis, S. irumu* and *Salmonella of group E 1* were only isolated from camel calves belonging to Mgmt R. *Salmonella fayed, S. subspecies I, S. poona, S. milwaukee, S. havana, S. montevideo* and *S. schwarzengrund* were only isolated from camel calves kept under Mgmt P.

Table 24: Comparison of *Salmonella* serotypes found in the two management systems

Salmonella sp.	**Management R**		**Management P**		**Total # of**
	n	%	n	%	n
S. bovismorbificans	47	100 %	0		47
S. butantan	31	100 %	0		31
S. typhimurium	**13**	**81 %**	**3**	**19 %**	**16**
S. kiambu	13	100 %	0		13
S. muenchen	11	100 %	0		11
S. fayed	0		4	100 %	4
S. subspecies I	0		4	100 %	4
S. subspecies IIIb	4	100 %	0		4
S. Poona	0		3	100 %	3
S. adelaide	**1**	**50 %**	**1**	**50 %**	**2**
S. milwaukee	0		2	100 %	2
S. derby	1	100 %	0		1
S. enteritidis	1	100 %	0		1
S. havana	0		1	100 %	1
S. irumu	1	100 %	0		1
S. montevideo	0		1	100 %	1
S. schwarzengrund	0		1	100 %	1
S. of group E1	1	100 %	0		1
TOTAL	**124**	**86 %**	**20**	**14 %**	**144**

4.3.3.3 Health status

Comparing the distribution of *Salmonella sp.* according to health status a total of 56 (38.9 %), 45 (31.3 %), 32 (22.2 %) and 11 (7.6 %) originated from healthy, diseased, convalescent and dead camel calves, respectively.

The only *Salmonella sp*. associated commonly (81%) with calf diarrhoea and present in camel calves of both management systems was *S. typhimurium* (see Annex 9.20).

4.3.4 Escherichia coli

4.3.4.1 Sample size

Out of 531 *E. coli* isolates, a total of 255 were analysed for virulence-associated genes such as *eae*, *hlyEHEC*, *stx, elt I1/Ib, est Ia/Ib* and *astA*. In 78 isolates virulence-associated genes were detected.

A total of 35 isolates were found positive for *eae* (13.7%), 27 for *astA* (10.6%), 11 for *hlyEHEC* (4.3%) and five for *stx* (2.0%). None of the isolates was found positive for *elt Ia/Ib* and *est Ia/Ib*.

4.3.4.2 Management system

213 isolates originated from calves belonging to Mgmt R (83.5%) and 42 isolates from calves kept under Mgmt P (16.5%).

While *eae* and *astA* could be detected in *E. coli* isolates from calves belonging to both management systems, *hlyEHEC* and *stx* was only found in isolates from calves belonging to Mgmt R.

The majority (92%) of the virulence-associated genes were found in camel calves kept in MgmtR (see Table 26).

Table 25: Detection of various *E. coli* virulence-associated genes according to the two management systems

Virulence-associated genes isolated	Mgmt R		Mgmt P		Total	
Eae	**31**	**(40%)**	**4**	**(5%)**	**35**	**(45%)**
astA	**25**	**(32%)**	**2**	**(3%)**	**27**	**(35%)**
hlyEHEC	**11**	**(14%)**	**0**		**11**	**(14%)**
Stx	**5**	**(6%)**	**0**		**5**	**(6%)**
Elt Ia/Ib	**0**		**0**		**0**	
Est Ia/Ib	**0**		**0**		**0**	
TOTAL	**72**	**(92%)**	**6**	**(8%)**	**78**	**(100%)**

4.3.4.3 Health status in relation to virulence-associated genes

Out of the 255 isolates, 146 (57.3%) were from healthy calves, 55 (21.6%) from calves suffering from diarrhoea, 51 (20%) from convalescent calves and three (1.2%) from dead calves.

According to Pearson-Chi-Square ($p<0.05$), there was no significant relationship between the health status and the detection of *eae* ($p=0.051$), *astA* ($p=0.100$), *hlyEHEC* ($p=0.572$) and *stx* ($p=0.655$) in the examined *E. coli* isolates. These virulence-associated genes were equally found in camels being healthy, with diarrhoea or convalescent. None of the virulence-associated genes could be detected in dead camel calves (see Table 27).

Table 26: Isolation of *E. coli* virulence-associated genes according to health status in camel calves

	eae		*astA*		*hlyEHEC*		*stx*		*TOTAL*	
	n	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
(1) Healthy	16	45.7	19	70.4	6	54.5	4	80	45	57.7
(2) Diarrhoea	6		1		4		0		11	
(3) Convalescent	13	54.3	7	29.6	1	45.5	1	20	22	42.3
(4) Dead	0		0		0		0		0	
TOTAL	**35**	**100**	**27**	**100**	**11**	**100**	**5**	**100**	**78**	**100**

More than half (57.7 %) of the virulence-associated genes were found in *E. coli* isolated from healthy carriers. There was no indication that *E. coli* play an important role in camel calf diarrhoea up to 12 weeks of age.

4.3.4.4 Detection of virulence associates genes according to age (first to 12th week of age)

The virulence-associated genes were found throughout the studied age groups. The mean age of camel calves carrying *E. coli* positive for virulence-associated genes varied between 40 days, 45 days, 34 days and 40 days for *eae, astA, hlyEHEC* and *stx*, respectively. The median was at 40 days, 54 days, 30 days and 38 days for *eae, astA, hlyEHEC* and *stx* in that order (see Annex 9.21).

Table 27: Mean, maximum, minimum, median, 25. and 75. percentile age of camel calves excreting *E. coli* with the detected virulence-associated genes

Virulence-associated gene		**Mean**	**Max.**	**Min.**	**Median**	**25. Percentile**	**75. Percentile**
Eae	**Neg.**	36	84	0	31	14	58
	Pos.	**40**	**75**	**3**	**40**	**27**	**54**
astA	**Neg.**	35	84	0	30	14	56
	Pos.	**45**	**80**	**1**	**54**	**27**	**62**
hlyEHEC	**Neg.**	36	84	0	33	14	58
	Pos.	**34**	**73**	**14**	**30**	**26**	**38**
Stx	**Neg.**	36	84	0	32	14	57
	Pos.	**40**	**60**	**26**	**38**	**30**	**50**

4.3.4.5 E. coli serotype O157

E. coli serotype O157:H7 was not isolated from the camel calves in the studied age group.

5 DISCUSSION

This study is the first one to comprehensively study the relative importance of bacterial and protozoan pathogens in the aetiology of camel calf diarrhoea.

5.1 Isospora orlovi

In the present study *Isospora* sp. was the most important pathogen found in the diarrhoea complex of camel calves.

Isospora sp. was regularly found in camel calves of the studied age group. The fact that almost every fourth calf (23%) suffering from diarrhoea was shedding *Isospora* sp. oocysts and that *Isospora* sp. only occurred in camel calves showing symptoms of diarrhoea (except for one convalescent case) reflects the importance of this pathogen in the diarrhoea complex of camel calves in the studied age group. This contradicts the previous findings of authors who hypothized that *Isospora* sp. found in camels belonged to wild birds and was only an accidental gut passenger (Pellérdy, 1974; Daruish and Golemansky, 1993). This study confirms the opinion of Raisinghani et al. (1987), who were the first ones to stress on the importance of clinical coccidiosis caused by *Isospora* sp. in Indian camels. Younan et al. (2002) and Kinne et al. (2002) both describe the involvement of *Isospora* sp. in the diarrhoea complex of camel calves in Kenya and the UAE, respectively. However, this is the first study that demonstrates the regular involvement of *Isospora* sp. in the aetiology of camel calf diarrhoea.

Isospora sp. infection was more prominent in camel calves belonging to pastoralist herds (61.5%) as compared to camel calves belonging to ranch herds (38.5%). *Isospora* sp. is known to be a stress-related disease agent (Steward and Soll, 1994). The competition between man and the camel calf for camel milk and the resulting stress for the calf due to milk deprivation might be a contributing factor to the higher prevalence of *Isospora* sp. in camel calves belonging to pastoralist herds. This was also reflected in the age distribution of the pathogen: *Isospora* sp. was found over a longer period in camel calves belonging to Mgmt P as compared to Mgmt R.

Isospora sp. excretion started within the second week of age with a peak between the third and fifth week of age. No *Isospora* sp. excretion was detected after week eight. This is similar to the findings of other authors whereby *Isospora* sp. positive camel calves were aged between two and eight weeks (Pellérdy, 1974, Younan et al., 2002, Kinne et al., 2002). The only exception is a case study by Raisinghani et al. (1987) who describe a camel calf suffering from diarrhoea and exhibiting *Isospora* sp. at six months of age. Thus, *Isospora* sp. might still occur beyond the eighth week but at significantly lower excretion density.

Table 28: *Isospora sp.* in camels, occurrence and age

Name	Sample size (n)	N# of samples positive for *Isospora* sp.	Min age	Max age
Tsygankov described by Pellérdy, 1974	19	10 (52.6%)	10 days (2nd week)	35 days (5th week)
Raisinghani et al., 1987	1	1	6 month (24th week)	6 month (24th week)
Younan et al., 2002	N/A	5	18 days (3rd week)	32 days (5th week)
Kinne et al., 2002	22	8 (36.4%)	21 to 28 days (4th week)	49 t 56 days (8th week)
Point prevalence study	197	13 (6.6%)	14 days (2nd week)	49 days (8th week)
Longitudinal study	277	12 (4.3%)	15 days (3rd week)	30 days (5th week)

The source of *Isospora* sp. infection for the newborn is latent carriers, which can shed the oocysts for months (Bowman, 1999). In piglets the infection with *I. suis* is maintained through contaminated farrowing crates (Lindsay et al., 1997). The "boma", where camels are kept at night most likely plays the same role as a stable. During its first week of life, the newborn camel calf spends almost all its time in the "boma", sufficient to be massively exposed to *Isospora* sp. infection. There might be a build-up of infection pressure in the calf environment ("boma") during the calving season leading to higher infection levels as the calving seasons carries on.

Sequence analysis of the SSU rRNA gene and ITS 1 confirmed that the Kenyan *Isospora* sp. isolates of this study belonged to the species *I. orlovi* and that the sequences were identical to Dubai isolates. Hence, it is confirmed that *I. orlovi* is a true camel pathogen in both the United Arab Emirates and Kenya (Morrison et al., 2004). *Isospora orlovi* most likely also plays an important role as a pathogen in the diarrhoea complex of the camel calf in other camel keeping countries of Africa and Asia.

5.2 *Salmonella* sp. and *Klebsiella pneumoniae*

The present study shows that *Salmonella* sp. and *Klebsiella pneumoniae* were common pathogens found in the diarrhoea complex of camel calves but might play a more important role in septicaemia of camel calves in the studied age group.

There seems to be an aetiological involvement of *Salmonella* sp. and *Klebsiella pneumoniae* in the diarrhoea complex of the studied camel calves. The point prevalence of *Salmonella* sp. and *Klebsiella pneumoniae* in camel calves suffering from diarrhoea was found to be 37.8% and 37.5%, respectively. However the majority (53.3% and

53.1% for *Salmonella* spp and *K. pneumoniae*, respectively) of positive samples were isolated from healthy camel calves. There was not significant relation between the health status of the sampled camel calves and the isolation of *Salmonella* sp. and/or *K. pneumoniae*.

In the longitudinal study the prevalence was higher (with 43.6% for *Salmonella* sp. and 25.3% for *K. pneumoniae*) in camel calves that were showing diarrhoea at least once during the observation period (Category B) than in those calves that remained healthy throughout (Category A) (22.7% and 12.5% for *Salmonella* spp and *K. pneumoniae*, in that order).

Salmonella prevalence from diarrhoeic camel calves reported in literature is by comparison much lower: The isolation rate of *Salmonella* sp. in one to ten weeks old diarrhoeic camel calves in the Moroccan Sahara was 13.6% (Berrada et al., 2000), while Salih et al. (1998) found 13% of diarrhoeic camel calves (birth till three months of age) *Salmonella* sp. positive in the Butana Region of Sudan. The sensitivity of the isolation method used may account for some of the differences.

This study reveals that there is a change in the prevalence of *Salmonella* sp. according to age and that it was especially high in the third week of age (54.5% point prevalence and 66.7% cumulative prevalence, respectively). The literature only shows that *Salmonella* sp. was present in camels of all ages (Berrada et al., 2000; Salih et al., 1998; Wernery, 1992), however to the author's knowledge this longitudinal study is the first one to describe the prevalence of *Salmonella* sp. in camel calves according to age.

Although the presence of *Klebsiella pneumoniae* in camels has been recognized in adult camels (Arora and Kalra, 1973) and diarrhoeic camel calves (Dia et al., 2000), this study is the first one to describe the commensal role of *K. pneumoniae* and its involvement in camel calf septicaemia. The age distribution of *K. pneumoniae* positive calves in the point prevalence study was comparable to the findings for *Salmonella* sp. and *Isospora* sp. *Klebsiella pneumoniae* was most prominent in the first three weeks of age, with peaks in week two (47.6%) and three (46.2%). The longitudinal study confirmed and specified the age distribution of *K. pneumoniae* in the studied group. *K. pneumoniae* was most prevalent in the first ten days of age (37.5% day 0 to 5 and 50% day 6 to 10) and less prevalent in older camel calves. However, *K. pneumoniae* was still isolated from older calves, which is a clear indication of its continuous saprophytic presence and excretion. It seems that the infection occurred at an early stage and calves become carriers and shed *K. pneumoniae* for a long time. The absence of *K. pneumoniae* isolations from faecal swabs in calves older than five weeks most likely reflects the limited sensitivity of the isolation method. But it also indicates a considerably lower presence of *K. pneumoniae* in the intestines of older calves.

5.3 *Salmonella* serotypes

This is the first study to describe the serotypes of *Salmonella* sp. found in Kenya. According to Faye (1997) the most important *Salmonella* serotypes in camels are *S. typhimurium, S. enteritidis, S. kentucky* and *S. saint-paul*. Wernery and Kaaden (2002) describe 69 different *Salmonella* serotypes that have been found in camels from various

countries so far, among them serotypes such as *S. typhimurium, S. bovismorbificans, S. muenchen, S. derby* and *S. enteritidis,* serotypes that were isolated in the present study as well.

In camel calves of the investigated age group *S. bovismorbificans* (32.6%) was the most common *Salmonella* serotype followed by *S. butantan* (21.5%), *S. typhimurium* (11.1%) *S. kiambu* (9.0%) and *S. muenchen* (7.6%).

The close contact between camels and other livestock such as cattle may explain the higher isolation rate of different *Salmonella* serotypes in camels kept on ranches. Camels belonging to pastoralists were usually kept separate from the other livestock species and migrate long distances, hence were less exposed. *Salmonella bovismorbificans* and *S. butantan* among eight other strains were only found in camel calves kept on ranches. Both mentioned serotypes have been isolated from camels in slaughterhouses in Ethiopia, however with a low prevalence rate, while *S. saintpaul* and *S. braenderup* were the most prevalent (Molla et al., 2003; Molla et al., 2004). Berrada et al. (2000) found four different *Salmonella* serotypes from camel calves suffering from diarrhoea in southern Morocco including *S. enteritidis* which was isolated only from one healthy calf in the present study.

In this study, only *S. typhimurium* and *S. adelaide* were isolated from calves in both management systems. It is important to point out that the majority of camel calves (75%) that were found positive for *S. typhimurium* were suffering from diarrhoea.

Salmonella bovismorbificans, *S. butantan* and *S. irumu* were isolated from post mortem cases. *Salmonella bovismorbificans* was the only relevant pathogen in two cases out of nine post mortems. Septicaemia in camel calves caused by *Salmonella* sp. has been described by Wernery and Kaaden (2002), however the serotypes involved were only given for llamas (*S. cholera-suis* and *S. typhimurium*).

5.4 *Klebsiella pneumoniae* capsular types

18 different capsular antigen types were identified out of 62 *K. pneumoniae* isolates from 32 individual camel calves with K60 (12.8%) being the most common. According to Podschun and Ullmann (1998) and Euzéby (2004) capsular type 1 and 2 are the most pathogenic types followed by 3 and 4. While capsular type 1 and 4 were not identified in this study, capsular type 2 and 3 were found in 8.5% and 2.1% of the 62 isolates, in that order. Two isolates of capsular type 2 were from a dead camel calf, where they were the only significant pathogen found. It can be concluded that this calf died of septicaemia caused by *K. pneumoniae* capsular type 2. The capsular type 3 isolate was isolated from a diseased calf.

To the author's knowledge, apart from capsular type 11 which was isolated from dead adult camels suffering from a respiratory disease (Arora and Kalra, 1973), none of the capsular types identified in this study have been described in camels before.

The sensitivity tests for the *K. pneumoniae* isolates showed a 61% resistance of the pathogen against Tetracycline. As Tetracycline is the most common antibiotic used by camel herders in the studied areas, failure in the treatment of septicaemia in camel calves must be common.

5.5 Escherichia coli

The present study reveals that *E. coli* does not play a prominent role as a pathogen in the diarrhoea complex of camel calves in the studied age group.

Escherichia coli was present in all camel calves throughout all age groups without significant prevalence variation and was never isolated as the only significant pathogen in the investigated post mortem cases (n=9).

This is the first study to analyse the virulence-associated genes of *E. coli* isolates from camel calves.

A total of 35 *E. coli* isolates were found positive for *eae* (13.7%), 27 for *astA* (10.6%), 11 for *hlyEHEC* (4.3%) and five for *stx* (2.0%) as virulence-associated genes of *E. coli*. None of the isolates was found positive for *elt Ia/Ib* and *est Ia/Ib*. More than half (57.7 %) of the virulence-associated genes were found in *E. coli* isolated from healthy carriers. The exception is *eae* which was mainly found in diarrhoeic and convalescent camel calves. *Eae* is found in *E. coli* O157:H7, a highly pathogenic strain of *E. coli* both in humans and animals. However, *E. coli* O157:H7 was not isolated from the studied group. There was no statistically significant relationship between the health status and the detection of *eae, astA, hlyEHEC* and *stx* in the *E. coli* isolates. The fact that only very few *E. coli* with virulence-associated genes (14%) were isolated from diarrhoeic camel calves and none from dead calves indicates that pathogenic *E. coli* do not play a prominent role in the diarrhoea complex of camel calves in the studied age group. This contradicts reports of other authors that regarded *E. coli* as major pathogen with 30% morbidity and 100% mortality rate (Schwartz and Dioli, 1992). These claims were only based on empiric field data and not backed up by any diagnostic investigations into the aetiology. The presented data on virulent *E. coli* also differ with the findings of Wernery and Kaaden (2002) who state that *E. coli* infection in camel calves starts later (four weeks of age) than in other livestock species such as cattle. In a review made by Abbas and Omer (2005) *E. coli* is quoted to be the major cause of diarrhoea in camel calves around the first week of life which differs with the present findings. Berrada et al. (2000) conclude that colibacillosis is one of the most important causes of diarrhoea in new born camel calves. All three cited studies, however, did not include the identification of virulence-associated genes in *E. coli*.

Surprisingly the vast majority of *E. coli* with virulence-associated genes was found in camel calves kept on ranches in Mgmt R (92%). Again the close contact between the camels and other ranch livestock species such as cattle and small ruminants might explain this striking difference. Cattle and small ruminant are known to harbour *E. coli* with virulence-associated genes.

Due to the use of the molecular differentiation of *E. coli* pathotypes used in this study a clear statement can be made that *E. coli* does not play an important role as a pathogen in the diarrhoea complex of camel calves in the studied age group.

Escherichia coli was also not found to be a common case of septicaemia in camel calves. But the number of the post mortem samples was very low in the present study and the role of *E. coli* as a cause of septicaemia in camel calves should be investigated further. Results of author authors (Bornstein et al., 2000, Wernery and Kaaden, 2002), describe septicaemia in camel calves caused by *E. coli,* but did not investigate the presence of virulence-associated genes.

5.6 Analysis of dead camel calves

The number of camel calf deaths presented in this study is extremely low. Still, the nine examined calves represented a miniature cross-section of deaths in the same age group, which was screened for presence of *Klebsiella* in the intestine. One third of the calf deaths examined were related to septicaemic *K. pneumoniae* infection, the same number as for *Salmonella sp.*. Debilitation due to diarrhoea appeared to be a contributing factor in these *Klebsiella* related septicaemia deaths.

Case No. 8 is of particular interest as only the demonstrated absence of virulence-associated genes in the isolated *E. coli* allowed for a conclusive bacteriological diagnosis (*K. pneumoniae* capsular type 2 septicaemia). The isolation of *K. pneumoniae* capsular type 2 from lungs and kidney of this camel calf is noteworthy as this capsular type is predominant in human clinical *Klebsiella* infections worldwide. Capsular type 2 is considered to be a very virulent serotype and is rarely encountered in the environment (Podschun and Ullmann, 1998). *Klebsiella pneumoniae* as causative agent of calf septicaemia in camels is comparable to the importance of the pathogen in horses. Septicaemia of foals is common. *Klebsiella pneumoniae* was isolated in every fifth (23%) case in a study carried out by Wilson and Madigan (1989). The authors describe the latent infected mare as main source of infection. The role of the mother camel as source of *K. pneumoniae* infection for the camel calf might be similar but remains to be investigated.

Calf No 1 had a history of meconium retention and respiratory symptoms from birth and died on the sixth day. This case showed some resemblance with newborn foals infected by *K. pneumoniae* intra-partum. Meconium retention is regularly seen in newborn camel calves (Koehler-Rollefson et al., 2001). Whether *K. pneumoniae* is involved in the aetiology of meconium retention in neonate camels remains to be seen.

An interesting observation is the absence of virulent *E. coli* in sample material from the dead camel calves. In neonatal equine foals pure *E. coli* septicaemia without concomitant involvement of *K. pneumoniae* or *Actinobacillus equuli* is rather exceptional (Dietz and Wiesner 1982). A comparable situation with regards to *E. coli* may exist in neonatal camel calves.

This study confirms reports from other camel keeping regions on the major importance of *Salmonella sp.* in septicaemia of young camel calves. The dominance of *Salmonella bovismorbificans* serovar 6,8:r:1,5 in the investigated deaths 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.

Earlier reports on *E. coli* infections causing death and septicaemia in young Kenyan camel calves were based on field observations and lacked laboratory confirmation (Schwartz and Dioli, 1992). Based on the presented findings one can conclude that the high mortalities in neonatal camel calves in Kenya ascribed to be *E. coli* infections (Schwartz and Dioli, 1992) may in fact be caused to a major part by *Salmonella sp.* and *K. pneumoniae*.

5.7 Helminths

Helminth infection (here with *Strongyloides* sp. and *Strongyle* sp.) did not play an important role in the diarrhoea complex of the investigated camel calf age group. Only in 8% of the diarrhoeic camel calves *Strongyloides* sp. eggs could be isolated while only four per cent of the calves were shedding *Strongyle* sp. eggs. The excretion for both parasites started with the sixth week of age, increasing constantly towards the eleventh week of age. *Strongyle* sp. eggs were also isolated from one camel calf in the second week of age. As camel calves become interested in their environment at this age they start to lick and taste plants and the ground (Kamoun, 1990). This might have lead to the ingestion of *Strongyle* sp. eggs shed by other camels, hence becoming an accidental gut passenger.

There was no difference in the age distribution or prevalence of *Strongyloides* sp. and *Strongyle* sp.between the two management systems during the point prevalence study.

Stronyle sp. eggs were more commonly isolated from calves belonging to Category B than *Stronyloides* sp. indicating a higher pathogenic role of *Strongyle* sp.. *Strongyloides* sp. was isolated in one camel calf aged 0-5 days and one camel calf aged 11-15 days. Both pathogens started to be prevalent in camel calves aged between 36-40 days, with a constant increase towards older age. Hence, *Strongyloides* sp. and *Strongyle* sp. become a contributing factor to the diarrhoea complex of camel calves after the sixth week of age, however with a very low prevalence up to twelve weeks of age.

5.8 Clinical findings of the Point Prevalence Study

Twenty three per cent of all camel calves investigated in the point prevalence study were suffering from diarrhoea at the time of sampling. This common occurrence of diarrhoea is in line with the findings of Kaufmann (1998) who describes diarrhoea among the three main diseases causing calf mortality in Northern Kenya. The 21.9 % prevalence of camel calf diarrhoea reported from Sudan (Agab and Abbass, 1999) is also comparable with the clinical observations of this study. The same authors stressed on the significance of diarrhoea as a cause of mortality and poor weight gain of camel calves and a contributing factor to slow herd growth in Sudan. In Niger, diarrhoea of camel calves is reported to have a morbidity of 80-90% and a mortality reaching 50% (Bada Alambedjir et al., 1992), rates which are much higher than the ones found in the present study. However, the data from Niger are purely based on herder interviews and represented an estimate rather than diagnostic findings. In Morocco, Bengoumi et al. (2000) describe a mortality rate of 20.2% in camel calves up to six months of age and diarrhoea is mentioned to

account for 72% of camel calf deaths.

The age distribution of diarrhoeic camel calves was similar in both management systems. Comparing clinical data from the two management systems investigated there was a higher prevalence of camel calves suffering from diarrhoea in Mgmt P (31.9%) than in Mgmt R (19.2%). To some extent these percentages may be an overestimate as herdsmen from Mgmt P preferred to present diseased camel calves to the researcher and cohort animals were not always available for every diseased calf sampled. However, due to the calf – human competition for milk in pastoralist camel herds, more calves suffer from stress and are more susceptible to diseases in general and diarrhoea in particular. These different stress levels are likely to account for part of the higher diarrhoea prevalence seen in Mgmt P as compared to Mgmt R. Kaufmann (1998) states that the competition between calf and man has an impact on calf development and growth but the difficulty remains in the quantification of this impact.

The age distribution of diarrhoeic camel calves clearly shows that diarrhoea prevalence is highest between the second and fourth weeks of age. High prevalence of diarrhoea during two to four weeks of age may be related to the decline of the passive immunity. The IgG blood level declines and reaches a very low level two weeks after birth, while the own antibody production does not start before the second week of age (Wernery, 2001). The author concluded that the critical period in camel calves for infections lies between two and five weeks, which is similar to the present findings. A separate increase in diarrhoea prevalence was seen towards the eleventh week of age and may be related to helminth infection.

This study focussed specifically on camel calves aged between birth and twelve weeks of age. Other studies of camel calf diarrhoea deal with pre-weaning camel calves (Kaufmann, 1998, Agab and Abass, 1999) aged from birth to 1.5 years. The Sudanese study found a peak of diarrhoea in early summer coinciding with the peak of the calving period, which may indicate that calf diarrhoea is higher in the new born calves. Abass and Omer (2005) state that diarrhoea in "young camel calves" is a problem.

5.9 Clinical finding of the Longitudinal Study

This is the only longitudinal study on diarrhoea in camel calves. It was carried out to complement the findings of the point prevalence study and to obtain a more conclusive picture of the age prevalence of different pathogens. Calves were differentiated depending on their health status history during the observation period. The data of the longitudinal study clearly showed that diarrhoea is a major threat to the health of the camel calves in the age group between birth and three months with a total of 39.5% of all calves sampled suffering from diarrhoea at least once during the observation period. Diarrhoea also is a contributing factor to camel calf losses in this age group.

Diarrhoea in the investigated camel calves occurred as early as day two of life. The peak of diarrhoea in camel calves belonging to Mgmt R layed between day 16 and 20. At this age mainly *Isospora* sp., *Salmonella* sp. and *K. pneumoniae* were isolated from the diarrhoeic camel calves. Abbas and Omer (2005) describe *Salmonella* and coccidia as major cause of diarrhoea in camel calves between two weeks and two months of age but did not mention the presence of *Isospora* sp. specifically. According to the same authors,

E. coli was the major cause of diarrhoea in camel calves around the first week of age but no data on the presence of *E. coli* pathogens were presented to back this claim. However, they also described *Campylobacter* and *Clostridium* as major causes for diarrhoea, two pathogens which were not monitored in this study.

The longitudinal study clearly shows that 43.6% of the camel calves belonging to Category B (= calves showing diarrhoea at least once during the observation period) were positive for *Salmonella* sp., compared to only 25.3% positive for *Klebsiella pneumoniae*. *Isospora* sp. represented a main cause for diarrhoea in camel calves with 92.3% of all camel calves shedding *Isospora* sp. suffering from diarrhoea on the day of sampling. Ten per cent of the camel calves in Category B were shedding *Isospora* sp. at an early age (between week three and eight) while no *Isospora* sp. excretion was found in Category A (=calves healthy throughout the observation period). This indicates that *Isospora* sp. is highly pathogenic for the investigated age group. With 12.5% *Strongyloides* sp. were mildly pathogenic while *Strongyle* sp. (21.7%) represents a pathogen for an older age group as *Strongyle* sp. eggs were found with high prevalence starting from week twelve.

6 SUMMARY

This study was conducted in order to investigate the prevalence of bacterial and protozoal intestinal pathogens in camel calves up to twelve weeks of age in Northern Kenya. A point prevalence study was conducted to describe the existing intestinal pathogens according to age groups and health status and to compare their occurrence between two camel management systems. A longitudinal study was carried out in the ranch management system in order to strengthen the findings of the point prevalence study and to describe the age prevalence of the existing pathogens more comprehensively.

Point prevalence study

Of the 229 individual camel calves sampled in both management systems, 67.7% were healthy, 23.1% diseased, exhibiting diarrhoea, 6.6% convalescent and 2.2% dead. A higher percentage of camel calves suffering from diarrhoea were found in pastoralist herds (31.9%) as compared to ranch herds (19.2%). Looking at the age prevalence of camel calves suffering from diarrhoea there was a peak within the second and third week of age in both management systems.

Of 197 individual camel calves investigated for parasitic infections, 6.6% were shedding *Isospora* sp. and *Strongyloides* sp. while only 4.6% were excreting *Strongyle* sp. eggs. *Isospora* sp. excretion was more prevalent in calves in pastoralist herds (12.9%) as compared to ranch herds (3.7%). *Strongyloides* sp. and *Strongyle* sp. were both more frequently isolated from pastoralist herds (9.7% and 6.5%) than from ranch herds (4.4% and 3.7%). *Isospora* sp. was found in 23% of calves suffering from diarrhoea. 92% of all calves shedding *Isospora* sp. suffered from diarrhoea. Excretion of *Isospora* sp. infection was most prevalent from the second week till the seventh week of age but no shedding was diagnosed at an older age.

Klebsiella pneumoniae was isolated in 26.9% (n=119) of calves sampled. In 19.1% (n=226), *Salmonella sp.* was isolated, while in 97.5% (n=200) *E. coli* was present. The point prevalences of *K. pneumoniae* and *Salmonella* sp. were particularly high in the first three weeks of age. No *K. pneumoniae* was isolated from camel calves older than six weeks while *Salmonella* sp. infection was constantly present up to the twelfth week of age. *E. coli* was constantly present in all age groups. There was no difference in *K. pneumoniae* point prevalences between the two management systems. Point prevalence of *Salmonella sp.* infections was similar in both management systems with a peak during the first three weeks of age. No *Salmonella* sp. was detected in ranch herds after the ninth week of age while there was constant excretion of the pathogen in pastoralist herds.

Longitudinal study

For the longitudinal study, a total of 323 samples were taken from 86 individual camel calves belonging to ranch herds. Category A camel calves (60.5%) were never recorded with diarrhoea while Category B camel calves (39.5%) were recorded with diarrhoea at least once during the observation period. In Category B 7% were dead calves with previous diarrhoea history.

The highest prevalence of calves recorded with diarrhoea was between weeks three and 12. The youngest calf sampled showing signs of diarrhoea was two days old, while the oldest was 132 days old.

The longitudinal study underlined the findings of intestinal pathogens in the point prevalence study. Infection with *Isospora sp.* was detected in camel calves from the second till the fourth week of age with a peak around the third week of age. All samples positive for *Isospora* sp. were taken from calves falling into Category B. Similar to the infection with *Strongyle sp*., there was a higher *Strongyloides sp.* prevalence with an older age in the camel calves. Both parasites were detected in calves falling into the two categories with a higher prevalence in Category B.

Klebsiella pneumoniae was more prevalent in young calves during their first ten weeks of age and less common in older camel calves. Comparing the two categories, there was a higher prevalence of *K. pneumoniae* infection in camel calves falling into Category B (25.3 %) as compared to Category A (12.5%).

Salmonella sp. prevalence was high with the beginning of the second week of age slowly decreasing towards an older age. The prevalence of *Salmonella* sp. infection was higher in camel calves falling into Category B (43.6%) than in Category A (22.7%).

Infections with *E.coli* were found in both categories and within the different age groups.

Additional differentiation of pathogens

Sequence analysis of the SSU rRNA gene and ITS 1 confirmed that the *Isospora* sp. isolates from this study belonged to the species *Isospora orlovi* and that the sequences were identical to isolates from Dubai.

Out of 32 *K. pneumoniae* positive camel calves a total of 62 *K. pneumoniae* were isolated, incl. multiple and repeat isolations. 42 of the 62 isolates were typed and 18 capsular antigens types identified: K2, K3, K5, K11, K13, K16, K26, K28, K31, K34, K36, K38, K54, K55, K60, K61, K64, K81, six isolates were untypable. According to Pearsons Chi-Square ($p<0.05$), there was no significant relation ($p=0.18$) between health status and capsular type of *K. pneumoniae* present. Of the 62 *K. pneumoniae* isolates, 62.9% originated from calves that had either suffered from diarrhoea or were found dead. In one third of the dead camel calves (n=9) *K. pneumoniae* was the only noteworthy pathogen present. Capsular type 28 and type 2 were involved in two cases, while in one case no capsular typing was carried out. *Klebsiella pneumoniae* isolates were resistant to Amoxicillin, Sulphonamide-TMP and Tetracycline but sensitive to Streptomycin.

Out of the 144 *Salmonella* sp. isolated 86.1% and 13.9% were from calves belonging to the ranches and pastoralist herds, respectively. *Salmonella bovismorbificans* was the most common serotype with 32.6%, followed by *S. butantan* (21.5%), *S. typhimurium* (11.1%), *S. kiambu* (9.0%) and *S. muenchen* (7.6%). *Salmonella typhimurium* and *S. adelaide* were the only two serotypes found in both management systems. Comparing the distribution of *Salmonella sp.* according to health status a total of 38.9%, 31.3%, 22.2% and 7.6% originated from healthy, diseased, convalescent and dead camel calves, respectively. The only *Salmonella sp*. associated commonly (81%) with calf disease and present in camel calves of both management systems was *S. typhimurium*.

Out of 531 *E. coli* isolates, a total of 255 were analysed for virulence-associated genes. In 78 isolates virulence-associated genes were detected: *eae* (13.7%), *astA* (10.6%), *hlyEHEC* (4.3%) and *stx* (2.0%). None was positive for *elt Ia/Ib* and *est Ia/Ib*. 83.5% of the isolates originated from calves belonging to ranch herds and 16.5% from calves kept in pastoralist herds. While *eae* and *astA* were found in *E. coli* isolates from both

management systems, *hlyEHEC* and *stx* were only found in isolates from ranch herds. The majority (92%) of the virulence-associated genes were found in camel calves kept in ranch herds. Virulence-associated genes were equally found in camels being healthy (57.7%), with diarrhoea or convalescent (42.3%). No virulence-associated genes were detected in dead camel calves. There was no indication that *E. coli* plays an important role in the diarrhoea-complex of camel calves up to twelve weeks of age. *Escherichia coli* serotype O157:H7 was not isolated from the camel calves in the studied age group.

„Prävalenz von bakteriellen und protozoären Darmpathogenen in Kamelkälbern in Nord Kenia“

7 ZUSAMMENFASSUNG

Diese Studie untersuchte die Prävalenz von bakteriellen und protozoären Darmpathogenen von bis zu zwölf Wochen alten Kamelkälbern in Nord Kenia. Eine Prävalenzstudie wurde durchgeführt, um vorhandene Darmpathogene der Kamelkälber in Relation zu ihrem Alter, ihrem Gesundheitszustand und im Vergleich zwischen zwei Managementsystemen zu beschreiben. Eine Verlaufsstudie wurde bei Ranch-Kamelkälbern durchgeführt um die Ergebnisse der Prävalenzstudie zu bestätigen und die Altersabhängigkeit der Pathogene detaillierter zu beschreiben.

Prävalenzstudie

Von den 229 untersuchten Kamelkälbern aus beiden Managementsytemen waren 67.7% gesund, 23.1% an Durchfall erkrankt, 6.6% rekonvaleszent und 2.2% tot. Der Vergleich der beiden Managemensysteme zeigte, dass mehr Kälber aus Pastoralistenherden (31.9%) an Durchfall erkrankt waren als aus Ranch-Herden (19.2%). Die Altersverteilung der Kamelkälber aus beiden Managementsystemen mit Durchfall wies ein Maximum in der zweiten und dritten Lebenswoche auf.

Von den 197 auf Parasiten untersuchten Kamelkälbern schieden 6.6% *Isospora* sp. und *Strongyloiden*-Eier aus und 4.6% *Strongyliden*-Eier. Die Prävalenz von *Isospora* sp. war in Pastoralistenherden mit 12.9% im Vergleich zu Ranch-Herden (3.7%) deutlich höher. *Strongyloiden*- und *Strongyliden* -Eier wurden in Pastoralistenherden (9.7% bzw. 6.5%) häufiger isoliert als in Ranch-Herden (4.4% bzw. 3.7%). *Isospora* sp. wurde von 23% der Kamelkälber mit Durchfall isoliert. 92% der Kamelkälber, die *Isospora* sp. ausschieden, hatten gleichzeitig Durchfall. Die Ausscheidungsrate von *Isospora* sp. wies die höchste Prävalenz von der zweiten bis zur siebten Lebenswoche auf, konnte jedoch in älteren Tieren nicht mehr nachgewiesen werden.

Klebsiella pneumoniae wurde in 26.9% (n=119), *Salmonella* sp. in 19.1% (n=226) und *E. coli* in 97.5% (n=200) der untersuchten Kamelkälber isoliert. Die Prävalenz von *K. pneumoniae* und *Salmonella* sp. war vor allem in den ersten drei Lebenswochen hoch. Nach der sechsten Lebenswoche konnte *K. pneumoniae* nicht mehr nachgwiesen werden. *Salmonella* sp. war präsent bis zur zwölften Lebenswoche. *Escherichia coli* wurde in allen Altersgruppen kontinuierlich nachgewiesen. Die Prävalenz von *K. pneumoniae* zeigte im Vergleich der beiden Managementsysteme keine Unterschiede auf. Die Prävalenz von *Salmonella* sp. wies in beiden Managementsystemen ein Maximum in den ersten drei Lebenswochen auf, konnte jedoch in Kamelkälbern von Ranch-Herden nach der neunten Lebenswoche nicht mehr nachgewiesen werden. In Pastoralistenherden wurde *Salmonella* sp. von Kälbern kontunierlich ausgeschieden.

Verlaufsstudie

Während der Verlaufsstudie wurden 323 Proben von 86 individuellen in Ranch-Herden gehaltenen Kamelkälbern von Geburt bis zum dritten Lebensmonat gesammelt.

Es wurde zwischen zwei Kategorien differenziert: in Kategorie A fielen Kamelkälber (60.5%), die während der gesamten Untersuchungsperiode nie mit Durchfall

diagnostiziert wurden; in Kategorie B fielen Kamelkälber (39.5%), die mindestens einmal während der Untersuchungperiode Durchfall aufwiesen. 7% der in Kategorie B fallenden Proben stammten von toten Kamelkälbern, die vorher an Durchfall erkrankt waren. Die höchste Prävalenz an Kamelkälbern mit Durchfall wurde zwischen der dritten und zwölften Lebenswoche gefunden. Das jüngste Kalb mit Durchfall war zwei Tage und das Älteste 132 Tage alt.

Die Verlaufsstudie bestätigt die Befunde zum Auftreten von Darmpathogenen der Prävalenzstudie. Die Infektion mit *Isospora* sp. trat bei Kamelkälbern im Alter von der zweiten bis zur vierten Lebenswoche auf. Alle Kamelkälber die *Isospora* sp. ausschieden, fielen in Kategorie B. Ähnlich wie bei der Infektion mit *Strongyliden* sp. war die Prävalenz von *Strongyloiden* sp. Infektion bei Kamelkälbern höher bei älteren Tieren. Beide Parasiten konnten von Tieren isoliert werden die in beide Kategorien fallen, jedoch war die Prävalenz geringgradig höher in Kamelkälbern die Kategorie B zugehörten.

Klebsiella pneumoniae wies eine höhere Prävalenz in Jungtieren bis zur zehnten Lebenswoche auf als in Kamelkälbern höheren Alters. Vergleicht man die beiden Kategorien, so war die Prävalenz von *K. pneumoniae* höher in Kamelkälbern, die in Kategorie B fielen (25.3% positiv im Vergleich zu 12.5% in Kategorie A). Die Prävalenz von *Salmonellen* sp. in Kamelkälbern war hoch in der zweiten Lebenswoche, mit einer stetigen Abnahme bei älteren Kälbern. Die Prävalenz von *Salmonella* sp. war höher bei Tieren die in Kategorie B (43.6%) fallen als in Kategorie A (22.7%). Es konnten keine Unterschiede in der Prävalenz von *E. coli* Infektionen zwischen Kategorie A und B nachgewiesen werden. *Escherichia coli* wurde in allen Altersgruppen nachgewiesen.

Weiterführende Erregerdifferenzierung

Die Sequenzanalyse der SSU rRNA Gene und ITS 1 Segmente bestätigte, dass die gefundenen *Isospora* sp. Isoloate dieser Studie der Spezie *Isospora orlovi* angehören und dass sie identisch mit Isolaten aus Dubai sind.

Von 32 *Klebsiella pneumoniae* positiven Kamelkälbern wurden insgesamt 62 *K. pneumoniae* Isolate isoliert, inklusive multipler und wiederholter Isolation. 47 der 62 Isoloate wurden typisiert und 18 Kapseltypen nachgewiesen: K2, K3 K5, K11, K13, K16, K26, K28, K31, K34, K36, K38, K54, K55, K60, K61, K64, K81, sechs Isolate waren nicht typisierbar. Nach Chi-Quadrat (Pearson) gab es zwischen dem Gesundheitsstatus des Kamelkalbes und den isolierten Kapseltypen keine signifikante Beziehung ($p<0.05$, $p=0.18$). Von den 62 *K. pneumoniae* Isolaten stammten 62.9% von Kamelkälbern, die an Durchfall erkrankt oder tot waren. Von einem Drittel der toten Kamelkälber (n=9) wurde *K. pneumoniae* als einziger aussagekräftiger Keim isoliert. Kapseltyp 28 und 2 wurden in zwei Fällen nachgewiesen, während im dritten Fall keine Kapseltypenbestimmung durchgeführt wurde. Die Isolate wiesen Resistenzen gegen Amoxicillin, Sulphonamide-TMP und Tetracycline auf und waren empfindlich gegenüber Streptomycin.

Von den 144 *Salmonella* sp. Isolaten wurden 86.1% und 13.9% von Kamelkälbern isoliert, die zu Ranch-Herden bzw. Pastoralisten-Herden gehörten. *Salmonella bovis-morbificans* war mit 32.6%, der häufigste Serotyp, gefolgt von *S. butantan* (21.5%), *S. typhimurium* (11.1%), *S. kiambu* (9.0%) und *S. muenchen* (7.6%). *Salmonella typhimurium* und *S. adelaide* waren die einzigen Serotypen, die in beiden Managementsystemen nachgewiesen wurden. Vergleicht man die Verteilung der

Salmonella sp. mit dem Gesundheitszustand der Kamelkälber, so waren 38.9 % der Kälber gesund, 31.3 % an Durchfall erkrankt, 22.2 % rekonvaleszent und 7.6 % tot. *Salmonella typhimurium* war die einzige Salmonelle, die häufig (81%) mit Kamelkälberdurchfall in beiden Managementsystemen assoziiert werden konnte.

Von den insgesamt 531 *E. coli* Isolaten wurden 255 auf das Vorkommen von virulenzassoziierten Genen untersucht. In 78 Isolaten konnten virulenzassoziierte Gene nachgewiesen werden: *eae* (13.7%), *astA* (10.6%), *hlyEHEC* (4.3%) und *stx* (2.0%). Keines der Isolate war positiv für *elt Ia/Ib* and *est Ia/Ib*. Die Isolate stammten zu 83.5% von Kamelkälbern, die auf Ranchen gehalten wurden und zu 16.5% von Kamelkälbern aus dem Pastoralistenherden. *Eae* und *astA* konnten von Kälbern aus beiden Managementsystemen isoliert werden, während *hlyEHEC* und *stx* nur in Isolaten von Ranch-Herden gefunden wurden. Die Mehrheit (92%) der virulenzassoziierten Gene wurden in Isolaten nachgewiesen, die von Kamelkälbern im Ranchmanagementsystem stammten. Die virulenzassoziierten Gene wurden gleichermassen von gesunden Tieren (57.7%), von an Durchfall erkrankten oder von rekonvaleszenten Tieren (42.3%) nachgewiesen. Keines der nachgewiesenen virulenzassoziierten Gene stammte von einem *E. coli* Isolat, das von einem toten Tiere gewonnen wurde. Es gab keine Hinweise darauf, dass *E. coli* eine wichtige Rolle im Durchfallkomplex von Kamelkälbern bis zu einem Alter von zwölf Wochen spielt. *Escherichia coli* Serotyp O157:H7 konnte von den untersuchten Kamelkälbern nicht isoliert werden.

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9 ANNEX

9.1 Media used

Blood Agar Base CM0055		Oxoid, Wesel, Germany
'Lab-Lemco' powder	10.0	g/l
Peptone Neutralised	10.0	g/l
Sodium chloride	5.0	g/l
Agar	15.0	g/l
pH 7.3 ± 0.2		

Brilliant Green Agar (Modified) CM0329 (Edel Kampelmacher Medium)		Oxoid, Wesel, Germany
'Lab-Lemco' powder	5.0	g/l
Peptone	10.0	g/l
Yeast extract	3.0	g/l
Disodium hydrogen phosphate	1.0	g/l
Sodium dihydrogen phosphate	0.6	g/l
Lactose	10.0	g/l
Sucrose	10.0	g/l
Phenol red	0.09	g/l
Brilliant green	0.0047	g/l
Agar	12.0	g/l
pH 6.9 ± 0.2		

BD BBL™ CTA Medium™ 211096 Becton Dickinson (BBL)

Water-blue Metachrome-yellow Lactose Agar acc. to Gassner, mod. (Gassner Agar) (TN1194)		Sifin, Berlin, Germany
Peptone from meat	4.4	g/l
Peptone from caseine	4.4	g/l
Yeast extract	2.7	g/l
Lactose	10.0	g/l
Saccharose	10.0	g/l
Sodium chloride	5.0	g/l

Metachrome-yellow	0.8	g/l
Water-blue	0.4	g/l
Agar	10.0	g/l
pH 7.0 ± 0.2		

Kligler Iron Agar CM0033 — Oxoid, Wesel, Germany

'Lab-Lemco' powder	3.0	g/l
Yeast extract	3.0	g/l
Peptone	20.0	g/l
Sodium chloride	5.0	g/l
Lactose	10.0	g/l
Glucose	1.0	g/l
Ferric citrate	0.3	g/l
Sodium thiosulphate	0.3	g/l
Phenol red	0.05	g/l
Agar	12.0	g/l
pH 7.4 ± 0.2		

LIM — Oxoid, Wesel, Germany

Agar	3.0	g/l
Bactopeptone	5.0	g/l
Yeast extract	3.0	g/l
Tryptose	15.0	g/l
NaCl	5.0	g/l
Bromcresol purple (1.6%)	0.016	g/l
Glucose	1.0	g/l
L-Lysine	5.0	g/l
pH 6.6		

Mc Conkey Agar CM 0007 — Oxoid, Wesel, Germany

Peptone	20.0	g/l
Lactose	10.0	g/l
Bile salts	5.0	g/l
Sodium chloride	5.0	g/l
Neutral red	0.075	g/l

Agar	12.0	g/l
pH 7.4 ± 0.2		

Nutrient Agar CM0003 — Oxoid, Wesel, Germany

'Lab-Lemco' powder	1.0	g/l
Yeast extract	2.0	g/l
Peptone	5.0	g/l
Sodium chloride	5.0	g/l
Agar	15.0	g/l
pH 7.4 ± 0.2		

Rappaport-Vassiliadis (RV) CM 0669 Enrichment Broth — Oxoid, Wesel, Germany

Soya peptone	5.0	g/l
Sodium chloride	8.0	g/l
Potassium dihydrogen phosphate	1.6	g/l
Magnesium chloride $6H_2O$	40.0	g/l
Malachite green	0.04	g/l
pH 5.2 ± 0.2		

Stuart Transport Medium CM0111 — Oxoid, Wesel, Germany

Sodium glycerophosphate	10.0	g/l
Sodium thioglycollate	0.5	g/l
Cysteine hydrochloride	0.5	g/l
Calcium chloride	0.1	g/l
Mehtylene blue	0.001	g/l
Agar	5.0	g/l
pH 7.4 ± 0.2		

Triple Sugar Iron Agar CM0277 — Oxoid, Wesel, Germany

'Lab-Lemco' powder	3.0	g/l
Yeast extract	3.0	g/l
Peptone	20.0	g/l
Sodium chloride	5.0	g/l
Lactose	10.0	g/l

Sucrose	10.0	g/l
Glucose	1.0	g/l
Ferric citrate	0.3	g/l
Sodium thiosulphate	0.3	g/l
Phenol red	0.024	g/l
Agar	12.0	g/l
pH 7.4 ± 0.2		

Tryptone Water CM0087 Oxoid, Wesel, Germany

Tryptone	10.0	g/l
Sodium chloride	5.0	g/l
pH 7.5 ± 0.2		

Urea Agar Base CM0053 Oxoid, Wesel, Germany

Peptone	1.0	g/l
Glucose	1.0	g/l
Sodium chloride	5.0	g/l
Disodium phosphate	1.2	g/l
Potassium dihydrogen phosphate	0.8	g/l
Phenol red	0.012	g/l
Agar	15.0	g/l
pH 6.8 ± 0.2		

Urea 40% Solution SR 0020 Oxoid, Wesel, Germany

XLD CM0469 Oxoid, Wesel, Germany

Yeast extract	3.0	g/l
L-Lysine HCl	5.0	g/l
Xylose	3.75	g/l
Lactose	7.5	g/l
Sucrose	7.5	g/l
Sodium desoxycholate	1.0	g/l
Sodium chloride	5.0	g/l
Sodium thiosulphate	6.8	g/l
Ferric ammonium citrate	0.8	g/l
Phenol red	0.08	g/l

Agar	12.5	g/l
pH 7.4 ± 0.2		
Poly A-E and A-67		
Saccharose Sucrose1.07687.1000	Merck, Darmstadt, Germany	
API 20 E	bioMérieux, Nürtingen, Germany	

9.2 Buffers used for DNA-DNA Hybridization

20 x SSC

NaCL	175	g
NaCitrate	88	g
Add Aqua Bidest	1000	ml
pH 7		

BRSS

NaCL	175	g

Pre-Hybridization Solution

20 x SSC	12.5	ml
BRSS	5	ml
10 % N-lauroylsarcosine	1	ml
10 % SDS	0.1	ml
Ad Aqua bidest	50	ml

Hybridization Solution

Pre-Hybridization Solution	2.5	ml
Probe	2.5	µl

Washing Buffer 1

20 x SSC	40	ml
10 % SDS	4	ml
Ad Aqua bidest	400	ml

Washing Buffer 2

20 x SSC	2	ml
10 % SDS	4	ml
Ad Aqua bidest	400	ml

Buffer 1

Maleic acid (1M)	100	ml
NaCl (1M)	150	ml
Tween 20	3	ml

Ad Aqua bidest	1000	ml
pH 7,5		
Buffer 2		
BRSS	10	ml
Ad Buffer 1 (without Tween)	100	ml
Buffer 3		
Tris-HCl (1M, pH 9,6)	10	ml
NaCl (1M)	10	ml
Ad Aqua bidest	100	ml
Antibody Conjugate		
Anti-Dig-AP	1	µl
Buffer 2	10	ml
Chemilumineszenz		
CSPD	1	µl
Buffer 3	100	µl
Stripping Buffer		
NaOH (3 mol/l)	33.3	ml
10 % SDS	5	ml
Ad Aqua bidest	500	ml
2 x SSC		
20 x SSC	50	ml
Ad Aqua bidest	500	ml

9.3 Age distribution according to health status of camel calves compared between the two management systems

		1		2		3		4	
Mgmt	***Age Group***	**Healthy**		**Diseased**		**Convalescent**		**Dead**	
	(d)	No	%	**No**	**%**	No	%	No	%
R	0-7	40	90.9%	**3**	**6.8%**			1	2.3%
	8-14	18	56.3%	**12**	**37.5%**			2	6.3%
	15-21	9	56.3%	**5**	**31.3%**	1	6.3%	1	6.3%
	22-28	10	71.4%	**3**	**21.4%**	1	7.1%		
	29-35	7	77.8%	**2**	**22.2%**				
	36-42	7	87.5%	**1**	**12.5%**				
	43-49	9	81.8%			1	9.1%	1	9.1%
	50-56								
	57-63	6	85.7%	**1**	**14.3%**				
	64-70	3	100.0%						
	71-77	1	50.0%	**1**	**50.0%**				
	78-84					1	100.0%		
P	0-7	3	75.0%	**1**	**25.0%**				
	8-14	3	100.0%						
	15-21	4	57.1%	**3**	**42.9%**				
	22-28	4	66.7%	**2**	**33.3%**				
	29-35	3	33.3%	**2**	**22.2%**	4	44.4%		
	36-42	4	66.7%	**1**	**16.7%**	1	16.7%		
	43-49	5	62.5%	**2**	**25.0%**	1	12.5%		
	50-56								
	57-63	7	58.3%	**3**	**25.0%**	2	16.7%		
	64-70								
	71-77	2	28.6%	**2**	**28.6%**	3	42.9%		
	78-84	1	100.0%						

9.4 Point prevalence of infection with *Isospora sp.*, *Strongyloides sp.* and other *Strongyle sp.* in camel calves according to age

	Isospora sp.				*Strongyloides sp.*				*Strongyle sp.*			
	Negative		Positive		Negative		Positive		Negative		Positive	
Age Group (d)	**No**	**%**	**No**	**%**	**No**	**%**	**No**	**%**	**No**	**%**	**No**	**%**
1 0-7	37	100			37	100			37	100		
2 8-14	32	97	**1**	**3**	32	97	**1**	**3**	32	97	**1**	**3**
3 15-21	15	83.3	**3**	**16.7**	18	100			18	100		
4 22-28	12	80	**3**	**20**	15	100			15	100		
5 29-35	16	88.9	**2**	**11.1**	17	94.4	**1**	**5.6**	18	100		
6 36-42	13	92.9	**1**	**7.1**	13	92.9	**1**	**7.1**	13	92.9	**1**	**7.1**
7 43-49	17	94.4	**1**	**5.6**	16	88.9	**2**	**11.1**	15	83.3	**3**	**16.7**
8 50-56												
9 57-63	17	100			15	88.2	**2**	**11.8**	14	82.4	**3**	**17.6**
10 64-70	2	100			2	100			2	100		
11 71-77	9	100			7	77.8	**2**	**22.2**	8	88.9	**1**	**11.1**
12 78-84	2	100			2	100			2	100		
No age noted	12	85.7	**2**	**14.3**	11	78.6	**3**	**21.4**	14	100		

9.5 Point prevalence of infection with *Isospora sp.*, *Strongyloides sp.* and *Strongyle sp.* according to age compared between Mgmt R and Mgmt P

		Isospora sp.				*Strongyloides sp.*				*Strongyle sp.*			
		Negative		Positive		Negative		Positive		Negative		Positive	
Mgmt	**Age Group (d)**	**No**	**%**	**No**	**%**	**No**	**%**	**No**	**%**	**No**	**%**	**No**	**%**
R	1 0-7	34	100			34	100			34	100		
	2 8-14	29	96.7	**1**	**3.3**	29	96.7	**1**	**3.3**	29	96.7	**1**	**3.3**
	3 15-21	13	92.9	**1**	**9.1**	14	100			14	100		
	4 22-28	10	83.3	**2**	**16.7**	12	100			12	100		
	5 29-35	9	100			9	100			9	100		
	6 36-42	8	100			7	87.5	**1**	**12.5**	7	87.5	**1**	**12.5**
	7 43-49	10	100			9	90	**1**	**10**	8	80	**2**	**20**
	8 50-56												
	9 57-63	7	100			5	71.4	**2**	**28.6**	7	100		
	10 64-70	2	100			2	100			2	100		
	11 71-77	2	100			2	100			1	50	**1**	**50**
	12 78-84	1	100			1	100			1	100		
	N/A	5	83.3	**1**	**16.7**								
P	1 0-7	3	100			3	100			3	100		
	2 8-14	3	100			3	100			3	100		
	3 15-21	2	50	**2**	**50**	4	100			4	100		
	4 22-28	2	66.7	**1**	**33.3**	3	100			3	100		
	5 29-35	7	77.8	**2**	**22.2**	8	88.9	**1**	**11.1**	9	100		
	6 36-42	5	83.3	**1**	**16.7**	6	100			6	100		
	7 43-49	7	87.5	**1**	**12.5**	7	87.5	**1**	**12.5**	7	87,5	**1**	**12,5**
	8 50- 56												
	9 57-63	10	100			10	100			7	70	**3**	**30**
	10 64-70												
	11 71-77	7	100			5	71.4	**2**	**28.6**	7	100		
	12 78-84	1	100			1	100			1	100		
	N/A	7	87.5	**1**	**12.5**								

9.6 Prevalence of infection with *Isospora sp.* according to age in camel calves kept under Mgmt R

	Isospora sp.			
	Negative		**Positive**	
Age (d)	No	%	**No**	**%**
0-5	21	100		
6-10	22	100		
11-15	19	95	**1**	**5**
16-20	13	81.3	**3**	**18.8**
21-25	8	66.7	**4**	**33.3**
26-30	19	90.5	**2**	**9.5**
31-35	13	100		
36-40	8	100		
41-45	12	100		
46-50	10	100		
51-55	11	100		
56-60	11	100		
61-65	12	100		
66-70	16	100		
71-75	9	100		
76-80	9	100		
81-85	11	100		
86-90	11	100		
91-95	9	100		
96-100	5	100		
>100	13	100		

9.7 Prevalence of infection with *Strongyloides sp*. according to age in camel calves kept under Mgmt R

	Strongyloides sp.			
	Negative		**Positive**	
Age (d)	No	%	**No**	**%**
0-5	20	95.2	**1**	**4.8**
6-10	22	100		
11-15	19	95	**1**	**5**
16-20	16	100		
21-25	12	100		
26-30	21	100		
31-35	13	100		
36-40	7	87.5	**1**	**12.5**
41-45	10	83.3	**2**	**16.7**
46-50	8	80	**2**	**20**
51-55	11	100		
56-60	11	100		
61-65	11	91.7	**1**	**8.3**
66-70	13	81.3	**3**	**18.8**
71-75	8	88.9	**1**	**11.1**
76-80	6	66.7	**3**	**33.3**
81-85	11	100		
86-90	7	63.6	**4**	**36.4**
91-95	4	44.4	**5**	**55.6**
96-100	2	40	**3**	**60**
>100	8	61.5	**5**	**38.5**

9.8 Prevalence of infection with other *Strongyle sp.* according to age in camel calves kept under Mgmt R

	Any other Strongyle sp.			
	Negative		**Positive**	
Age (d)	No	%	**No**	**%**
0-5	21	100		
6-10	22	100		
11-15	19	95	**1**	**5**
16-20	14	87.5	**2**	**12.5**
21-25	11	91.7	**1**	**8.3**
26-30	20	95.2	**1**	**4.8**
31-35	13	100		
36-40	5	62.5	**3**	**37.5**
41-45	11	91.7	**1**	**8.3**
46-50	7	70	**3**	**30**
51-55	10	90.9	**1**	**9.1**
56-60	7	63.6	**4**	**36.4**
61-65	6	50	**6**	**50**
66-70	15	93.8	**1**	**6.3**
71-75	7	77.8	**2**	**22.2**
76-80	3	33.3	**6**	**66.7**
81-85	8	72.7	**3**	**27.3**
86-90	8	72.7	**3**	**27.3**
91-95	9	100		
96-100	4	80	**1**	**20**
>100	6	46.2	**7**	**53.8**

9.9 Age prevalence of *Isospora sp.* in camel calves falling into Category B

	Category B (*Isospora sp.*)			
	Negative		Positive	
Age (d)	No	%	**No**	**%**
0-5	9	100		
6-10	9	100		
11-15	12	92.3	**1**	**7.7**
16-20	5	62.5	**3**	**37.5**
21-25	3	42.9	**4**	**57.1**
26-30	9	81.8	**2**	**18.2**
31-35	6	100		
36-40	4	100		
41-45	6	100		
46-50	3	100		
51-55	5	100		
56-60	4	100		
61-65	4	100		
66-70	5	100		
71-75	5	100		
76-80	2	100		
81-85	3	100		
86-90	3	100		
91-95	1	100		
96-100	2	100		
> 100	6	100		
Not recorded age	2	100		

9.10 Age prevalence of *Strongyloides sp.* infection in camel calves compared between Category A and B

	CALF HEALTH STATUS							
	Category A				Category B			
	Negative		**Positive**		Negative		**Positive**	
Age (d)	No	%	**No**	**%**	No	%	**No**	**%**
0-5	11	91.7	**1**	**8.3**	9	100		
6-10	13	100			9	100		
11-15	6	85.7	**1**	**14.3**	13	100		
16-20	8				8	100		
21-25	5				7	100		
26-30	10				11	100		
31-35	7				6	100		
36-40	4				3	75	**1**	**25**
41-45	5	83.3	**1**	**16.7**	5	83.3	**1**	**16.7**
46-50	6	85.7	**1**	**14.3**	2	66.7	**1**	**33.3**
51-55	6				5	100		
56-60	7				4	100		
61-65	8				3	75	**1**	**25**
66-70	9	81.8	**2**	**18.2**	4	80	**1**	**20**
71-75	3	75	**1**	**25**	5	100		
76-80	5	71.4	**2**	**28.6**	1	50	**1**	**50**
81-85	8				3	100		
86-90	7	87.5	**1**	**12.5**			**3**	**100**
91-95	3	37.5	**5**	**62.5**	1	100		
96-100	2	66.7	**1**	**33.3**	2	100		
> 100	5	71.4	**2**	**28.6**	3	50	**3**	**50**
Not recorded age	1	100			3	75	**1**	**25**

9.11 Age prevalence of Strongyle sp. infection in camel calves compared between Category A and B

	CALF HEALTH STATUS							
	Category A				Category B			
Age (d)	Negative		Positive		Negative		Positive	
	No	%	No	%	No	%	No	%
0-5	12	100			9	100		
6-10	13	100			9	100		
11-15	6	85.7	1	14.3	13	100		
16-20	8	100			6	75	2	25
21-25	5	100			6	85.7	1	14.3
26-30	10	100			10	90.9	1	9.1
31-35	7	100			6	100		
36-40	1	25	3	75	4	100		
41-45	6	100			5	83.3	1	16.7
46-50	5	71.4	2	28.6	2	66.7	1	33.3
51-55	5	83.3	1	16.7	5	100		
56-60	6	85.7	1	14.3	1	25	3	75
61-65	5	62.5	3	37.5	1	25	3	75
66-70	11	100			4	80	1	20
71-75	4	100			3	60	2	40
76-80	3	42.9	4	57.1			2	100
81-85	6	75	2	25	2	66.7	1	33.3
86-90	7	87.5	1	12.5	1	33.3	2	66.7
91-95	8	100			1	100		
96-100	3	100			1	50	1	50
> 100	4	57.1	3	42.9	2	33.3	4	66.7
Not recorded age	1	100			3	75	1	25

9.12 Point prevalence of infection with *K. pneumoniae, Salmonella sp.* and *E. coli* in camel calves according to their age

	Klebsiella pneumoniae				*Salmonella sp.*				*E. coli*			
	Negative		**Positive**		Negative		**Positive**		Negative		**Positive**	
Age Group (d)	**No**	**%**	**No**	**%**	**No**	**%**	**No**	**%**	**No**	**%**	**No**	**%**
1 0-7	16	64	**9**	**36**	39	81.3	**9**	**18.7**	2	4.5	**42**	**95.5**
2 8-14	11	52.4	**10**	**47.6**	27	77.1	**8**	**22.9**	1	3	**32**	**97**
3 15-21	7	53.8	**6**	**46.2**	10	45.5	**12**	**54.5**			**22**	**100**
4 22-28	9	90	**1**	**10**	18	90	**2**	**10**			**18**	**100**
5 29-35	5	62.5	**3**	**37.5**	14	77.8	**4**	**22.2**			**14**	**100**
6 36-42	6	100			12	85.7	**2**	**14.3**			**11**	**100**
7 43-49	9	100			16	88.9	**2**	**11.1**	1	6.8	**14**	**93.3**
8 50-56												
9 57-63	10	100			16	84.2	**3**	**15.8**			**15**	**100**
10 64-70	3	100			3	100					**3**	**100**
11 71-77	6	100			8	88.9	**1**	**11.1**			**9**	**100**
12 78-84	1	100			2	100					**2**	**100**
No age recorded	4	57.1	**3**	**42.9**	16	88.9	**2**	**11.1**	1	7.1	**13**	**92.9**

9.13 Point prevalence of *K. pneumoniae, Salmonella sp.* and *E. coli* infection according to age compared between Mgmt R and P

		Klebsiella pneumoniae				*Salmonella sp.*				*E. coli*			
		Negative		**Positive**		Negative		**Positive**		Negative		**Positive**	
Mgmt	**Age Group (d)**	**No**	**%**	**No**	**%**	**No**	**%**	**No**	**%**	**No**	**%**	**No**	**%**
R	1 0-7	15	62.5	**9**	**37.5**	35	79.5	**9**	**20.5**	2	4.9	**39**	**95.1**
	2 8-14	11	55	**9**	**45**	25	78.1	**7**	**21.9**	1	3.2	**30**	**96.8**
	3 15-21	4	66.7	**2**	**33.3**	8	53.3	**7**	**46.7**			**15**	**100**
	4 22-28	4	100			14	100					**12**	**100**
	5 29-35	1	50	**1**	**50**	7	77.8	**2**	**22.2**			**8**	**100**
	6 36-42	4	100			8	100					**8**	**100**
	7 43-49	2	100			9	90	**1**	**10**			**9**	**100**
	8 50-56												
	9 57-63					7	100					**4**	**100**
	10 64-70	3	100			3	100					**3**	**100**
	11 71-77					2	100					**2**	**100**
	12 78-84					1	100					**1**	**100**
P	1 0-7	1	100			4	100					**3**	**100**
	2 8-14			**1**	**100**	2	66.7	**1**	**33.3**			**2**	**100**
	3 15-21	3	42.9	**4**	**57.1**	2	28.6	**5**	**71.4**			**7**	**100**
	4 22-28	5	83.3	**1**	**16.7**	4	66.7	**2**	**33.3**			**6**	**100**
	5 29-35	4	66.7	**2**	**33.3**	7	77.8	**2**	**22.2**			**6**	**100**
	6 36-42	2	100			4	66.7	**2**	**33.3**			**3**	**100**
	7 43-49	7	100			7	87.5	**1**	**12.5**	1	16.7	**5**	**83.3**
	8 50- 56												
	9 57-63	10	100			9	75	**3**	**25**			**11**	**100**
	10 64-70												
	11 71-77	6	100			6	85.7	**1**	**14.3**			**7**	**100**
	12 78-84	1	100			1	100					**1**	**100**

9.14 Age prevalence of *K. pneumoniae* in camel calves screened in a longitudinal study

	Klebsiella pneumoniae.			
	Negative		**Positive**	
Age (d)	No	%	**No**	**%**
0-5	10	62.5	**6**	**37.5**
6-10	5	50	**5**	**50**
11-15	7	63.6	**4**	**36.4**
16-20	4	50	**4**	**50**
21-25	8	100		
26-30	13	86.7	**2**	**13.3**
31-35	7	77.8	**2**	**22.2**
36-40	7	77.8	**2**	**22.2**
41-45	8	80	**2**	**20**
46-50	7	87.5	**1**	**12.5**
51-55	6	85.7	**1**	**14.3**
56-60	11	100		
61-65	8	100		
66-70	4	80	**1**	**20**
71-75	7	100		
76-80	7			
81-85	6			
86-90	4			
91-95	3			
96-100	1			
>100	2	75	**1**	**25**

9.15 Age prevalence of *Salmonella sp.* in camel calves screened in a longitudinal study

	Salmonella sp.			
	Negative		**Positive**	
Age (d)	No	%	**No**	**%**
0-5	19	73.1	**7**	**26.9**
6-10	18	72	**7**	**28**
11-15	13	56.5	**10**	**43.5**
16-20	6	35.3	**11**	**64.7**
21-25	4	33.3	**8**	**66.7**
26-30	14	56	**11**	**44**
31-35	8	50	**8**	**50**
36-40	7	70	**3**	**30**
41-45	6	40	**9**	**60**
46-50	7	70	**3**	**30**
51-55	8	72.7	**3**	**27.3**
56-60	10	83.3	**2**	**16.7**
61-65	11	84.6	**2**	**15.4**
66-70	14	82.4	**3**	**17.6**
71-75	6	60	**4**	**40**
76-80	9	90	**1**	**10**
81-85	11	91.7	**1**	**8.3**
86-90	11	100		
91-95	9	100		
96-100	5	100		
>100	9	69.2	**4**	**30.8**

9.16 Age prevalence of *E. coli* in camel calves screened in a longitudinal study

	E. coli			
	Negative		**Positive**	
Age (d)	No	%	**No**	**%**
0-5	2	8.3	**22**	**91.7**
6-10			**23**	**100**
11-15			**21**	**100**
16-20			**17**	**100**
21-25			**10**	**100**
26-30			**24**	**100**
31-35			**15**	**100**
36-40			**10**	**100**
41-45	1	6.7	**14**	**93.3**
46-50			**10**	**100**
51-55			**9**	**100**
56-60			**12**	**100**
61-65	1	7.7	**12**	**92.3**
66-70			**13**	**100**
71-75			**10**	**100**
76-80	1	12.5	**7**	**87.5**
81-85			**12**	**100**
86-90	1	9.1	**10**	**90.9**
91-95			**8**	**100**
96-100			**5**	**100**
>100			**13**	**100**

9.17 Age prevalence of *K. pneumoniae* infection in camel calves compared between Category A and B

	Category A				Category B			
	Negative		Positive		Negative		Positive	
Age (d)	No	%	**No**	**%**	No	%	**No**	**%**
0-5	7	70	**3**	**30**	3	50	**3**	**50**
6-10	4	80	**1**	**20**	1	20	**4**	**80**
11-15	3	60	**2**	**40**	4	66.7	**2**	**33.3**
16-20	3	60	**2**	**40**	1	33.3	**2**	**66.7**
21-25	4	100			4	100		
26-30	6	85.7	**1**	**14.3**	7	87.5	**1**	**12.5**
31-35	4	100			3	60	**2**	**40**
36-40	5	100			2	50	**2**	**50**
41-45	3	100			5	71.4	**2**	**28.6**
46-50	6	85.7	**1**	**14.3**	1	100		
51-55	3	100			3	75	**1**	**25**
56-60	6	100			5	100		
61-65	3	100			5	100		
66-70	3	75	**1**	**25**	1	100		
71-75	2	100			5	100		
76-80	5	100			2	100		
81-85	3	100			3	100		
86-90	3	100			1	100		
91-95	3	100						
96-100					1	100		
> 100	1	100			1	50	**1**	**50**
Without age					1	100		

9.18 Age prevalence of *Salmonella sp.* infection in camel calves compared between Category A and B

	Category A				Category B			
	Negative		Positive		Negative		Positive	
Age (d)	No	%	**No**	**%**	No	%	**No**	**%**
0-5	12	70.6	**5**	**29.4**	7	77.8	**2**	**22.2**
6-10	11	73.3	**4**	**26.7**	7	70	**3**	**30**
11-15	8	88.9	**1**	**11**	5	35.7	**9**	**64.3**
16-20	5	55.6	**4**	**44.4**	1	12.5	**7**	**87.5**
21-25	3	50	**3**	**50**	1	16.7	**5**	**83.3**
26-30	10	83.3	**2**	**16.7**	4	30.8	**9**	**69.2**
31-35	5	62.5	**3**	**37.5**	3	37.5	**5**	**62.5**
36-40	3	60	**2**	**40**	4	80	**1**	**20**
41-45	2	33.3	**4**	**66.7**	4	44.4	**5**	**55.6**
46-50	5	71.4	**2**	**28.6**	2	66.7	**1**	**33.3**
51-55	5	83.3	**1**	**16.7**	3	60	**2**	**40**
56-60	5	71.4	**2**	**28.6**	5	100		
61-65	7	87.5	**1**	**12.5**	4	80	**1**	**20**
66-70	9	75	**3**	**25**	5	100		
71-75	5	100			1	20	**4**	**80**
76-80	8	100			1	50	**1**	**50**
81-85	7	87.5	**1**	**12.5**	4	100		
86-90	8	100			3	100		
91-95	8	100			1	100		
96-100	3	100			2	100		
> 100	6	85.7	**1**	**14.3**	3	50	**3**	**50**
Without age	1	50	**1**	**50**	5	100		

9.19 Age prevalence of *E. coli* infection in camel calves compared between Category A and B

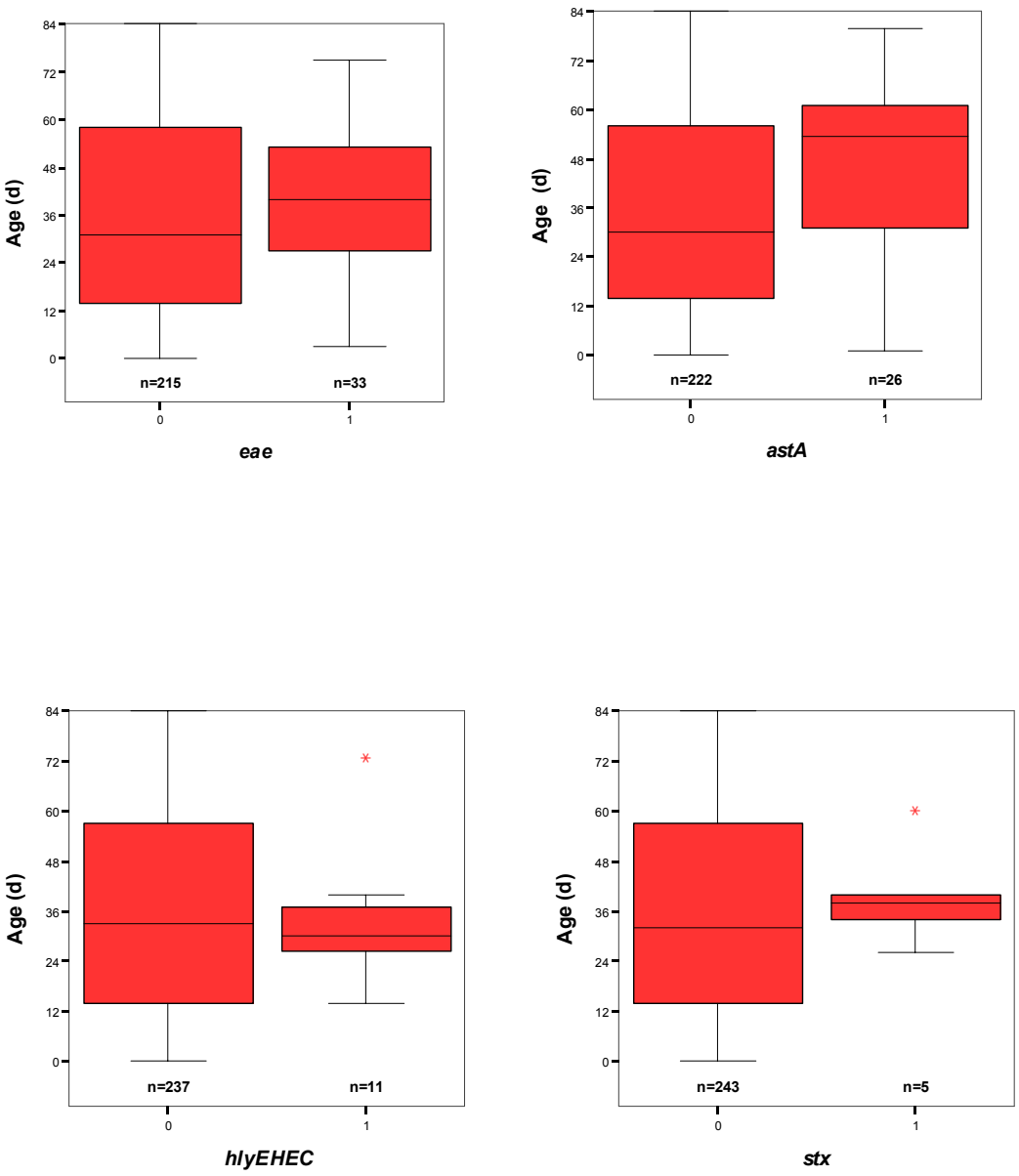
	Category A				Category B			
	Negative		Positive		Negative		Positive	
Age (d)	No	%	**No**	**%**	No	%	**No**	**%**
0-5	2	13.3	**13**	**86.7**			**9**	**100**
6-10			**14**	**100**			**9**	**100**
11-15			**8**	**100**			**13**	**100**
16-20			**9**	**100**			**8**	**100**
21-25			**4**	**100**			**6**	**100**
26-30			**12**	**100**			**12**	**100**
31-35			**7**	**100**			**8**	**100**
36-40			**5**	**100**			**5**	**100**
41-45			**6**	**100**	1	11.1	**8**	**88.9**
46-50			**7**	**100**			**3**	**100**
51-55			**5**	**100**			**4**	**100**
56-60			**7**	**100**			**5**	**100**
61-65			**8**	**100**	1	20	**4**	**80**
66-70			**10**	**100**			**3**	**100**
71-75			**5**	**100**			**5**	**100**
76-80			**7**	**100**	1	100		
81-85			**8**	**100**			**4**	**100**
86-90			**8**	**100**	1	33.3	**2**	**66.7**
91-95			**7**	**100**			**1**	**100**
96-100			**3**	**100**			**2**	**100**
> 100			**7**	**100**			**6**	**100**
Without age			**1**	**100**			**5**	**100**

9.20 Distribution of *Salmonella* serotypes according to health status

***Salmonella* sp.**	**Health status**				**TOTAL**	**(2) + (3) + (4)**
	(1) Healthy	**(2) Diseased**	**(3) Convalescent**	**(4) Dead**		
S. bovismorbificans	15 (31.9 %)	13 (27.7 %)	11 (23.4 %)	8 (17 %)	47 (100 %)	32 (68.1 %)
S. butantan	15 (48.4 %)	6 (19.4 %)	8 (25.8 %)	2 (6.4 %)	31 (100 %)	16 (51.6 %)
S. typhimurium	**3 (18.8 %)**	**12 (75.0 %)**	**1 (6.2 %)**	**0**	**16 (100 %)**	**13 (81.2 %)**
S. kiambu	9 (69.2 %)	0	4 (30.8 %)	0	13 (100 %)	4 (30.8 %)
S. muenchen	3 (27.4 %)	4 (30.8 %)	4 (30.8 %)	0	11 (100 %)	8 (61.6 %)
S. fayed	2 (50 %)	2 (50 %)	0	0	4 (100 %)	2 (50 %)
S. subspecies I	1 (25 %)	2 (50 %)	1 (25 %)	0	4 (100 %)	3 (75 %)
S. subspecies IIIb	0	2 (50 %)	2 (50 %)	0	4 (100 %)	4 (100 %)
S. Poona	1 (33.3 %)	1 (33.3 %)	1 (33.3 %)	0	3 (100 %)	2 (66.6 %)
S. adelaide	1 (50 %)	1 (50 %)	0	0	2 (100 %)	1 (50 %)
S. milwaukee	2 (100 %)	0	0	0	2 (100 %)	0
S. derby	1 (100 %)	0	0	0	1 (100 %)	0

***Salmonella* sp.**	**Health status**				**TOTAL**	**(2) + (3) + (4)**
	(1) Healthy	**(2) Diseased**	**(3) Convalescent**	**(4) Dead**		
S. enteritidis	1 (100 %)	0	0	0	1 (100 %)	0
S. Havana	1 (100 %)	0	0	0	1 (100 %)	0
S. irumu	0	0	0	1 (100 %)	1 (100 %)	0
S. montevideo	0	1 (100 %)	0	0	1 (100 %)	1 (100 %)
S. schwarzengrund	0	1 (100 %)	0	0	1 (100 %)	1 (100 %)
S. of group E1	1 (100 %)	0	0	0	1 (100 %)	0
TOTAL	56 (38.9 %)	45 (31.3 %)	32 (22.2 %)	11 (7.6 %)	144 (100 %)	88 (61.1 %)

9.21 Age distribution of camel calves found positive with *E. coli* carrying virulence-associated genes *eae, astA, hlyEHEC* and *stx*.



0 = number of negative cases

1 = number of positive cases

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I hereby declare, that the work for this dissertation was done by me and only with the help of the mentioned sources and references.

(Hiermit erkläre ich, dass ich die vorliegende Arbeit selbständig angefertigt habe. Ich versichere, dass ich ausschliesslich die angegebenen Quellen und Hilfen in Anspruch genommen habe.)

Berlin, June 2007

Ilona Viktoria Glücks