Central Veterinary Research Laboratory
Dubai (United Arab Emirates)
Scientific Director: Priv. Doz. Dr. Dr. habil. U. Wernery

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Institut für Hygiene und Technologie der
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(Lehrstuhl Prof. Dr. E. Märtlbauer)
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Hygienic status of camel milk in Dubai (United Arab Emirates) under two different milking management systems

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Hygienischer Status von Kamelmilch in Dubai (Vereinigte Arabische Emirate) unter Berücksichtigung zweier verschiedener Milchgewinnungssysteme

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To my family
and to my friends who supported
me during the last years

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ABBREVIATIONS

A. pyogenes	*Arcanobacterium pyogenes*
B. abortus	*Brucella abortus*
B. cereus	*Bacillus cereus*
B. melitensis	*Brucella melitensis*
BPLS	Brilliantgreen-Phenolred-Lactose-Agar
C. burnetii	*Coxiella burnetii*
C. dromedarius	*Camelus dromedarius*
C. bactrianus	*Camelus bactrianus*
C. perfringens	*Clostridium perfringens*
C. striatum	*Corynebacterium striatum*
CAMP	Christie, Atkins and Munch-Petersen
cfu	Colony forming units
CIRAD	Centre de Coopération internationale en recherche agronomique pour le développement
CMT	California Mastitis Test
CNS	Coagulase negative staphylococci
CPS	Coagulase positive staphylococci
CVRL	Central Veterinary Research Laboratory
DNA	Deoxyribonucleic acid
E. coli	*Escherichia coli*
EC	Electrical conductivity
EFSA	European Food Safety Authority
EHEC	Enterohaemorrhagic *Escherichia coli*
F. necrophorum	*Fusobacterium necrophorum*
FAO	Food and Agriculture Organisation of the United Nations
FMD	Foot-and-mouth disease
AMK	Aerobe mesophile Keimzahl
GLIPHA	Global Livestock Production and Health Atlas
HC	Haemorrhagic colitis
HUS	Haemolytic uremic syndrome
KbE	Koloniebildende Einheiten
KPS	Koagulasepositive Staphylokokken

L. glama	*Lama glama*
L. guanicoe	*Lama guanicoe*
L. ivanovii	*Listeria ivanovii*
L. monocytogenes	*Listeria monocytogenes*
L. pacos	*Lama pacos*
LF	Leitfähigkeit
LFGB	Lebensmittel- und Futtermittelgesetzbuch (National Code for Food and Feed)
LMBG	Lebensmittel- und Bedarfsgegenständegesetz (National Law for Food and Commodities)
M. africanum	*Mycobacterium africanum*
M. avium	*Mycobacterium avium*
M. bovis	*Mycobacterium bovis*
M. kansasii	*M. kansasii*
MLCB	Mannit-Lysin-Cristalviolet-Brilliantgreen-Agar
NAGase	N-Acetyl-ß-D-glucosamindase
OIE	Office International des Epizooties (World Organisation for Animal Health)
P. aeruginosa	*Pseudomoas aeruginosa*
P. haemolytica	*Pseudomonas haemolytica*
PEMBA	Polymyxin-Pyruvate-Eggyolk-Mannite-Bromine-Thymolblue-Agar
PGRP	Peptidoglycan Recognition Protein
RER	rough endoplasmatic reticulum
Resp.	Respectively
S. agalactiae	*Streptococcus agalactiae*
S. aureus	*Staphylococcus aureus*
S. bovis	*Streptococcus bovis*
S. dysgalactiae	*Streptococcus dysgalactiae*
S. epidermidis	*Staphylococcus epidermidis*
S. Enteritidis	*Salmonella* Enteritidis
S. hyicus	*Staphylococcus hyicus*
S. intermedius	*Staphylococcus intermedius*
S. Typhimurium	*Salmonella* Typhimurium

S. uberis	*Streptococcus uberis*
SCC	Somatic cell count
TBC	Total bacteria count
TSYEA	Tryptone-Soya Agar Base with Yeast Extract
TTP	Thrombotic thrombocytopenic purpura
UAE	United Arab Emirates
V. vicugna	*Vicugna vicugna*
VRBA	Violet-Red Bile Agar
VTEC	Verotoxinogenic *Escherichia coli*
WHO	World Health Organisation

1. INTRODUCTION

The dromedary camel (*Camelus dromedarius*, one-humped camel) is the most important livestock animal in the semi-arid areas of Northern and Eastern Africa as well as in the deserts of the Arabian Peninsula. It is a multipurpose animal, used for its supply of milk, meat, hides and transport (BURGEMEISTER, 1974; KAPPELER, 1998).

Camel milk is one of the most valuable food resources for pastoral people in arid and semi-arid areas. In the last years milk consumption among urban population was increasing (FARAH, 2004; CHAIBOU, 2005). On the other hand, there are still few countries as the United Arab Emirates, Saudi Arabia, Mauritania and Kazakhstan where camel dairies exist and camel milk and milk products are produced for placing on the market (ABEIDERRAHMANE, 1997; WERNERY et al., 2002).

The main objective of this doctoral thesis was to determine the hygienic status of dromedary milk in the United Arab Emirates comparing camels kept and milked in a traditional environment and in a modern dairy, where camels were milked by machine. As in most countries, there are no limits for bacterial contamination of camel milk (SEMEREAB & MOLLA, 2001) in the United Arab Emirates. Therefore, one aim of this investigation was to provide basis values for orientation. The emphasis on the investigated bacteria was set on the determination of the total bacterial count (TBC), coagulase positive staphylococci, coliforms and *Escherichia coli*, *Salmonella* spp., *Listeria* spp. and *Bacillus cereus*. As verotoxinogenic *Escherichia coli* play an important role in food borne diseases in many countries, the presence of VTEC was examined in the faeces of the dairy camels.

The second point was to investigate whether or not common mastitis screening tests currently used in cows, ewes and goats are applicable to camel milk. The sensitivity and specificity of the electrical conductivity, of California mastitis test (CMT) and the correlation of somatic cell count with CMT were examined.

In addition, the two indicator enzymes for the proof of pasteurisation and higher heat treatments - alkaline phosphatase and peroxidase - were investigated on their adequacy for testing the same treatments in camel milk.

2. LITERATURE REVIEW

2.1 Dromedaries as milking animals

2.1.1 Taxonomy and breeds

In zoological taxonomy, camelids are classified in the suborder *Tylopoda* (pad-footed animals) that represents with the suborders *Suiformes* (pig-like) and *Ruminantia* (ruminants) the order *Artiodactyla* (even-toed ungulates). This makes obvious that camelids (family *Camelidae)* as ruminating animals are classified in proximity to ruminants but developed in parallel and are not part of the suborder *Ruminantia*. Some differences as foot anatomy, stomach system and the absence of horns underline this fact (SCHWARTZ & DIOLI, 1992; FOWLER, 1998; WERNERY, 2003).

The family *Camelidae* is divided into three genera: The old world camels (genus *Camelus*) and the new world camels (genus *Lama* with the species *L. glama, L. guanicoe*, *L. pacos* and genus *Vicugna* with the species *V. vicugna*) (WILSON & REEDER, 2005). In the older literature (e. g. LEGEL, 1990) sometimes only two genera (*Camelus* and *Lama*) have been described. Two domesticated species of old world camels exist: the dromedary or one-humped camel (*Camelus dromedarius*, Table 2.1*)* that has its distribution in the hot deserts of Africa and Asia and the Bactrian or two-humped camel (*Camelus bactrianus)* that can be found in the cold deserts and dry steppes of Asia. In the desert Gobi there is still a population of wild two-humped camels classified as *Camelus ferus* (RAO et al., 1970; PETERS, 1997; FOWLER, 1998).

The Bactrian camel was named after the area of Bactriana in Central Asia. The name of the dromedary has derived from the Greek word “dromeus” which means runner or “droma” - running (JASSIM & NAJI, 2002). The one-humped camel was probably domesticated in the region of today’s Yemen and Oman about 3.000 to 4.000 years ago (FOWLER, 1998). The wild Arabian camel became extinct (LENSCH, 1999).

Table 2.1: Genealogy of the dromedary camel (WILSON, 1984)

Order	*Artiodactyl*a (even-toed ungulates)
Suborder	*Tylopoda* (pad-footed animals)
Family	*Camelidae*
Subfamily	*Camelinae*
Genus	*Camelus*
Species	*Camelus dromedarius*

Camel breeds are not as differentiated and classified as breeds in other livestock. Systematic selection for productive traits has never been done in camels, except for racing animals (KAPPELER, 1998). Nevertheless, there are different breeds used for different purposes like riding, meat or milk production. Dromedaries for riding are daintier compared to burden dromedaries whose body can vary from small to tall, but is always of heavy weight (BURGEMEISTER, 1974). The breed most common in the UAE is the 'Al-Khawar' breed. It is mainly known for its racing performances but also bred for milk production. (CIRAD, 2006). The weight of a riding or light burden dromedary is given with approximately 400 kg (FARAH, 2004). In the following, the term "camel" without further details will be used exclusively for dromedary camels.

2.1.2 Physiology of reproduction in dromedary camels

The sexual cycle of dromedary camels begins at 24 months (PUSCHMANN, 1989). Different to ruminants, camels are seasonal polyoestrous animals. Usually the ovulation of the female dromedary is induced by copulation or the presence of a male (WILSON, 1984). Camel bulls show their sexual cycle during 3 - 4 months in winter season, beginning in December (RAO et al., 1970; FAZIL & HOFMAN, 1981).

The mean gestation period is reported to be between 315 - 360 days (PUSCHMANN, 1989) up to 370 -375 days (RAO et al., 1970; FAZIL & HOFMAN, 1981; ARTHUR, 1992). Generally, camels are mated for the first time at the age of 3 - 4 years. It is possible to breed with camels up to 25 - 30 years leading to 8 - 10 calves in a lifetime for pure milking camels. In most countries, it is customary to breed female camels in alternate years only (HASSAN, 1967, RAO et al., 1970; ARTHUR, 1992, FARAH, 2004).

2.1.3 Camel population in the world

According to FAO statistics (Global Livestock Production and Health Atlas - GLIPHA, 2006) the world population of camels is about 20 million animals, mainly in arid zones, of which 15 million camels live in Africa and 5 million in Asia (GLIPHA, 2006). In 2001, the total camel population was 19 million of which 17 million were dromedaries (*C. dromedarius*) and 2 million were Bactrian camels (*C. bactrianus*) (FARAH, 2004). In most countries, the camel population is increasing after a period of decreasing number due to the introduction of modern transport facilities (FARAH, 2004). An overview is given in Tables 2.2 and 2.3.

Table 2.2: Development of the dromedary population in some countries in Asia (GLIPHA, 2006)

Asia	**Count (n)**		
	1995	**1999**	**2003**
Afghanistan	201.000	290.384	175.000
Bahrein	900	915	920
India	1.030.000	820.000	900.000
Iran	143.000	143.000	146.000
Iraq	5.400	8.500	7.600
Israel	5.000	5.300	5.300
Jordan	18.000	18.000	18.000
Kuwait	3.400	3.600	9.000
Lebanon	490	450	440
Oman	94.400	117.000	124.700
Pakistan	1.1000.000	800.000	800.000
Qatar	48.483	50.305	51.000
Saudi Arabia	421.700	255.475	260.000
Syrian Arab Republic	6.711	13.330	13.500
Turkey	2.000	1.400	900
UAE	**158.264**	**207.446**	**250.000**
Yemen	231.000	246.000	264.000

Table 2.3: Development of the dromedary population in some countries in Africa (GLIPHA, 2006)

Africa	Count (n)		
	1995	**1999**	**2003**
Algeria	126,350	220,000	245,000
Burkina Faso	13,300	14,473	15,600
Chad	613,450	715,000	730,000
Djibouti	64,010	67,790	69,000
Egypt	131,000	134,000	120,000
Eritrea	71,000	75,000	75,000
Ethiopia	340,000	527,340	326,500
Kenya	787,700	811,500	830,000
Libyan Arab Jamahiriya	101,000	42,000	47,000
Mali	292,000	466,900	470,000
Mauritania	1,113,000	1,206,000	1,292,000
Morocco	37,000	36,000	36,000
Niger	380,000	404,000	420,000
Nigeria	14,881	18,000	18,000
Senegal	5,000	4,000	4,000
Somalia	6,100,000	6,925,500	7,000,000
Sudan	2,903,000	3,031,000	3,200,000
Tunisia	231,000	231,000	231,000

2.1.4 Importance of the camel today and in the past

As dromedaries are very drought tolerant, they thrive in arid zones of many countries in the world and provide food, hides and transport. Therefore, there has even been an increasing interest in the dromedary in arid countries, where other domesticated animals have difficulties to survive. Camels can graze on low productive pastures on which the production of milk is possible and economically profitable. For this reason, camels may reduce the dependence of pastoralists on other livestock that usually is much more vulnerable to drought than camels (YAGIL, 1982; MORTON, 1984; WILSON, 1984; FARAH, 1993; SEMEREAB & MOLLA, 2001; SELA et al., 2003; FARAH, 2004).

With the process of settlement in many countries, one-humped camels lost a part of their importance as nomad livestock but have taken an important place as farm animals (CHAFFER et al., 2000). In addition to this, dromedary camels are bred on a large scale in most countries of the Arabian Peninsula as camel racing has a high socio-economic importance in the Arabian Gulf where a new industry developed. Approximately 200.000 racing camels are living in the UAE. An average racing camel can participate in races until the age of 6 years and more (SNOW, 1992; HAYDN-EVANS & WERNERY, 1995).

The Bactrian camel is also used for providing milk, meat, hides and wool as well as being a mean of transport (CHAPMAN, 1985).

2.1.4.1 Food and other products

2.1.4.1.1 Milk and milk products

Milk

Camel milk is one of the most valuable food resources for nomads in arid regions and can contribute to a better income for pastoralists, especially as in the last years milk consumption among the urban population was increasing (FARAH, 2004; CHAIBOU, 2005).

The fact that it is mainly consumed in its raw state (boiling of the milk is not common as it is known to remove its "goodness"), the high ambient temperature and the lack of refrigeration facilities in many arid areas are the main reasons for hygienic problems (RADWAN et al., 1992; SEMEREAB & MOLLA, 2001).

Camel milk is considered a useful component of the diet for individuals that show allergic reactions to the protein fraction of cow, ewe or goat milk, as camel milk does not contain ß-lactoglobulin and the content of alpha-casein is much lower than in milk of the other herbivores mentioned (RESTANI et al., 1999).

A trial on patients with multi-resistant tuberculosis showed that camel milk (compared to cow milk) has a positive effect on the general condition of the tested individuals (MAL et al., 2001). In addition, camel milk appears to have a reducing effect on blood sugar level and increases quality of life of people affected by type I diabetes mellitus allowing the reduction

of the insulin dose if camel milk is consumed every day (AGRAWAL et al., 2005). The controlling effect on hyperglycaemia is probably due to the content of insulin and the slower coagulum formation in the stomach which results in a faster stomach passage (YAGIL et al., 1998; AGRAWAL et al., 2003). However, in the investigation of BREITLING (2002) no blood sugar reducing effect was observed.

There are few countries as the UAE, Saudi Arabia, Mauritania and Kazakhstan where camel dairies exist and camel milk and milk products are produced in pasteurised form for placing on the national market (ABEIDERRAHMANE, 1997; WERNERY et al., 2002).

Milk products

Camel milk can be transformed into cheese with satisfactory organoleptic qualities. This way of conservation is widely used in industrial and manual production processes. Camel milk coagulates after addition of calf rennet or synthetic coagulating enzymes. As the ability of coagulation is much lower in camel milk than in the milk of cows, ewes or goats (GAST et al., 1969; OTTOGALLI & RESMINI, 1976), the concentration of the coagulating additives has to be four times higher than the concentration for cow milk, but can be reduced by adding calcium salts. One problem of the production of camel milk cheese is the high moisture content that contributes to a lower density of the cheese. As the quality can be improved bynew technologies, camel milk cheese can be an important source of food in arid zones (OTTOGALLI & RESMINI, 1976; RAMET, 1987; RAMET, 1989; KAMOUN & BERGAOUI, 1989).

Pastoralists produce fermented milk called “Susa” or “Al-Garss” without heating, which leads to a product of varying taste and usually poor hygienic quality. Improvements of storing surplus milk in good (rainy) seasons by small-scale farmers were also investigated (FARAH et al., 1990).

Some attempts were made to produce butter from camel milk but gave no satisfactory result concerning consistency and taste (GAST et al., 1969; FARAH et al., 1989; ABU-LEHIA, 1997).

According to a Kenyan investigation, it is possible to lengthen the durability of camel milk by producing sweet condensed milk (FIELD et al., 1997).

2.1.4.1.2 Meat and meat products

Meat

Besides milk, meat is one of the most important products of the camel. It compares favourably with other livestock in yield and quality of the carcasses. But camels are still not systemically bred for meat production in many regions as camels are considered too valuable. For this reason, usually males and infertile female camels are sold as slaughter animals by pastoralists. Nevertheless, the sale of these camels for meat production can present an important source of income.

According to BURGEMEISTER (1974), MORTON (1984); FARAH (2004) and FINKE (2005) there has been an increasing demand of camel meat in people and societies that do not breed camels, thus leading to a higher number of camel abattoirs and butcheries in several countries that mainly slaughter young animals.

Meat products

Traditional camel meat products in Africa and Asia are mainly made of dried and salted meat (ULMER & FISCHER, 2004).

2.1.4.1.3 Other products

Camel wool is one of the world's most expensive natural animal fibres. In some countries, camels are kept in the backyards of cities to gain wool, besides milk and meat. An adult camel usually produces 2 - 3 kg per shearing (RADWAN et al., 1992; WERNERY, 2003). Camel hides are known for their strength and durability. They are used by camel breeders, but also as fashion accessories (WERNERY, 2003). Other products used are: dung as fertiliser and source of fuel for pastoralists and bones for production of jewellery or bone-meal for fertilising purposes (KÖHLER-ROLLEFSON, 2000).

2.1.5 Infectious diseases of food safety importance

2.1.5.1 Zoonoses

The zoonotic risk arising from camel milk should be considered because camel milk is usually consumed in its raw state (RADWAN et al., 1992; YOUNAN, 2004).

Brucellosis

Brucellosis is one of the most important zoonoses and affects human welfare and livestock health worldwide. It exists especially in the Mediterranean Basin, the Arabian Peninsula (see below), the Indian Subcontinent and parts of Central and South America. The disease is caused by bacteria of the genus *Brucella* (*B.*) which includes different species (mainly *B. abortus* and *B. melitensis*) that vary in their affinity and virulence to several hosts (FAO, 2004a; FAO, 2004b).

Old world camels are susceptible to *B. abortus* (bovine brucellosis) and *B. melitensis* (ovine/caprine brucellosis) (STRAUSS, 1995; FAO, 2004a). Both may cause widespread animal health problems in the Arabian Peninsula, occurring regularly in the UAE, as well as in Saudi Arabia, Yemen, Qatar, Kuwait and since 2002 also in Bahrain (OIE, 2004; OIE, 2006). Yet no human cases were reported in the last 9 years (OIE, 2004). However, several reports exist describing a human infection caused by consuming fresh camel milk.

BURGEMEISTER et al. (1975) found the presence of *B. abortus*-antibodies of 7.7 % in dromedaries in Tunisia, whereas TESHOME & MOLLA (2002) proved a total seroprevalence of *B. melitensis* in camels of 5.9 % in different regions of Ethiopia. Also RADWAN et al. (1992) and WERNERY et al. (2007a) reported a seroprevalence of *B. melitensis* in camels in Saudi Arabia and the UAE. As camel milk is often consumed in its raw state, the presence of *Brucella* spp. has to be taken as a serious health risk even if it seems that the excretion rate of *Brucella* organisms is lower than in goats and these organisms are not capable of growing in milk (HEESCHEN, 1994; YOUNAN, 2004). Epidemiologically, brucellosis in camels seems to be related to the prevalence of *B. melitensis.* According to YOUNAN (2004) it appears that there is a clear correlation between infections of sheep and goats with *B. melitensis* and infections of camels. In the above described study farmers and milkers were examined with the result that 20 % of them showed Malta fever due to *B. melitensis*.

Bovine tuberculosis

Tuberculosis is a chronic disease - caused by bacteria of the genus *Mycobacterium* (*M.*) - that affects many animal species. It is characterised by development of tubercles in the organs of most species. Bovine tuberculosis is caused by *M. bovis* and is a significant zoonotic disease (FAO, 2004d). As *M. bovis* is inactivated by pasteurisation, mainly raw camel milk plays a role in transmission of tuberculosis to humans (FAO, 2004d; YOUNAN, 2004), even if *M.*

bovis is not capable of growing in milk. This can be the case, if camels are kept in close contact to other livestock sensible to tuberculosis (EFSA, 2003; FAO, 2004d). In camel necropsy examinations *M. bovis*, *M. avium* and *M. kansasii* were isolated (STRAUSS, 1995). One outbreak of tuberculosis in camels caused by *M. bovis* has been reported since 1996 in the UAE (WERNERY et al., 2007b). Bovine tuberculosis is also endemic in Bahrain (last confirmed case in 2003) and Qatar (last confirmed case in 2002) (OIE, 2004; OIE, 2006). In 2006 one case of camel tuberculosis caused probably by a representative of the *M. africanum* subtype 1 has been described by KINNE et al. (2006).

Paratuberculosis (Johne's disease)

M. avium subsp. *paratuberculosis* is of worldwide concern in milk production due to the issue of its potential role in Crohn's disease in humans. An investigation of raw bulk milk samples and pasteurised cow milk in the United Kingdom showed, that *M. paratuberculosis* is occasionally present in raw and correctly pasteurised cow milk (72 - 74 °C for 25 s, phosphatase-negative) (GRANT et al., 2002). Few is known about paratuberculosis in camels but infections with *M. avium* subsp. *paratuberculosis* are reported in old world camels (BURGEMEISTER et al., 1975; FAZIL & HOFMAN, 1981; KINNE et al., 2007). According to OIE (2004) and OIE (2006) the last confirmed case of paratuberculosis in the UAE and in Oman occurred in 1999 in ovines, however, one male dromedary in the UAE died recently from camel paratuberculosis and represents the first case in camels in this country for 13 years (KINNE et al., 2007).

Q fever

Q fever is an infectious disease caused by *Coxiella* (*C.*) *burnetii*. It is of public health importance as it can be transmitted to humans by milk - frequently milked from clinically inapparent domesticated animals, but it is inactivated by pasteurisation (FAO, 2004c). *C. burnetii* seems to be wide-spread in camels according to STRAUSS (1995). This complies with the findings of BURGEMEISTER et al. (1975) who proved 17.3 % of serological positive reagents in Tunisia. Some non-confirmed cases of Q fever in animals have been reported in Bahrain from 1997 - 2000 and in Oman 2003 and 2004. No case in the UAE has been reported in the last years (OIE, 2004; OIE, 2006).

2.1.5.2 Foot-and-mouth disease (FMD)

Reports on FMD in old world camels are contradictory. It appears that dromedaries can contract the disease through close contact to FMD-contaminated livestock as well as in some cases following experimental infection (ABD EL-HAKIM, 2005; WERNERY & KAADEN, 2004, WERNERY et al., 2006c). According to these findings, camelids are considered being little susceptible to FMD by the World Organisation for Animal Health (OIE, 2002). According to WERNERY & KAADEN (2004) they do not become FMD virus carriers and do not transmit the disease to other susceptible animals (WERNERY, 2007) whereas ABD EL-HAKIM (2005) proved the transmission from camel to camel and towards cattle in a recent study carried out in Egypt. In this investigation, most camels were clinically unapparent but able to spread the virus (serotypes O and A). However, more research is needed to clarify this subject, especially as the answer to this question could be important for international trade of camel products as mainly in developing countries FMD is frequently endemic (WERNERY & KAADEN, 2004). FMD is generally occurring on the Arabian Peninsula, especially in cattle. Since 1996 cases are reported annually in the UAE excluding the years 2002, 2004 and 2005 (OIE, 2004; OIE, 2006).

2.2 Characteristics of lactation and camel milk

2.2.1 Anatomy of the camel udder

The camel udder consists of four quarters, each with two, sometimes three separated glandular complexes leading into one teat. So in each teat there are two (or three) milk canals. (YAGIL, 1985; WEBER, personal communication, 2003). The left and right halves of the camel udder are separated by a groove as the udder is suspended by fibro-elastic tissue, leading from the linea alba to the prepubic tendon (SMUTS & BEZUIDENHOUT, 1987). As one-humped camels are not systematically bred for milk production, there is a great variety in different udder and teat shapes and sizes. Additionally the shape can vary according to age and stage of lactation (TIBARY & ANOUASSI, 2000; ALBRECHT, 2003; WERNERY et al., 2004).

2.2.2 Characteristics of camel milk

Camel milk has a white opaque colour, a faintly sweetish odour and a sweet but sharp taste. It is thinner than cow or buffalo milk (OHRI & JOSHI, 1961; ABDURAHMAN, 1996a). Camel milk has a much slower natural creaming rate than cow milk - in its raw state and heat treated (FARAH & RÜEGG, 1991; FARAH, 1993).

2.2.3 Lactation

She-camels are capable to produce more milk than a young camel calf need. The length of the lactation period depends on race, parturition, climatic and food conditions and is reported to average between 12 (BURGEMEISTER, 1974; FARAH, 2004) or 18 months (RAO et al., 1970), 24 months are also mentioned (YAGIL, 2000). The natural frequency of calf-suckling is 8 x / 24 h (5 x during daytime, 3 x during the night) with a total duration of 214.8 $\pm$ 56.7 s (SAMBRAUS, 1995; SIMPKIN et al., 1997b).

2.2.4 Milk yield

Camels are considered as animals with the ability to give more milk than other herbivores in the same keeping conditions (FARAH et al., 1990). During the first 3 months of lactation their milk yield increases significantly. After a peak during the 4^{th} to 5^{th} month, it starts to decrease (BASMAEIL & BAKKAR, 1987; SIMPKIN et al., 1997a; GAILI et al., 2000; WERNERY et al., 2004).

The fact that there are various milking strategies and management conditions in different countries is likely to have an effect on the milk secretion rate and on the accuracy of milk yield estimation. In camels separated from their calves between milking times the total milk yield increases clearly (SIMPKIN et al., 1997a; SIMPKIN et al., 1997b).

The milk yield varies between the different dromedary breeds or types and between individual camels of the same breed. High milking frequency and good, adequate feed have also a positive effect on milk yield. Therefore the camel should not be considered a priori as species with low milk production (KNOESS et al., 1986; KAMOUN & BERGAOUI, 1989; ALSHAIK & SALAH, 1994; WERNERY et al., 2004). Some dairy breeds are characterised

by a milk production capability of more than 2.090 kg up to 4.000 kg per lactation (305 d) under natural grazing conditions (WARDEH, 1998).

BEKELE et al. (2002) reported that camels that lost their calf give less milk (3.8 l/d) than camels whose calves survived (4.2 l/d), whereas WERNERY et al. (2004) stated that separation from or death of the calf has no negative effect on milk yield.

An overview on the reported milk yields is given in Table 2.4. The average daily yield lies between 2.9 l in Niger (CHAIBOU, 2005) and a maximum value of 18.7 l in Pakistan (KNOESS et al., 1986). The usual range of daily yield is given with 2.4 - 11.9 l. There is also a great variety in the reported average lactation yield: 1.220 kg - 5.695 kg (Table 2.4).

Table 2.4: Milk yield in camels reported from various sources

Author(s)	**Country**	**Yield (kg/d)**	**Yield range (kg/d)**	**Average Yield (kg/lact.[1])**	**Yield range (kg/lact.[1])**
BEKELE et al., 2002	Ethiopia	4.1	-	1.422	-
BASMAEIL & BAKKAR, 1987	Saudi Arabia	5.5	2.4 - 7.6	-	-
BURGEMEISTER, 1974	Tunisia	4.0	-	1.220	-
CHAIBOU, 2005	Niger	2.9	up to - 7.7	-	-
EL-BAHAY, 1962	Egypt	-	3.5 - 4.5	-	1.600 - 2.000
GAILI et al., 2000	Saudi Arabia	-	-	-	1.048 - 2.576
GAST et al., 1969	Algeria	-	4.0 - 10.0	-	-
KAMOUN & BERGAOUI, 1989	Tunisia	6.1	3.0 - 11.0	1.860	915 - 3.355
KNOESS et al., 1986	Pakistan	18.7	-	5.695	-
SIMPKIN et al., 1997a	Kenya	-	5.7 - 6.6	2.670	1.386 - 4.146
WERNERY et al., 2004	UAE	5.0	3.0 - 7.0	-	-
WERNERY et al., 2006a	UAE	4.7	3.1 - 7.5	-	-

[1] In most articles the average lactation period is given with 305 d.

2.2.5 Milk contents and pH

The constituents of camel milk are well investigated since several years (Table 2.5). Moisture is given with 86.0 - 90.5 % (GAILI et al., 2000), which can be compared to that of cow, goat or human milk (FOX & MCSWEENY, 1998; FARAH, 2004).

Fat content is reported with values between 2.0 and 4.2 % (HASSAN, 1967; ALSHAIK & SALAH, 1994). Different to cow milk, camel milk fat contains few short-chain fatty acids (C_4 - C_{12}). The fatty acid pattern contains more long-chain fatty acids (C14:0, C16:0 and C18:0) (FARAH, 1993) and resembles in this point to human milk fat (LAXA, 1934). GAST et al. (1969) claim that the value of camel milk is to be found in the high concentrations of linoleic acid and polyunsaturated acids, which are essential for human nutrition, whereas STAHL (2005) and STAHL et al. (2006) report similar fat acid patterns in camel and cow milk.

With its protein content of 2.5 to 4.0 % (OHRI & JOSHI, 1961; ALSHAIK & SALAH, 1994) camel milk can be compared to goat milk. The lactose values are reported with 3.8 to 5.7 % (ALSHAIK & SALAH, 1994; FIELD et al., 1997) which compares to cow or ewe's milk and is little less than human milk. Finally the ash content is given with 0.7 to 1.2 % (GNAN & SHERIHA, 1986; MERIN et al., 1998) which can be compared with the ash content in milk of cows, goats and sheep (FARAH, 2004). The detailed values are displayed in Table 2.5. For comparison the composition of milk of other animal species and humans is given in Table 2.6.

Table 2.5: Chemical composition and pH of camel milk

Author(s)	Country	Moisture (%)	Fat (%)	Protein (%)	Lactose (%)	Ash (%)	pH
ALSHAIK & SALAH, 1994	Saudi Arabia	89.9	2.0 - 3.9	2.5 - 2.8	3.8 - 4.2	-	-
EL-BAHAY, 1962	Egypt	87.9	3.8	3.5	3.9	0.8	6.6
FIELD et al., 1997	Kenya	84.9	2.4	3.0	5.7	0.8	6.6
GNAN & SHERIHA, 1986	Libya	87.0 - 87.3	3.3 - 3.7	3.3 - 3.5	5.6 - 4.2	0.8 - 1.2	6.2 - 6.8
GAILI et al., 2000	Saudi Arabia	86.0 - 90.5	2.5 - 3.9	2.5 - 3.4	5.0 - 5.6	0.8 - 0.9	-
GULIYE, 1996	Israel	88.5	3.4	2.8	4.8	0.8	6.5
HASSAN, 1967	Sudan	-	4.2	3.7	4.1	0.8	-
KAMOUN & BERGAOUI, 1989	Tunisia	88.5	2.8	-	4.7	0.9	-
MERIN et al., 1998	Israel	89.2	2.6 - 3.1	2.7 - 2.8	-	0.7 - 0.8	-
OHRI & JOSHI, 1961	India	86.4	3.8	4.0	4.9	1.0	6.7
SELA et al., 2003	Israel	-	2.6	2.7	4.6	0.8	

Table 2.6: Chemical composition of milk of other animal species and humans (FOX & MCSWEENY, 1998; FARAH, 2004)

Species	Moisture (%)	Fat (%)	Protein (%)	Lactose (%)	Ash (%)
Cow	86 – 88	3.7 - 4.4	3.2 - 3.8	4.8 - 4.9	0.7 - 0.8
Goat	87 – 88	4.0 - 4.5	2.9 - 3.7	3.6 - 4.2	0.8 - 0.9
Sheep	79 – 82	6.9 - 8.6	4.5 - 6.7	4.3 - 4.8	0.9 - 1.0
Human	87.8 – 88.4	3.3 - 4.7	1.0 - 1.3	6.8 - 7.0	0.2 - 0.3

The average pH of camel milk is reported with values between 6.2 and 6.8 (GNAN & SHERIHA, 1986). The average value reported is 6.6 pH (EL-BAHAY, 1962; FIELD et al., 1997) and can be compared to the pH of ewe's milk (YAGIL, 1982). It can increase up to 7.2 in case of clinical mastitis (TUTEJA et al., 2003).

Camel milk is rich (24 - 36 mg/l) in vitamin C compared to cow milk (3 – 23 mg/kg) (FIELD, et al., 1997; KAPPELER 1998; JASSIM & NAJI, 2002).

The water content in camel milk is increasing during lactation and with parities (GULIYE, 1996; GAILI et al., 2000; EL-HATMI et al., 2004). On the other hand, the fat content decreases with the progress of lactation (GAILI et al., 2000; EL-HATMI et al., 2004). According to YAGIL & ETZION (1980), YAGIL et al. (1998) and YAGIL (2000) the water content increases also under conditions of dehydration whereas DAHLBORN et al. (1997) and MERIN et al. (1998) could not confirm this observation. Other reasons reported for variation in milk contents are age, race, and lactation stage (FARAH, 1993). According to MERIN et al. (1998) and EL-HATMI et al. (2004) the contents of camel milk vary with husbandry conditions: Protein and fat contents decrease under domestic keeping conditions (free access to water, addition of concentrate feed) while ash content increases and water content does not change. Milk of all four quarters seems to have the same composition. Its contents are similar to human milk except for lactose content, the milk is therefore considered suitable for infant feeding (OHRI & JOSHI, 1961; RESTANI et al., 1999).

According to GNAN et al. (1998) camel milk has a high antimicrobial activity compared to cow milk which can be attributed to compounds that are more active in camel milk whey than in casein. FARAH (2004) underlines that the main difference between cow and camel milk lies in the different physicochemical characteristics of the individual components as protein, lipids and ash.

2.3 Bacteria in camel milk

Milk is a good medium for several bacteria to develop. The growth of bacteria in milk depends mainly on temperature and the presence of other bacteria (HEESCHEN, 1994). As camel milk is usually consumed in its raw state, the presence of pathogenic bacteria may be of public health importance besides its influence on animal health (SAAD & THABET, 1993; YOUNAN, 2004). Generally, bacteria in milk can occur through colonisation of the teat canal or an infected udder (clinical or subclinical mastitis), resp., or as contaminants.

Contamination

Normally, the first contamination of milk takes place in the moment of milking during the passage of the teat canal and by milking equipment or milking personal. Further on contamination is possible during transport and storage of the milk. The main reason for spoilage of milk are saprophytic microorganisms. Mastitis pathogens, as far as they are zoonotic, are of public health concern as some of them are capable of producing toxins or causing infections in man (HEESCHEN, 1994; SEMEREAB & MOLLA, 2001).

Mastitis

A high percentage of subclinical mastitis in camels is reported by several authors (BARBOUR et al., 1985; ABDURAHMAN et al., 1995; GULIYE, 1996; OBIED et al. 1996; ALMAW & MOLLA, 2000). The pathogenic bacteria found by different scientific groups are similar to bacteria reported in mastitis of cows or other animals kept in traditional nomadic environment or camel farms (BARBOUR et al., 1985; ALMAW & MOLLA, 2000). The examination for pathogenic microrganisms is considered as the most reliable screening test for mastitis detection (CHAFFER et al., 2000) besides somatic cell count (see 2.4.1) , whereas electrical conductivity (see 2.4.2) and N-acetyl-ß-D-Glucosaminidase (NAGase, see 2.4.3) appear to be not suitable for mastitis diagnosis in camels (YOUNAN et al., 2001).

Udder defence mechanisms against pathogenic bacteria

In many cases of infections of camel udders with pathogenic bacteria, the latter are present at counts lower than 3.0 x 10^3 cfu/ml. A minority exceeds this count which may lead to the conclusion that the camel udder protects itself from infection and multiplication of these bacteria by an effective immune system (BARBOUR et al., 1985). Additionally BARBOUR et al. (1984), EL AGAMY et al. (1992) and KAPPELER (1998) found enzymes as lactoferrin, lactoperoxidase and lysozyme, known for their antimicrobial activity in cow milk (EL AGAMY et al., 1992; NAIDU, 2000) as well as peptidoglycan recognition protein (PGRP) in dromedary milk that also shows antimicrobial activity (KAPPELER et al., 1994).

Hygiene requirements

Up to now there is no legislation in the UAE or the European Union laying down hygienic standards for camel milk.

2.3.1 Total bacteria count (TBC)

The TBC of camel milk is reported with values that vary between 10^2 and 10^8 cfu/ml (SEMEREAB & MOLLA, 2001; WERNERY et al., 2002; SELA et al., 2003; YOUNAN, 2004). These differences underline the fact that TBC depends on several parameters: The camel milk itself, contamination of the camel udder and contamination of milking personal, containers etc. The relation of the different sources of contamination varies according to the keeping and milking conditions of the camels. Under pastoral production conditions, environmental contamination is likely to play a bigger role in the hygiene of raw camel milk than initial bacterial contamination of the camel milk (YOUNAN, 2004). If the total bacterial count is low, raw milk was observed not to turn sour for 4 days, when it was kept in a clean container and refrigerated (YOUNAN, 2004). In Table 2.7 different average TBC in camel milk are displayed.

Table 2.7: Average TBC in camel milk

Author(s)	**Country**	**TBC (cfu/ml)**
SEMEREAB & MOLLA, 2001	Ethiopia	4×10^5 - 10^5
SELA, et al., 2003	Israel	8.0×10^4 - $5.3\ 10^8$
WERNERY et al., 2002	UAE, bowl samples	94.1 % $\leq 1.0 \times 10^5$
YOUNAN, 2004	Kenya, udder samples	10^2 - 10^4
YOUNAN, 2004	Kenya, bucket samples	10^3 - 10^5

2.3.2 Staphylococci

Staphylococci are small Gram-positive cocci belonging to the family of *Micrococcaceae*. The species can be subdivided into two groups showing either coagulase positive or coagulase negative reactions (KLOOS & SCHLEIFER, 1986). The results of investigations carried out by OBIED et al. (1996), ALMAW & MOLLA (2000), SENA et al. (2000) and ABDEL GADIR et al. (2005) showed that coagulase positive (CPS) and negative staphylococci (CNS) are the bacteria most frequently isolated from camels and can be considered as main reason for subclinical mastitis in dromedaries.

The following Table 2.8 gives an overview on the prevalence of staphylococci in camel milk.

Table 2.8: Staphylococci in camel milk

Author(s)	Country	*S.aureus* (%)	CNS (%)	Samples (n)	Camels (n)
Healthy camels					
ABDEL GADIR et al., 2005	Ethiopia	24.6	> 56	956	253
ABDURAHMAN, 1998	Sudan	x	x	-	
AL-ANI & AL-SHAREEFI, 1997	Iraq	x	x	50	x
ALMAW & MOLLA, 2000	Ethiopia	0.6	3.6	753	195
BARBOUR et al., 1985	Saudi Arabia	17.1	-	205	205
CHAFFER et al., 2000	Israel	8.8	20.4	137	35
EL-JAKEE, 1998	Egypt	5.0	10.0	100	100
GULIYE, 1996	Israel	x	-	86	86
KOSPAKOV, 1976 [1]	Kazakhstan	x	-	-	
OBIED et al., 1996	Sudan	-	11.7	757	757
SAAD & THABET, 1993	Egypt	5.9	-	40	40
SEMEREAB & MOLLA, 2001	Ethiopia	14.9	31.7	130	130
TUTEJA et al., 2003	India	20.9	27.8	282	71
WERNERY et al., 2002	UAE	0.5	-	1313	14
YOUNAN et al., 2001	Kenya	11.0	-	1242	207
Healthy camels and mastitis cases					
SENA et al., 2000	India	14.0	-	150	x
Mastitis cases					
EL-JAKEE, 1998	Egypt	17.0	13.0	100	100
HAFEZ et al., 1987	Saudi Arabia	50.0		62	62
RAMADAN et al., 1987	Saudi Arabia	x	-	1	1

x Presence
[1] Bactrian camels

The prevalence of staphylococci varies according to the different studies, but there is nearly no investigation on the bacteriological hygiene of camel milk where staphylococci are not mentioned. The prevalence of CPS is given with 0.5 - 24.7 % in samples from clinically healthy camels (ABDEL GADIR et al., 2005; WERNERY et al., 2002) and up to 50 % in cases of clinical mastitis (HAFEZ et al., 1987). CNS are reported with a prevalence of 3.6 to

over 56 % of the samples in clinically inapparent udders (ABDEL GADIR et al., 2005; ALMAW & MOLLA, 2000) and with 13 % in cases of clinical mastitis (EL-JAKEE, 1998). In most investigations both CNS and CPS were isolated from the milk of the same camels.

2.3.2.1 Coagulase positive staphylococci (CPS)

Generally, the term "CPS" describes *Staphylococcus (S.) aureus*. Other CPS like *S. intermedius* or *S. hyicus* may occur in camel milk (ABDEL GADIR et al., 2005). In cow and goat milk these bacteria apparently do not play an important role in milk and milk products (SCHNELLHARDT, 1998). BARBOUR et al. (1985) and YOUNAN (2004) stress that the mastitis in milking dromedary is not only of veterinary interest but represents a direct threat to human health considering that *S. aureus* can produce heat stable enterotoxins that are not inactivated during pasteurisation of milk or production of milk products and can provoke food intoxication (vomiting and diarrhoea).

AL-ANI & AL-SHAREEFI (1997) point out, that *S. aureus* is the main cause of chronic mastitis in camels in Iraq. BARBOUR et al. (1985), HAFEZ et al. (1987) and EL-JAKEE (1998) report that *S. aureus* is one of the most common bacteria isolated from mastitis cases in camels in Saudi Arabia and Egypt. CHAFFER et al. (2000), GULIYE (1996) and TUTEJA et al. (2003) found a clearly increased somatic cell count in milk in which *S. aureus* was proved and considered it also as a main cause for clinical and subclinical mastitis.

One of the main risk factors for production of staphylococcal enterotoxin is the storage of milk at ambient temperature after milking. Already a short storage time can lead to enhanced growth of CPS and can present a serious problem to human health if the strain produces enterotoxin (NOLETO & BERDOLL, 1980).

For goat milk, several authors state that a high number of clinically asymptomatic goats were infected with enterotoxinogenic staphylococci and conclude, that there is a permanent health risk emanating from goat milk and its products (SCHNELLHARDT, 1998) which shows the importance of investigation on these bacteria.

2.3.2.2 Coagulase negative staphylococci (CNS)

CNS are the main cause of subclinical mastitis what goes conform with investigations in goat milk. The CNS most often isolated from camel milk is *S. epidermidis* (TUTEJA et al., 2003; ABDEL GADIR et al., 2005).

2.3.3 Coliforms and *Escherichia coli*

Coliforms, and *Escherichia (E.) coli* are often used as marker organisms. While the proof of coliforms is usually used as an indicator for poor manufacturing hygiene, *E. coli* is a marker for faecal contamination due to the fact that it is a commensal of the intestinal tract (SCHMIDT-LORENZ & SPILLMANN, 1988). However, this holds true more for water than for food (BUSSE, 1985). Moreover, both groups are known as mastitis pathogens in cows (SCHALM et al., 1971). Coliforms have been reported in camel milk from several authors - mainly in clinically healthy udders. Table 2.9 shows the prevalence of coliforms and *E. coli* in camel milk which is situated between 1.0 and 17.3 % in samples taken from healthy camels (EL-JAKEE, 1998; ABDEL GADIR et al., 2005) and at 1.4 % up to 29.4 % for coliforms in general (SAAD & THABET, 1993; WERNERY et al., 2002).

2.3.3.1 *Escherichia coli*

E. coli is also known as pathogenic bacteria causing severe intestinal and extraintestinal diseases in man (KAPER et al., 2004) as well as mastitis in cows (BRADLEY & GREEN, 2001). A peracute case of mastitis in she-camels due to *E. coli* following a caesarean section was reported by KAPUR et al. (1982). ABDEL GADIR et al. (2005) isolated *E. coli* mainly (99.0 % of the isolates) from camel quarters that showed signs of subclinical mastitis. They also reported one case of clinical mastitis caused by *E. coli*.

Table 2.9: Number of samples positive for coliforms and *E. coli* in camel milk

Author(s)	Country	Coliforms (%)	*E. coli* (%)	Samples (n)	Camels (n)
Healthy camels					
ABDEL GADIR et al., 2005	Ethiopia	-	17.3	956	253
AL-ANI & AL-SHAREEFI, 1997	Iraq	-	-	50	50
BARBOUR, et al., 1985	Saudi Arabia	-	1,5	205	205
EL-JAKEE, 1998	Egypt	3.0	1.0	100	100
GULIYE, 1996	Israel	-	-	86	86
SAAD & THABET, 1993	Egypt	29.4	-	40	40
SEMEREAB & MOLLA, 2001	Ethiopia	x	8,3	130	130
WERNERY et al., 2002	UAE	1.4	-	1313	14
Healthy and mastitis camels					
SENA et al., 2000	India	-	9.3	150	x
Mastitis cases					
EL-JAKEE, 1998	Egypt	11.0	6.0	100	100
KAPUR et al., 1982	India	x	x	1	1

x Presence

2.3.3.2 Coliforms

Coliforms are a heterogeneous group of *Enterobacteriaceae* (as *E. coli*, *Klebsiella*, *Enterobacter*, lactose positive biotypes of *Citrobacter*, *Serratia* and *Hafnia*). A high percentage of biotypes of these species originate from soil or water, some come from faecal contamination (SCHMIDT-LORENZ & SPILLMANN, 1988).

Coliforms besides *E. coli* that are reported in camel milk are *Klebsiella pneumoniae* (0.5 - 7.1 % of the milk samples) and *Citrobacter freundii* (0.6 - 3.0 %). In most cases these bacteria are present in clinically healthy camel udders, whereas 2 authors isolated these bacteria from cases of severe mastitis (Table 2.10)

Table 2.10: Coliform species in camel milk

Author(s)	Country	*Citrobacter freundii* (%)	*Klebsiella pneumo-niae* (%)	Samples (n)	Camels (n)
No clinical signs of mastitis					
ABDEL GADIR et al., 2005	Ethiopia	-	0.9	956	253
BARBOUR et al., 1985	Saudi Arabia	-	0.5	205	205
EL-JAKEE, 1998	Egypt	3.0	2.0	100	100
SEMEREAB & MOLLA, 2001	Ethiopia	0.6	7.1	130	130
Clinical signs of mastitis					
KAPUR et al., 1982	India	-	100	1	1
EL-JAKEE, 1998	Egypt	1.0	-	100	100

2.3.3.3 Verotoxinogenic *Escherichia coli* (VTEC)

In the last 25 years, VTEC gained importance as foodborne causative agents of human intestinal infections. Mainly the serovar O157:H7 provoked several outbreaks in Northern America, Europe, Japan, and other countries. Beside some other factors the principal virulence factor of VTEC is the production of toxins (shiga toxins or verotoxins) which are responsible for haemorrhagic colitis (HC), haemolytic uremic syndrome (HUS) and thrombotic thrombocytopenic purpura (TTP) (KARMALI, 1989; NATARO & KAPER, 1998; KAPER et al., 2004; SAYED & ABDEL HAFEZ, 2005).

Besides other ruminants cattle are considered as the main reservoir for VTEC as they excrete *E. coli* mostly without showing symptoms. Outbreaks through raw milk, pasteurised milk and raw milk cheese are reported (KARMALI, 1989; BEUTIN et al., 1993; SCHREPF, 1998; BÜLTE, 2004; SAYED & ABDEL HAFEZ, 2005). As mentioned above, VTEC are linked to three types of disease: HC that is characterised by severe bloody diarrhoea, HUS, a severe complication of HC in cases in children with ischemia in kidneys, the central nervous system and other organs of which 10 % end fatally. TTP is a disease with similar symptoms as HUS, but without previous diarrhoea that can also end fatal (KARMALI, 1989; SCHREPF, 1998). VTEC infections through sheep or goat milk have rarely been reported, EL-HADY et al.

(1995) proved *E. coli* O157:H7 in raw sheep milk in Egypt. To our knowledge no cases of infections in camels have yet been reported. The WHO (WHO, 2005a), however, mentions the camel as reservoir for VTEC serotype O157:H7.

2.3.4 *Bacillus cereus*

Bacillus (*B.*) *cereus* is a Gram-positive facultative anaerobe rod of the genus *Bacillus*. It is a widespread bacterium with the ability to form spores with high resistance against environmental influences. *B. cereus* is the cause of two different types of foodborne disease in man: a diarrhoeal type due to the production of enterotoxins in the small intestine and an emetic type, which is caused by the ingestion of a toxin (cereulide) produced in the foodstuff (WEGSCHNEIDER, 2004; BECKER et al., 2005; EFSA, 2003). *B. cereus* is also known as cause for acute mastitis in cows (BROWN & SCHERER, 1957; TERPLAN, 1957) and is often found in milk. The presence of *B. cereus* in camel milk is reported by SAAD & THABET (1993) with a prevalence of 29.4 % in the milk samples of healthy camels in Egypt. ABDEL GADIR et al. (2005) proved the presence of *B. cereus* in 9.1 % of 956 quarter milk samples taken from 253 traditionally managed lactating camels in Ethiopia. ALBRECHT (2003) reported the presence of *B. cereus* in the sand of a camel dairy farm in the UAE (CVRL).

2.3.5 *Salmonella* spp.

Salmonella spp. are Gram-negative, facultative anaerobe rods with more than 2500 known serovars that belong to the family *Enterobacteriaceae*. *Salmonella* spp. are of high importance in food safety being able to provoke severe intestinal infections in humans which can lead to death especially in elderly people (KLEER, 2004; WHO, 2005b). As in most animals, salmonella infections are common in camels in countries all over the world. Whereas some of the affected animals show clinical symptoms; others do not (FAZIL & HOFMAN, 1981; WERNERY, 2000; SEMEREAB & MOLLA, 2001). BURGEMEISTER (1974) proved the presence of a serological reaction to *Salmonella* (*S.*) Typhimurium and *S.* Enteritidis antigens each in 5.8 % of the examined camels. No cases of lactogenic transmission from camels to humans have yet been reported (YOUNAN, 2004). The most frequent reason for the presence of *Salmonella* spp. in milk is through faecal contamination after heat treatment as salmonellae are inactivated during pasteurisation (KLEER, 2004). No outbreaks of intestinal salmonellosis

have been reported in animals in the UAE since 1996 whereas intestinal salmonella infections are occurring in livestock in Saudi Arabia and Kuwait (OIE, 2004) and salmonellae are regularly isolated from healthy camels in the UAE (WERNERY, 1992; CVRL, 2006).

2.3.6 *Listeria* spp.

Listeriae are Gram-positive widespread rods with a high resistance against environmental influences as cold, drought and solar radiation and are growing well in cold environment (TERPLAN et al., 1986). Of the *Listeria* genus, mainly *Listeria* (*L.*) *monocytogenes* is of health importance for animals and humans, whereas other species as *L. ivanovii* and *L. seeligeri* are of minor importance in this respect or are considered as non pathogenic as *L. innocua*. The most common symptoms of listeriosis caused by *L. monocytogenes* are the dysfunction of the central nervous system, abortion and diarrhoea with possible lethal outgoing, especially in predisposed individuals like pregnant women, children, elderly and immunosuppressed people. Very few is reported about listeria infections in old world camels. According to BURGEMEISTER et al. (1975), a serological positive reaction was observed in 34.6 % of the tested camels in Tunisia, whereas listeriae do not seem to be of high importance in the Arabian Peninsula as no cases of listeriosis in animals and humans have been reported in the Arabian Peninsula in the last 10 years (OIE, 2004).

2.3.7 Other bacteria

2.3.7.1 *Streptococcus* spp.

The presence of *Streptococcus* spp. is mentioned in most articles in connection with the hygiene of camel milk. When a differentiation was done, mainly *Streptococcus* (*S.*) *agalactiae*, *S. dysgalactiae* and *S. uberis* were found in camel milk (see Table 2.11). *S. agalactiae* is reported as one of the main cause for clinical mastitis in camels and a potential human pathogen, causing infections mainly in newborns (ALMAW & MOLLA, 2000; YOUNAN, 2004). ABDEL GADIR et al. (2005) isolated *S. uberis* out of 7.0 % of 956 quarter-milk samples, of which 95 % were taken from cases of subclinical mastitis and 5 % of clinical mastitis.

Table 2.11: *Streptococcus* spp. in camel milk

Author(s)	Country	*Streptococcus* spp. (%)	*S. agalactiae* (%)	*S. dysgalactiae* (%)	Samples (n)	Camels (n)
Healthy camels						
ABDEL GADIR et al., 2005	Ethiopia	*S. uberis*: 7.0	2.6	-	956	253
AL-ANI & AL-SHAREEFI, 1997	Iraq	x	-	-	50	-[1]
ALMAW & MOLLA, 2000	Ethiopia	-	1.5	-	753	195
BARBOUR et al., 1985	Saudi Arabia	4.9	-	-	205	205
CHAFFER et al., 2000	Israel	1.5	-	-	137	35
EL-JAKEE, 1998	Egypt	12.0 (*S. uberis* 4.0)	8.0	-	100	100
GULIYE, 1996	Israel	-	-	x	86	86
KINNE & WERNERY, 2002	UAE	-	x	-	1	1
SEMEREAB & MOLLA, 2001	Ethiopia	7.8	1.8	1.2	130	130
TUTEJA et al., 2003	India	20.9	10.4	10.4	282	71
YOUNAN et al., 2001	Kenya	-	12.0	-	1242	207
Healthy camels and mastitis cases						
SENA et al., 2000	India	-	67.4	-	150	-[1]
Mastitis cases						
EL-JAKEE, 1998	Egypt	28.0 (*S. uberis* 12.0)	16.0	-	100	100
HAFEZ et al., 1987	Saudi Arabia	33.3	-	-	62	62

x Presence
[1] No number of examined camels is given

2.3.7.2 Further bacteria

Besides the above mentioned bacteria, other species are also reported. Table 2.12 gives an overview of these microorganisms isolated from camel milk. Six of seven authors detected *Micrococcus* spp. in the milk of clinically inapparent udders with a prevalence of 0.5 - 25.4 %, whereas one of six authors (EL-JAKEE, 1998) found these bacteria in 19 % of the milk samples of examined mastitis cases.

The prevalence of *Pasteurella* (*P.*) *haemolytica* is given with 1.5 - 6.0 % by six out of seven authors in the milk of clinically inapparent camels and with 3.0 % by one author (EL-JAKEE, 1998) in samples of camels with clinical signs of mastitis.

HAFEZ et al. (1987) did not specify the subspecies of the 16.7 % *Pasteurella* spp. isolated from 62 mastitis cases.

Pseudomonas (*P.*) *aeruginosa* was isolated by four of seven authors from healthy camels with a prevalence of 1.0 - 17.7 %. No isolation of samples from camel udders with clinical mastitis is reported.

The prevalence of *Arcanobacterium* (*A.*) *pyogenes* is given with 1.0 - 10.0 % in milk of clinically inapparent camels by four authors and 2.0 % in samples of camels with mastitis by EL-JAKEE (1998).

EL-JAKEE (1998) also reported on the following anaerobic bacteria in samples of camel milk: *Clostridium* (*C.*) *perfringens* (56 % toxigenic), *Peptostreptococcus* spp. and *Fusobacterium* (*F.*) *necrophorum*, both from inflamed udders and udders that show no sign of mastitis (Table 2.13).

Table 2.12: Other bacterial findings in camel milk

Author(s)	Country	Samples (n)	Camels (n)	*Micrococcus* spp. (%)	*P. haemolytica* (%)	*P. aeruginosa* (%)	*A. pyogenes* (%)
Healthy camels							
ABDEL GADIR et al., 2005	Ethiopia	956	253	0.5	2.1	-	-
ALMAW & MOLLA, 2000	Ethiopia	753	195	x	x	-	x
AL-ANI & AL-SHAREEFI, 1997	Iraq	50	-[2]	x	x	-	x
BARBOUR et al., 1985	Saudi Arabia	205	205	25.4	1.5	1.0	1.0
EL-JAKEE, 1998	Egypt	100	100	17.0	6.0	5.0	10.0
SAAD & THABET, 1993	Egypt	40	40	23.5	-	17.7	-
SEMEREAB & MOLLA, 2001	Ethiopia	130	130	-	5.4	4.8	1.2
Camels with Clinical Mastitis							
EL-JAKEE, 1998	Egypt	100	100	19.0	3.0	-	2.0
HAFEZ et al., 1987	Saudi Arabia	62	62	-	16.7[1]	-	-
HASSANEIN et al., 1984	Egypt	1	1	-	-	-	x
KAPUR et al., 1982	India	1	1	-	-	-	-
KINNE & WERNERY, 2002	UAE	1	1	-	x	-	-
RAMADAN et al., 1987	Saudi Arabia	1	1	-	x	-	-

[1] *Pasteurella* spp.
[2] No number of examined camels is given
x Presence

Table 2.13: Anaerobic bacteria in camel milk from Egypt (EL-JAKEE, 1998)

Samples (n)	**Camels (n)**	***C. perfringens* (%)**	***Peptostreptococcus* spp. (%)**	***F. necrophorum* (%)**
100	100 (no mastitis)	15.0	18.0	8.0
100	100 (mastitis)	3.0	10.0	5.0

2.3.8 Yeasts

EL-JAKEE (1998) found in 4 % of camels with clinical signs of mastitis the presence of *Candida albicans*. In none of the healthy udders, *Candida albicans* was found. In most investigations on camel milk, no yeasts were detected or were not within the scope of examination.

2.4 Further diagnostic means in camel milk

2.4.1 Somatic cells and cell fragments in camel milk

Apparently there is a significant positive correlation between positive CMT results and the presence of clinical mastitis in dromedaries (BARBOUR et al., 1985; KINNE & WERNERY, 2002). This leads to the presumption that the camel - like the cow - has phagocytic cells as one of the essential defence mechanisms of the mammary gland against pathogenic microorganisms (SCHALM et al., 1971; BARBOUR et al., 1985; ABDURAHMAN et al., 1992; SAAD & THABET, 1993).

According to ABDURAHMAN (1998) and ABDURAHMAN et al. (1996b) somatic cells in milk of Bactrian camels are composed of macrophages (8.8 - 11 µm) in the first place, neutrophils (8.3 - 10.2 µm) and lymphocytes (4.4 - 8.8 µm). In addition to those cells a large number of cell fragments can be found in camel milk that are surrounded by plasma membranes, containing mitochondria and abundant rough endoplasmatic reticula (RER), but do not have a nucleus. They appear as particles with a high variety in diameter, structure and cytoplasmatic density and are similar to the fragments seen in goat milk. These particles may have an influence on the diagnostic of udder health by determination of the somatic cell

count, as they could be counted by mistake as cells using direct microscopy. These cell fragments appear not to react with some mastitis indicators as CMT what can be explained by the absence of DNA in the fragments in goat and camel milk (see 2.4.1.1) (SCHALM et al., 1971; ABDURAHMAN et al., 1992).

Besides other influences on SCC like age and stage of lactation of a she-camel (OBIED et al., 1996) the mean somatic cell count appears to increase if pathogenic bacteria can be isolated (SAAD & THABET, 1993; CHAFFER et al., 2000; SEMEREAB & MOLLA, 2001; TUTEJA et al., 2003 and KOSPAROV, 1976 (*C. bactrianus*)). Therefore CHAFFER et al. (2000) come to the conclusion that the SCC can be used as effective marker for inflammation as well as indicator for udder infections in camels. The fact that the Coulter Counter counts all particles (cells and cell fragments) in the milk seems to have no significant negative influence on these results.

2.4.1.1 Semi-quantitative California mastitis test (CMT)

The principle of this reaction is based on the fact that the DNA of the somatic cells in the milk builds a complex with the testing reagent with the result of a higher viscosity of the milk SCHALM, 1960).

SENA et al. (2000) defined the health status of camel udders on the basis of CMT results as follows (Table 2.14):

Table 2.14: Health status defined with the aid of CMT (SENA et al., 2000)

CMT		**Definition**	**Udder health**
-	(1)	no slime formation	normal milk
+	(2)	slime formation that disappears with shaking	subclinical mastitis
++	(3)	sticky, persistence of slime, gel formation	clinical mastitis (grade I)
+++		pH > 9,5, gel formation thickened towards the centre	clinical mastitis (grade II)

ALMAW & MOLLA (2000) (Ethiopia) state that 87.7 % of the camel udders with CMT

positive results showed infections with pathogenic bacteria (Table 2.15). Going conform with these findings, ABDURAHMAN (1998) states that 100 % of the CMT positive samples were tested positive with pathogenic bacteria (no number of camels is given, Table 2.15), whereas YOUNAN et al. (2001) observed all udders with negative CMT result containing 77 % *S. agalactiae* and 68 % *S. aureus*.

Table 2.15: Correlation of CMT and the detection of pathogenic bacteria in camel milk

Author(s)	Country	CMT positive + pathogenic bacteria (%)	CMT positive - pathogenic bacteria (%)	Samples (n)	Camels (n)
ALMAW & MOLLA, 2000	Ethiopia	87,7[1]	10,8[1]	753	195
ABDURAHMAN, 1998	Sudan	100	-	-	-

[1] 1,5 % (1 camel) was not included in the description of the CMT positive results

2.4.1.2 Somatic cell count (SCC)

One investigation on milk of Israeli camels using Coulter Counter resulted in a mean SCC of 3.1 x 10^5 cells/ml. No significant difference in SCC was detected in milk samples without pathogenic bacteria and samples contaminated with *S. aureus* (CHAFFER et al., 2000). BHATT et al. (2004) reported average SCC values of 2.6 x 10^5 and 2.5 x 10^5 cells/ml before treatment with antibiotics and counts of 2.1 x 10^5 and 1.6 x 10^5 cells/ml in camel milk after antibiotic treatment. The SCC was done according to SCHALM et al. (1971) with Giemsa stain.

KOSPAKOV (1978) reports mean SCC of 1.3 x 10^6 cells/ml in healthy Bactrian camels and 1.3 x 10^7 cells/ml in milk samples that showed a positive (+ to +++) CMT value.

AL-ANI & AL-SHAREEFI (1997) and TUTEJA et al. (2003) compared SCC and CMT results as shown in Table 2.16:

Table 2.16: CMT - correlation to somatic cell count

Author(s)	Country	CMT	SCC (cells/ml)	Samples (n)	Camels (n)
AL-ANI & AL-SHAREEFI, 1997[1]	Iraq	+/- + ++ +++ ++++	400.000 600.000 800.000 1.300.000 1.600.000	50	50
TUTEJA et al., 2003[2]	India	- +/- + ++ +++	480.000 990.000 1.720.000 6.560.000 12.230.000	282	71

[1] SCC determined according to COLES, 1986
[2] SCC determined according to SCHALM et al., 1976 (Giemsa stain)

According to the results of SENA et al. (2000) there is a significant positive correlation between pH, CMT and total leukocyte count (TLC). ABDURAHMAN (1998) and ABDEL GADIR et al. (2005) reported a significant positive correlation between the mean values for CMT and SCC and the presence of major pathogen bacteria in camel milk samples. Considering these findings CMT appears to be the most suitable diagnostic measure available for detecting udder inflammation.

2.4.2 Electrical conductivity in cow and camel milk

Electrical conductivity is defined as the resistance of a material to electric current. Within milk production it is widely used as a simple and effective tool for mastitis diagnosis in cows. In case of mastitis, the cell membranes of the udder parenchyma are damaged. This increases the permeability of the barrier between blood and milk. The content of chloride (Cl^-) and sodium (Na^+) is increasing and the content of lactose and potassium (K^+) decreasing which leads to a higher electrical conductivity of the milk. The average conductivity of cow milk ranges between 4 and 5.8 mS/cm. It depends on lactation stage, age, milking interval and race of the individual animal (NIELEN et al., 1992; WALZEL, 1997; BILLON et al., 2001). General values for cow milk are given in Table 2.17.

Table 2.17: Values of electrical conductivity of cow milk (ANONYMOUS, 2001)

Assessment of the quarters	Difference to the quarter with the lowest electrical conductivity (mS/cm)	Absolute values of electrical conductivity in cows (mS/cm)
Normal	< 0.6	< 5.5
Suspect	0.6 - 0.9	5.5 - 6.4
Subclinical mastitis	≥ 1.0	≥ 6.5

The electrical conductivity was not considered adequate as method for mastitis diagnosis in camels by YOUNAN et al. (2001) and BHATT et al. (2004) as no significant change can be proved in case of mastitis. The values given by YOUNAN et al. (2001) are displayed in Table 2.18. In contradiction to these results, the content of chloride was found increased (> 14 %) in clinical cases of mastitis compared to healthy udders or subclinical mastitis (< 14 %) (SENA et al., 2000).

Table 2.18: Electrical conductivity of camel milk (YOUNAN et al., 2001)

CMT	negative	positive
Samples (n)	309	15
Absolute conductivity in healthy udders (mS/cm)	6.5 ± 3.0	-
Conductivity differences between non-infected quarters of one camel udder (mS/cm)	0.9 - 5.1	-
Absolute conductivity in udders with bacterial infections (mS/cm)	-	7.5 ± 1.4
Conductivity differences between infected quarters of one camel udder (mS/cm)	-	0.0 - 3.8

2.4.3 N-acetyl-ß-D-Glucosaminidase (NAGase)

NAGase is a lysosomal enzyme released from damaged epithelial and other somatic cells in the mammary gland estimated as a good indicator of mastitis in bovine and ovine milk. In camel milk NAGase activity is significantly higher than in cow milk which might be due to the high count of cell fragments. It does not clearly correlate with bacterial findings in contrary to SCC (ABDURAHMAN, 1995; GULIYE, 1996; CHAFFER et al., 2000).

Therefore NAGase activity was not investigated as a diagnostic mean for infections or inflammations of camel udders.

2.4.4 Indicator enzymes for heat treatment of milk

As contamination of camel milk with pathogenic bacteria cannot be completely avoided but only reduced by good milking practice, pasteurisation is an important tool for inactivation of bacteria, resulting in an extended shelf life and improved safety. Ultra high heat treatment of camel milk appears to be no alternative, as sediment is formed by denatured proteins if the milk is not cooled (FARAH et al., 2004).

In the former German Milk Regulation (ANONYMOUS, 2000b) two possibilities for pasteurisation of cow milk were mentioned:
- Continuous heating at +62 to +65 °C for 30 to 32 min
- Short time heating at +72 to +75 °C for 15 to 30 s

For the control of this treatment, it is stated that the alkaline phosphatase has to be inactivated, but the peroxidase shall still be active. The control parameter alkaline phosphatase is also laid down in Regulation (EG) 853/2004 (ANONYMOUS 2004). Peroxidase, however, is not further mentioned.

For high temperature heating, the control can be made by the proof of peroxidase inactivation. It appears that camel milk whey proteins are less susceptible to heat treatment than whey proteins of cow milk (FARAH, 1986; MOHAMED & LARSSON-RAZNIKIEWICZ, 1991; MONTET, 1997). This means that whey proteins of camel milk are denatured at higher temperatures which is important for further observation of the behaviour of enzymes in heat treated camel milk.

LOISEAU et al. (2001) and WERNERY et al. (2006b) showed that alkaline phosphatase is not suitable as indicator for pasteurisation, as it is not totally inactivated even after heat-treatment of camel milk for 30 s at 90 °C whereas no detailed data is known on peroxidase. It seems that the enzyme is stable after heat-treatment of 76 °C for 16 s (LOISEAU et al., 2001).

3. OWN INVESTIGATIONS

3.1 Materials

3.1.1 Animals

All samples originated from former racing camels (*Camelus dromedarius*) of the common UAE-breed. The age of the she-camels was estimated over 12 years following the method of PUSCHMANN (1989). The lactation stages varied between 1 to 16 months and the daily milk yields varied between 1 and 12 l per day.

3.1.2 Samples

All camel milk samples of this investigation were taken during the winter period 2002/2003 (October - April) from a dromedary herd consisting of 25 animal kept at the Central Veterinary Research Laboratory (CVRL) and four desert camps in the Emirate of Dubai, United Arab Emirates. The examination took place in the CVRL. 47 samples for examination for *Salmonella* spp. and 50 samples for *Listeria* spp. were collected in August and September 2003, frozen directly after collection and sent to the "Lehrstuhl für Hygiene und Technologie der Milch" in Munich for examination. For the trial 168 samples of hand milked milk, 260 bulk milk samples of machine milked milk from the CVRL herd and 468 quarter-milk samples of camels from the same herd milked by machine were examined.

Table 3.1: Characterisation of the animals

Character	Milked by hand	Milked by machine
Samples	1 sample of all 4 quarters per camel	Herd samples, Quarter-milk samples
Number of animals	18	25
Previous use	Former racing camels	Former racing camels
Living conditions	In desert camps, with free access to the desert.	In groups of 6 camels in a confined space of approximately 80 m² for each group at CVRL
Milking frequency	Semi-daily	Semi-daily
Calves	Stayed with mother camels, separated some hours before milking	Were allowed to drink 1 h directly after each milking and 9 - 10 h on week-ends
Calf with mother	12 months	Until end of lactation
Soil	Sand	Sand
Feed	Desert herbs, hay	Alfalfa (Lucerne), barley, hay daily and occasionally dates
Water	At least semi-daily	Free access
Disease control	Free of brucellosis	Free of brucellosis, tuberculosis, Salmonella and Q-fever
Owners	Different owners	CVRL

3.1.3 Milking

3.1.3.1. Machine milking

- Westfalia mobile bucket milking machine
- ‘Stimopuls CP Pulsator’
- ‘Classic 300’ claw, standard
- Liners ‘Stimulor L 25 grün’
- Light milking unit channels
- Sight glasses
- Short milk tubes (made from silicon) with 10 mm inner diameter
- Short pulse tubes

- Long milk tube with 16 mm inner diameter
- Long pulse tube
- Milk bucket
- Plastic containers with 9 and 20 l content

3.1.3.2. Hand Milking

- Large steel bowls, content 5 l
- Aluminium foil as cover
- Clean latex gloves

3.1.4 Sampling

- Clean latex gloves
- Sterile sample tubes, content: 80 ml
- Sterile pipette

3.1.5 Examination scheme

Table 3.2: Examination scheme

Examination	**Hand milked samples**	**Machine milked samples**	**Quarter-milk samples**
Total bacterial count	x	x	x
Coagulase positive staphylococci	x	x	x
Coliforms	x	x	x
Bacillus cereus	x	x	-
Salmonella spp.	x	x	-
Listeria spp.	x	x	-
Pathogenic bacteria on blood agar	-	-	x
CMT	x	x	x
Electrical conductivity	-	-	x

3.1.6 Bacteria in camel milk - culture media and reagents

3.1.6.1. Preparation of samples and dilutions

- Ringer Solution Tablets	Oxoid BR 52

3.1.6.2. Total bacterial count (TBC)

- Milk Plate Count Agar	Oxoid CM 681

3.1.6.3. Coagulase positive staphylococci (CPS)

- Baird-Parker Agar Base	Oxoid CM 275
- Egg Yolk-Potassium-Tellurite-Emulsion	Oxoid SR 54
- Hydrogen peroxide (3 % solution for catalase test)	Merck 1.11351
- Slidex™ Staph-Kit	bioMérieux 73112
- BBL CRYSTAL®, Gram-Positive ID System	Becton Dickinson 245240
- Distilled Water	CVRL
- Gram Stain	

3.1.6.4. Coliforms and *Escherichia coli*

- Violet-Red Bile Agar (VRB)	Oxoid CM 107
- Nutrient Agar	Oxoid CM 3
- Oxidase Test Sticks	Oxoid BR 64
- BBL Enterotube II	Becton Dickinson 273176
- Kóvacs Indole Reagent	Merck 1.09293
- VP 1 40 % Potassium hydroxyde	Becton Dickinson 266651
- VP 2 5 % alpha (1-) Naphtol	Becton Dickinson 266661

3.1.6.5. Verotoxinogenic *Escherichia coli* (VTEC) in faecal samples

- Trypton Soya Broth with Novobiocin, Cefsulodin & Mitomycin	Heipha, 1055r
- Verotoxin Veterinär Test Kit: NOVITEC®	Hiss Diagnostics 900-

3.1.6.6. *Bacillus cereus*

- *Bacillus cereus* Agar Base – PEMBA	Oxoid CM 617
- *Bacillus cereus* Selective Supplement	Oxoid SR 99
- Egg Yolk Emulsion	Oxoid SR 47

3.1.6.7. *Salmonella* spp.

- Buffered Peptone Water	Oxoid CM 509
- *Salmonella* Enrichment Broth Rappaport Vassiliadis (RVS)	Merck 1.07700
- Difco® Selenite Cystin Broth	Becton Dickinson 268740
- Brilliant Green Agar modified (BPLS)	Oxoid CM 329
- MLCB Agar	Oxoid CM 783
- Nutrient Agar	Oxoid CM 3
- Oxidase Test Sticks	Oxoid BR 64
- *Salmonella* Test Serum Polyvalent I	Dade Behring ORMT11
- *Salmonella* Test Serum Polyvalent II	Dade Behring ORMU11
- Potassium hydroxide Pellets (3 % solution for KOH test)	Merck 1.05033
- BBL Enterotube II	Becton Dickinson 273176

3.1.6.8. *Listeria* spp.

- Fraser Broth	Oxoid CM 895
- Half Fraser Supplement	Oxoid SR 166
- Fraser Supplement	Oxoid SR 156
- ALOA Medium	AES 520079
- PALCAM Agar Base	Oxoid CM 877
- PALCAM Selective Supplement	Oxoid SR 150
- Tryptone Soya Agar	Oxoid CM 131
- Yeast Extract	Oxoid L 21
- Columbia Agar with Sheep Blood plus	Oxoid PB 5039
- Potassium hydroxide Pellets (3 % solution for KOH Test)	Merck 1.05033
- Hydrogen peroxide (3 % solution for catalase test)	Merck, 1.11351

- D (-) Mannitol Merck 1.05987
- D (+) Xylose Merck 1.08692
- L (+) Rhamnose Merck 1.07361
- Test strains: Beta-hemolytic strain of *Staphylococcus aureus, Rhodococcus equi, Listeria monocytogenes*, *L. ivanovii* and *L. innocua* from the Culture Collection of the "Lehrstuhl für Hygiene und Technologie der Milch", Munich

3.1.6.9. Other bacteria

- Blood Agar Base No 2 Oxoid CM 271
- Sheep Blood, 7 %, defibrinated CVRL
- Nutrient Agar Oxoid CM 3
- Oxidase Test Sticks Oxoid BR 64
- Kóvacs Indole Reagent Merck 1.09293
- API 20 E bioMérieux 20100
- API 20 NE bioMérieux 20050
- BBL CRYSTAL®, Gram-Positive ID System Becton Dickinson 245240)

3.1.7 Somatic cells and cell fragments in camel milk

3.1.7.1. Semi-quantitative California mastitis test (CMT)

- Test Solution with pH Indicator Hauptner 96101
- Test Bowl with 4 Test Fields Hauptner 96100

3.1.7.2. Somatic cell count (SCC)

- Glass Slides Menzel-Gläser Superfrost
- Giemsa Stain Wescor A - C
- Rinse solution Wescor D
- Distilled Water CVRL
- Light Microscope Nikon labophot 239635

3.1.8 Electrical conductivity

- Portable “Mastitron-Kontrollsystem”	Milku, Neukrichen-Vluyn

3.1.9 Indicator enzymes for heat treatment of milk

3.1.9.1. Peroxidase

- Traventol® Test	Bionic Niebüll

3.1.9.2. Alkaline phosphatase

- Lactognost® Test	Heyl

3.2 Methods

3.2.1 Milking

3.2.1.1 Machine milking

Milking procedure

The camels were milked 2 times daily during the week and one time per day on week-ends. Before milking the camels were led into a single milking stand as described by ALBRECHT (2003). At the beginning of the milking procedure the udders were observed for signs of clinical mastitis. If no signs were detected, the teats were manually stimulated for 60 to 120 s until swelling of the teats and beginning of the milk let-down. Before the milking cups were put on, the teats were cleaned with a wet disinfectant udder tissue (Westfalia Stimuclean Euterpapier, Nr. 593558). First the teat body then the top of the teat was cleaned with another tissue or a clean part of the initial tissue. As the camel udders in an arid environment are usually clean and only covered with a thin layer of sand, the first aim was to take off this layer, as it may be contaminated with pathogenic bacteria as *B. cereus* or *S. aureus*. After putting the milking cups in place, machine stimulation followed with 300 cycles for 20 s. No pre-milking was done as the milk let-down lasted only a very short time. The milking frequency was 90 cycles/min with a pulsation rate of 60:40 and vacuum pressure of 38 kPa as described by ALBRECHT (2003). The total duration of stimulation and milking was 5 - 9 min.

The milk of 2 to 6 camels was milked into one bucket and transferred into sterilised containers of 9 and 20 l. No teat dipping took place as the milk quality was satisfactory and the milk canals were estimated to be closed before the camels lay down in the sand, as they were fed directly after milking. In addition to this, the calves were allowed to stay with the she-camels and drink after milking for 1 h during the week and for 8 h during week-ends.

Hygiene during milking

As described above, the teats were cleaned with convenient udder tissues after manual stimulation and before machine milking. During stimulation, the milkers wore new clean latex gloves for each camel. The contamination of the milk with sand was inhibited as far as possible although the milking machine was standing on sand during milking. The milking

personal was wearing clean overalls during milking that were changed every 2 - 3 days. During the transfer of the milk from the bucket to the containers, the responsible person was wearing a clean white smock and latex apron. This transfer took place in an air-conditioned milking room that was cleaned thoroughly twice daily.

Cleaning and sterilisation of the milking utensils

The cleaning of the milking machine was done directly after milking by rinsing the pipes and bucket with 20 l of cold water (approximately 30 °C), 10 l of cold water with in addition 20 ml of 5 % Mucasol®-solution (Brand Wertheim) and 20 l of hot water (70 - 80 °C).

Once a week, the parts of the milking machine with milk contact were cleaned, dried and sterilised in an autoclave for 15 min at 121 °C. The sight glasses were sterilised by boiling during 5 min in water.

Storing and cooling of the milk

After milking, the milk was transported in containers (see above) to the CVRL laboratory building (distance: 500 m). From each container, a sample for bacterial examination of 60 ml was taken. After cooling, the milk was transferred into 1 l-plastic bottles, closed, labelled and stored at 6 °C until consumption.

3.2.1.2 Hand milking

First, the camels were controlled for signs of clinical mastitis. If not detectable, manual stimulation of the udder and teats followed until milk let-down began. The cleaning of the teats and the clothing of the milkers were as described in 3.2.1.1. The camels were milked by two men simultaneously into a sterilised milking bowl (separately for each camel) and transported to the CVRL (see 3.2.2.1).

3.2.2 Sampling, transport, examination

In Table 3.3 a summary of the sampling procedure is given.

Table 3.3: Summary of the sampling procedure

Samples	Hand milked samples	Machine milked samples	Quarter milk samples
Source of samples	Milk bowl	Milk bucket of the milking machine	Teat
Sampling container	Sterile plastic tube	Sterile plastic tube	Sterile plastic tube
Begin of cooling (*x* h after sampling)	1 - 3	0.5 - 1	0.1 - 1
Cooling temperature (°C)	6	6	6
Examination (*x* h after sampling)	3 - 15	3	1 - 3

3.2.2.1 Hand milked samples

After milking, the steel bowl was covered thoroughly with aluminum foil and cooled down to 6 °C. When the milking procedure was completed, the bowls were transported during 30 min at outside temperature (26 - 44 °C) - in a car - to the CVRL, where a sample of 60 ml was taken with a sterile pipette into a sterile sample tube, numbered and cooled until examination. The sampling took place in a cool, clean room.

3.2.2.2 Machine milked herd samples

The milk of 2 to 6 camels was milked into one milking bucket and transferred into a sterilised plastic container (9 or 20 l of content) stored at ambient temperature (26 - 44 °C) for up to 1 h. After milking 15 - 20 camels, the sampling took place as described in 3.2.2.1.

3.2.2.3 Quarter-milk samples of machine milked camels

Before sampling, the udder was examined for the presence of lesions, swelling or other signs for a clinical mastitis. If no abnormality could be detected, the quarter-milk samples were collected after stimulation of the udder, directly before machine-milking. The person taking the sample was wearing clean latex gloves.

The teats were cleaned with a wet disinfectant udder tissue (see 3.2.1.1), first

the teat body, than - with another tissue - the top of the teat. 2 to 3 milk streams of foremilk were discarded. After this, 60 ml were milked directly into a sterile tube. From each camel, 4 samples (one of each teat) were collected, examined for the presence of clots, flakes, blood or abnormal colour and cooled down to 6 °C within 5 - 30 min.

The examination started 1 - 3 h after sampling. For the examination, only milk of camels that showed no signs of clinical mastitis was selected.

3.2.3 Bacteria in camel milk

3.2.3.1. Preparation of the samples

The preparation of the samples for quantitative determination of Total Bacteria Count (TBC), coagulase positive staphylococci, *Bacillus cereus*, coliforms and *E. coli* was done following the method L 01.00-1 “General guidance for the preparation of test samples, initial suspensions and decimal dilutions for microbiological examination of milk and milk products" of the Official Collection of Methods of Analysis According to § 64 LFGB (former § 35 LMBG). The samples were mixed thoroughly, then 10 ml of each sample were added to 90 ml of a Ringer solution and mixed thoroughly. After preparation of this initial suspension a series of decimal dilutions was done by adding 1 ml of the previous dilution to 9 ml of Ringer solution. Directly after adding 1 ml of the previous dilution, the tubes were shaken with a shaking instrument for culture tubes.

3.2.3.2. Total bacteria count (TBC)

To determine the count of aerobic mesophilic bacteria in the camel milk, the method L 00.00-88 "Examination of food – Horizontal method for the enumeration of microorganisms - Colony-count technique at 30 °" of the Official Collection of Methods of Analysis According to § 64 LFGB (former § 35 LMBG) was followed.

Therefore twice 1 ml of the 1st three to five dilutions - depending on the expected TBC - were pipetted each into a sterile Petri dish, then covered and mixed with 12 to 15 ml of Milk-Plate-Count-Agar that was kept liquid at a temperature of 44 - 47 °C. For each dilution, a new sterile pipette was used. When the agar was solidified, the plates were incubated for 72 h at 30

°C.

3.2.3.3. Coagulase positive staphylococci (CPS)

The determination of coagulase positive staphylococci was done following a modification of the method L 00.00-55 "Examination of food - Horizontal method for the enumeration of coagulase positive staphylococci (*Staphylococcus aureus* and other species) - Part 1: Technique using Baird-Parker agar medium" of the Official Collection of Methods of Analysis According to § 64 LFGB (former § 35 LMBG). From the sample and the first 2 dilutions, two times 0.1 ml were each transferred with a sterile pipette onto Baird-Parker Agar and spread equally with a spatula until total absorption. For each dilution, a new sterile pipette was used. If low counts of CPS were expected, two times 3 Baird-Parker Agar plates were inoculated with 1 ml - distributed on the 3 plates - of the sample. The incubation took place for 24 and 48 h at 37 °C. For confirmation of suspect colonies on the Baird- Parker agar medium the catalase test and an agglutination test (Slidex™) followed. In doubtful cases further analysis was made with the BD BBL CRYSTAL™ Gram-positive ID system.

3.2.3.4. Coliforms and *Escherichia coli*

The quantitative determination of coliforms was performed according to the method L 01.00-3 "Examination of food - Determination of coliform bacteria in milk, milk products, butter, cheese and ice-cream – Method with solid medium" of the Official Collection of Methods of Analysis According to § 64 LFGB (former § 35 LMBG)., A decimal dilution series of the sample was prepared, as described in 3.2.2.1. Two times 0.1 ml of the sample and the first 2 dilutions were transferred each into an empty sterile Petri dish and mixed by gentle circular horizontal movements with minimum 10 ml of Violet Red Bile Agar (VRBA) that was added with a temperature of 44 - 47 °C. After solidifying of the agar, the plates were incubated at 30 °C for 22 h.

In parallel to this testing, two times 0.1 ml of the sample were streaked each onto a plate of solidified VRBA. If there were coliforms detectable, a pure culture was prepared from every different-shaped colony on Nutrient agar. With these pure cultures, the oxidase test was done. If negative, the culture was subjected to biochemical testing with BBL Enterotube II.

3.2.3.5. Verotoxinogenic *Escherichia coli* (VTEC) in faecal samples

The faeces samples were taken before milking, when the camels were already standing in the milking stands. On 4 different days, 42 rectal samples were taken from 22 camels with a clean latex glove. The samples were stored for a short time (30 min) in a sterile faecal sample container. Then a swab was taken from these samples and transferred into a transport liquid (0.9 % NaCl-solution). 50 µl of this solution were added to 5 ml m-TSB-medium. The culture tubes were incubated for 16 – 18 h in a shaking incubator (with 180 rpm) at 37 °C.

From this culture, the "Enzyme-linked Immuno-absorbent Assay (ELISA) for quantitative identification of Verotoxins (Shigatoxin 1 and 2) in faeces and food samples" was done following the instructions of the NOVITEC® operation manual. This ELISA was evaluated by visual method.

3.2.3.6. *Bacillus cereus*

The enumeration of *B. cereus* was carried out according to the method L 00.00 25 "Examination of food - Determination of presumptive *Bacillus cereus* in food - Part 1: Colony count technique of the Official Collection of Methods of Analysis According to § 64 LFGB (former § 35 LMBG). Two times 0.1 ml of the milk sample and of the 2 first dilutions were each distributed evenly on *Bacillus cereus* selective agar (PEMBA) with a spatula until total absorption and incubated at 37 °C for 24 - 48 h.

Further going analysis was effected with the BD BBL CRYSTAL™ Gram-positive ID system.

3.2.3.7. *Salmonella* spp.

The frozen milk samples were thawed slowly and examined following the joint ISO | IDF Standard "IDF 93 | ISO 6785 – Milk and milk products – Detection of *Salmonella*" For the pre-enrichment, 25 ml of the sample were incubated in 225 ml of buffered peptone water for 16 to 20 h at 37 °C. 10 ml of this culture were transferred each into 2 different selective enrichment broths:

- Selenit cystin broth that was incubated for 24 and 48 h at 37 °C

- Rappaport-Vassiliadis broth that was incubated for 24 and 48 h at 41.5 °C.

After 24 and 48 h each culture was streaked on Brilliant green agar modified and MLCB agar. After incubation for 24 h at 37 °C, suspect colonies were confirmed by biochemical and serological tests:
- Oxidase test
- KOH test
- BBL Enterotube II
- Agglutination with polyvalent *Salmonella* test sera.

3.2.3.8. *Listeria* spp.

The frozen milk samples were thawed slowly and examined according to the method L 00.00-32 "Examination of food – Horizontal method for the detection and enumeration of *Listeria monocytogenes* – Part 1: Detection method" of the Official Collection of Methods of Analysis According to § 64 LFGB (former § 35 LMBG).

Therefore 25 ml of the sample were incubated in 225 ml of 1/2 Fraser selective enrichment broth for 24 h at 30 °C. 0,1 ml of this culture were transferred into 10 ml of Fraser selective enrichment broth and incubated for 24 h at 37 °C (not 48 h as noted in L 00.00-32). The remaining culture in the 1/2 Fraser broth was incubated for another 24 h at 30 °C. After incubation for 48 h of the 1/2 Fraser broth and 24 h of the Fraser broth, of each enrichment a streak on PALCAM agar and on ALOA agar was done and incubated for 48 h at 37 °C.

The assessment of the colonies followed after 24 and 48 h. Suspect colonies were streaked on tryptone soya agar with yeast extract (TSYEA) and incubated for 24 h at 37 °C. From typical colonies (detected by view under normal light and Henry light) a pure culture was prepared and used for further testing for:
- Catalase reaction
- KOH test
-CAMP test (on blood agar plates for assessment of the hemolysis with strains of *Rhodococcus equi*, *Staphylococcus aureus* and control strains for comparison: *L. monocytogenes, L. innocua* and *L. ivanovii*)
- Metabolisation of L-Rhamnose and D-Xylose.

3.2.3.9. Other bacteria

From every sample and the first decimal dilution 0.1 ml each were distributed evenly with the aid of a spatula on a blood agar plate until total absorption and incubated for 24 h at 37 °C. The colonies were assessed on their morphology, haemolysis and considering the findings on the selective agar plates. If there were suspect colonies (e. g. streptococci-like colonies), further investigation followed from pure subculture on blood agar and Nutrient agar:

- Oxidase and Catalase test
- Gram stain
- BB-Gram positive if Gram positive
- Enterotube, api NE and api E if Gram negative

3.2.4 Somatic cells and cell fragments in camel milk

3.2.4.1 Semi-quantitative California mastitis test (CMT)

From each quarter-milk sample kept in 60 ml bottles, 3 ml of milk were transferred with a pipette into one of the 4 shallow paddles of the black coloured CMT test bowl. 3 ml of the CMT test liquid were added into each test field and mixed by tender circular horizontal movements of the bowl. The results were interpreted following the specification given in Table 3.4.

Table 3.4: Grading and interpretation of the CMT in cow milk (according to SCHALM et al, 1971)

Symbol	Suggested meaning	Description of visible reaction	Interpretation for cow milk[1]
-	Negative	Mixture remains liquid	0 - 200.000 cells/ml
T (+/-)	Trace	A slight slime forms and is seen to best advantage by tipping the paddle back and forth and observing the mixture as it flows over the bottom of the cup. Trace reactions tend to disappear with continued movement of the fluid.	150.000 - 500.000 cells/ml
1 (+)	Weak	A distinct slime but with no tendency toward gel formation. With some milks the reaction is reversible, for with continued movement of the paddle the slime may disappear.	400.000 - 1.500.000 cells/ml
2 (++)	Distinct positive	The mixture thickens immediately with gel formation. As the mixture is caused to swirl, it tends to move as a mass around the periphery of the cup, leaving the bottom of the cup exposed. When the motion is stopped, the mixture levels out again and covers the bottom of the cup.	800.000 - 5.000.000 cells/ml
3 (+++)	Strong positive	A gel is formed which causes the surface of the mixture to become convex. Usually there is a central peak which remains projecting above the main mass after the motion of the paddle has been stopped. Viscosity is geatly increased so that there is a tendency for the mass to adhere to the bottom of the cup.	Cell numbers generally over 5.000.000 cells/ml
x	Alkaline milk pH 7.0 or over	This symbol should be added to the CMT score whenever the reaction is distinctly alkaline as indicated by a contrasting deeper purple color.	An alkaline reaction reflects depression of secretory activity. This may occur either as a result of inflammation or in drying-off of the gland.

y	Acid milk	Bromcresol purple is distinctly yellow at pH 5.2. This symbol should be added to the score when the mixture is yellow.	Distinctly acid milk in the udder is rare. When encountered, it indicates fermentation of lactose by bacterial action within the gland.

[1] The cell counts for cow milk are noted exclusively as a guide for interpretation
[2] When in doubt as to the correct score of a reaction, always assign the lesser score thus indicating the weaker reaction between two choices.

The results were compared with the results of the bacterial examination and electrical conductivity of these samples. The results were also compared with values of electrical conductivity of cow milk.

3.2.4.2 Somatic cell count (SCC)

For determination of the somatic cell count, the procedure L 01.01-3 "Examination of food – Enumeration of somatic cells in raw milk – Microscopic enumeration of somatic cells" of the Official Collection of Methods of Analysis According to § 64 LFGB (former § 35 LMBG) was modified as follows: As described in L 01.01-3, 0.01 ml of raw milk were distributed with a micro-syringe onto a thoroughly cleaned glass slide on a surface of 1 cm² (0.5 x 20 mm). Of each sample 2 to 4 smears were prepared, completely dried and stained with Giemsa stain. Afterwards, the stained cells (without consideration of the fragments) were counted at a magnification of 1000 and multiplied with a working factor as described in L 01.01-3 for calculation of the cells in 1 ml. The counts were compared with the results of the California mastitis test.

3.2.5 Electrical Conductivity

From each quarter-milk sample - taken during the beginning of the daily milking, as described above - the electrical conductivity was determined within 1 to 2 h with the portable Mastitron control system. For this purpose, the measuring cell of the instrument was filled with milk of the sample; the value - shown by the instrument in mS/cm - was noted and interpreted in comparison with standards for cow milk and for correlation between electrical conductivity and the results of California mastitis test as well as the results of the bacteriological examination on blood agar (mastitis pathogens).

3.2.6 Indicator enzymes for heat treatment of milk

3.2.6.1 Peroxidase

As Lactoperoxidase can be used as indicator for high and ultra high heat treatment and sterilisation of cow milk, the aim was to investigate if this can be transferred to camel milk. Therefore a test series with milk that has been heated between 5 s and 40 min at temperatures between 30 and 90 °C in a water bath was established. Following the instructions of the manufacturer of the Traventol® test, 2 drops were added to 5 ml of the samples into a culture tube. The reaction was evaluated after 2 min with regard to the colour change.

3.2.6.2 Alkaline phosphatase

As the test of the activity of alkaline phosphatase in milk is the usual method to confirm the proper pasteurisation of cow milk, here we investigated, if the same method can be applied for camel milk. For this purpose a series of milk heated in culture tubes in a water bath at temperatures between 40 and 95 °C for durations between 2 s and 60 min was tested with the Lactognost® test kit following the manufacturer's instructions. The reagents were added and mixed until completely solved. In the following the samples were incubated for 1 h at 37 °C and evaluated after 10 min reaction time. The results were evaluated considering the colour change.

3.3 Results

3.3.1 Animals

Heath status

All camels were tested during the study for the following animal diseases with negative results: salmonellosis (serology), bovine tuberculosis (serology), paratuberculosis (serology) and brucellosis (serology).

Udder shape

Every camel tested had different udder and teat size as well as shape.

Milk yield

An exact record of the milk yield was not in the scope of this investigation. Generally the daily yield varied between 1 and 10 l with an average of around 5 l/day

Behaviour

At the beginning of the study, most of the camels at CVRL were already used to machine milking in the above described milking stands. New camels adapted between 5 and 14 days to the milking procedure. Once adapted, the camels were calm and appeared relaxed. Nevertheless, two camels did not enter the milking stands but accepted machine milking without problems. Generally, all camels were more nervous if changes in their environment occurred, like unknown persons or noises.

Hand milking was generally well accepted in the camels of this study.

Before milking, the teats were stimulated by udder massage that was generally well accepted by the camels. After a short adaptation period, the milk let-down started after 30 to 60 s.

During machine milking the camels were fed with hay or alfalfa (lucerne). This led to a calm behaviour and appeared to encourage the camels to enter the milking stands. Some of the camels tended to lie down during milking,. especially when there were changes in the environment or if milking took too long. Generally, after an adaptation period of three weeks up to three months, camels were calmer.

3.3.2 Bacteria in camel milk

3.3.2.1. Total bacteria count (TBC)

The geometric mean of TBC (Table 3.5) was 9.2 x 10^2 cfu/ml in machine milked herd samples, 1.1 x 10^2 cfu/ml in hand milked camel samples and 9.2 x 10^2 cfu/ml in quarter-milk samples. The median of machine milked samples was with 9.6 x 10^2 cfu/ml similar to the geometric mean, whereas the median of quarter-milk samples (1.7 x 10^3 cfu/ml) was little higher than the geometric mean and the median of hand milked samples (1.3 x 10^2 cfu/ml) was again similar to the geometric mean. The difference between the geometric means of TBC in maschine and hand milked samples, resp., were statistically significant (*t*-test; Excel 2003). In Figure 3.1 the distribution of the TBC of the different sample types is summarised.

Figure 3.1 shows that in 6 % of the hand milked samples the TBC was lower than 5.0 x 10^2 cfu/ml whereas 76.2 % of the machine milked samples showed values between 5.0 x 10^2 and 5.0 x 10^3 cfu/ml (20 % had a TBC between 1.0 x 10^2 and 5.0 x 10^2 cfu/ml). 44.2 % of the quarter-milk samples had a TBC between 5.0 x 10^3 and 1.0 x 10^4 cfu/ml whereas the remaining 55.8 % were distributed equally on the other segments of TBC.

Table 3.5: Total bacteria count in camel milk samples

Samples	**n**	**TBC (cfu/ml)[1]**	**Quartiles**		**Minimum / Maximum TBC (cfu/ml)**
			Median ($x_{0.50}$) (cfu/ml)	**$x_{0.25}$ / $x_{0.75}$ (cfu/ml)**	
Hand milked	168	1.1 x 10^2	1.3 x 10^2	4.7 x 10^1 / 2.8 x 10^2	0.2 x 10^1 / 4.2 x 10^3
Machine milked	260	9.2 x 10^2	9.6 x 10^2	5.3 x 10^2 / 1.6 x 10^3	2.2 x 10^1 / 8.5 x10^5
Quarter-milk samples	468	9.2 x 10^2	1.7 x 10^3	4.8 x 10^2 - 3.6 x 10^3	0.2 x 10^1 / 6.7 x 10^4

[1] Geometric mean

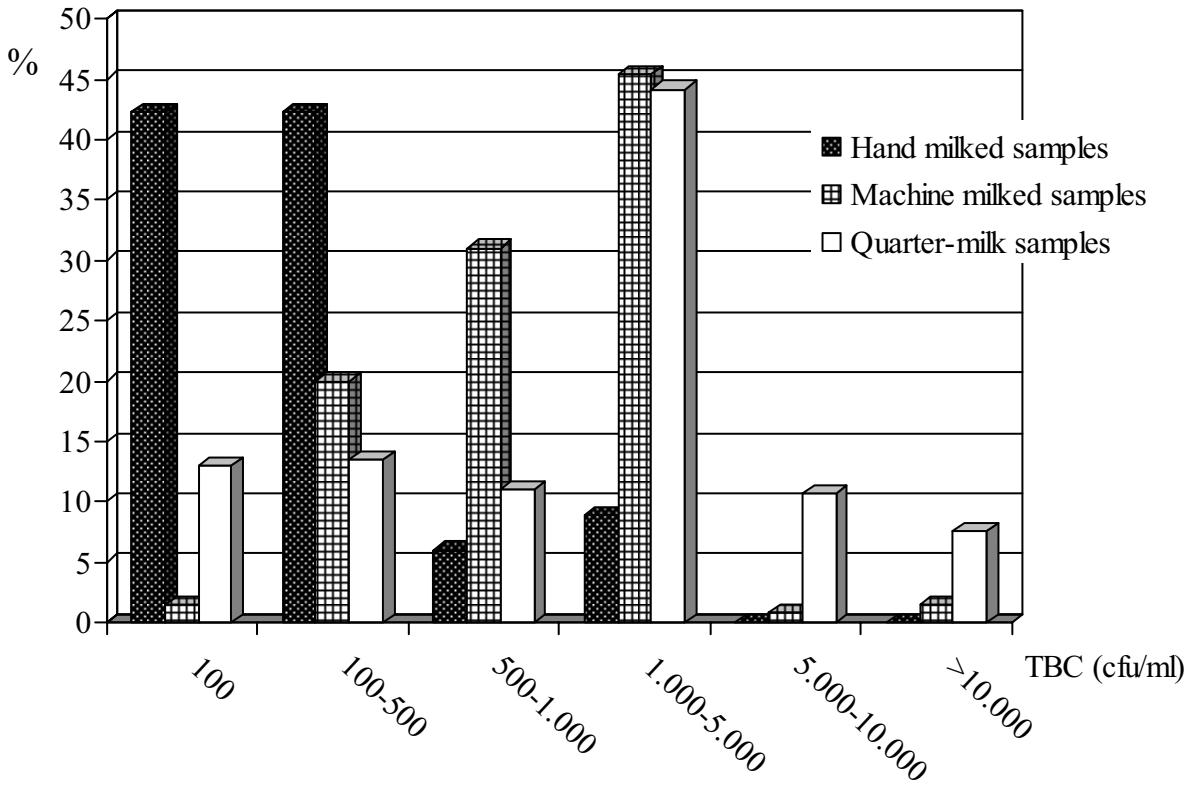

Figure 3.1: Total bacteria count in different camel milk samples

3.3.2.2. Staphylococci

In 74 (28.5 %) of the machine milked samples the presence of coagulase positive staphylococci was found whereas 9 (5.4 %) of the hand milked samples showed the presence of coagulase positive staphylococci and 21 (4.5 %) of the quarter-milk samples (Table 3.6). CPS were found in the quarter-milk samples of 4 camels (22.2 %). The infected quarters were - with one exception - front quarters.

Table 3.6: Prevalence of coagulase positive staphylococci in milk samples

Samples	**n**	**Samples positive**		**Maximum count**
		n	**%**	**(cfu/ml)**
Hand milked	168	9	5.4	3.9×10^2
Machine milked	260	74	28.5	1.8×10^2
Quarter-milk samples	468	21	4.5	1.4×10^3

From most milk samples, coagulase negative staphylococci could be isolated.

3.3.2.3. Coliforms and *Escherichia coli*

3.3.2.3.1 Coliforms

In Table 3.7 the prevalence of coliforms in camel milk samples is displayed. 17 (6.5 %) of the machine milked samples showed the presence of coliforms, of which 7 isolates (41.2 %) were *Citrobacter freundii*, 2 (11.8 %) *Escherichia coli*, 4 (23.5 %) *Enterobacter cloacae* as well as 4 (23.5 %) *Klebsiella pneumoniae*. 14 (8.3 %) of the hand milked samples were positive for coliforms; all isolates were *Serratia marcescens*. The quarter-milk samples were free from coliforms.

Table 3.7: Prevalence of coliforms in different camel milk samples

Samples	**n**	**Prevalence**		**Maximum Count**
		n	**%**	**(cfu/ml)**
Hand milked	168	14	8.3	2.8×10^2
Machine milked	260	17	6.5	8.4×10^2
Quarter-milk samples	468	0	0	-

3.3.2.3.2 *Escherichia coli*

No *E. coli* was isolated from the examined herd milk and quarter-milk samples of clinically healthy camels kept at the CVRL.

One camel developed a severe clinical mastitis during the study due to *E. coli* that involved the 2 left quarters beginning from the hind quarter (see Attachment II). This camel could be treated but the two affected quarters ceased milk production.

3.3.2.3.3 Verotoxinogenic *Escherichia coli* (VTEC) in faecal samples

In 42 faecal samples of 22 camels, no verotoxin and VTEC, resp., could be detected.

3.3.2.4. *Bacillus cereus*

No hand milk sample was found positive for *B. cereus*. Of the 260 machine milked herd samples, in 9 samples (3.1 %), *B. cereus* was present (Table 3.8). Quarter-milk samples were not examined for *B. cereus*.

Table 3.8: Prevalence of *B. cereus* in camel milk samples

Samples	**n**	**Samples positive**		**Maximum count**
		n	**%**	**(cfu/ml)**
Hand milked	168	-	-	-
Machine milked	260	9	3.1	4.7×10^1

3.3.2.5. *Salmonella* spp.

In 24 hand milked samples and 23 machine milked samples no *Salmonella* spp. were detected in 25 ml each.

3.3.2.6. *Listeria* spp.

In 26 hand milked samples one sample (3.8 %) showed presence of *Listeria welshimieri* in 25 ml. From the 24 machine milked bucket samples no *Listeria* spp. could be isolated in 25 ml each.

3.3.2.7. Other bacteria

From most of the milk samples coagulase negative staphylococci could be isolated. A detailed description is given in 3.3.3.1.1 in comparison with CMT test results.

3.3.2.8. Cases of clinical mastitis

In total, 5 cases of clinical mastitis were observed during the study, of which one case included 1 quarter, 2 cases two quarters and 2 cases included 3 quarters (Table 3.9).

Table 3.9: Cases of clinical mastitis

Case No	Quarters involved	CMT	Bacteria isolated
1	3	+ / ++ / +++	*Streptococcus (S.). bovis*, *S. aureus*, *Burkholderia (B.) cepacia*
2	1	+++	*S. aureus*
3	3	+ / ++ / ++	*S. bovis*, *B. cepacia*
4	2	++ / +++	*Streptococcus (S.) agalactiae*, *B. cepacia*
5	2	++ / +++ [1]	*E. coli*

[1] One sample was tested +++, afterwards milk production ceased

After local and systemic antibiotic treatment, in 4 of the 5 camels, multiresistant *Burkholderia (B.) cepacia* was isolated. This appeared to irritate the udder as CMT did not normalise in these camels after disappearance of the clinical symptoms. They were excluded from further milking. In case of mastitis camels were treated systemically and with udder injectors. The application of the local antibiotic was possible but more laborious than in cows because the antibiotic had to be administered into each affected eighth.

3.3.3 Further diagnostic means in camel milk

3.3.3.1. Somatic cells

3.3.3.1.1 Semi-quantitative California mastitis test (CMT)

468 quarter milk samples were examined for their somatic cell count using semi-quantitative CMT. Hereof, 341 (72.9 %) samples showed no reaction, 76 samples (16.2 %) were described with +/-, 42 (9.0 %) with + and 9 samples (1.9 %) with ++ (Table 3.10). Camels that showed high CMT (+++) results were not integrated into the daily milking process. Results are displayed in Table 3.9.

Table 3.10: CMT of all quarter-milk samples

CMT	-		+/-		+		++		+++
	n	**%**	**n**	**%**	**n**	**%**	**n**	**%**	
Quarter-milk samples	341	72.9	76	16.2	42	9.0	9	1.9	-[1]

[1] No camel during routine sampling showed CMT +++, it was observed in 3 cases of clinical mastitis (Table 3.9)

In 15 (11.8 %) of the 127 CMT positive (+/-, +, ++) samples pathogenic bacteria were found. Of the two samples with CMT +/-, *S. aureus* and *S. agalactiae* were isolated. *Corynebacterium striatum* was found in all 11 samples that were taken from one hind quarter of one camel (9 CMT +, 2 CMT ++). In addition, from one CMT ++ sample *S. aureus* was isolated, another contained *Corynebacterium striatum* and the third *S. aureus* and *C. striatum*. In 27 (7.9 %) of the 341 CMT negative samples pathogenic bacteria were detected (16 *S. aureus*, 2 *S. aureus* and 2 *S. bovis*, 4 *C. striatum*, 1 *B. cepacia* and 2 *S. agalactiae*).

3.3.3.1.2 Somatic cell count (SCC)

The somatic cell count was evaluated with the microscopic method on Giemsa stained slides. In Table 3.11 the somatic cell count is displayed in comparison to CMT results.

Table 3.11: Somatic cell count by microscopic method in comparison to CMT results

CMT	**-**	**+/-**	**+**	**++**	**+++**
n	10	10	10	10	5
Mean[1] SCC (cells/ml)	6.3×10^4	1.4×10^5	3.7×10^5	8.4×10^5	1.8×10^6
SCC range (cells/ml)	2.0×10^4 - 1.4×10^5	8.6×10^4 - 2.9×10^5	2.2×10^5 - 6.6×10^5	6.0×10^5 - 1.2×10^6	1.5×10^6 - 2.2×10^6
SCC median (cells/ml)	8.0×10^4	1.4×10^5	3.7×10^5	9.0×10^5	1.7×10^6

[1] Geometric mean

3.3.3.2. Electrical conductivity

The electrical conductivity of 468 quarter-milk samples was between 5.5 and 10.6 mS/cm (one sample each). The average conductivity was 7.7 ± 0.6 mS/cm, whereas 34.4 % of the values were situated between 8.0 and 8.9 mS/cm and 4.7 % of the values were over 9.0 mS/cm. 16.3 % (76) of the values were under 7.0 mS/cm and 0.9 % (4) under 6.0 mS/cm (Figure 3.2).

The average difference between the highest and lowest value in one camel in one examination of all 4 quarters was 0,5 ± 0.26 mS/cm. 22 camels (18.8 %) had a difference of the electrical conductivity between the 4 quarters of ≥ 0.8 mS/cm, and 46 camels (39.3 %) of ≥ 0,2. The maximum difference was observed with 2.0 mS/cm in a camel with 4 completely inapparent quarters, the lowest was 0.1 mS/cm. The results showed that the values mainly depended on the individual camel on one day of examination (see Attachment I). No differences between front or hind quarters were detectable.

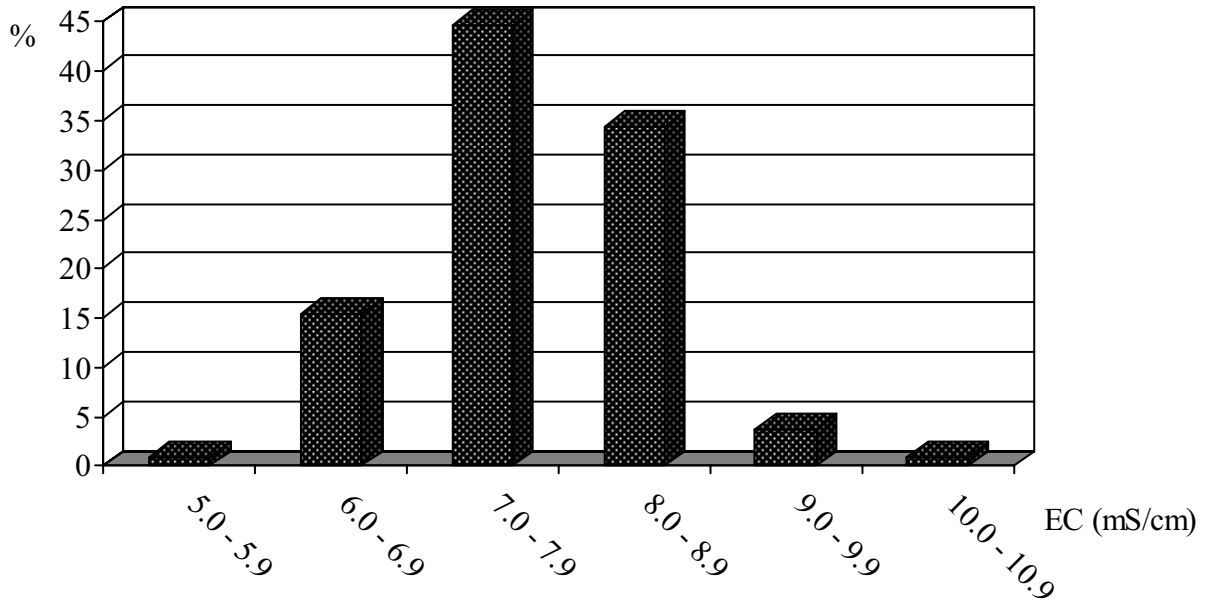

Figure 3.2: Electrical conductivity in quarter-milk samples

Compared with the bacterial count and CMT score, no clear correlation was detectable between electrical conductivity and subclinical or clinical mastitis (Table 3.12). The conductivity of the milk of 3 camels that developed clinical mastitis with high CMT score did not change. One camel with severe mastitis (CMT +++; 8.8 mS/cm) in one quarter showed

nearly the same conductivity on all 4 quarters (8.5 - 8.7 mS/cm), another camel revealed lower conductivity (7.7 mS/cm) in the milk of the affected (CMT +++) than in the healthy quarters (7.8; 8.9; 8.4 mS/cm).

Table 3.12: Comparison of CMT, electrical conductivity (EC) and pathogenic bacteria in camel milk

CMT	**-**	**+/-**	**+**	**++**
n	341	76	42	9
Pathogenic bacteria	27 (7.9%)	2 (2.6%)	10 (23.8%)	3 (33.3%)
Average EC (mS/cm)	7.7 ± 0.8	7.6 ± 0.8	7.8 ± 0.7	7.8 ± 0.7
Minimum EC (mS/cm)	5.5	6.0	6.4	6.2
Maximum EC (mS/cm)	10.6	9.6	9.2	8.9

Table 3.13: Mean electrical conductivity of the camels examined – part 1

Camel [1]	**1**	**2**	**3**	**4**	**5**	**6**
Analyses (n) [2]	1	13	8	11	6	8
Mean conductivity (mS/cm)	8.2 ± 0.2	7.8 ± 0.4	7.4 ± 0.3	7.6 ± 0.4	8.6 ± 0.7	7.7 ± 0.6
Maximum	7.9	7.0	6.6	6.9	7.0	6.3
Minimum	8.5	9.2	8.5	8.9	10.6	9.5

Table 3.14: Mean electrical conductivity of the camels examined - part 2

Camel [1]	**7**	**12**	**16**	**17**	**18**	**20**
Analyses (n) [2]	1	4	10	10	5	3
Mean conductivity (mS/cm)	7.0 ± 0.2	7.5 ± 0.6	7.8 ± 0.7	8.4 ± 0.4	6.8 ± 0.5	6.3 ± 0.2
Maximum	6.1	6.9	6.3	7.4	5.5	5.9
Minimum	6.7	8.9	9.4	9.0	7.9	6.7

Table 3.15: Mean electrical conductivity of the camels examined - part 3

Camel [1]	**21**	**22**	**23**	**27**	**30**	**36**
Analyses (n) [2]	1	9	12	8	5	1
Mean conductivity (mS/cm)	6.7 ± 0.3	7.8 ± 0.6	8.3 ± 0.4	7.5 ± 0.3	7.1 ± 0.6	6.7 ± 0.0
Maximum	6.3	6.2	7.3	6.9	6.0	6.7
Minimum	7.2	8.8	9.2	8.2	8.4	6.8

[1] The camel numbers given in Tables 3.13 to 3.15 are ID numbers of individual camels
[2] For every analysis samples of all four quarters were tested

3.3.4 Indicator enzymes for heat treatment of milk

3.3.4.1 Peroxidase

The activity of peroxidase was clearly positive (++) in raw camel milk and stayed active after heating the milk at 65 °C for 40 min. A first reduction of the colour reaction was observed after a heat-treatment at 67 °C for 10 min. After heat treatment at 70 °C for 3 min and 72 °C for 1 min, as well as at 75 °C the peroxidase was clearly negative (Table 3.16).

Table 3.16: Peroxidase activity in raw and heat treated camel milk

Heating time	raw	62 °C	65 °C	67 °C	70 °C	72 °C	75 °C	80 °C
5 s	++	++	++	++	++	++ [1]	neg.	neg.
20 s	++	++	++	++	++	+ [1]	neg.	neg.
40 s	++	++	++	++	+	+/- [1]	neg.	-
1 min	++	++	++	++	+	neg.	neg.	-
2 min	++	++	++	++	+/-	neg.	-	-
3 min	++	++	++	++	neg.	neg.	-	-
5 min	++	++	++	++	neg.	neg.	-	-
10 min	++	++	++	+	neg.	-	-	-
20 min	++	++	++	+	neg.	-	-	-
30 min	++	++	++	+	neg.	-	-	-
40 min	++	++	++	+	neg.	-	-	-

[1] Slower reaction

3.3.4.2 Alkaline phosphatase

The activity of alkaline phosphatase remained heating at 72 °C. A reduction (+) of the reaction was observed after heat-treatment for 1 min at 75 °C and was more pronounced (+/-) after 20 min. Following to heat-treatment of the milk for 5 s at 80 °C, the reaction was slightly reduced (+) and less visible (+/-) after heating for 40 s. After 5 min of heating at 80 °C the reaction was clearly negative (Table 3.17).

Table 3.17: Alkaline Phosphatase activity in raw and heat treated camel milk

Heating time	raw	65 °C	67 °C	70 °C	72 °C	75 °C	80 °C	90 °C
5 s	++	++	++	++	++	++	+	neg.
20 s	++	++	++	++	++	++	+	neg.
40 s	++	++	++	++	++	++	+/-	neg.
1 min	++	++	++	++	++	++	+/-	neg.
2 min	++	++	++	++	++	+	+/-	-
3 min	++	++	++	++	++	+	+/-	-
5 min	++	++	++	++	++	+	neg.	-
10 min	++	++	++	++	++	+	neg.	-
20 min	++	++	++	++	++	+/-	neg.	-
30 min	++	++	++	++	++	+/-	-	-
40 min	++	++	++	++	++	+/-	-	-

4. DISCUSSION OF THE RESULTS

4.1. Animals

4.1.1. Dromedaries as milking animals

Health status

All camels of the study were tested with negative results for salmonellosis, bovine tuberculosis and paratuberculosis. Considering the data given by OIE (2004) and OIE (2006) brucellosis could be expected. A regular testing on *Brucella abortus* and *B. melitensis* should generally be integrated in the testing procedure, especially as camel milk is generally consumed in its raw state. Concerning bovine tuberculosis and paratuberculosis the negative test results confirm the documentation of OIE (2004) and OIE (2006).

Udder shape

Different to the udders of cows, the udders of the camels milked in this study showed a considerable variety in shape as described under 3.3.1. The teat length varied and the size of udders and teats varied considerably, too. A finding that is mainly due to the fact that - until today - no systematic breeding has been done with the aim to produce a milk camel suitable for automatic milking (ALBRECHT, 2003; WERNERY et al., 2004). Therefore a correct position of the milking cups could not be assured. This can be the source of udder inflammations and the exclusion of a camel from automatic milking.

More information on udder anatomy and its defence mechanisms as well as improved breeding methods for milking dromedaries is surely needed. If in the next years an increasing number of commercial camel dairies develop (WERNERY et al., 2004), more interest will probably be attributed to this matter.

Behaviour

According to the present study, camels are easily adaptable to machine milking (see 3.3.1) in contradiction to the observations of RADWAN et al. (1992). However, if they were disturbed by unknown noises or new or too many people, some camels stopped milk let-down for the next hours. Therefore, a calm clean environment and permanent milking personnel should be assured.

4.1.2. UAE breed

Camels of the common UAE breed - also called 'Al-Khawar' (CIRAD, 2006) - can be milked with satisfactory result. Compared to pure milking animals, all camels in the present study started late with calving as they were previously used as racing animals. The primary selection of the camels was done according to their racing performance. However, with an average milk yield of 5 l/day (see 3.3.1) the performance of this breed can be compared to other breeds used for milking (BURGEMEISTER, 1974, Tunisia; BASMAEIL & BAKKAR, 1987, Saudi Arabia; KAMOUN & BERGAOUI, 1989, Tunisia; BEKELE et al., 2002, Ethiopia).

4.2. Milking

4.2.1. Milking and calf management

Feeding

According to SAMBRAUS (1995), camels do not graze during feeding their calves. This observation leads commonly to the opinion that camels cannot or should not be fed during milking. In the present study all camels were calm and less anxious when fed (with hay or alfalfa) during milking (see 3.3.1). However, it has to be assured that persons that have contact with the feed are not directly involved in milking to prevent contamination of the milk.

Milking

According to the investigations of YAGIL et al.(1999) and FARAH (2004) it seems to be impossible to stimulate camel milk let-down by manual udder massage but only by the calf, whereas in the present study manual stimulation of the camel udder was possible when the camels were adapted to this procedure (see 3.3.1). The need of adaptation is made obvious by the fact that the stimulation duration is longer in the first three months than in the remaining time of lactation as reported by WERNERY et al. (2004). Stimulation of the teats only by milking machine is not considered adequate by ALBRECHT (2003) and WERENERY et al. (2004) as the camels developed oedema and were more susceptible to mastitis. Therefore manual udder massage appears to be the most suitable method for stimulating the milk let-down in camels.

Generally, the expenses of camel keeping and milking facilities are low - under both, intensive and traditional husbandry - as few precautions are needed in contrast to dairy farms for cows in hot and arid areas. Milking parlours can greatly facilitate the milking process as the camels are fixed but are not necessarily needed especially for small scale enterprises. If milking machines are introduced, this will give an impetus to the camel herders to breed camels with standard sized udder and teats. Moreover, milking machines will not only guarantee higher milk production and better milk hygiene, but would also improve the social status of camel farmers.

Calf management

During the present investigation calves were allowed to suckle one hour after milking and nine to ten hours on week-ends (see 3.1.2). Compared to other studies (YOUNAN et al., 2001) this is a short period of time. Nevertheless, it allowed to minimise udder contamination and mastitis e. g. by calves suckling different mothers, besides the positive effect on milk yield and the reduced risk of teat lesions (AL-ANI & AL-SHAREEFI, 1997) or by anti suckling devices frequently used to prevent calves from drinking (ABDEL GADIR et al., 2005). Removing the calf after parturition is very controversal, but should be achieved after several generations of selective breeding. The current practise favours the separation after several hours prior to milking. Some camel owners believe that camels have a significantly lower daily milk yield after having lost their calves in comparison to those whose calves stayed alive. However, in future the objective should be to remove the calf entirely.

4.2.2. Risk factors for bacterial contamination of milk

The main risk factor for poor hygienic quality of camel milk is bacterial contamination (see 3.2.1.1 and 3.3.1), a finding also reported generally in milk production in arid countries by FAYE & LOISEAU (2002). The possibility of contamination can emanate from the lactating camel through subclinical mastitis, contaminated udder skin or camels urinating during milking which may increase the risk of faecal contamination of the milk. Although most of the camels started sometimes to urinate during milking, in very few samples *Enterobacteriaceae* were found. Compared to cow faeces camel faeces are hard and dry and, therefore, normally do not contaminate hind legs and tail. Some of the camels in this study started to lie down during milking procedure (see 3.3.1), a behaviour that can risk the introduction of sand or faeces into the milking gear and therefore should be avoided e. g. by

using belts, especially during the adaptation period.

The main supposed risk factors for (subclinical) mastitis in the camels of the present study were inadequate adaptation to the milking machine (stimulation, respray, vacuum, blind-milking, irritation by non-adapted milking cups) as well as suckling calves or anti-suckling-devices. The latter has also been determined as risk factor by ALMAW & MOLLA (2000). One problem of anti-suckling-devices may present the exchange between camels without washing or disinfection.

Contamination of teats with environmental bacteria by the hands of milkers as described by FAYE & LOISEAU (2002) was minimised by wearing gloves that were changed after each camel (see 3.2.1.1) but cannot be excluded. In addition, the milking personnel changed milking clothes every second day.

Also badly cleaned milking and packing utensils have been defined as risk for contamination by FAYE & LOISEAU (2002). In this study the milking machine could have been the cause for contamination of the milk as there was a lack of hot cleaning water on some days. The risk of introduction of bacteria by contaminated milking utensils was minimised by autoclaving the milking gear and hand milking bowls. For transportation, only autoclaved buckets were used.

4.2.3. Mastitis control

In addition to hygienic milking conditions and techniques, mastitis control is a key element in milking management (SEMEREAB & MOLLA, 2001). The camels of the present investigation were controlled before every milking for signs of clinical or subclinical mastitis (see 3.2.1.1 and 3.2.1.2). If mastitis was detected, the concerned camels were excluded from the usual milking procedure and milked separately. Additionally, antibiotic treatment was applied.

The camels of the present study were easily treated with udder injectors even if the application of the local antibiotic was more laborious than in cows as the antibiotic has to be applied into each affected eighth (see 3.3.2.8) as camels have two to three milk canals (WEBER, personal communication, 2003). Therefore and because of a remaining insecurity

of the local treatment a combination with systemically administered antibiotics was done and is generally considered useful. No systematic investigation was done in the present study on the success of mastitis treatment as the scope was the hygiene of milk from healthy animals.

4.3. Bacteria in camel milk

The results of bacterial contamination of camel milk in this investigation matches in general with the findings of other authors (see 2.3). The main pathogenic bacteria occurring in raw camel milk appear to be similar to the findings in cow milk (SCHALM et al., 1971; SEDDEK et al., 1999; WORKINEH et al., 2002; ABDEL GADIR et al., 2005) and goat and ewes milk (EL IDRISSI et al., 1994; SCHNELLHARDT, 1998; NDEGWA et al., 2000). Most authors consider coagulase positive (CPS) and negative staphylococci (CNS) as main microbiological contaminants of camel milk. With 4,5 % to 28.5 % of CPS-positive samples and most of the samples positive for CNS, this can be confirmed by the present study.

4.3.1. Total bacterial count (TBC)

In the present investigation 100 % of the hand milked samples, 97.7 % of the machine milked and 81.8 % of the quarter-milk samples showed total bacterial counts lower than 5.0 x 10^3 cfu/ml (Figure 3.1) and therefore amount only to 5 % of the limit of 1 x 10^5 cfu/ml laid down by the European Union for raw cow milk (ANONYMOUS, 2004f). The geometric mean of the TBC of hand milked samples was 1.1 x 10^2 cfu/ml (Table 3.5). This goes conform with the results of WERNERY et al. (2002) who proved values of 10^2 to 10^4 cfu/ml but they are lower than the values given by YOUNAN (2004) who determined a TBC of 10^3 - 10^5 cfu/ml in hand milked bucket samples. In the present study, quarter-milk samples showed a mean value of 9.2 x 10^2 cfu/ml (Table 3.5) and lie therefore a little below the values given by YOUNAN (2004).

The geometric mean TBC of machine milked samples was 9.2 x 10^2 cfu/ml (Table 3.5). The statistically significant differences to the mean of TBC in hand milked samples can be explained with a probable contamination during automatic milking as well as by the storage time at ambient temperature (see 3.2.2.2).

The result is not comparable with previous studies on machine milked dromedary milk as in

the investigation carried out by WERNERY et al. (2006a) only the suitability for human consumption - with a TBC under the maximum value of 1.0 x 10^5 cfu/ml laid down in the European Union - was investigated. However, the value lies significantly below common TBC in machine milked cow milk (WALZEL, 1997). Compared to the average TBC in goat (6.7 x 10^4) and ewes (5.8 x 10^4) milk (SCHNELLHARDT, 1998) the TBC of machine milked camel milk is also lower.

The mean TBC of hand milked samples of traditionally managed camels lies significantly lower than the mean values described above and the mean TBC for hand milked camel milk samples given by SEMEREAB & MOLLA (2001), WERNERY et al. (2002) and SELA, et al. (2003) (Table 2.7).

One explanation for the relatively high TBC of quarter-milk samples could be the presence of bacteria on the teat skin when samples were taken. Even after discarding the first milk streams it could be possible that this milk still contains more bacteria from a contamination of the teat canal. However, before sampling, the teats were thoroughly cleaned and disinfected. Therefore, it is unlikely that a significant contamination of the teat skin still occurred. Other sources for milk contamination as the skin of milking personnel were prevented by gloves.

4.9 % of the samples showed now growth of bacteria (6.9 % in quarter-milk samples, 7.2 % in hand milked samples and none in machine milked herd samples). This was also reported by GULIYE (1996) in 18.6 % of the examined quarter-milk samples and appears to occur regularly in camel milk samples.

The generally low mean values of TBC in camel milk could be explained by high UV radiation in the UAE combined with a dry environment (GAST et al., 1969). Furthermore, the udder is located in a high position due to the length of camel hind legs. In addition, faeces of camels are dry and prevent the udder from faecal contamination.

A second explanation could be the high antimicrobial activity of camel milk through enzymes as lactoferrin, lactoperoxidase or peptidoglycan recognition protein (PGRP) (BARBOUR et al., 1984; EL AGAMY et al., 1992; KAPPELER, 1998) and an active immune system (BARBOUR et al., 1985). This bacteriostatic effect decreases significantly after heat treatment of the milk (BERNKERROUM et al., 2004). SARWAR & ENBERG (2001) proved

a higher lysozyme activity in milk from multipare and older camels. The camels of the present study were all older than 12 years.

4.3.2. Staphylococci

4.3.2.1 Coagulase positive staphylococci (CPS)

With a prevalence of 28.5 % in machine milked samples, 5.4% in hand milked and 4.5 % in quarter-milk samples (Table 3.6), CPS were the major pathogenic bacteria most often isolated in this study. The fact that machine milked samples show a higher prevalence of CPS could probably be due to the intermittent excretion of *S. aureus* from some clinically inapparent camels. As in quarter-milk samples of 12 machine milked camels no CPS were found, it is probable that the presence of CPS in the examined herd milk samples was caused by the 4 CPS-positive camels. As the infected quarters were most often front quarters, a contamination of the front milking cups was suspected. However, the milking gear was thoroughly cleaned twice a day and sterilised once a week.

In several investigations on milk of healthy camels the CPS results were contradictory: WERNERY et al. (2004) proved CPS in 0.5 % and ALMAW & MOLLA (2000) in 0.6 % of the examined milk samples. These values are very low compared to the prevalence in the present study that goes more conform with the results of CHAFFER et al. (2000) and EL-JAKEE (1998) with a prevalence of CPS of 8.8 % and 5.0 %, respectively. The prevalence of CPS in 24.6 % of camel milk samples given by ABDEL GADIR et al. (2005) is similar to the results of the machine milked samples in the present study.

In addition, comparison is made to an investigation on goat and ewes milk in which a total prevalence of CPS of 6.8 % was found in ewes and goat milk samples milked by hand and in 10.3 % for machine milk samples (SCHNELLHARDT, 1998). Similar to this report, the prevalence of CPS in the examined camel milk was significantly higher in machine milked than in hand milked samples. Nevertheless, the absolute values are considerably lower in goat milk. One influencing parameter therefore could be a lower ambient temperature as the investigation of SCHNELLHARDT (1998) was carried out in Germany.

No analysis has been done on the toxin producing characteristics of the CPS in the camel milk

of this investigation. Considering the heat stability of some staphylococcal enterotoxins (BALABAN & RASOOLY, 2000), this should be the subject of further investigations.

4.3.2.2 Coagulase negative staphylococci (CNS)

From most of the milk samples CNS were isolated (data not shown). Although known as facultative (or ”minor”) pathogens especially isolated from subclinical mastitis (SCHNELLHARDT, 1998; SEMEREAB & MOLLA, 2001; ABDEL GADIR et al., 2005) these staphylococci did not show a measurable influence on milk yield, CMT or clinical symptoms.

As mentioned under 4.3.1 an explanation for the frequent occurrence of CNS is most probable the contamination of the samples by the teat canal or teat skin. However, before sampling the first streams of milk were discarded and the teats were thoroughly cleaned and disinfected to minimise contamination as far as possible.

4.3.3. Coliforms and *Escherichia coli*

The fact that coliforms were only isolated from herd or camel samples having more contact to the environment than quarter-milk samples, may lead to the conclusion that - with one exception - the source was external contamination and not infection of the udders. Possible sources could be unclean hands or clothes of the milking personal, urination of the camels during milking (as the urine passes along the hind legs, faecal contamination could be possible) or the introduction of sand.

4.3.3.1. Coliforms

In the present study the prevalence of coliforms in machine milked samples was 6.5 % and 8.3 % in hand milked samples (Table 3.7). As the prevalence in healthy camels is given with values between 1.4 % (WERNERY et al., 2002) and 29.4 % (SAAD & THABET, 1993) no significant comparison can be made. As coliforms can be a sign of insufficient hygienic conditions and to a minor degree of faecal contamination (BÜLTE, 2004) the prevalence may vary considerably according to hygiene conditions. The fact that no coliforms were detected in quarter-milk samples in the present investigation underlines this observation.

In the machine milked samples Citrobacter freundii, Escherichia coli, Enterobacter cloacae and Klebsiella pneumoniae were isolated. In 8.3 % of the hand milked samples Serratia marcescens was detected. With exception of Enterobacter cloacae and Serratia marcescens these coliforms were also described by other authors (BARBOUR et al., 1985; EL-JAKEE, 1998; SEMEREAB & MOLLA, 2001; ABDEL GADIR et al., 2005).

4.3.3.2. *Escherichia coli*

No *E. coli* was isolated from quarter-milk samples of healthy camels in this study. The prevalence of *E. coli* has been reported by other authors between 1.0 and 17.3 % in samples taken from healthy camels (EL-JAKEE, 1998; ABDEL GADIR et al., 2005). As *E. coli* is a common intestinal bacterium (BÜLTE, 2004), its presence in milk cannot be totally avoided but minimised by good hygiene practice. As camel faeces are dry and normally do not contaminate the udder skin, this factor can be largely excluded in comparison to cows.

One camel developed a severe clinical mastitis caused or promoted by *E. coli* during the study (pictures 6 and 7 in attachment II). Clinical cases of mastitis in camels caused by *E. coli* were also reported by KAPUR et al. (1982), EL JAKEE (1998) and ABDEL GADIR et al. (2005).

4.3.3.3. Verotoxinogenic *Escherichia coli* (VTEC)

No cases of infections of camels or camels as carriers of VTEC have yet been reported but WHO (WHO, 2005a) mentions the camel as reservoir for EHEC serovar O157:H7. Therefore the prevalence of VTEC in camel faeces was investigated in this study. The negative results underline the theory that VTEC does not play a major role in the Arabian Peninsula but attention should be paid to this problem as the impact on public health can be severe (BÜLTE, 2004).

4.3.4. *Bacillus cereus*

B. cereus was present in 3.1 % of the machine milked herd samples (Table 3.8) what can be attributed to the possibility of contamination of the milk with sand, as *B. cereus* was found in sand samples in the investigation of ALBRECHT (2003). The results of all hand milked samples were negative for *B. cereus* what is probably due to a good milking hygiene. No case

of mastitis caused by *B. cereus* was seen in the present investigation what complies with the results of most investigations stating that *B. cereus* is not a pathogen of importance in camel milk. The high prevalence of *B. cereus* that is reported by SAAD & THABET (1993) could be due to poor milking hygiene and contamination from soil.

4.3.5. *Salmonella* spp.

All 24 hand-milked and 23 machine-milked samples were negative for *Salmonella* spp. in 25 ml. The animals were tested serologically negative for salmonellae during the study and apparently no contamination with these bacteria from other sources took place. Considering that salmonella infections are occurring in neighbouring countries of the UAE (OIE, 2004), and the findings of EL-ZINEY & AL-TURKI (2006) who isolated salmonella in 24 % of the tested camel milk samples in Egypt, the examination for salmonellae should be integrated in the examination scheme if camel milk is produced in a large scale dairy farm.

4.3.6. *Listeria* spp.

Considering that listeriae are ubiquitous bacteria and *L. welshimeri* is not considered as pathogenic (FAO, 2005), the proof of *L. welshimeri* in one milk sample poses no risk to human heath. It is probably due to an external contamination, as other samples of this camel were negative for listeriae.

4.3.7. Other bacteria

4.3.7.1 *Streptococcus* spp.

In the present study, *S. agalactiae* has been detected in the quarter-milk sample of one camel that showed signs of clinical mastitis (Table 3.9), a result which is conform with the findings of EL-JAKEE (1998). Also ABDEL GADIR et al. (2005) isolated *S. agalactiae* from camel milk, but these camels showed no signs of clinical or subclinical mastitis (Table 2.11).

From one quarter milk sample with positive CMT, *S. bovis* was isolated. To our knowledge no *S. bovis* in camel milk has been reported before, nevertheless, it has been isolated from cow and goat milk (POUTREL & RYNIEWICZ, 1984; HÖHN, 2006).

4.3.7.2 *Burkholderia cepacia*

In one CMT-negative quarter milk sample as well as in three CMT positive samples of camels with clinical mastitis, multi-resistant *Burkholderia* (*B*.) *cepacia* was isolated after antibiotic treatment of these camels. In these cases, CMT did not become negative again. *B. cepacia* are ubiquitous Gram-negative rods with high intrinsic resistance against several antibiotics. It can produce pulmonary infections, especially in immune-suppressed persons (HUBER, 2002; WOLF, 2003), but has yet not been reported from camel milk.

4.3.7.3 *Corynebacterium striatum*

Of 11 CMT positive samples taken from one quarter, *Corynebacterium striatum* has been isolated. These Gram-positive bacilli can usually be found in the flora of skin and cutaneous membranes and cause infections of the urinary tract, of injuries or endocarditis mainly in immune-suppressed humans (MARTINEZ-MARTINEZ et al., 1995). This facultative pathogen has not been reported in camel milk before.

4.4. Further diagnostic means in camel milk

BILLON et al. (2001) proposed as additional diagnostic mean of subclinical mastitis in cows the close observation of the daily milk yield. In camels the implementation of this idea could be difficult as dromedaries react very sensible to their environment with yield variation. But generally it is important to verify the udder health and general health status of the camels. As camels are very sensitive to udder pain the development of clinical mastitis can be easily detected. The following diagnostic means for subclinical mastitis were investigated.

4.4.1 Somatic cells

4.4.1.1. Semi-quantitative California mastitis test (CMT)

According to the present investigation 72.9 % of the milk samples were CMT negative. In 7.9 % of these samples pathogenic bacteria were found against 2.6 % in CMT +/-, 23.8 % in CMT + and 33.3 % in CMT ++ samples (Table 3.10). A clear but not very pronounced correlation was observed between pathogenic bacteria and CMT result (Table 3.12) that is

also reported by ABDEL GADIR et al. (2006). In several studies CMT is recognised as an adequate sensitive screening test for subclinical mastitis in camels (BEKELE & MOLLA, 2001; YOUNAN et al. 2001; BHATT et al., 2004).

Considering the results of the present study, CMT can be used as screening method for udder irritations and combined with other parameters like testing for pathogenic bacteria for subclinical mastitis. It is obviously not adequate as a single diagnostic test - a result that goes conform with the findings of WINTER & BAUMGARTNER (1999) in goat milk and of MIDDLETON et al. (2004) in cow milk.

4.4.1.2. Somatic cell count (SCC)

The SCC in CMT-negative milk samples in the present investigation were with an geometric mean of 6.3 x 10^4 cells/ml (Table 3.11) considerably lower than those stated by AL-ANI & AL-SHAREEFI (1997), TUTEJA et al. (2003) and BHATT et al. (2004) (Table 2.16), whereas the SCC of samples with CMT +/- to CMT + were between 1.4 x 10^5 and 3.4 c 10^5 cells/ml which resembles the average counts of these authors. A clear increase of the SCC in the present study was noted in samples with CMT ++ and +++, a finding also reported by TUTEJA et al. (2003). However, the mean values of CMT ++ and CMT +++ samples were significantly higher in the study of TUTEJA et al. (2003) than in the present study (Tables 2.16 and 3.11). As in the investigations of SAAD & THABET (1993), CHAFFER et al. (2000) and SEMEREAB & MOLLA (2001) the present study showed that from samples with high SCC more often pathogenic bacteria were isolated (Table 3.12).

The present examination - similar to the studies of TUTEJA et al. (2003) and BHATT et al. (2004) - was done with Giemsa staining and light microscopy and therefore minimised the risk of counting particles other than somatic cells.

The positive correlation between SCC and CMT was not very pronounced, in contradiction to ABDEL GADIR et al. (2006) for camel milk and KALOGRIDOU-VASSILIADOU et al. (1992) for goat milk. Considering in addition that it is difficult to fix camel milk on object slides, determination of SCC by counting cells on slides remains a method difficult to interpret. According to MORONI et al. (2005) determination of the SCC in goat milk is also possible by using automated fluorescent microscopic somatic cell counter with specific

binding of the DNA by ethidium bromide dye. Investigation on SCC-determination by automated fluorescent microscopic cell counter should be considered and further research should be done in this field.

4.4.2 Electrical conductivity (Tables 3.13 - 3.15)

With a mean value of 7.7 mS/cm (Figure 3.2 shows the frequency distribution of EC in quarter-milk samples) EC is significantly higher than in cow (NIELEN, et al., 1992; WALZEL, 1997; BILLON et al., 2001) and goat milk (PARK, 1991). The single EC values in the present study seem to correlate in some camels with a positive CMT reaction but show no correlation to CMT and TBC or pathogenic bacteria in other camels or in the same camels on other days (Attachment I). For this reason, the EC can not be considered an adequate (additional) screening method for subclinical mastitis in camels - a result that goes conforms with the results of YOUNAN et al., (2001) and BHATT et al. (2004). Similar findings are reported for goat milk (PARK, 1991).

4.5. Indicator enzymes for heat treatment of milk

In the examined raw camel milk samples alkaline phosphatase and peroxidase activity was clearly positive as described in cow milk (ANONYMOUS, 2000b). Different to cow milk, alkaline phosphatase was inactivated only if heated at 80 °C for 5 min or 90 °C for 5 s. Even heat treatment for 40 min at 75 °C did not totally inactivate the alkaline phosphatase Table 3.17), a finding that goes confirm with the results of WERNERY et al. (2006b).

Peroxidase was inactivated in the present study after 3 min heat treatment at 70 °C and after 1 min at 72 °C (Table 3.16). Considering these findings, peroxidase could be an adequate indicator for pasteurisation in combination with alkaline phosphatase – as indicator for higher heat-treatment - or in combination with other enzymes. According to LOISEAU et al. (2001), there are at least two milk enzymes that are suitable for controlling correct heat treatment in camel milk:

- Glutamyltranspeptidase, that loses > 70 % of its activity after 30 s of treatment at 75 °C and is completely inactive after heating at 89 °C for 16 s.
- Leucine arylamidase is inactivated at 75 °C for 28s or heating at 80°C for 7 s.

Definitely more investigation on this subject is needed to lay down values for the proof of pasteurisation of camel milk.

4.6. Camel milk in legislation

As camel milk is commonly produced outside the European Union (with some non-commercial exceptions) it is not in the scope of specific EU legislation, whereas import conditions could be of importance. In the UAE, camel milk is not included in food safety legislation. Therefore, one aim of this investigation was to build a basis for the development of such legislation in the UAE and to contribute some figures for microbiological criteria for camel milk.

5. SUMMARY

During a five month period the milk of 43 former racing camels in Dubai (United Arab Emirates) was examined for total bacteria count (TBC), pathogenic bacteria, California mastitis test (CMT), somatic cell count (SCC), electrical conductivity (EC) and activity of indicator enzymes for heat treatment. The results of milk milked in the traditional way by hand (196 samples of 18 camels) were compared with milk of camels kept in intensive husbandry and milked by machine (260 samples of 25 camels). Additionally, 468 quarter-milk samples of the 25 camels mentioned above were examined.

The geometric mean of TBC of the hand milked samples was 1.1 x 10^2 cfu/ml, of machine milked samples 9.2 x 10^2 cfu/ml and of quarter-milk samples 9.2 x 10^2 cfu/ml. 100 % of the hand milked samples, 97.7 % of the machine milked and 81.8 % of the quarter milk samples showed results lower than 5.0 x 10^3 cfu/ml and therefore amount only to 5 % of the limit of 1 x 10^5 cfu/ml laid down by the European Union for raw cow milk.

The prevalence of coagulase positive staphylococci (CPS) was 28.5 % in machine milked samples, 5.4 % in hand milked and 4.5 % in quarter-milk samples. CPS were the major udder pathogenic bacteria most often isolated in this study. In most samples coagulase negative staphylococci were determined. No effect of these minor pathogenic bacteria on udder health was detected.

None of the hand milk samples showed a positive result for *Bacillus cereus*. In 9 (3.1 %, maximum 4.7 x 10^1 cfu/ml) of the machine milked samples *B. cereus* was present. Quarter-milk samples were not examined for *B. cereus*.

In 17 (6.5 %) of the machine milked samples coliforms were detected. 7 of these isolates (41.2 %) were *Citrobacter freundii*, 2 (11.8 %) *Escherichia coli*, 4 (23.5 %) *Enterobacter cloacae* and 4 (23.5 %) *Klebsiella pneumonia.* 14 (8.3 %) of the hand milked samples were positive for coliforms - all *Serratia marcescens*. In quarter-milk samples coliforms were not detected.

Other (partly facultative) pathogenic bacteria found were *Streptococcus agalactiae*,

Streptococcus bovis, Corynebacterium striatum and *Burkholderia cepacia.*

All 47 samples had a negative result for *Salmonella* spp. Of the 61 samples tested for listeriae only one sample revealed a positive result for *Listeria welshimeri*.

Besides pathogenic bacteria the adequacy of EC and CMT for mastitis screening was tested. No distinct correlation was found between EC and the presence of pathogenic bacteria. CMT results also did not correlate distinctly with the above mentioned test results. Therefore, CMT is not considered adequate as single mastitis screening method. For such a screening, CMT should be accompanied by other tests particularly by the determination of udder pathogenic bacteria.

The geometric mean of SCC for a negative CMT was 6.3×10^4 cells/ml. It increased to 1.4×10^5 cell/ml with CMT +/-, 3.7×10^5 with CMT +, 8.4×10^5 at CMT ++ and 1.8×10^6 cells/ml with CMT +++.

The activity of the indicator enzymes peroxidase and alkaline phosphatase for heat treatment of milk was determined after heat treatment at different temperatures and times. Both enzymes showed different inactivation temperatures than in cow milk. However, they could be suitable for the proof of pasteurisation (peroxidase) or for higher heat treatment (alkaline phosphatase) if combined with other heat sensible parameters.

In addition to the milk samples 42 faecal samples of 22 camels were examined for the presence of verotoxinogenic *Escherichia coli* by ELISA. All of them revealed a negative result.

The study shows that all in all the milk from the camel herds examined was of good hygienic quality, fit for human consumption and quite comparable to the quality of cow milk. Moreover, the results of the present study show that the microbiological criteria for cow milk regulated by law in the European Union are also applicable for camel milk.

6. ZUSAMMENFASSUNG

Hygienischer Status von Kamelmilch in Dubai (Vereinigte Arabische Emirate) unter Berücksichtigung zweier verschiedener Milchgewinnungssysteme

Über einen Zeitraum von fünf Monaten wurde die Milch von 43 ehemaligen Rennkamelen in Dubai (Vereinigte Arabische Emirate) auf die aerobe mesophile Keimzahl (AMK) und die Anwesenheit pathogener Bakterien, auf die somatische Zellzahl und mit dem California Mastitis Test (CMT), auf die elektrische Leitfähigkeit (LF), sowie hinsichtlich der Eignung von Indikatorenzymen für den Nachweis einer Wärmebehandlung untersucht. Die Ergebnisse von traditionell mit der Hand ermolkener Milch (196 Proben von 18 Kamelen) wurden mit denen von Milch intensiv gehaltener und maschinell gemolkener Kamele (260 Proben von 25 Kamelen) verglichen. Weiterhin wurden 468 Viertelgemelksproben der 25 oben genannten Kamele in die Untersuchungen einbezogen.

Das geometrische Mittel der AMK der von Hand ermolkenen Milch lag bei 1,1 x 10^2 KbE/ml, das der maschinell ermolkenen Milch bei 9,2 x 10^2 KbE/ml, und das der Viertelgemelksproben ebenfalls bei 9,2 x 10^2 KbE/ml. Die AMK von 100 % der von Hand ermolkenen Milchproben, von 97,7 % der maschinell ermolkenen Milchproben und von 81,8 % der Viertelgemelksproben lag unter 5,0 x 10^3 KbE/ml und betrug somit weniger als 5 % des in der Europäischen Union für rohe Kuhmilch festgelegten Grenzwertes.

Koagulasepositive Staphylokokken (KPS) wurden in 28,5 % der maschinell ermolkenen Milchproben (Maximalwert 1,8 x 10^2 KbE/ml), in 5,4 % der von Hand ermolkenen Milchproben (Maximalwert 3,9 x 10^2 KbE/ml) sowie in 4,5 % der Viertelgemelksproben (Maximalwert 1,4 x 10^3 KbE/ml) nachgewiesen. KPS waren damit die am häufigsten isolierten wichtigen Mastitiserreger („major pathogens“). Aus den meisten Milchproben wurden koagulasenegative Staphylokokken isoliert. Ein Einfluß dieser minderpathogenen Mastitiserreger („minor pathogens“) auf die Eutergesundheit wurde nicht beobachtet.

In keiner der von Hand ermolkenen Milchproben wurde *Bacillus cereus* nachgewiesen, wohingegen der Keim aus 9 (3,1 %, Maximalwert 4,7 x 10^1 KbE/ml) der mit maschinell ermolkenen Proben isoliert werden konnte. Viertelgemelksproben waren nicht auf *B. cereus*

untersucht worden.

In 17 (6,5 %) der maschinell ermolkenen Milchproben konnten coliforme Keime nachgewiesen werden (Maximalwert 8,4 x 10^2 KbE/ml). Bei 7 (41, 2 %) dieser Isolate handelte es sich um *Citrobacter freundii*, bei 2 (11,8 %) um *Escherichia coli*, bei 4 (23,5 %) um *Enterobacter cloacae* und bei 4 weiteren (23,5 %) um *Klebsiella pneumoniae*. Aus 14 (8,3 %) der von Hand ermolkenen Proben wurden Coliforme (*Serratia marcescens*) isoliert (Maximalwert 2,8 x 10^2 KbE/ml), während sich diese Keime in keiner der Viertelgemelksproben fanden.

Außerdem konnten folgende potentiell pathogene Mikroorganismen nachgewiesen werden: *Streptococcus agalactiae*, *Streptococcus bovis, Corynebacterium striatum* und *Burkholderia cepacia.* 47 Proben wurden mir einem negativen Ergebnis auf die Anwesenheit von *Salmonella* spp. geprüft. In einer von 61 auf Listerien untersuchten Milchproben konnte *Listeria welshimeri* nachgewiesen werden.

Neben den Untersuchungen auf pathogene Mikroorganismen wurde die Eignung der LF und des CMT als Suchtests für Mastitiden geprüft. Eine eindeutige Beziehung zwischen der LF und dem Nachweis pathogener Mikroorganismen ergab sich nicht. Die Ergebnisse des CMT zeigten ebenfalls keine eindeutige Beziehung zu den oben genannten Testergebnissen, so dass dieser nicht als alleiniger Suchtest für Mastitiden empfohlen werden kann. Er sollte daher durch weitere Tests, insbesondere den auf euterpathogene Mikroorganismen, ergänzt werden.

Für CMT-negative Milchproben wurde ein geometrisches Mittel der somatische Zellzahl von 6,3 x 10^4 Zellen/ml errechnet. Die Zellzahl stieg auf einen Wert von 1,4 x 10^5 Zellen/ml bei Proben mit CMT +/-, auf 3,7 x 10^5 bei Proben mit CMT +, auf 8,4 x 10^5 bei Proben mit CMT ++ und auf 1,8 x 10^6 Zellen/ml bei CMT +++.

Weiterhin wurde die Aktivität der bei der Kuhmilchuntersuchung als Indikatorenzyme für eine Wärmebehandlung verwendeten Peroxidase und alkalischen Phosphatase ermittelt, wobei die Kamelmilch bei unterschiedlichen Temperatur-/Zeitkominationen erhitzt wurde. Beide Enzyme unterschieden sich hinsichtlich ihrer Inaktivierungskinetik von den entsprechenden Enzymen der Kuhmilch. Dennoch könnte eine Bestimmung ihrer Aktivität für den Nachweis einer Pasteurisierung (Peroxidase) oder einer intensiveren (z. B.

Hocherhitzung) Wärmebehandlung (alkalische Phosphatase), eventuell in Kombination mit weiteren geeigneten Parametern, eingesetzt werden.

Zusätzlich zu den Milchproben wurden 42 Kotproben von 22 Kamelen mit einem enzymimmunologischen Verfahren auf die Anwesenheit von verotoxinbildenden *Escherichia coli*-Stämmen geprüft. Alle Untersuchungen verliefen negativ.

Die Ergebnisse der vorliegenden Arbeit zeigen, dass die Milch der untersuchten Kamelherden von guter hygienischer, der Kuhmilch vergleichbarer Qualität und für den menschlichen Verzehr geeignet ist. Darüber hinaus zeigen die Ergebnisse der vorliegenden Studie, dass die in der Europäischen Union für Kuhmilch festgelegten mikrobiologischen Kriterien auch auf Kamelmilch anwendbar sind.

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LEGISLATION AND METHODS

Amtliche Sammlung von Untersuchungsverfahren nach § 64 LFGB (§ 35 LMBG).
L 01.00-3: Untersuchung von Lebensmitteln; Bestimmung der coliformen Keime in Milch, Milchprodukten, Butter, Käse und Speiseeis; Verfahren mit festem Nährboden Verfahrensprinzipien:Verfahren mit festem Nährboden.
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L 01.01-3: Untersuchung von Lebensmitteln; Zählung somatischer Zellen in Rohmilch; Mikroskopische Zählung somatischer Zellen
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L 00.00-32: Untersuchung von Lebensmitteln - Horizontales Verfahren für den Nachweis und die Zählung von *Listeria monocytogenes* - Teil 1: Nachweisverfahren
Beuth Verlag GmbH, Berlin, Germany

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L 01.00-1: Untersuchung von Lebensmitteln - Allgemeiner Leitfaden für die Vorbereitung von Untersuchungsproben und die Herstellung von Anschüttelungen und Dezimalverdünnungen für mikrobiologische Untersuchungen von Milch und Milchprodukten
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L 01.00-72: Untersuchung von Lebensmitteln - Bestimmung präsumtiver *Bacillus cereus* in Milch und Milchprodukten - Teil 1: Koloniezählverfahren bei 37 °C
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ATTACHMENT I

Table I.1 Electrical Conductivity in the milk of the camels of the study on different days - Part 1

Camel	**EC [mS/cm]**	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7**	**Day 8**	**Day 9**	**Day 10**	**Day 11**	**Day 12**	**Day 13**
1	Average	8.2	-	-	-	-	-	-	-	-	-	-	-	-
	Max. diff	0.6												
2	Average	7.9[1]	8.4[1]	7.2	7.3	7.3	7.7	7.8	8.3	7.7[1]	7.5[3]	8.4[3]	8.1[2]	7.7[1]
	Max. diff.	1.3	1.8	0.3	0.6	0.3	0.3	0.3	0.4	0.8	0.6	0.6	0.9	0.7
3	Average	7.2	7.0	7.3	6.8	7.5	7.5	7.5	8.1[1]	-	-	-	-	-
	Max. diff.	0.4	0.3	0.3	0.5	0.4	0.5	0.3	0.9					
4	Average	7.6	7.3	7.0	7.3	7.4[1]	7.3	7.31	7.1	8.5	8.4[2]	8.0[1]	-	-
	Max. diff.	0.2	0.1	0.2	0.4	0.4	0.3	0.3	0.2	0.2	1.1	0.7		
5	Average	8.1	8.9	8.8	9.4	7.4	9.3	-	-	-	-	-	-	-
	Max. diff.	0.9	1.0	1.2	1.9	1.1	2.0							
6	Average	7.8	7.2	7.0	7.8	8.0	6.4	8.1	8.9	-	-	-	-	-
	Max. diff.	0.3	0.8	0.6	0.2	0.2	0.2	0.3	1.2					
7	Average	6.4	-	-	-	-	-	-	-	-	-	-	-	-
	Max. diff.	0.6												
12	Average	8.7	7.0	7.4	7.1[1]	-	-	-	-	-	-	-	-	-
	Max. diff.	0.5	0.3	0.5	0.3									
16	Average	9.2	8.1	8.7	8.8	7.7	7.9	6.4	6.9	7.2	7.6	-	-	-
	Max. diff.	0.4	0.4	0.3	0.4	0.5	0.6	0.2	0.5	0.4	0.5			

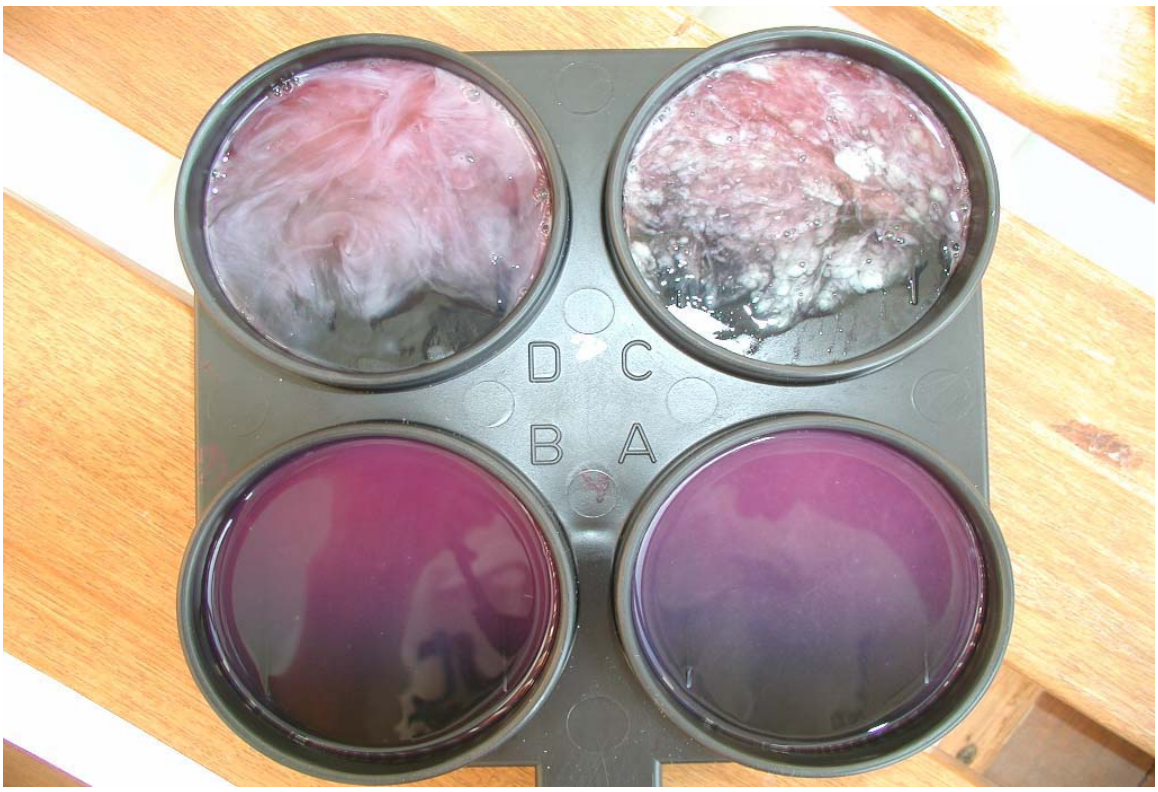
[1] One quarter CMT positive
[2] 2 - 3 quarters CMT positive
[3] All quarters CMT positive

Table I.2 Electrical Conductivity on different days in the camels of the study - Part2

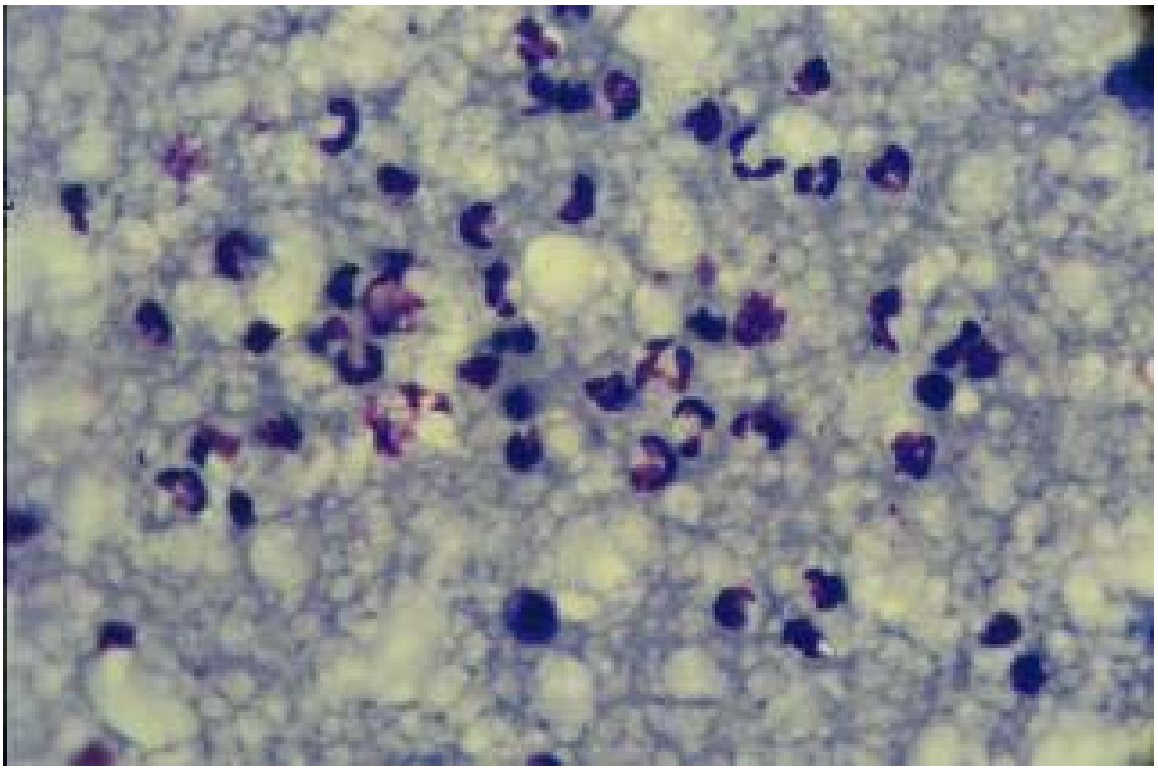
Camel	EC [mS/cm]	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13
17	Average Max. diff.	7.9 0.1	8.5 0.1	8.3 0.5	8.5 0.4	8.8 0.1	8.8 0.2	8.3 0.7	8.0 0.1	9.0 0.1	7.7 0.9	-	-	-
18	Average Max. diff.	7.0 0.8	6.8 0.3	5.8 0.7	6.6 0.5	7.8 0.2	-	-	-	-	-	-	-	-
20	Average Max. diff.	6.3* 0.4	6.5 0.5	6.3* 0.6	-	-	-	-	-	-	-	-	-	-
21	Average Max. diff.	6.7* 0.9	-	-	-	-	-	-	-	-	-	-	-	-
22	Average Max. diff.	8.1 1.0	7.4 0.2	8.3** 0.3	8.4* 0.2	7.9 0.4	8.5 0.2	6.4 0.3	7.1 0.4	7.8** 0.6	-	-	-	-
23	Average Max. diff.	8.0* 0.4	8.1* 0.4	8.5* 0.2	8.8* 0.4	8.5 0.7	7.8* 0.6	8.0* 0.8	8.3* 0.4	8.9* 0.3	8.2* 0.5	7.5* 0.5	8.9* 0.6	-
27	Average Max. diff.	7.4 0.5	7.1 0.3	7.2*** 0.3	7.5 0.3	8.0 0.3	7.5 0.8	7.6** 1.0	7.7* 0.2	-	-	-	-	-
30	Average Max. diff.	7.0 0.5	6.7* 0.1	7.5 0.4	8.1 0.6	6.1 0.2	-	-	-	-	-	-	-	-
36	Average Max. diff.	6.7 0.1	-	-	-	-	-	-	-	-	-	-	-	-

[1] One quarter CMT positive
[2] 2 - 3 quarters CMT positive
[3] All quarters CMT positive

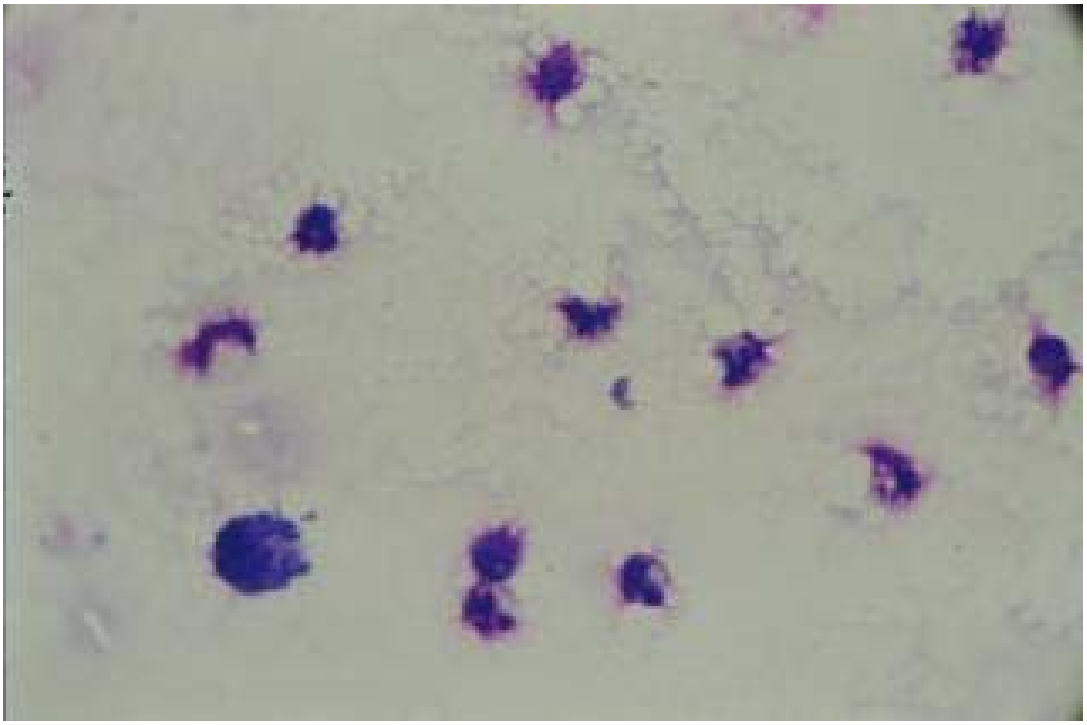
ATTACHMENT II



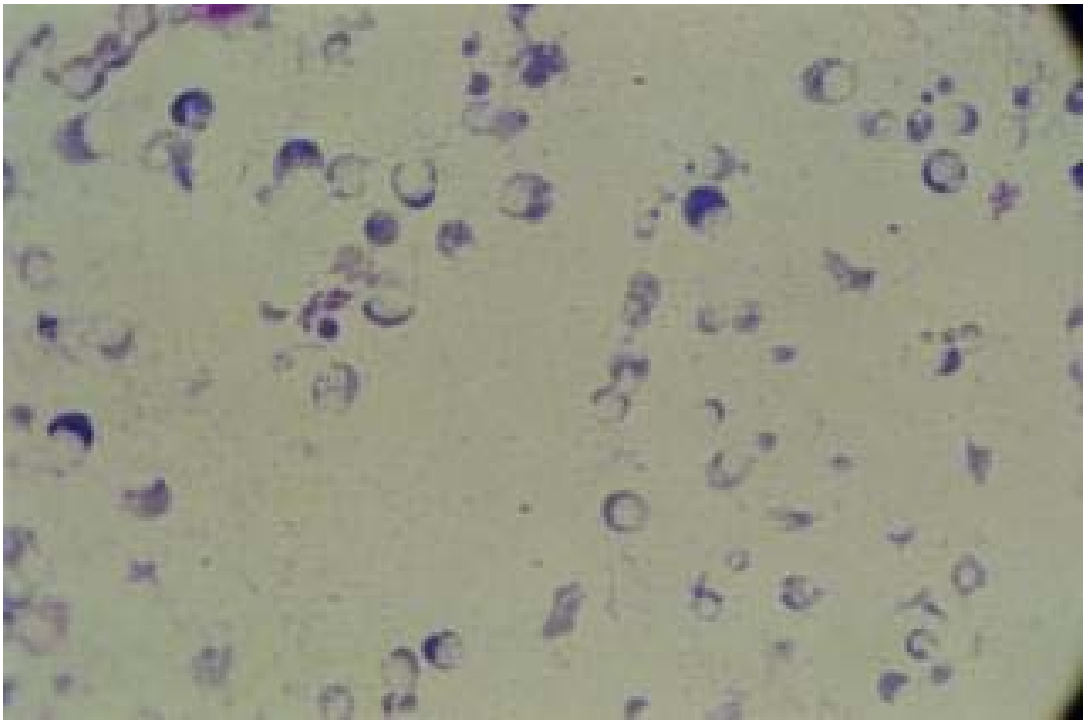
Picture 1: Case of clinical mastitis (E. coli) A: CMT – B: CMT +/- C: CMT +++ D: CMT+



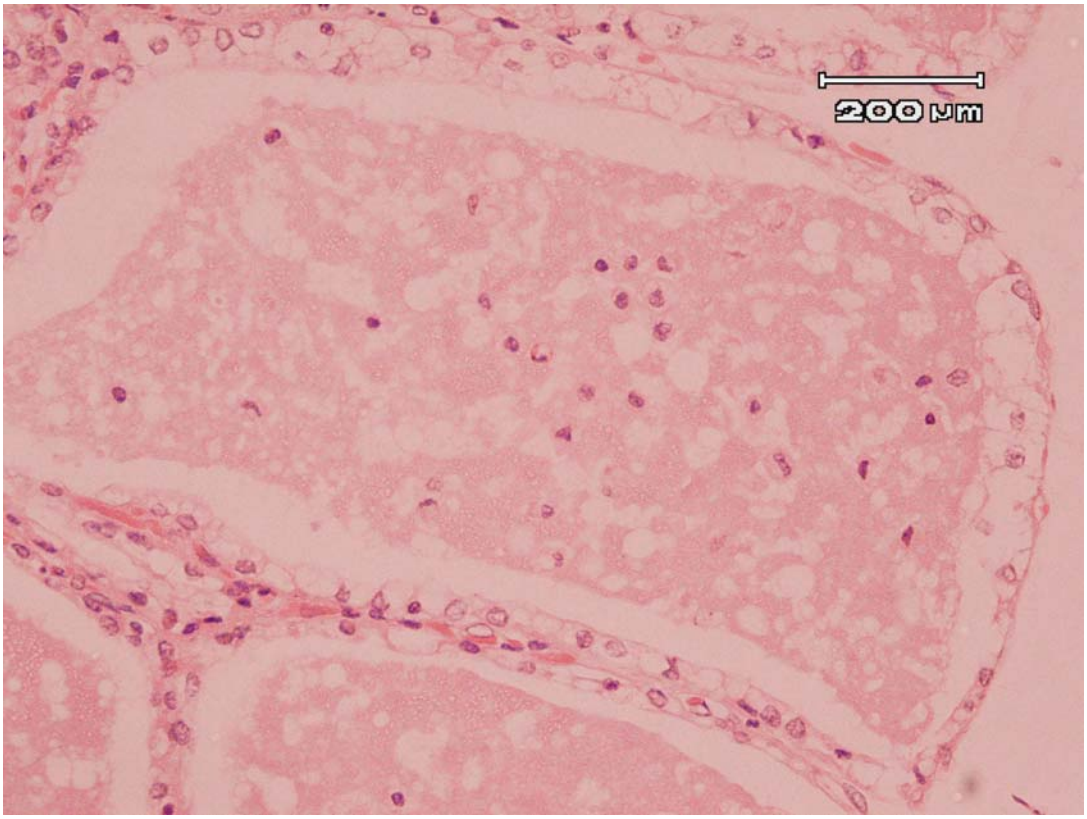
Picture 2: Somatic cells (Giemsa stain) for counting according to PRESCOTT and BREED



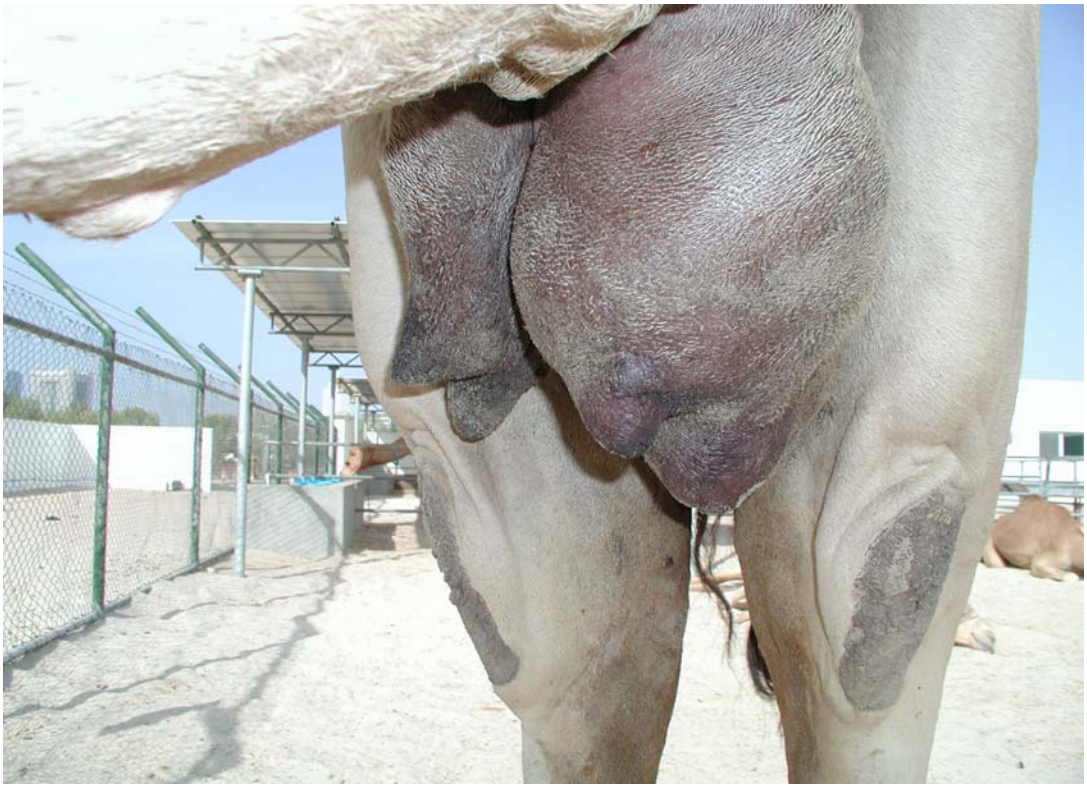
Picture 3: Camel milk cells after centrifugation (Giemsa stain)



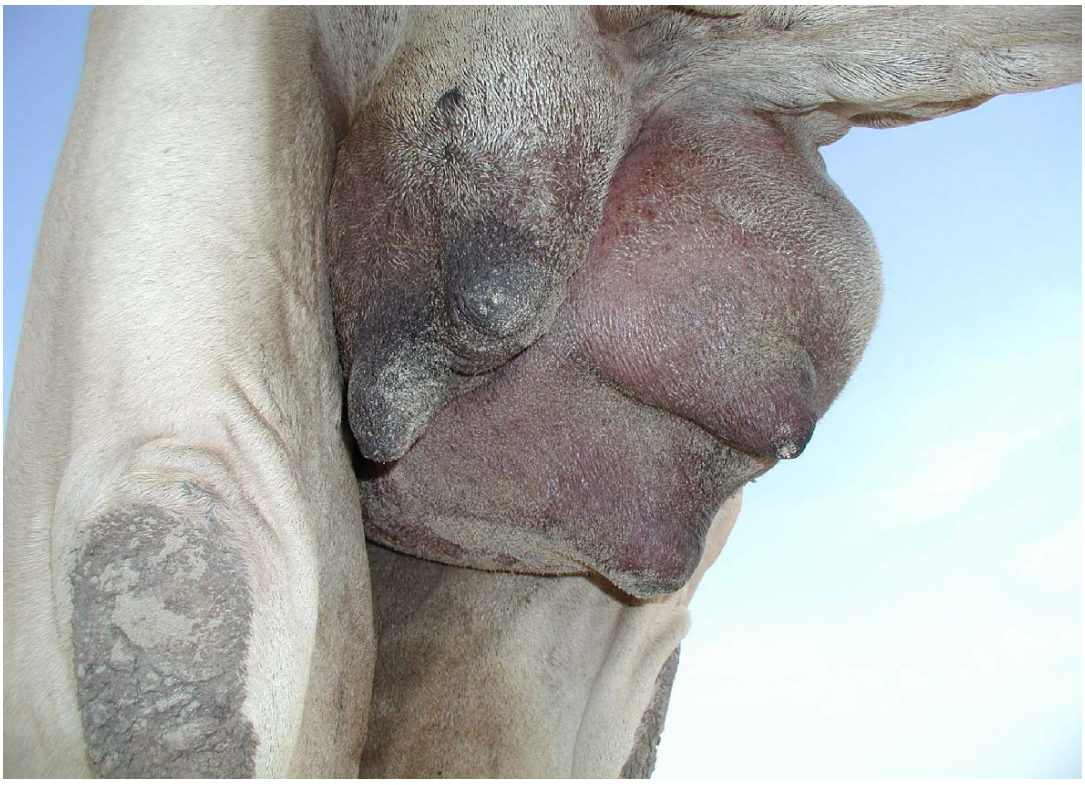
Picture 4: Fragments in centrifuged camel milk (Giemsa stain)



Picture 5: Section of milk acini of a lactating camel with cells



Picture 6: Camel with severe clinical mastitis on the two left quarters



Picture 7: Same camel, healthy and inflamed quarters compared

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