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CASEOUS LYMPHADENITIS (*Pseudotuberculosis*) IN CAMELIDS

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ABSTRACT

Corynebacteria are pyogenic bacteria causing a variety of suppurative diseases. The virulence of *Corynebacterium pseudotuberculosis* is attributed to the haemolytic toxin which possesses phospholipase activity and to cell wall lipids. The pathogen causes pseudotuberculosis or lymphadenitis in sheep, goats and camelids, but it is rare in other animal species. The infection is spread via ingestion, inhalation or wounds, and pathognomic for the disease are cold, closed painless abscesses up to the size of a lemon or orange in external lymph nodes especially at the base of the neck and in prescapular lymph nodes. *Corynebacteria* are sensitive to several antibiotics but the abscess prevents the medication from reaching the bacteria. It is therefore recommended to perform both, surgical and antibiotic treatment. Commercial vaccines are available for sheep and goats, but have not been evaluated for camelids. These vaccines do not provide complete protection against the development of abscesses but a significant reduction in the number of abscesses. After mange Caseous lymphadenitis (CLA) remains the most important skin disease of camelids.

Key words: CLA, NWCs, OWCs, pseudotuberculosis, serology, vaccine

Caseous lymphadenitis (CLA) or pseudotuberculosis in sheep and goats occurs worldwide. It is a chronic disease caused by *Corynebacterium (C.) pseudotuberculosis*. It is characterised by abscessation of one or more superficial lymph nodes. It sometimes also causes pneumonia, hepatitis, mastitis, arthritis, orchitis, and meningitis. *C. pseudotuberculosis* also affects horses ("Pigeon Fever") and produces an ulcerative lymphangitis in cattle. Pseudotuberculosis is widespread in Old World Camels (OWCs), and certainly after mange the most prevalent skin disorder. Entire dromedary herds can be affected by this disease. The organism has also been isolated from abscesses of New World Camels (NWCs).

Aetiology

The French veterinarian Nocard first described *C. pseudotuberculosis* in 1888. It is a short, irregular ovoid, Gram-positive rod almost resembling a coccus. In smears made from abscesses, the bacteria shows a marked pleomorphism. For routine isolation, sheep or ox blood is used and the plates should be incubated at 37°C for at least 48 h. *C. pseudotuberculosis* colonies are small, white and dry and can be surrounded by a narrow zone of haemolysis. There are two proposed biotypes, ovine/caprine and equine/bovine. Although very rare, the pathogen can cause human infections with the sheep/goat strain among farm and abattoir workers. Infected people show chronic lymphomegaly and normally require surgical treatment.

Epidemiology

Camel pseudotuberculosis has been observed in Iran, Egypt, Ethiopia, Kenya, Australia, Saudi Arabia, India, Russia, China, UAE and East Africa (Wernery *et al*, 2012, in press).

Only the ovine/caprine strain has been found in camels. The isolation of *C. pseudotuberculosis* from abscesses poses certain difficulties as the colonies resemble streptococcal colonies and are frequently overgrown by accompanying bacteria.

The infection is spread via ingestion, inhalation or directly through wounds. *C. pseudotuberculosis* invades also intact skin, and then the pathogens are engulfed by macrophages and taken to a draining lymph node. There, an abscess might form. *C. pseudotuberculosis* is a pyogenic, facultative intracellular bacterium which penetrates the tissue and produces filterable toxins. At least three toxins, a toxic cell-wall lipid, an exotoxin, phospholipase-D (PLD) which damages mammalian cell membranes and a haemolysin play essential roles in the development of CLA. The toxic cell-wall lipid is

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associated with the virulence of the bacterium and the haemolysin causes haemorrhages, increased vascular permeability and enhanced bacterial invasion.

In contrast to pseudotuberculosis in sheep and goats, *C. pseudotuberculosis* is not always the only bacteria isolated from the abscesses in camels. Many different bacterial species other than *C. pseudotuberculosis* have been isolated including:

Streptococcus sp.

Staphylococcus aureus

Staphylococcus sp.

C. pyogenes

C. equi.

Afflicted animals often concurrently suffer a severe tick infestation (*Hyalomma dromedarii*) from which *C. pseudotuberculosis* is often isolated.

Spesivtseva and Noskov (1959) and Dalling *et al* (1966) purport that *Histoplasma farciminosum* is responsible for an outbreak of pseudotuberculosis among Bactrian camels in the Soviet Union. The disease occurred in 1958 when camels were walked from Central Asia to several farms near Moscow. The lesions were observed in the pre-shoulder lymph nodes. *Mycelium* and *Cryptococcus*-like organisms were detected in the draining lymph nodes. *Cryptococci* were also observed in macrophages.

Skin lesions caused by acacia thorns, ticks, contaminated injection needles and nodular worms may inadvertently result in damage to the skin and thus create portals of entry for *Corynebacteria*.

The mucous membranes of the oral cavity might be damaged by acacia thorns and/or by dry and hard stems from desert plants. Following its entry through the skin or mucous membrane, *C. pseudotuberculosis* bacteria are phagocytosed by leucocytes. Instead of being destroyed by the cells, the outer lipid wall of the bacterium allows it to survive within the leukocyte which transports the pathogen via the lymphatic drainage to the regional lymph node. In the lymph node the bacteria multiply in the host cell which may liberate many bacteria. This recurring process of bacterial multiplication and cell death leads to formation of classical CLA. In some cases, these superficial abscesses are responsible for the lymphatic and haematogenous spread of infection to other sites like lung, mediastinal lymph nodes, liver, kidney and brain. Lymphogenous and haematogenous distribution of the infection from the primary site to internal organs and tissues may occur latently.

Afzal *et al* (1996) isolated pure cultures of *C. pseudotuberculosis* from 11 racing camels from the UAE suffering from lymphadenitis. Six of the camel isolates and a sheep strain used as control, produced necrosis of rabbit skin and redness. In an experiment, one of each isolate (with and without dermonecrosis and the sheep strain) was inoculated into the base of the ear of experimental camels. Camels infected with the sheep strain and the dermonecrotic isolate produced lymph node swelling only, whereas the strain without dermonecrosis produced multiple abscesses in the experimental camels 40 days after infection. Re-infection of the experimentally infected dromedaries after they had recovered from the disease did not produce any lesions. It has been found that strains of *C. pseudotuberculosis* which do not possess the gene for phospholipase D are unable to produce lymph node abscesses.

Several new publications have stressed the importance of CLA in NWCs and OWCs. Muenchau (2006) reported that the disease has appeared in Europe for the first time in camelids in 2003 after CLA was introduced into the sheep industry in the UK in 1991. Mortality in OWCs can reach 15% and in NWCs 22% mainly when internal organs are involved. It has also been shown that camelids suffering from CLA have increased stomach and intestinal ulcers. Several cases of CLA in dromedaries are reported from the Canary Island where animals are kept for the tourist industry. A rare case of pleuritis, arthritis and periarthritis in association with *C. pseudotuberculosis* and *S. enterica* infection was diagnosed in a dromedary in Gran Canaria, Spain by Tejedor-Junco *et al* (2009). The same authors described several more cases of CLA on the Canary Islands. At several locations of the dromedary body, lymph nodes were enlarged, cold, soft and painless. Samples were collected from unruptured lymph node abscesses, and pure 16 isolates of *C. pseudotuberculosis* were cultured which exhibited 4 different biochemical patterns. In one case involving a young dromedary, *C. ulcerans* was isolated from the affected dorsal and ventral superficial lymph nodes of the left cervicothoracic region. Not only on the Canary Island of Spain, CLA is a severe disease in dromedaries affecting the tourist industry, also on mainland Europe many dromedaries and Bactrian's purchased from animal traders for breeding purpose were affected by the disease which spread to other animals when introduced into herds. Increased intermingling of animals of different species for show performances and trading pose a severe risk for the spread of CLA. Even though the rate of

mortality is not very high, the economic losses can be severe as many animals especially camelids are valuable companion animals. In Australia, where several hundred thousand feral dromedaries are roaming through the interior, unsightly lymph node abscesses have been observed by many people.

CLA has not only affected OWCs but has also been reported from NWCs from many different parts of the world. Several authors have reported CLA in Peru even in altitudes above 4000m. Natural infections with *C. pseudotuberculosis* in Andean alpacas produced mastitis and abscesses in superficial lymph nodes of the bodies as well as abscess formation mainly in the renal lymph nodes. It is interesting to note that abscesses in NWCs are different to the ones described in sheep. Instead, there is a liquefactive necrosis and a severe granulomatous reaction surrounded by a thick layer of connective tissue, which is also observed in dromedaries. Furthermore, the authors assume that the pathogen is disseminated through mastitis to suckling crias and not through skin wounds (shearing).

Cases of CLA have also been reported from North America but they are rare. The authors described CLA in 5 alpacas. The diagnosis was made on the basis of microbiological culture, and the treatment was successful through anti-microbial therapy and excision of the abscesses. In Italy, Beghelli *et al* (2004) reported an outbreak of CLA in central Italy after 54 alpacas were imported from Germany into an established herd of 28 animals. Despite all efforts, the disease spread through the imported quarantined herd and jumped into the Italian nucleus herd. In total 24 alpacas had shown signs of CLA, of which 18 died. In 2003, alpacas from Germany went also to Sweden causing considerable problems in an alpaca herd due to *C. pseudotuberculosis* infection.

Experimentally infection of adult alpacas was performed by Braga (2007) to follow the clinical and pathologic course of disease, and study the humoral response to infection. Nine alpacas were inoculated with 1.1×10^6 CFUs of *C. pseudotuberculosis* from llama (n = 4) or alpaca (5) origin, and 4 alpacas were sham inoculated as controls. The alpacas were clinically observed after inoculation and euthanised on days 16, 58, 93, or 128 after inoculation. An indirect ELISA, which made use of the *C. pseudotuberculosis* cell wall as the antigen, was used to measure antibody titers in serum samples. The alpacas developed persistent fever, a local severe inflammatory response, and leucocytosis (>30×10^9/L). Internal abscesses that localised mainly in the renal lymph node were observed, but no lung lesions. Initial lesions were typical pyogranulomas with central caseous necrosis, whereas later lesions consisted of connective tissue, mononuclear cells, abundant neutrophils, and liquefactive necrosis. Infected alpacas had detectable serum antibody titers starting on day 16 that persisted until day 93 after inoculation. The different strains were pathogenically indistinct from each other.

In the UAE, CLA is also very common and effects mainly dromedaries browsing in the desert. Over several years Wernery *et al* (not published) isolated 21 *C. pseudotuberculosis* strains from dromedary CLA cases in the Emirate Dubai which underwent different laboratory tests. It was found that with the exception of nitrate production they reacted biochemically similar to known ruminant isolates, were all positive for phospholipase D production, were all sensitive to Penicillin and were characterised using the pulsed-field gel electrophoresis (PFGE) with the enzymes Sfi I and Asc I. However, the patterns did not only differ from isolates of small ruminants, but also from each other. It is therefore believed that genetically different *C. pseudotuberculosis* populations may exist on host-related basis, and the use of strain-specific vaccines from other host species may not work.

Clinical signs and pathology

The incubation period of *C. pseudotuberculosis* abscesses ranges from 25 to 40 days in sheep and goats. In camelids the incubation period is much longer and abscesses can form even after more than 6 months. Reports show that animals become infected even after more than one year after the last positive CLA case, indicating that either carriers exist or the environment is contaminated. After 40 days, Afzal *et al* (1996) observed multiple abscess formation in camels experimentally infected with *C. pseudotuberculosis*. Extensive caseous necrosis in lymph nodes and other organs (especially lung) develop in sheep and goats. In comparison, pathological changes in the internal organs due to *C. pseudotuberculosis* are rare in camels. The generalised cutaneous form is also seldom observed. Beside abscesses in muscles and subcutaneous tissues of the thigh, shoulder, elbow, base of the neck, axilla region, under the jaw and on the joints multiple large abscesses in internal organs, particularly in the lungs can also be found.

Pathognomonic for the disease are cold, closed, painless abscesses up to the size of a lemon or orange

in the external lymph nodes (Figs. 1 a, b, c), especially at the base of the neck and in the prescapular lymph nodes. However, it can appear at many different locations (Fig 2).

If opened, the abscess extrudes thick, yellow cream-like pus. Most abscesses are enveloped by well-developed connective tissue capsules. In most cases a concentrically lamellated (onion ring) pattern of the abscess develops in sheep and goats. However, these pathological changes have never been described in camelids. A few cases have been seen in dromedaries whereby the abscesses break through the ribs and the organism enters the lung, producing severe bronchopneumonia with pulmonary caverns (Fig 3).

The microscopic lesions consist of caseous necrosis of the lymph nodes with a lymphoid and epitheloid reaction. Giant cells are not observed. Histopathological examinations of the affected lymph nodes reveal acute serous suppurative and chronic suppurative lymphadenitis. Pseudotuberculosis occurs primarily in camels more than 3 years old.

Treatment and control

Affected animals serve as reservoirs of infection and if a CLA case has emerged, the following procedures should come immediately into effect:

- remove all infected animals immediately
- disinfect and clean stables and pens rigorously, remove the dung, bedding, and topsoil from barns and pens
- if superficial abscesses are lanced, provide strict aseptic methods and destroy contaminated equipment and disinfect instruments thoroughly
- administer autogenous killed vaccines
- animal housing should be free from wire or other causes of skin trauma
- external parasites must be controlled
- purchase of animals should only be allowed from herds with no history of abscessation
- serological screening of the entire herd and removal and treatment of reactors
- no serological positive animal should be detected after the second screening
- replace animals only with infection-free camelids

In small ruminants, the general measure of control is achieved by culling all animals with enlarged lymph nodes. This procedure is not practical with companion animals. The organism is susceptible to antibiotics but treatment is not always rewarding because abscesses are encapsulated, the pathogen is intracellular and response to antibiotics is therefore often poor. Subcutaneous ripe abscesses must therefore be treated with either complete extirpation or surgical drainage. To achieve the most effective therapy, it is therefore recommended to carry out both, surgical and antibiotic treatment.

Corynebacteria are extremely sensitive to penicillin, tetracyclines and cephalosporines, yet the pus in the abscess prevents the medication from reaching the bacteria. Since erythromycin is more able to penetrate the tissues, Bergin (1986) suggests a combination of penicillin and erythromycin to treat pseudotuberculosis in camels. Another possibility of treating pseudotuberculosis in adult dromedaries is the intravenous injection of 20 ml dimethyl sulfoxide (DMSO) and 20 ml Baytril® for 12 days. The abscess will eventually subside with no relapse.

Vaccines against CLA for sheep and goats are commercially available, but are often not licensed in countries in which they are not produced. Therefore, they are not available for camelids. These vaccines are formulated from concentrated formalin-inactivated *C. pseudotuberculosis* culture supernatants containing phospholipase D. GlanvacTM 3 from Australia is a multicomponent adjuvant vaccine containing ultrafiltered antigens of *C. pseudotuberculosis*, *Clostridium perfringens* type D and *C. tetani*. Attenuated mutant vaccines are also available. The commercial vaccines do not provide complete protection against the development of abscesses but a significant reduction in the number of abscesses. Immunity to CLA is associated with antitoxin activity and primary cell-mediated. After vaccination and challenge inoculation, vaccinated sheep had significantly less external, internal and total abscesses than control sheep. Antibodies were analysed to the somatic antigens of *C. pseudotuberculosis* and to the exotoxin. The sheep become positive after vaccination, whereas control sheep remain seronegative.

Vaccination appears less successful in goats and, although it protects against experimental challenge, there has been little protection from natural infections. Colostral immunity also effects the development of immunity from vaccination and lamb in flocks with a high prevalence of CLA should not be vaccinated before 12 weeks of age.

Several scientists have started research in the production of autogenous vaccines against

Fig 1a. Lemon-size CLA abscess in a one-year-old dromedary.

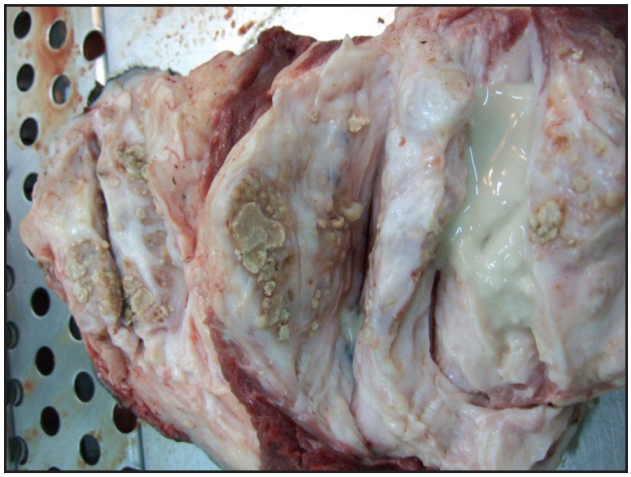

Fig 1b. Multiple abscesses caused by CLA in a Kenyan dromedary.

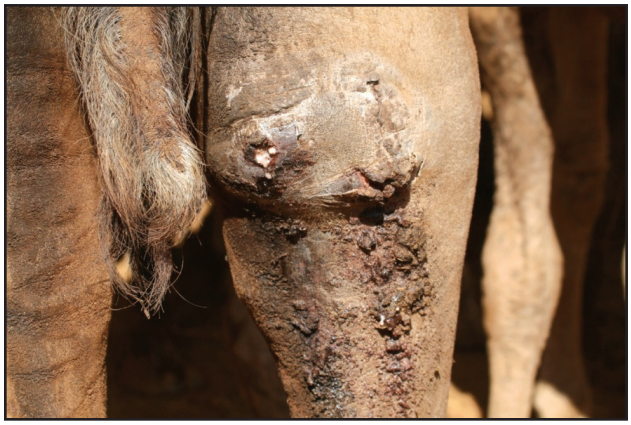

Fig 1c. An open CLA abscess.

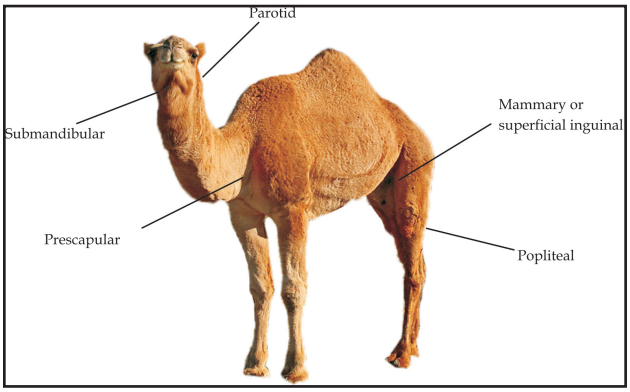

Fig 2. Lymph node sites at which CLA abscesses may be found.

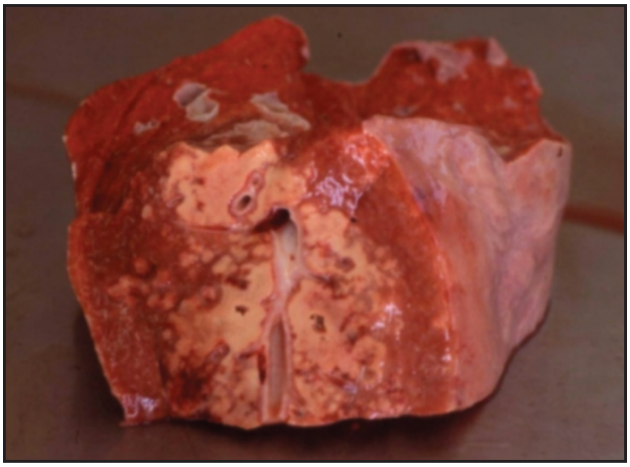

Fig 3. Severe bronchopneumonia with pulmonary caverns.

Fig 4. Granuloma caused after CLA vaccination.

pseudotuberculosis. During the CLA-outbreak in Italy in alpacas, Beghelli *et al* (2004) used an autogenous, inactivated and adjuvanted vaccine following failure of controlling the outbreak by isolation and treatment. Several strains of *C. pseudotuberculosis* from abscesses of different alpacas that had died from CLA were incorporated into the vaccine. The autovaccine was administered to 64 animals subcutaneously 3 times (day 0, 21, 150) into the thoracic skin fold caudal to the elbow. Four months after beginning of vaccination campaign, no more fatalities occurred, but several new cases of CLA lymphadenopathy and abscessation of mainly the retromandibular lymph nodes emerged.

Kobera and Poehle (2004) also used a herd specific vaccine against CLA which stopped the fatalities in 2 alpaca herds and also decreased the

number of abscesses. Similar results were achieved with commercial vaccines.

Braga (2007) evaluated the immune potential of cell wall and toxin components of *C. pseudotuberculosis* from alpaca origin using 20 µg/ml of muramyl dipeptide as adjuvant. Twelve adult alpacas were inoculated in the left flank with vaccines composed of low and high doses of bacterial crude antigens, cell wall: 250 and 500 µg/ml and toxin: 133 and 265 µg/ml, respectively.

After 3 weeks, immunised and naive alpacas were challenged intradermally in the right flank with 1×10^6 colony forming units (CFU) of *C. pseudotuberculosis*. Non vaccinated control animals developed persistent fever and abscesses at the inoculation site, regional and internal lymph nodes. Only alpacas vaccinated with high dose of toxin, were free of abscesses. In contrast, the alpacas vaccinated with a low dose of toxin showed abscesses at the inoculation site, regional, and renal lymph nodes. The cell wall vaccinated alpacas even showed a lesser degree of protection than the other groups vaccinated with a high and low dose of toxin, developing superficial and internal abscesses. In addition, a robust and early humoral response was observed in all vaccinated alpacas after challenge, lasting at least 3 months. A high doses toxin vaccine (300µg/ml), produced at CVRL is currently used in Kenya in dromedaries on an experimental basis with different adjuvants.

Most of the dromedaries which were injected at the base of the neck with commercial vaccines develop granulomas of different size (Fig 4).

Novel vaccines may provide enhanced protection in the future against CLA, but much more work is required. Therefore, other methods of controlling and eradicating the disease must be considered seriously in the future.

Serology

Several different serological tests have been tried for the serodiagnosis of CLA, but have not been validated for camelids. They include haemagglutination, haemagglutination inhibition, Agargel test and ELISAs. Most of these tests have a low sensitivity. However, a new indirect double antibody sandwich ELISA, an interferon gamma assay and a western blot are said to possess a high specificity and sensitivity at herd level in goats and sheep. The most effective of these tests detect antibodies to the PLD exotoxin.

Some of these tests are being used alone or as combination in some countries for the eradication of CLA. CLA can be eradicated when any sheep that demonstrates clinical signs of CLA or tested positive for PLD with ELISA or western blot is removed from the herd. Piontkowski and Shivvers (1998) used two distinct ELISAs to measure antibodies to the somatic antigens of *C. pseudotuberculosis* and to the exotoxin.

In several European countries with a strong camelid society a programme exists to test all NWCs regularly on a voluntary basis for antibodies to CLA. Several farms have followed this approach to eradicate the disease through adspection, palpation and serology. However, during the investigations it was observed that more than 40% of the tested NWCs showed antibodies to *C. pseudotuberculosis* without showing any clinical signs. This confirms that a high number of NWCs had come in contact with the pathogen or are carriers without showing clinical signs. Direct ELISAs for the detection of IgG antibodies in sera from sheep and goats with CLA are commercially available (e.g. Hyphen BioMed, France; ELITEST CLA). They detect anti-PLD antibodies with the help of HRP-labelled mouse monoclonal anti-goat/ sheep conjugate. These ELISAs are not validated for use in camelids. A non-commercial ELISA for the detection of antibodies to CLA was developed using a method for the extraction and concentration of immunoreactive excreted *C. pseudotuberculosis* proteins described by Paule *et al* (2004) and Seyffert *et al* (2009). Preliminary serological investigation conducted in the UAE for serodiagnosis of CLA in dromedaries with both ELISAs (in house ELISA using immunoreactive proteins and Hyphen-Biomed ELISA) of which the anti-sheep/goat conjugate was replaced by protein A, showed encouraging results when 21 CLA-positive dromedaries from which the pathogen was isolated were serologically positive, and 1119 dairy dromedaries were negative. None of the dromedaries on the Dubai Camel Dairy Farm displayed any clinical signs of CLA over a period of 5 years. The results also showed that there were no carriers of CLA on the farm. Braga (2007) investigated the antibody development in vaccinated alpacas as well as in alpacas suffering from natural CLA with their in house indirect ELISA using HRP-labeled protein-A conjugate. The indirect ELISA made use of the *C. pseudotuberculosis* cell wall antigen. Experimentally infected alpacas developed antibodies on day 16 p.i. that persisted for 93 days after inoculation.

Pseudotuberculosis remains one of the most important bacterial diseases in camelids with an

infection rate between 10% and 60%, and in Eastern Kenya many thousands dromedaries suffer from CLA.

References

Afzal M, Sakir M and Majid Hussain M (1996). *Corynebacterium pseudotuberculosis* infection and Lymphadenitis (Toloa or Mala) in the camel. Tropical Animal Health and Prodution 28:158-162.

Beghelli D, D'Alterio GL, Severi G, Moscati L, Pezzotti G, Foglini A, Battistacci L, Cagiola M and Ayala-Vargas C (2004). Evaluation of the immune response to vaccination against *C. pseudotuberculosis* in an alpaca herd in Italy: Preliminary results. In 4th European Symposium on South American Camelids/DECAMA European Seminar, Goettingen, Germany, 07-09 October.

Bergin TJ (1986). *Corynebacterium pseudotuberculosis* and "Mala" (lymphadenitis) in camels, in FAO the camel: development and research. Proceedings of Kuwait Seminar, Kuwait 20-23.10.1986.

Braga WU (2007). Protection in alpacas against *Corynebacterium pseudotuberculosis* using different bacterial components. Veterinary Microbiology 119(2-4):297-303.

Dalling T, Robertson A, Boddie G and Spruell J (1966). Diseases of camels. In The Int. Encyclopaedia of Veterinary Medicine, W. Green and Son, Edinburgh, 1:585.

Kobera R and Poehle D (2004). Case reports in South American camelids in Germany. In Proc. 4th European Symposium on SACs and DECAMA Europ. Seminar, 7.-9.10.2004, Goettingen, Germany 151.

Muenchau B (2006). Pseudotuberkulosis bei Kamelen. In: Proceedings of DGZWE. pp 61-73.

Paule BJA, Meyer R, Moura-Costa LF, Bahia RC *et al* (2004). Three-phase partitioning as an efficient method for extraction/concentration of immunoreactive excreted-secreted proteins of *Corynebacterium pseudotuberculosis*. Protein Expression and Purification 34:311-316.

Piontkowski MD and Shivvers DW (1998). Evaluation of a commercially available vaccine against *Corynebacterium pseudotuberculosis* for use in sheep. Journal of American Veterinary Medical Association 212(11):1765-1768.

Seyffert N, Guimaraes AS, Pacheco LGC, Portela RW, Bastos BL, Dorella FA, Heinemann MB, Lage AP, Gouveia AM, Meyer R, Miyoshi A and Azevedo V (2009). High seroprevalence of caseous lymphadentitis in Brazilian goat herds revealed by *Corynebacterium pseudotuberculosis* secreted proteins-based ELISA. Research in Veterinary Science 88(1):50-55.

Spesivtseva NA and Noskov AI (1959). Epizootic lymphangitis in camels. Trudy Vses. Inst. Vet. sanit. Ectoparasit. 14:86.

Tejedor-Junco MT, Lupiola P, Caballero MJ, Corbera JA and Gutierrez C (2009). Multiple abscesses caused by Salmonella enterica and *Corynebacterium pseudotuberculosis* in a dromedary camel. Tropical Animal Health and Prodution 41:711-714.

Wernery U, Kinne J and Schuster R (2012, in press). Infectious Disorders of Camelids. Will be published by OIE.