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EFFICACY OF HERD-SPECIFIC AUTOGENOUS VACCINE AGAINST CONTAGIOUS LYMPHADENITIS IN DROMEDARIES - PRELIMINARY REPORT

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ABSTRACT

In a first experiment of this kind in dromedaries, the efficacy of a herd specific autogenous vaccine containing a formalin inactivated *C. pseudotuberculosis* culture supernatant was assessed in Kenya. This vaccine which contained 300µg/ml PLD was subcutaneously injected thrice at the neck of 117 dromedaries of which 26 were calves between 4 to 6 months. All dromedaries suffered from generalised CLA with abscess formation mainly in front of the shoulder. All dromedaries possessed antibodies to CLA. After 2 vaccinations, no more abscesses developed and existing abscesses receded in adult dromedaries whereas a similar success was observed in young calves after the 3rd vaccination when their age had reached 12 months. It is believed that herd-specific strains and the amount of PLD in the vaccine are important factors for the efficacy.

Key words: Caseous lymphadenitis, dromedaries, vaccine

Caseous lymphadenitis (CLA) or pseudotuberculosis is caused by *Corynebacterium* (C.) *pseudotuberculosis*, a Gram-positive rod. The disease is characterised by abscessation of one or more superficial lymphnodes but can also cause pneumonia, hepatitis and mastitis, arthritis, orchitis and meningitis. Pseudotuberculosis is widespread in Old World Camels (OWCs) and certainly after mange the most prevalent skin disorder because entire herds can be affected by the disease. The pathogen has also been isolated from New World Camels (NWCs) (Wernery, 2012). *C. pseudotuberculosis* may affect farm and abattoir workers, produces ulcerative lymphangitis in cattle (ULA) and can assume several disease forms in horses. Deep intramuscular abscesses in horses were first reported to occur in California, USA in 1915. Since that time, the disease commonly referred to as "pigeon fever" is one of the most frequent infectious diseases in horses in Western USA. Two other clinical forms of the disease include internal organ involvement and infection of the limbs and as in bovines are also termed ULA. High environmental temperatures associated with drought conditions have affected tens of thousands of horses in the past decade in the US (Spier, 2012). It is considered a re-emerging disease in the USA and Canada. *Corynebacteria* are very sensitive to different antimicrobials but the abscess often prevents the medication from reaching the bacteria. The abscess can reach lemon or even orange size and often need both antimicrobial and surgical treatment. Extruding pus is extremely contagious and every possible precautionary method should be implemented to clean and / or dispose contaminated material properly. Veterinarians and handlers of the infected animals should take great caution because of the zoonotic potential of this organism.

Because of the great difficulty to avoid and to treat CLA, we started to produce a CLA vaccine which efficacy is reported here.

Materials and Methods

Several *C. pseudotuberculosis* isolates were cultured from external abscesses from Kenyan dromedaries which suffered from CLA. The pus swabs were streaked on blood agar and a secondary sub-culture were made to obtain pure colonies. The strains were then stored at -80°C in cryobank beads (Cryobank™ MAST, Germany). The procedure of the vaccine production followed recommendations by Braga (2007) with some small alterations. For vaccine production the beads from one isolate were streaked onto Brain Heart Infusion agar (BHI) (Oxoid, England, CMO 225) which contained 5% sheep blood.

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The plates were incubated at 37°C for 48 hours to obtain a confluent growth over the entire plates. The bacterial layer of four plates was washed off from the agars and suspended in 1000 ml BHI broth containing 0.05% Tween 80. The following steps were applied to the broth:

- Incubate the inoculated 1 litre broth at 37°C for 72 hours with a magnetic stirrer
- Place the broth into a refrigerator for 24 hours
- Centrifuge the broth at 13,000g at 4°C for 30 min, discard the sediment (bacteria)
- Add 5ml (0.5%) of a 10% formalin solution to 1 litre broth supernatant
- Mix the supernatant containing formalin thoroughly and place it into a refrigerator
- Check the supernatant for sterility by plating 100µl of fluid onto blood agar and incubate plates at 37°C at least for 48 hours.
- The protein content (PLD) was measured using colorimetric assay (Cobas 311) and was found to contain 300µg/ml
- When the supernatant is sterile, add 250ml ADVAX^{TM}CXL adjuvant (Vaxine Pty Ltd, Australia) to 1 litre sterile broth and mix well on shaker for 30 minutes at room temperature
- Perform a final sterility check before application

The killed vaccine was subcutaneously (sc) applied. Camel calves received 2.5ml and adult dromedaries 5 ml and were boostered 3 to 4 weeks later and again after 6 months.

The dromedaries originated from 2 herds in Northern Kenya and were tested with an indirect ELISA for antibodies against CLA (Wernery *et al*, 2012). They consisted of 79 adult dromedaries plus 26 calves between the ages of 4 to 6 months. The second herd consisted of 12 non-castrated bulls used for safaris. All dromedaries in the first herd including the calves exhibited multiple abscesses at different body locations especially in front of the shoulders. Three bulls of the second herd did not show any abscesses. They were all vaccinated thrice as mentioned above.

Results

Only 2% of the 117 vaccinated dromedaries showed a swelling at the injection site which receded after 3 to 5 days. This occurred after each vaccination. The vaccine was applied on the left side of the neck twice and once on the right side. In all 91 adult dromedaries no new abscesses appeared and after the booster injection (second vaccination) abscesses receded.

Of the 26 camel calves, 8 (31%) developed small closed abscesses at the base of the shoulder even after the second vaccination at the age of 6 to 8 months.

During adspection of all 117 dromedaries six weeks after the 3rd vaccination, none of the dromedaries had developed new abscesses and all existing abscesses did not open and were much smaller than before the vaccination started. The 3 bulls in the second herd which exhibited no abscesses at all remained without abscesses also after the 3 vaccinations. All 26 camel calves which were more than a year old when vaccinated a third time, did not develop anymore abscesses and the existing abscesses receded.

Discussion

C. pseudotuberculosis is a pyogenic, facultative intracellular pathogen which penetrates tissue and produces filterable toxins. At least three are known: a toxin cell-wall lipid, an exotoxin-phospholipase-D (PLD) which damages mammalian cell membranes and a haemolysin. They all play essential roles in the development of CLA. Afzal *et al* (1996) found that strains of *C. pseudotuberculosis* which were devoid of the gene for PLD were unable to produce lymphnode abscesses. Two biotypes of *C. pseudotuberculosis exist* which are the ovine/caprine and equine/bovine strains and only the ovine/caprine strain has been identified in camelids.

Preliminary investigations of different *C. pseudotuberculosis* strains have shown (Wernery, 2012) that genetically different *C. pseudotuberculosis* isolates on host related basis exist but that they also differ from herd to herd. This finding however should be further substantiated in future.

Vaccine against CLA for sheep and goats are commercially available but have only been tried in dromedaries in a small experimental trial at CVRL. These vaccines are formulated from concentrated formalin-inactivated *C. pseudotuberculosis* culture supernatant containing PLD. They do not provide complete protection against the development of abscesses in small ruminants but a significant reduction in the number of abscesses. Several scientists have started research in the production of autogenous vaccines against pseudotuberculosis especially during the CLA-outbreak in Italy in alpacas (Beghelli *et al*, 2004). Braga (2007) who used a high dose of toxin (500µg/ml) in a challenge trial in alpacas observed no abscesses.

CVRL vaccine is also a formalin-inactivated *C. pseudotuberculosis* culture supernatant formulation which contains PLD. PLD is responsible for the production of lymphnode abscesses and it is believed that the vaccine produces antibodies against this toxin. Important seems to be the amount of the toxin in the vaccine. Low amounts of PLD may reduce the number of abscesses but cannot prevent the disease. As mentioned before the strain used for the vaccine is also of uppermost importance. Commercial vaccines which were injected in dromedaries at CVRL into the base of the neck developed granulomas of different size (Wernery, 2012) and are therefore not suitable. Kinne *et al* (2012) showed that the adjuvant ADVAX XL™ was the most suitable adjuvant for camels because it did not induce local reactions after application of different antigens. This Australian adjuvant was used for the CVRL vaccine with no side-effect.

Our preliminary results showed that a herd-specific autogenous vaccine containing a formalin-inactivated *C. pseudotuberculosis* culture supernatant with a protein content of 300µg/ml protected adult dromedaries from developing new abscesses after 3 applications. Furthermore, existing abscesses receded. In young dromedary calves, the success rate of the vaccine was less impressive which may be explained by the possible interference of maternal antibodies and or by the immature immune status of 4 to 6 month-old calves. However, when these animals reached the age of 12 months the vaccine worked well.

Further research is necessary to investigate the efficacy of this autogenous vaccine in challenge trials as it has been performed in NWCs.

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