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CAMELID BRUCELLOSIS - CLINICAL FEATURE, EXCRETION PATTERN, SEROLOGICAL AND BACTERIOLOGICAL DIAGNOSIS : REVIEW*

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Human brucellosis remains one of the most common zoonotic diseases worldwide with more than 500.000 new cases annually (Wernery, 2016). Infection prevalence in animal reservoirs determines the incidence of human cases. That means, where there is animal brucellosis also human brucellosis is diagnosed.

Clinical Feature

In general, *Brucella*–infected animals do not show any clinical signs and therefore it is very difficult to convince the owner, that his animal has a severe disease and has to be culled. This is one of the big challenges veterinarians face, at least with dromedary camels on the Arabian Peninsula. Most of the dromedaries are used for racing and are very valuable animals. The owner will not agree to euthanise a serological positive animal. Compensation does not exist.

CVRL proposed to the government the following:

- Chip all positive dromedaries and keep a record
- Castrate positive bulls
- Never breed with positive females

The WOAH (World Organisation for Animal Health) Manual of Diagnostic Tests and Vaccines for Terrestrial Animals – earlier known as the OIE Manual – is the bible of infectious diseases and it has been upgraded since many years. The 2022 online version of the manual is now available and contains the amended Brucellosis chapter which includes *B. melitensis, B. abortus* and *B. suis*.

This chapter was revised by experts and it gives the newest scientific research results. It is a great piece of work and more or less all answers to any question, will be found in this chapter. WOAH chapters, are regularly revised by experts mainly from WOAH reference laboratories, but also by others, now in total 9 times.

Camelid brucellosis is now in the general chapter of cattle, sheep, goats and swine. CVRL approached the WOAH to also add a separate brucellosis chapter on camelids, but this was declined for the moment.

Brucellosis in canines has become a concern over the years as a zoonotic disease and will be added in future in a special chapter.

Brucellosis has been reported in dromedary camels (*Camelus dromedarius*), the two humped or Bactrian camels (*Camelus bactrianus*) as well as in South American New World camels: Llama, alpaca, guanaco and vicuna. They contract infection when intermingling mainly with small ruminants infected with *B. abortus* and/or *B. melitensis*, but mainly *B. melitensis* (OIE, 2012).

Brucellosis in camelids occurs in all of the known forms described in ruminants. Abortion is the most obvious manifestation. Infections also result in still born calves, retained placenta and reduced milk yield. Hygromas are rare (Fig 1).

Orchitis and epididymitis have been described. Pathological lesions have been rarely described but they include lymphocytic and histiocytic placentitis with oedema and necrosis. Aborted foetuses show subcutaneous oedema, interstitial pneumonia and liver degeneration.

In general, abortions occur only in the first pregnancy and infected dams are healthy. Abortion can occur at any pregnancy stage.

Interestingly, camel calves born to infected dams always remain sero negative (Von Hieber, 2010) although the pathogen is intermittently excreted through milk.

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Infection occurs via the mucous membranes: oral - nasopharyngeal, conjunctival, genital mucosa, also through cutaneous abrasions (handlers), because *Brucella* are tiny bacteria.

Excretion Patterns

Camels become infected through:

- Contaminated feed
- Colostrum and milk
- Contaminated water
- Licking or sniffing at placenta and aborted foetuses
- Very rarely through semen

Shedding of the pathogen is through placenta, aborted foetus and milk, not through nasal discharge, urine or faeces. Through placenta, *Brucella* bacteria are excreted in the hundred millions (10^{12} to 10^{13}) (Juhasz *et al,* 2019).

Camelids do not ingest their placentas (placentophagy) as many other animal species do (Juhasz *et al,* 2019). The placenta dries and wind can bring the pathogen to other farms 500m away. Airborne infections are well known also in laboratories.

Serological diagnosis

An important sentence in the WOAH Manual chapter is the following statement, which is cited herewith:

"No single serological test is appropriate in each animal species and therefore each test should be validated for its fitness in the corresponding animal species".

This means, that the existing brucellosis tests have to be evaluated for use in camelids, which is very important, as camelids possess a special immune system devoid of light chains, named *nanobodies*. During one of the meetings at the WOAH Headquarters in Paris, WOAH representatives asked CVRL to perform such an evaluation.

For the evaluation of *Brucella* serological tests in dromedaries, H.H. Sheikh Mohammed Bin Rashid Al Maktoum, Vice President and Prime Minister of U.A.E, Ruler of Dubai donated 14 *Brucella* negative dromedaries which were infected intranasally and intratracheally with a *B. melitensis* strain, previously isolated from a dromedary camel (Figs 2 and 3).

Over a period of 12 months serological and culture methods including PCR were used for the diagnosis of these 14 experimentally infected *B. melitensis* dromedaries. The details are shown in Table 1.

The development of *Brucella* antibodies in the experimentally infected dromedaries over a period of 12 months by comparing 15 different serological tests (Soellner *et al,* 2018) showed the following results after hundreds of test results were analysed:

Only 2 serological tests were characterised by a high degree of sensitivity for the diagnosis of brucellosis in dromedaries. These 2 tests are:

- Rose Bengal Test (RBT) from Vircell, Spain
- Competitive ELISA from Ingenasa, Spain

The RBT from Vircell produced for the diagnosis of human Brucellosis gave the best results as the concentration of the antigen was ideal, not too thick and nor too thin. If the concentration of the antigen is too thick the 1+ reaction will be missed.

Bacteriological diagnosis

Seven of the *B. melitensis* experimentally infected dromedaries were euthanised and properly investigated. In total, from each dromedary 43 samples were taken and examined (Johnson *et al,* 2018).

- Method of isolation:

1. Direct culture method from tissue samples.
2. Concentration method includes mincing, maceration and homogenising the samples in PBS and using the centrifuged sediment for culture.
3. Enrichment method in which the sediment from concentration method is inoculated into Tryptic Soy Broth (TSB) with Brucella supplement.

Farrell's agar, Brain-Heart infusion Agar (BHI) with *Brucella* supplement and Tryptic Soy agar

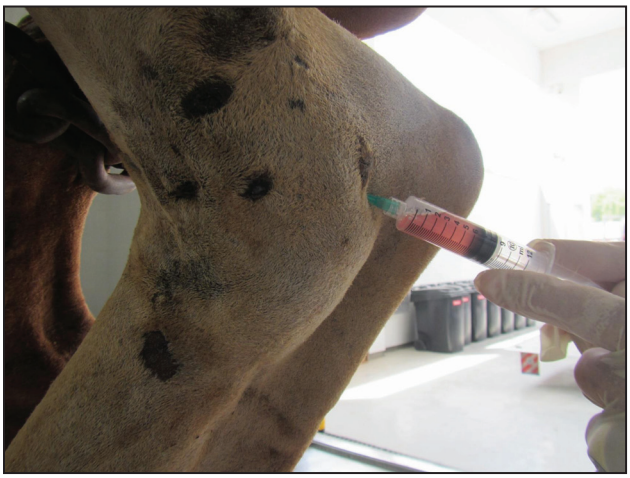

Fig 1. Hygroma caused by *B. melitensis*.

Fig 2. Intranasal infection of a dromedary camel with *B. melitensis*.

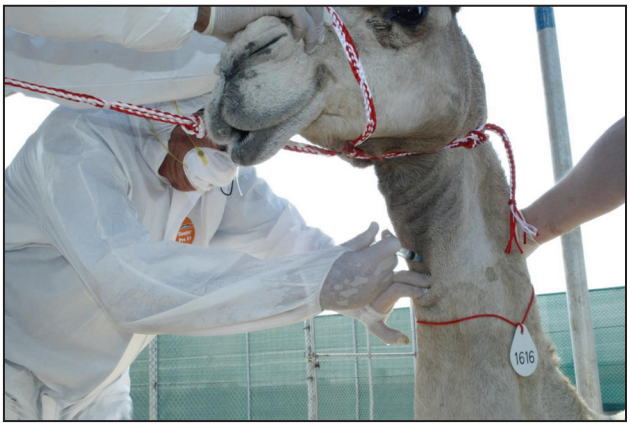

Fig 3. Intratracheal infection of a dromedary camel with *B. melitensis*.

Table 1. Overview of all 15 serological tests used in the diagnosis of brucellosis in experimentally infected dromedaries.

Test	Name/Antigen	Conjugate	Species	Company	Country
RBT	Rose Bengal Antigen	-	Animals	APHA Scientific	UK
RBT	BENGATEST	-	Animals	Synbiotics Europe/ Zoëtis	France
RBT	Pourquier®Rose Bengal Ag	-	Animals	IDEXX	USA
RBT	Rose Bengal	-	Humans	Vircell	Spain
CFT	*Brucella abortus* antigen	-	Animals	APHA Scientific	UK
SAT	*Brucella abortus* antigen	-	Animals	APHA Scientific	UK
Lateral flow test	*B. melitensis/abortus/suis*/ Antigen	Monoclonal anti-camel-IgG	Animals	MEDLINK, MSA™	UAE
Lateral flow test	*Brucella* sLPS (*B. melitensis/ abortus/suis*)	Protein G	Bovine, cattle, sheep, goat & human	Genomix	India
i-ELISA	*Brucella abortus* antigen	Anti-ruminant	Cattle	IDEXX	USA
i-ELISA	*Brucella* LPS	Anti-multi-species-IgG-HRP conjugate	Bovine, ovine, caprine, porcine	ID. vet	France
i-ELISA	Anti-*Brucella* ELISA Camel (IgG), *Brucella* LPS	No Information	Camel	EUROIMMUN	Germany
i-ELISA	Argentina Antigen	Protein A	Animals	Inhouse	-
c-ELISA	*Brucella abortus* antigen	Goat anti-mouse IgG antibody	Bovine, ovine, caprine, porcine	Svanova Boehringer Ingelheim	Sweden
c-ELISA	*Brucella abortus* antigen	Monoclonal antibody specific to the epitope C of LPS of *Brucella*	Small ruminants, bovine, porcine	Ingenasa	Spain
c-ELISA	*Brucella melitensis* LPS extract	Monoclonal anti-*B. melitensis* LPS antibody	Cows, sheep & goats	APHA Scientific	UK

without supplement are inoculated with samples from the above mentioned methods and incubated for 6 days in 5% CO_2 at 37°C. BHI is the preferable agar as after 6 days of incubation the colonies are usually bigger and easier differentiated compared to others.

The results were as follows:

- The isolation of *Brucella* organisms is the gold standard, PCR often failed when less than 3 colonies grew on culture media.
- The organs for the culture of *B. melitensis* are lymph nodes but which ones is unpredictable. However, the pathogen was mostly found in pre-scapular lymph nodes, udder lymph nodes, lung-associated lymph nodes, submandibular and pharyngeal lymph nodes. Interestingly the pathogen was not found in the uterus.
- The remaining infected camels are still serologically positive even after 5 years, may be lifelong.

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