Brucella melitensis CAUSED ABORTION IN A SEROLOGICALLY POSITIVE DROMEDARY CAMEL

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ABSTRACT

An abortion case in a dromedary camel caused by a *B. melitensis* strain belonging to the East-Mediterranean genetic group (GT42 by multiple-locus variable-number tandem-repeat analysis (MLVA8) and GT108 by MLVA11) is reported. Earlier, the dromedary was seronegative for Brucellosis for 6 years. The animal got infected between 112 and 204 days of gestation from an unknown source, developed bacteriaemia and the acute infection resulted in the abortion at 249 days of gestation. The pathological findings in the foetus and the placenta were not pathognomonic for Brucellosis. In the foetus, both pleural and abdominal cavities were filled with bloody fluid and pleurisy was observed. In the placenta, oedema, diffuse mineralisation and focal detachment of the trophoblast were detected without inflammatory changes. The *Brucella* pathogens were excreted to the environment with the aborted foetus and the placenta. The bacteria disappeared from the blood and the uterus of the dam within a short time after abortion and were isolated only from some of the lymph nodes. The *Brucella* seropositive camel in its acute phase of the disease did not infect other contact animals. In addition, the transmission of the disease to other dromedaries at the time of abortion was also prevented with appropriate biosecurity measures.

Key words: Abortion, brucellosis, B. melitensis, dromedary camel

Brucellosis in breeding camels occurs in all of the known forms described in ruminants and abortion is its most obvious manifestation (Acosta et al, 1972; Agab et al, 1996; Fazil and Hofmann, 1981; Radwan et al, 1995; Wilson et al, 1982). Infections may also result in stillborn calves, retained placenta and reduced milk yield, as it is common in bovine and ovine. However, retained placentas have not been reported in Camelidae (Wernery et al, 2014). Literature is scarce on the pathological changes caused by Brucella organisms in camelids. In serological brucellosis-positive male camels, orchitis and epididymitis have been described (Ahmed and Nada, 1993). Pathological lesions in foetuses of ten B. abortus naturally infected dromedary camels have also been reported (Narnaware et al, 2016). They included subcutaneous oedema, interstitial pneumonia, liver degeneration and mononuclear infiltration in the kidney. The placentas were oedematous, showing necrosis and mononuclear infiltration. Pathological changes were also reported (Gidlewski et al, 2000; Gilsdorf et al, 2001) in a pregnant llama which was experimentally

infected with *B. abortus* through the conjunctival sac. They included lymphocytic and histiocytic placentitis with marked loss of trophoblastic epithelial cells.

B. melitensis and *B. abortus* organisms have been isolated from different tissues from Old World camels (OWCs) and New World camels (NWCs) including milk, lymph nodes, foetal stomach, placentas, vaginal swabs, hygromas and testis (Wernery *et al*, 2014).

This paper highlights the pathological alterations caused by *B. melitensis* infection in the aborted foetus and placenta as well as in its serologically positive dam. Culture and PCR results of specimens collected from the aborted foetus, placenta and dam as well as the load of *Brucella* in the specimens of the aborted foetus and placenta are described.

Materials and Methods

An aborted foetus and placenta from a *Brucella* seropositive dam from a camel farm in the UAE were submitted for necropsy to the Pathology Department of the Central Veterinary Research Laboratory (CVRL)

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and evaluated for macroscopic lesions. At necropsy, the carcass was sexed, weighed and measured. Samples from lung, liver, umbilical cord, pleural fluid, abdominal fluid and compartment one (C1) content were collected from the aborted foetus. Samples from placenta were also collected and included for further testing.

Three weeks after the abortion, the Brucella seropositive dam was humanely euthanised using Xylazin (Ilium Xylazil, Troy Laboratories, Australia) and T61 containing Embutramide, Mebezonium and Tetracaine (MSD Animal Health, The Netherland). Then, the carcass was submitted to CVRL where a full necropsy was performed including sampling from liver, udder, uterus and body lymph nodes. The 11 years old dromedary dam was serologically tested positive for Brucella antibodies, 45 days prior to abortion with Rose Bengal Test (RBT), then Brucellosis was confirmed with Complement Fixation Test (CFT), Serum Agglutination Test (SAT), Rose Bengal Test (RBT) and competitive Enzyme-linked Immunosorbent Assay (cELISA) 41 days prior to the abortion. B. melitensis was isolated from sodium citrate blood collected on the same day. This animal was repeatedly tested negative for brucellosis during the previous 6 years, from 2012 until 2018 (Table 3) and aborted after 249 days of pregnancy.

Formalin fixed, paraffin embedded samples of both aborted foetus and dam as well as placenta were processed for routine histopathological investigation.

Culture Methodology

All investigations were carried out in a level 2 biosafety cabinet in CVRL's high security Level 3 facility. Seven specimens collected at necropsy from the aborted foetus and placenta of the Brucella seropositive dromedary dam were cultured for the detection of *Brucella*. The specimens included organs (liver, right and left lung and umbilical cord), body fluids (pleural and abdominal fluid) and C1 content. From the dam, 20 specimens were collected for Brucella culture which included 11 lymph nodes, liver, 2 uterine tissues, 4 udder tissues, mammary secretion and blood in sodium citrate blood tubes. Blood was withdrawn from the jugular vein into EDTA tube and serum tube for PCR and serological testing, respectively. All 7 specimens from the foetus and placenta as well as the 20 specimens from the dam were cultured for the isolation of Brucella spp.

For the culture of specimens from the dam, 3 methods were used: direct, concentration and enrichment as described previously (Johnson *et al*,

2018). However, for the culture of aborted foetus specimens, only direct and concentration methods were used.

Two selective agars and an enrichment broth also described previously (Johnson *et al*, 2018) were used for culture. They were Farrell's media (*Brucella* medium base CM0169, Oxoid, supplemented with filtered horse serum SHS100, E and O Laboratories, UK and *Brucella* selective supplement SR0083A, Oxoid), Brain-Heart-Infusion agar (Brain Heart Infusion CM1135, Oxoid, with 1% bacteriological agar and supplemented with filtered horse serum SHS100, E and O Laboratories, UK and *Brucella* selective supplement, SR0083A, Oxoid) and Trypticase soy broth supplemented with *Brucella* selective supplement.

All inoculated agar plates and enrichment broths were incubated at 37°C in an atmosphere of 5% CO₂ for 6 days. After 6 days of incubation, all plates were examined for growth of typical *Brucella* colonies and the enrichment broths were well homogenised and 0.1 ml of each broth was quadrant streaked on *Brucella* selective agars. The streaked plates were incubated for another 6 days at 37°C in an atmosphere of 5% CO₂. After 6 days of incubation, the plates were examined for growth of typical *Brucella* colonies.

Culture for enumeration

Approximately 1 g/ml each of the 7 specimens collected from the foetus and placenta was also cultured to determine the load of *Brucella* in each specimen. For this, 1 gram of each organ sample was weighed, finely minced and homogenised in 9 ml PBS (1:10 dilution). The sample homogenates as well as 1 ml of the original body fluids (pleural and abdominal fluid) and C1 content were further serial diluted in PBS up to 10⁷ dilutions and 0.1 ml of each dilution was spread plate cultured on two *Brucella* selective agars mentioned above. All inoculated plates were incubated for 6 days at 37°C in an atmosphere of 5% CO₂. *Brucella* colonies grown on the selective agars from each dilution plate were counted and cfu/g/ml were calculated.

DNA extraction and PCR

PCR for the detection of *Brucella* antigen was only performed on original samples and not on concentrated or enriched samples.

For DNA extraction, 20 mg tissue was incubated in ATL buffer (Qiagen, Germany) at 56°C for 10 min and the lysate was then loaded onto the MagNA Pure automated extraction platform (Roche Diagnostics

Ltd, UK). DNA was extracted using Magna Pure LV DNA extraction kit according to the manufacturer's instructions. The PCR was performed according to the method described previously (Probert *et al*, 2004), using Light Cycler® 2.0 instrument (Roche, Germany). PCR cycling conditions used were: initial denaturation at 95°C for 10 min followed by 45 cycles of 95°C for 15sec and 57°C for 1 min. Samples with a fluorescence signal observed before 40 cycles were scored as positive.

Serology

The dromedary dam was periodically monitored for brucellosis for the last 6 years with Rose Bengal Test (antigen - APHA Scientific-RAA0060, UK) according to methods described previously (OIE, 2018).

Clinical and molecular epidemiology

As part of the Brucella monitoring program, all adult dromedaries on the farm have been subjected to RBT 2-4 times a year. The positive dam was in contact with another 78 pregnant animals in 2 separate locations during a 3 month period between the time of the negative (20.3.2018) and the positive (20.6.2018) Brucella RBT tests. All these camels have been tested repeatedly with RBT (last date of testing: 22.11.2018). After testing positive for Brucellosis, the pregnant camel was immediately separated into a relatively small paddock (10x15 m) far from the pregnant group (> 400 m). Another camel had been moved to the same location approx. 2 weeks before the positive dam aborted. The abortion took place on 4th of August 2018 spontaneously without assistance. This contact animal has been under continuous monitoring by weekly blood samples for RBT (the time of the last testing more than 3.5 months after the abortion). In addition,



Fig 1. Female aborted foetus with greyish, oedematous placenta and twisted umbilical cord.

DNA extract of the isolated *B. melitensis* strain was examined by multiple-locus variable-number tandem-repeat (VNTR) analysis (MLVA) with different typing schemes (MLVA 8, 11 and 16) as described earlier (Gyuranecz *et al*, 2016). MLVA 8 (consisting of loci of panel 1) and MLVA 11 (including loci of panels 1 and 2A) was used to compare the genetic relatedness of this strain to previously isolated strains from the same herd. MLVA 8 uses only 8 allels. MLVA 8 is a very robust but less discriminative method, while the MLVA 11 uses 11 allels and is more discriminative method. The raw MLVA data were analysed and phylogenetic trees were constructed using neighbour-joining method with the MEGA 7 software.

Results

Pathology

Aborted foetus

The aborted female foetus (13 kg, 50 cm crownrump length) was in a fair and fresh condition at necropsy. On gross examination the placenta appeared greyish and oedematous and the umbilical cord was six times twisted (Fig 1). Both pleural and abdominal cavities were filled with bloody fluid. The lungs of the foetus were covered with a net of red fibrin which was attached to the costal pleura (Fig 2). The right lung was more affected showing marbled appearance of the surface and massive congestion of the caudal lobe. Pleurisy was observed. The liver was very soft and the C1 contained red fluid with small clusters of brownish soft material. Histopathology revealed marked oedema and diffuse severe mineralisation of the superficial chorionic stroma of the placenta with focal detachment of trophoblasts (Fig 3). However, no inflammation, no

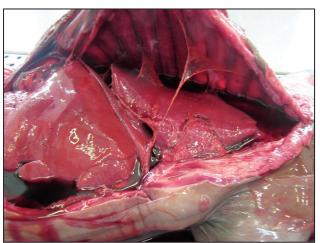


Fig 2. Pleural cavity of the aborted foetus filled with bloody fluid. The lung is covered with a net of red fibrin that is also attached to the costal pleura.

fungus, no parasites, no viral inclusions were seen. The lung showed cellular debris in subpleural alveoli and bronchioli (Fig 4).

Dromedary dam

During necropsy of the female camel (534 kg) many swollen body lymph nodes including mediastinal and intestinal lymph nodes were seen. The udder was in lactation and the lung showed massive congestion. Histopathology of the lymph nodes revealed marked proliferation of follicular and parafollicular lymphatic tissue as well as central oedema with small haemorrhages. Udder and uterus did not show any signs of inflammation.

Culture and PCR

Heavy growth of *Brucella* spp. was observed in all 7 foetal specimens and placenta when cultured by direct and concentration methods. Hence, culture from the enrichment broth tubes was not performed.

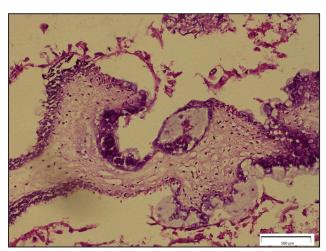


Fig 3. Light micrograph of the placenta with focal detachment of trophoblasts with a lot of cellular debris (H&E stain).

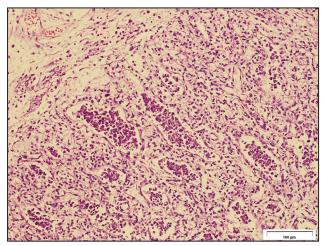


Fig 4. Light micrograph of the foetal lung with a lot of cellular debris in subpleural alveoli and bronchioli (H&E stain).

All 8 tested specimens showed 'mat growth' on both the selective agars. *B. melitensis* was also detected in all the specimens, i.e. both lungs, liver, umbilical cord, pleural and abdominal fluid, C1 content and placenta by PCR.

The culture and PCR results of specimens from dam and the enumeration results of specimens from aborted foetus and placenta are summarised in Tables 1 and 2.

Table 1 summarises the results of *B. melitensis* culture and PCR from the 20 necropsy specimens and EDTA blood from the dam. From a total of 20 specimens cultured, *B. melitensis* was isolated from 9 specimens: different body lymph nodes and the right

Table 1. *B. melitensis* culture and PCR results of 20 specimens and EDTA blood from the dam.

S. No	Specimens	Culture Results (Enrichment method)	PCR Results (Original specimens)
1	Right submandibular lymph node	Isolated	Not detected
2	Left submandibular lymph node	Not isolated	Not detected
3	Pharyngeal lymph node	Isolated	Not detected
4	Left pharyngeal lymph node	Isolated	Not detected
5	Lateral retropharyngeal lymph node	Isolated	Not detected
6	Prescapular lymph node (dorsales)	Isolated	Not detected
7	Prescapular lymph node (ventrales)	Isolated	Not detected
8	Lymph node mediastinales medii	Not isolated	Not detected
9	Lnn tracheobronchales medii	Isolated	Not detected
10	Lnn tracheobronchales sinistri	Not isolated	Not detected
11	Lung lymph node	Isolated	Not detected
12	Liver	Not isolated	Not detected
13	Right uterine horn	Not isolated	Not detected
14	Left uterine horn	Not isolated	Not detected
15	Milk	Not isolated	Not detected
16	Udder left hind quarter	Not isolated	Not detected
17	Udder right hind quarter	Isolated	Detected
18	Udder left front quarter	Not isolated	Not detected
19	Udder right front quarter	Not isolated	Not detected
20	Sodium citrate blood	Not isolated	Not detected
21	EDTA blood	Not applicable	Not detected

Lnn = lymph nodes

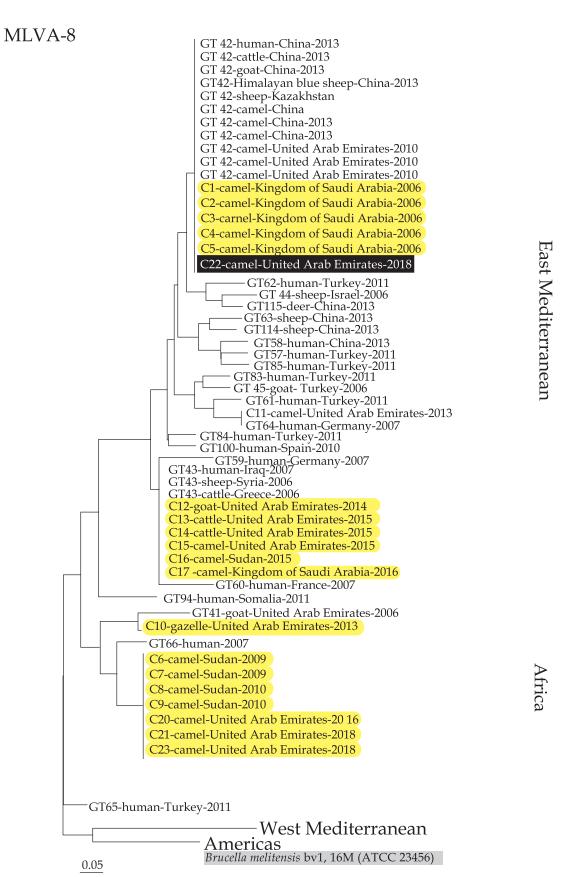


Fig 5. Neighbour joining analysis of *Brucella melitensis* isolate using multiple-locus variable-number tandem repeat analysis (MLVA) data. Isolate from the study are typed black while previous strains are highlighted in yellow (Gyuranecz *et al.*, 2016).

hind udder tissue. *B. melitensis* was only detected in the right hind udder tissue by PCR.

Colony forming units of *B. melitensis* isolated per g/ml of each specimen from the aborted foetus and placenta are presented in Table 2 as mean counts obtained from 2 selective agars. In all specimens, *Brucella* was found in very high concentration ranging from $10^6 - 10^{10}$ cfu/g/ml. The highest concentration of bacteria was found in the placenta with 10^{10} cfu/g followed by C1 content and pleural fluid with 10^9 cfu/ml each.

Serology

Table 3 summarises the results of periodical serological screening of the dam for brucellosis throughout a period of 6 years (2012-2018). The dam was serologically negative for brucellosis by RBT over a period of 6 years. In June 2018, the dam suddenly tested positive for brucellosis and thereafter in July and August 2018, after the abortion.

Clinical and molecular epidemiology

All 78 pregnant camels that were in contact with the positive dam before or during the time of infection, remained negative for Brucellosis with RBT (last date of testing: 22.11.2018). In addition, the contact animal that was kept together with the infected dam at the time of abortion also remained negative until the time of the last testing (22.11.2018. i.e. more than 3.5 months after the abortion). The genotype of this strain was identified as MLVA8 GT42 and MLVA11 GT108. Very similar or identical strains have been described earlier in dromedary camel (Gyuranecz et al, 2016). The genetic relatedness of this Bruce1lla melitensis strain to previous isolates is shown on the neighbour-joining analysis of MLVA8 in Fig 5. This strain clustered into the main East-Mediterranean genetic group.

Discussion

Old World camels are frequently infected with brucellosis, particularly when they come into contact with infected ruminants (Wernery, 2016). Brucellosis in NWCs is rare, but outbreaks with classical signs of brucellosis have been described (Fowler, 2010). Humans are at risk through the consumption of unpasteurised milk (Wernery *et al*, 2014) but modes of transmission occur also by contact through skin with animal tissues, blood, urine, vaginal discharge and aborted foetuses, especially placentas. The pathogen is excreted in large numbers in aborted foetuses, foetal membranes and uterine discharge.

Table 2. *B. melitensis* colony forming units from aborted foetus specimens and placenta.

Specimens	Colony forming units per gram/ millilitre (cfu/g/ml)	
Left Lung	1.8×10^8	
Right Lung	1.4×10^8	
Liver	1.2×10^6	
Umbilical cord	4.6×10^7	
Pleural fluid	6.0×10^9	
Abdominal fluid	4.0×10^{8}	
C1 content	3.0×10^9	
Placenta	1.46×10^{10}	

Table 3. Brucellosis serum RBT results obtained since 2012 from the dam.

Order	Date	Test location	Result	
1	20-Nov-2012	CVRL	Negative	
2	10-Jun-2013	CVRL	Negative	
3	11-Dec-2013	CVRL	Negative	
4	29-Apr-2014	CVRL	Negative	
5	20-Nov-2014	CVRL	Negative	
6	6-Jun-2015	CVRL	Negative	
7	5-Dec-2015	CVRL	Negative	
8	17-May-2016	CVRL	Negative	
9	1-Sep-2016	DM VSS	Negative	
10	18-Dec-2016	Farm in house	Negative	
11	15-Feb-2017	DM VSS	Negative	
12	16-Jul-2017	Farm in house	Negative	
13	11-Sep-2017	DM VSS	Negative	
14	14-Dec-2017	Farm in house	Negative	
15	20-Mar-2018	Farm in house	Negative	
16	27-Mar-2018	DM VSS	Negative	
17	20-Jun-2018	Farm in house	Positive	
18	24-Jun-2018	CVRL	Positive	
19	25-Jun-2018	Farm in house	Positive	
20	26-Jun-2018	CVRL	Positive	
21	27-Jun-2018	Farm in house	Positive	
22	5-Jul-2018	Farm in house	Positive	
23	5-Aug-2018	CVRL	Positive	
24	6-Aug-2018	Farm in house	Positive	

CVRL = Central Veterinary Research Laboratory
DM VSS = Dubai Municipality Veterinary Service Section

In cattle, it is known that abortion is associated with the shedding of 10^{12} to 10^{13} *Brucella* bacteria per gram material. Survival of the organisms in the environment is enhanced by cool temperatures and high humidity, but in one case, it was proven that in the hot desert environment, 2 dromedaries in a herd

became infected with *B. melitensis* most probably through contaminated dust particles from aborted camel foetuses 500 metres away (Wernery *et al*, 2014). Air borne infections occur in animal pens, stables and laboratories (Schulze zur Wiesch *et al*, 2010). Many placental mammals, including herbivores, participate in placentophages, in which camelids are a noted exception; this fact may contribute to the spread of *Brucella* through wind.

As described in cattle and most probably in domestic small ruminants and wild ruminants, also in dromedary camels proven by this investigation, hundred million of Brucella pathogens per gram or millilitre are excreted with the aborted foetus. This will consequently contaminate the area where the abortion takes place which is under desert conditions mainly sand. Lochia and afterbirth material will quickly dry, but as camels are gregarious animals and very curious, they will lick and sniff at placenta and the aborted foetus. Infection occurs mainly via the mucous membranes of the respiratory tract and most probably also through conjunctiva. Despite this, in the present case, it seems that the contact animal did not get infected due to biosecurity measures. The staff of the farm immediately removed the foetus, placenta and the contaminated soil and also disinfected the area. This could be the reason why the other camel did not contract the disease in spite of high bacterial load of the aborted foetus.

Brucellosis is an important zoonotic disease and in humans, is characterised by recurrent fever, night sweats, joint and back pain and general weakness. People at greatest risk are those who drink unpasteurised milk and attend parturient animals. It is mainly a disease of professionals working with livestock.

As described earlier (Johnson *et al*, 2018), enrichment of tissue samples and culture on BHI agar gave the most reliable results for the isolation of the pathogen. *B. melitensis* was isolated, from 9 (45%) out of 20 specimens of the dam, mainly from lymph nodes of the respiratory tract area. It was unexpected that the left and right uterine horns were already free of the pathogen 3 weeks after the abortion. However, *B. melitensis* was cultured from the right hind quarter of the mammary gland.

According to previous studies (Johnson *et al*, 2018), PCR results were negative when specimens were tested directly without concentration or enrichment. In this study, the right hind udder tissue of the dam was positive by PCR (Table 1). However,

PCR became positive when the original specimen contained high concentration of *B. melitensis*. This is evident from the positive PCR results of the aborted foetus specimens in which *Brucella* was present in high numbers (Table 2). The same was observed in the right hind udder tissue of the dam (Table 1), the only organ from which *Brucella* bacteria was isolated in high numbers. Culture remains the "gold standard" for the diagnosis of brucellosis.

The dam was from a closed camel dairy farm with no connection to any large or small ruminants. The herd is professionally managed and the entire herd is regularly tested for different infectious diseases including brucellosis using serological tests. One of the most reliable tests for camel brucellosis is the Rose Bengal Test from Vircell, Spain. Different serological tests with *B. melitensis* experimentally infected dromedaries were evaluated (Söellner, 2018). It remains obscure, why in 2018 suddenly after 6 years of being brucellosis negative the dromedary camel became positive (Table 3). Three weeks after the abortion, it was decided to euthanise the dam. *B*. melitensis was predominantly cultured from different lymph nodes where they hide as previously described (Wernery et al, 2007) in non-pregnant lactating camels.

The pathology of the placenta and the foetus was not pathognomonic for brucellosis. Similar findings have been described for abortions not caused by Brucellosis. Besides the greyish and oedematous placenta, the most striking pathological lesion seen on the aborted foetus was the 6 times twisted umbilical cord. Similar to most camel abortions with twisted umbilical cord seen at CVRL, both, the chest and abdominal cavities were filled with bloody fluid. Histopathology revealed marked oedema and diffuse mineralisation of the superficial chorionic stroma of the placenta with focal detachment of the trophoblast. Surprisingly, no inflammation was seen in the placenta. This is very similar to histopathological findings described in llamas and alpacas after abortion (mainly mineralisation and low incidence of placentitis) (Schaefer et al, 2012). The lung showed cellular debris in subpleural alveoli and bronchioli. However, similar lesions in aborted foetuses caused by natural B. abortus infection have been described (Narnaware et al, 2016), additionally with necrotising placentitis and foetal pneumonia during mid to last trimester of pregnancy.

Abortion caused by *B. abortus/melitensis* is associated with massive replication of the pathogen within the chorioallantoic trophoblasts of the placenta.

This extensive intracellular replication ruptures the infected trophoblasts releasing the bacteria and infecting the foetus. The infection of the calf and loss of placental integrity leads to the abortion or the birth of weak, infected calves (Saegerman *et al*, 2010).

The necropsy of the dam revealed no lesions besides many swollen lymph nodes, showing marked proliferation of follicular and parafollicular lymphatic tissue as well as central oedema with small haemorrhages in histopathology. Udder and uterus did not show any signs of inflammation.

Very little is known about the pathological changes caused by Brucella organisms in camelids. Previous studies (Abu Damir et al, 1984) found lesions after artificial infection of non-pregnant dromedaries with B. abortus in the cranial and genital lymph nodes, which showed follicular hyperplasia of cortical and paracortical areas of medullary cords and sinusoidal congestion. A mild interstitial hepatitis was also observed. The other authors (Nada and Ahmed, 1993; Wernery et al, 2007) described lesions in serologically and culture positive but non-pregnant natural infected adult dromedaries. They included inflammation with reddening of the uterus lining, fibrosis of the endometrium and atrophy of the uterine glands. Hydrobursitis was also observed, enlarging the bursa which was then filled with clear amber coloured fluid. Histopathological investigations are rare and were mainly found in lymph nodes, from which B. melitensis was isolated (Wernery et al, 2007). They showed marked sinusoidal oedema, activated follicles and histiocytosis, similar alterations as seen in this case.

In conclusion, we described an abortion case in a dromedary camel caused by a B. melitensis strain belonging to the East-Mediterranean genetic group (GT42 by MLVA8 and GT108 by MLVA11). The dromedary was infected during mid gestation from an unknown source, developed bacteriaemia and the acute infection resulted in abortion approx. 2 months after the infection. This specific strain of the bacteria has never been isolated on the farm but was identical or closely related to strains isolated 12 years earlier from camels originating from Kingdom of Saudi Arabia. The pathological findings in the foetus and the placenta were not pathognomonic for Brucellosis. Surprisingly, no inflammatory changes, only oedema, diffuse mineralisation and focal detachment of the trophoblast were detected in the placenta. But, hundreds of billions of Brucella pathogens were excreted to the environment with the aborted foetus and the placenta. Within a short time after abortion, the bacteria disappeared from the blood and the uterus of the dam and was isolated only from some of the lymph nodes and right hind udder tissue. The *Brucella* seropositive camel in its acute phase of the disease did not infect other contact animals. In addition, with appropriate biosecurity measures we could prevent the transmission of the disease to other dromedaries at the time of abortion. Successful prevention required precise laboratory diagnosis and thorough and continuous monitoring of the animals.

References

- Abu Damir H, Kenyon AJ, Khalafalla AE and Idris OF (1984). Brucella antibodies in Sudanese camels. Tropical Animal Health and Production 16:209-212.
- Acosta M, Ludena H, Barreto D and Moro Sommo M (1972). Brucellosis in alpacas. Rev. Invest. Pecu. 1(1):37-49.
- Agab H, Abbas B, El Jack Ahmed H and Mamoun IE (1996). First report on the isolation of *Brucella abortus biovar* 3 from camels (*Camelus dromedarius*) in Sudan. Camel Newsletter 12(9):52-55.
- Ahmed WM and Nada AR (1993). Some pathological affections of testis and epididymis of slaughtered camels (*Camelus dromedarius*). International Journal of Animal Science 8:33-36.
- Fazil MA and Hofmann RR (1981). Haltung und Krankheiten des Kamels. Tierärztliche Praxis 9:389-402.
- Fowler ME (2010). Medicine and Surgery of Camelids, 3rd ed. Wiley-Blackwell, Ames, IA. pp 207-208.
- Gidlewski T, Cheville NF, Rhyan JC, Miller LD and Gilsdorf MJ (2000). Experimental *Brucella abortus* induced abortion in a llama: pathologic effects. Veterinary Pathology 37:77-82.
- Gilsdorf MJ, Thoen CO, Temple RMS, Gidlewski T, Ewalt D, Martin B and Henneger SB (2001). Experimental exposure of llamas (*Lama glama*) to *Brucella* abortus: humoral antibody response. Veterinary Microbiology 81:85-91.
- Gyuranecz M, Wernery U, Kreizingera Zs, Juhász J, Felde O and Nagy P (2016). Genotyping of *Brucella* melitensis strains from dromedary camels (*Camelus dromedarius*) from the United Arab Emirates with multiple-locus variable-number tandem repeat analysis. Short Communication. Veterinary Microbiology 186:8-12.
- Johnson B, Kinne J, Jose Sh, Pfeffer M, Shanmugaraja R, Pandarakandy Sh, Ali F, Maio E, Rodriguez Caveney M, Söllner NK and Wernery U (2018). Recovery of *Brucella melitensis* from artificially infected dromedaries. Austin Journal of Veterinary Science and Animal Husbandry 5(2):1046.
- Nada, AR and Ahmed WM (1993). Investigations on brucellosis in some genital abnormalities of she-camels (*Camelus dromedarius*). International Journal of Animal Science 8(1):37-40.
- Narnaware SD, Dahiya SS, Kumar S, Tuteja FC, Nath K and Patil NV (2016). Pathological and diagnostic

- investigations of abortions and neonatal mortality associated with natural infection of *Brucella abortus* in dromedary camels. Comparative Clinical Pathology. DOI: 10.1007/s00580-016-2348-4.
- OIE, World Organization for Animal Health (2018). Brucellosis (*Brucella abortus, B. melitensis and B. suis*) infection with *B. abortus, B. melitensis* and *B. suis*. Manual of Diagnostic Tests and Vaccines for Terrestrial Animals, 8th Ed. pp 1-44.
- Probert WS, Schrader KN, Khuong NY, Bystrom SL, Graves MH (2004). Real-time multiplex PCR assay for detection of Brucella spp., *B. abortus* and *B. melitensis*. Journal of Clinical Microbiology 42(3):1290-1293.
- Radwan AI, Bekairi SI, Mukayel AA, Albokmy AM, Prasad PVS, Azar FN and Coloyan ER (1995). Control of Brucella melitensis infection in a large camel herd in Saudi Arabia using antibiotherapy and vaccination with Rev 1 vaccine. Bulletin de l'Office International Des Épizooties 14(3):719-732.
- Saegerman C, Berkvens D, Godfroid J and Walravens K (2010). Bovine Brucellosis, in: Lefèvre, P.C., Blancou, J., Chermette, R., Uilenberg, G. (Eds.), Infectious and Parasitic Diseases of Livestock. Lavoisier, France. pp 991-1021.
- Schaefer DL, Bildfell RJ, Long P and Löhr CV (2012). Characterisation of the microanatomy and histopathology of placentas from aborted, stillborn, and

- normally delivered alpacas (*Vicugna pacos*) and llamas (*Lama glama*). Veterinary Pathology 49(2):313-321.
- Schulze zur Wiesch J, Wichmann D, Sobottka I, Rohde H, Schmoock G, Wernery R, Schmiedel S, Burchard GD and Melzeer F (2010). Genomic tandem repeat analysis proves laboratory-acquired brucellosis in veterinary (camel) laboratory in the United Arab Emirates. Zoonoses Public Health 57(5):315-317.
- Söllner NK (2018). Evaluation of serological tests for the diagnosis of Brucellosis in *Brucella melitensis* experimentally infected dromedary camels. Doctoral Thesis, Veterinary Faculty of Leipzig University, Germany (in press).
- Wernery U, Kinne J, Joseph M, Johnson B and Nagy P (2007).

 Where do Brucella organisms hide in serologically positive lactating dromedaries, In: Proc. Int. Camel Conference, College of Veterinary and Animal Science, Rajasthan Agricultural University, Bikaner, India. pp 68-72.
- Wernery U, Kinne J and Schuster RK (2014). Camelid Infectious Disorders. OIE Book. pp 135-162.
- Wernery U (2016). Camelid Brucellosis: A Review. Journal of Bacteriology and Mycology 3(1):1019.
- Wilson AJ, Schwartz HJ, Dolan R, Field CR, Roettcher D (1982). Epidemiologische Aspekte bedeutender Kamelkrankheiten in ausgewählten Gebieten Kenias Der Praktische Tierarzt 11:974–987.