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Infectious causes of reproductive loss in camelids

A. Tibary^{a,*}, C. Fite^a, A. Anouassi^{b,c}, A. Sghiri^b

^a College of Veterinary Medicine and Center for Reproductive Biology, Washington State University, Pullman, WA 99164-6610, USA

^b Institut Agronomique et Vétérinaire Hassan II, Rabat, Morocco

^c Veterinary Research Center, Abu Dhabi, UAE

Abstract

Reproductive losses in camelids are due to infertility, pregnancy loss, udder diseases and neonatal mortality caused by a variety of infectious diseases. Uterine infection and abortion represent the major complaint in camelid veterinary practice. The major infectious organisms in endometritis and metritis are *E. coli* and *Streptococcus equi* subspecies *zooepidemicus*. Abortion rates due to infectious diseases vary from 10% to more than 70% in some areas. Leptospirosis, toxoplasmosis and chlamydiosis have been diagnosed as the major causes of abortion in llamas and alpacas. In camels, brucellosis and trypanosomiasis represent the major causes of infectious abortion in the Middle East and Africa. Mastitis is rare in South American camelids. The prevalence of subclinical udder infection in camels can reach very high proportions in dairy camels. Udder infections are primarily due to *Streptococcus agalactiae* and *Staphylococcus aureus*. Neonatal mortality is primarily due to diarrhea following failure of passive transfer and exposure to *E. coli*, rotavirus, coronavirus, Coccidia and *Salmonella*. This paper reviews the etio-pathogenesis of these causes of reproductive losses, as well as the major risk factors and strategies to prevent their occurrence.

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1. Introduction

Camelids are an important animal production resource in many areas of the world. The reproductive efficiency of camelidae, particularly the Bactrian and dromedary camels, is generally considered low [1–3]. In camels, birthing rates rarely exceed 40% in nomadic herds and 70% in more intensive herds [1,2]. In addition to low birthing rates, camel herds suffer from high neonatal loss sometimes reaching epizootic proportions [2,3]. In South American camelids (SAC), llamas and alpacas, birthing rates are slightly better, but high rates of pregnancy loss and infertility represent a major

complaint in practice [4]. In alpacas, the mean annual fertility reported is 50% [5], whereas in llamas the mean birth rate is 45.9% [6]. Low fertility in alpacas seems to be due to the 50–57.8% reported embryo mortality up to 30 d of gestation [7].

Infectious organisms are responsible for a myriad of diseases that directly or indirectly affect reproductive success in camelidae. Infections of the genital tract may lead to temporary or permanent infertility in the male and female. In the pregnant female, infectious diseases may lead to abortion as well as prenatal and neonatal loss. In the postpartum female, the main concern is mastitis, which may also lead to poor neonatal survival. The goal of the present paper is to review the epidemiology, etio-pathogenesis, diagnosis and prevention of the major reproductive loss-causing infectious diseases. Aspects of biosecurity in breeding operations are also discussed.

* Corresponding author. Tel.: +1 509 335 1963;
fax: +1 509 335 0880.

E-mail address: tibary@vetmed.wsu.edu (A. Tibary).

2. Infectious cause of infertility in the female

Uterine infections are considered to be the most common cause of reproductive failure in camelidae [4,8–15]. However, there are limited data on the incidence of endometritis and metritis, as well as the pathogenesis and treatment of uterine infections, in camelidae.

2.1. Pathogenesis and predisposing factors

As in many other domestic animal species, the uterus becomes contaminated by a variety of organisms from the caudal genitalia or the environment during breeding, parturition or genital manipulations. In most females, infectious organisms are eliminated by natural uterine defense mechanisms. However, in a proportion of the females, these defense mechanisms fail partially or completely, allowing establishment of an infection. Although no studies have been carried out on uterine defense mechanisms in camelidae, they are likely to be similar to what is known in other species. The major mechanisms used by the female to clear uterine infection are local immunity, phagocytosis and mechanical clearance by myometrial contractions. These mechanisms are more effective during the follicular phase when blood estrogen concentrations are high and the uterus has maximal contraction [12,16].

The single most important factor causing damage and contamination of the uterus is repeated mating. As camelidae are induced ovulators, aggressive mating during the “wrong” phase of follicular developmental phase can induce severe uterine inflammation [4,17]. Resistance of the uterus to infection and its ability to rid itself of microorganisms is also diminished in the presence of degenerative changes in the endometrium (fibrosis) or repeated heavy infection with pathogenic strains [8,18,19].

In our experience, the major contributing factors to uterine infection are overbreeding, postpartum complications (retained placenta and rectal-vaginal tears) and unsanitary gynecological manipulation [4,8]. In camelidae, during mating, the penis penetrates the cervical canal and enters deep into the uterine cavity [17]. Repeated insults of the uterus due to improper mating practices can lead to inflammation and loss of the ability to resist infection. Many breeders who are not familiar with the reproductive physiology of the female camelidae rely exclusively on “receptive” behavior for breeding. However, studies have shown that receptivity is not necessarily correlated with ovarian activity [20,21]; this results in multiple matings that

have little chance of achieving pregnancies, but cause damage to the endometrium and cervix [4,21–23].

2.2. Etiology and diagnosis

The approach to diagnosis of infertility problems in general, and uterine infection in particular, has been thoroughly described elsewhere [4,8,18,23]. Breeding history and detailed examination of the reproductive tract including palpation, ultrasonography, vaginoscopy, uterine cytology, uterine culture and uterine biopsy should be performed on all barren females [18,24].

2.2.1. Clinical evaluation

Uterine infection should be suspected in any animal with a history of repeat breeding, early embryonic death or abortion. The barren female with endometritis or metritis may have a history of recent abortion, retained placenta, dystocia or uterine or vaginal prolapse. Systemic signs are usually absent in cases of chronic endometritis. However, fever, depression and signs of toxic shock may accompany acute postpartum metritis [25]. In these cases, supportive therapy should be started immediately. Examination of the perineum and vulva may reveal mucopurulent discharge. In some cases, dried flakes of vaginal discharge may be present at the base of the tail. In the postpartum female, a thick, pinkish or white postpartum discharge (lochia) from the vagina is normal and may persist for up to 1 week after parturition. However, a profuse, watery or smelly discharge should be considered abnormal and a sign of postpartum metritis. Conformation of the vulva and perineum are very important in the evaluation of the barren female. An incompetent vulva or vestibulo-vaginal sphincter due to tears or laceration may be the primary cause of contamination of the vagina and uterus. Excessive vulvar edema may be associated with overbreeding, but can also result from excessive estrogen therapy [4,8,18].

Transrectal ultrasonography may reveal fluid accumulation in the uterine cavity, thickening of the uterine wall, or both. In the postpartum female, uterine infection should be suspected if there is a delay in uterine involution, which should be complete by 15 d in SAC and 25 d in camels. Pyometra is usually seen in the immediate postpartum period. In the non-parturient female, pyometra is generally associated with vaginal or cervical adhesions following dystocia or obstetrical manipulation trauma [4,12,13]. Transrectal ultrasonography during uterine flushing is a good technique to visualize echodense material in the uterus, as well as

intraluminal adhesions or abscesses and to evaluate the uterine wall.

Vaginoscopy using a small mare tube speculum for llamas and camels or a sigmoidoscope for alpacas may reveal vaginal discharge or inflammation [18,24]. Small quantities of thick mucus may be present in the anterior vagina. The cervix should be evaluated for signs of inflammation (cervicitis) and discharge. The normal cervix may be hyperemic and bleed immediately after mating.

2.2.2. Uterine cytology, culture and biopsy

Cytological examination of the uterus can be performed using a direct double-guarded swab, a cytology brush or by recuperating saline solution infused into the uterine cavity. The latter technique should be performed after taking a uterine swab for microbiological evaluation [4,18]. Uterine culture and cytology samples are preferably taken when the female is at peak follicular phase and the cervix is open. In large camelidae (dromedary, bactrian and llama) all of these procedures are performed using the same rectovaginal techniques used in the bovine. In alpacas, passage of the swab or pipette requires direct visualization and insertion into the cervix with the assistance of a vaginoscope. This is relatively easy to do when the alpaca has a mature follicle (uterus contracted and patent cervix) [4,23]. The uterus flushing technique is by far the best, but has the disadvantage of being time consuming. For this technique, the uterus is flushed with a small quantity of sterile saline using either a Foley catheter or a mare insemination pipette. The fluid is collected, fixed in 40% ethanol and centrifuged to concentrate cells. Smears are prepared from the sediment, air-dried and stained with Wright–Giemsa, Papanicolau stain or Hematoxyline and Pollack's trichrome. The degree of inflammation is assessed by evaluation of the amount and morphology of polymorphnuclear (PMNs) leucocytes. The presence of 3–5 PMNs per high power field is usually relevant in the diagnosis of endometritis. The causative microorganism may sometimes be observed in the cytology evaluation [4,23].

Bacteriological examinations can be done from direct uterine culture swabs, a fragment of uterine biopsy, cervical discharge or uterine flushing medium. Direct uterine cavity swabbing is the most widely used technique. The uterine culture swab should be double-guarded to avoid unwanted contamination [4,23]. Samples should be examined routinely for aerobic and anaerobic bacteria, ureaplasma, mycoplasma, as well as for fungi [14,26]. Samples from camels should also be examined for *Trichomonas* sp. and *Campylobacter* sp.

which have been reported to cause reproductive failure in dromedaries [27].

Interpretation of microbiological results of uterine swabs is very difficult, given the wide range of bacteria that can be isolated (Table 1). Some of these organisms are part of the normal vaginal flora, whereas others are opportunistic and can become pathogens if the appropriate conditions are present. The bacteria responsible for endometritis in the camel are essentially similar to those found in cattle and horses [14,15,27–29]. The most common bacteria isolated from the uterus of camelids with endometritis are *Escherichia coli*, *Streptococcus zooepidemicus*, β -haemolytic *Streptococci*, *Enterococcus*, coagulase negative *Staphylococcus*, *Proteus* spp., *Enterobacter aerogenes*, *Klebsiella pneumoniae*, and *Arcanobacter pyogenes* [15,26–28,30,31]. *Pseudomonas aeruginosa*, *Campylobacter fetus*, and *Tritrichomonas fetus* have been isolated from infertile camels and may be associated with venereal transmission and should be considered as possible causes in infertility or abortion outbreaks [14]. *Aspergillus* spp. and *Mucor* sp. have been isolated from female dromedaries with endometritis. Confirmation of uterine infection should also be done on the basis of uterine cytology and biopsy as some chronic uterine infections may not result in a positive culture.

Uterine biopsy is a very reliable technique for the evaluation of modifications of the endometrium due to inflammatory, degenerative, or neoplastic processes. The technique used in camels and llamas is similar to that used in the mare. A mare biopsy punch is inserted through the cervix, either by direct vaginal or transrectal manipulation. The senior author prefers to take samples from the left uterine horn unless a lesion is visualized on the right horn [12,26,32]. Uterine biopsy in alpacas is more difficult. Here, a smaller curved biopsy punch is often used in our clinic.

Endometrial epithelial cells can be simple cuboidal, columnar, tall columnar, or a combination of these. Beneath the epithelium is a variably loose or dense area of connective tissue, the upper lamina propria, which contains a few uterine glands or gland necks. Sub-epithelial hemorrhage is very frequent. The number of endometrial glands are very variable but are lower than that observed in the mare. The number of glands increases in the deeper lamina propria, which has less-dense connective tissue. The glandular epithelium is columnar in the superficial part and cuboidal in the deeper layers. Intussusception of epithelial cells into the gland lumens is a common artifact [26]. Inflammatory changes of the endometrium are characterized by leucocyte infiltration with differing degrees of intensity,

Table 1
Organisms isolated from the genital tract of female camelidae

Ref.	Samples	Organisms isolated	Remarks
[147]	Abattoir (dromedary)	<i>E. coli</i> , micrococci, corynebacteria, sarcina, <i>Str. zymogenes</i> , anthracoids, proteus spp., <i>Strdurans</i> , <i>C. pyogene citrobacter</i> , <i>Staph. aureus</i> , gaffkya, <i>Pasteurella multocida</i> , <i>Staph. epidermidis</i> and molds	High probability that many of these organisms are contaminants
[148]	Abattoir (dromedary)	<i>Micrococcus pyogenes</i> , <i>C. pyogenes</i> , <i>Pseudomonas aeruginosa</i> , <i>Staph. albus</i> and hemolytic <i>Streptococcus</i>	Isolated from cases of endometritis and pyometra
[149]	Abattoir (dromedary)	<i>Proteus</i> spp., <i>Serratia</i> , enterobacter, <i>Klebsiella</i> , <i>E. coli</i> , enterococci, anthracoids, <i>C. renale</i> , <i>Staph. aureus</i> , micrococci, <i>Str. pyogenes</i>	Dromedaries with endometritis
[31]	Abattoir (dromedary)	<i>Staph. epidermidis</i> , <i>M. luteus</i> , <i>M. roseus</i> , <i>Pr. morgani</i> , <i>Staph. caseolyticus</i> mixed with <i>B. anthracoides</i> , <i>Provid. stuartii</i> and unidentified corynebacterium (between 4.8 and 23.8% were found in apparently normal uteri)	From apparently healthy uteri, 63.6% had bacterial growth
	Abattoir (dromedary)	Many bacterial pathogens were isolated from endometritis of infected camels and showed high isolation rate. <i>E. coli</i> , <i>Str. pyogenes</i> , <i>Staph. aureus</i> , <i>Kl. oxytoca</i> , <i>Ps. aeruginosa</i> , <i>H. somnus</i> and <i>C. pyogenes</i> represented the most frequent types in order of their frequency	In uteri with signs of endometritis, 93.3% contained specific microorganisms
[26]	Biopsy samples (llamas)	<i>Actinomyces pyogenes</i> , <i>Bacillus</i> sp., <i>Staphylococcus</i> sp., <i>Escherichia coli</i> , <i>Streptococcus</i> sp., <i>Bacteroides</i> sp., <i>Fusobacterium necrophorum</i>	Bacteriologic culture results were consistent with histological findings in 18 llamas (66.7%)
[29]	Endometritis (dromedary)	<i>Campylobacter fetus</i>	
[27]	Uterine swabs (dromedary)	Aerobic bacilli	Females without endometritis had 65% sterile samples
	Uterine swabs (dromedary)	<i>E. coli</i> , <i>Staph aureus</i> <i>Tritrichomonas foetus</i>	Females with endometritis
[14]	Uterine swabs (dromedary)	<i>Staphylococcus</i> sp., <i>Staphylococcus aureus</i> , <i>Streptococcus</i> sp., aerob Bacilli, <i>Diplococcus</i> , <i>Escherichia coli</i> , <i>Clostridium sporogenes</i>	Healthy females
	Uterine swabs (dromedary)	<i>Campylobacter fetus</i> , <i>Pseudomonas aeruginosa</i> , <i>Klebsiella ozaenae</i> , <i>Salmonella</i> sp., <i>Serratia marcescens</i> <i>Micrococcus</i> sp.	Organisms isolated from females with endometritis (in addition to the species cited above)
[150]	Abattoir (dromedary)	<i>Corynebacteria</i> Anthracoids, Micrococci, <i>Sarcina</i> , <i>Gaffkya</i> and Gram-negative bacilli	Isolated from non-pregnant and pregnant uteri (<i>Gaffkya</i> was not isolated from any pregnant organ)

as well as the presence of degenerative changes, e.g. fibrosis [24].

Acute endometritis is characterized by an infiltration of predominantly polymorphnuclear leucocytes (PMNs) in the sub-epithelial zone of the stratum compactum. This endometritis can become suppurative and cause desquamation of the endometrium. The exudate is composed mainly of PMNs with a small number of lymphocytes and plasma cells [33]. Sub-acute endometritis is characterized by hypertrophy and thickening of the blood vessels, subepithelial infiltration of fibroblasts, histiocytes and lymphocytes, as well as slight leucocytic infiltration around the endometrial glands.

Chronic endometritis is characterized by a predominantly lymphocytic infiltration with the occasional presence of plasmacytes, macrophages, eosinophiles or mast cells. Siderophages may be present in the postpartum uterus or following abortion or embryo loss.

Chronic inflammation may be localized or generalized and may involve the stratum spongium. Chronic degenerative endometritis is characterized by irreversible changes including atrophy of the endometrial glands and fibrosis; the diameter and secretory activity of the endometrial glands are decreased and the glandular epithelium cells have pyknotic nuclei. These degenerative changes are mainly due to the presence of periglandular or peri-vascular fibrosis. In severe cases, there is nesting with cystic dilation of the endometrial glands or lymphatic cysts. The severity of fibrosis is evaluated by the number of fibrotic layers present: slight (1–3 layers), medium (4–10 layers), severe (>10 layers) [23,26,33].

A special form of chronic endometritis is described in dromedary camels. This is characterized by diffuse lymphocytic infiltrates and by the presence of granulomatous foci, consisting of small lymphocytes, histiocytes, and reticular cells. Most of these granulo-

matous infiltrates are located in the sub-epithelial zone. These lesions are similar to those described in cattle in cases of campylobacteriosis or tuberculosis. They could also be due to fungal infection [12,34]. A recent preliminary report suggests that these histopathological observations may reflect normal lymphoid tissue in the uterus, although further studies are needed to confirm this hypothesis [35].

A classification system, similar to that used in the equine, has been proposed for fertility prognosis based on results of endometrial biopsies [26]. This classification has also been adopted for dromedary endometrial biopsies and includes categories (or grades) according to the type and severity of lesions observed and their potential effect on pregnancy rate and rate of embryo loss (Table 2) [24].

2.2.3. Treatment

Uterine infections can lead to irreversible damage (e.g. salpingitis), resulting in a total loss of fertility [13]. There is no clinical trial comparing the efficacy of different treatments of endometritis in camelidae. Most practitioners use treatments proposed for the bovine or equine species. These include uterine lavage or flushing, intrauterine antibiotic infusion, systemic antibiotic treatments or a combination. Intrauterine infusion of homologous blood plasma twice at 24 h intervals has been used in llamas and alpacas [10,23].

Uterine lavage is generally done with warm isotonic saline solution or a weak antiseptic solution. The objective of this treatment is to remove organisms and cellular debris and improve uterine clearance by promoting endometrial contraction and increased local blood flow. The ideal antiseptic solution to be used for uterine flushing should not contain more than 1–3% of a

0.5% povidone iodine solution. A lavage with saline or lactate ringer solution (LRS) at the end of the treatment to remove all antiseptic from the uterus is highly recommended. Oxytocin (5–10 IU for alpacas and 20 IU for camels) may be given to improve uterine clearance. Uterine lavage should be done carefully in the case of septic endometritis to avoid complications.

Intra-uterine antibiotic infusion is done after uterine lavage and removal of all the purulent material and cellular debris. Antibiotic choice should be based on culture and sensitivity results. The most common antibiotics used are penicillin K (1.5×10^6 U for SAC, 5×10^6 U for camels), Gentamicin sulfate (200–300 mg for SAC, 500–1000 mg for camels), Ticarcillin (3 g for dromedaries, particularly for *Pseudomonas* infections), amikacin sulfate (2 g for camels infected with *Pseudomonas* and *Klebsiella*) and ceftiofur sodium (250–500 mg for SAC, 1 g for camels). The third generation cephalosporin, ceftiofur, has a broad spectrum of action and is effective against both Gram-negative and Gram-positive bacteria. Antibiotics should be diluted in sterile water or saline solution (20–30 mL for SAC, 60 mL for camels). Treatment should be continued daily for 5–7 d [8]. The success of treatment for endometritis is variable and depends on the duration and endometrial changes observed in the uterine biopsy. Pregnancy rates after treatment range from 30% to 60% [15,26]. Treated females should be re-examined after a period of sexual rest of at least 2 weeks.

Sexual rest following treatment and adoption of a “minimum contamination breeding technique” (MCBT) will help prevent re-infection of the uterus. The duration of sexual rest varies from 2 to 4 weeks, depending on the severity of infection. Minimum contamination breeding technique requires ultrasound monitoring of ovarian

Table 2
Classification of endometrial biopsy and potential effect on fertility (adapted from Powers et al. [26])

Categories	Characteristics	Effect on fertility	Prognosis
Grade 1A	Normal endometrium	Normal	Very good
Grade 1B	Few lymphocytes within the endometrium. Siderophages (postpartum or postabortion). Low-grade infection, inflammation due to mating	Slightly decreased	Good if treated promptly
Grades 2A and 2B	Active and acute, chronic, or chronic active endometritis. Chronic inflammation is more deeply located in the endometrium, compared with active and chronic active inflammation	Reduced conception rate, increased early embryo death	Good if recent, poor if the female has been barren for >1 year
Grade 3A	Chronic endometritis with glandular fibrosis	Increased early embryonic loss or abortion	Poor
Grade 3B	Uterine neoplasia		Poor

activity, breeding only once when the follicle is mature, followed by intrauterine infusion of antibiotics 24 h after mating. In some situations, induction of ovulation using hCG (750 IU in alpaca and llamas, 1500–2000 IU in camels) or GnRH (0.5–1 mg) or an analogue (e.g. buserelin: 4–8 µg for llamas, 15–20 µg for camels) help ensure ovulation. Susceptible females should not be bred more frequently than once a week. Females bred using the MCBT and induction of ovulation should be checked for pregnancy or receptivity 12–14 d after mating [4,8,17].

2.2.4. Prevention

Prevention of uterine infection requires sound individual and herd reproductive management. Pre-breeding examinations should be performed on all maiden animals to avoid breeding animals that are too young or have no follicular activity [4]. Only females that are exhibiting strong receptivity (as opposed to submissive behavior) should be bred [17]. Detection of receptivity requires the owner to be familiar with each individual female's "normal" behavior. In hand-mating situations, breeders should be aware that aggressive males could force themselves upon females even if they were not receptive. Also, females may assume the sternal position on command by the trainer, without regard to their ovarian status. This is frequently seen in racing/riding camels that are trained to respond to commands. Ideally, breeding should only take place when a mature follicle has been detected on the ovary (12–18 mm in camels, 8–12 mm in llamas and alpacas); this is particularly important for females that are susceptible to infection.

A complete gynecological evaluation should be performed before breeding on all females with a history of infertility, obstetrical problems or postpartum complications. This allows early detection and treatment of uterine infections as well as prevention of venereal transmission of organisms.

The incidence of endometritis can be reduced by observing strict rules of hygiene during breeding and parturition. Parturition should take place in a clean area. If obstetrical manipulations are required during birth, they should be accomplished by experienced persons after washing thoroughly with a mild antiseptic soap. Early treatment of complications of parturition, e.g. retained placenta and postpartum infections, reduces the risk of irreversible damage to the uterus.

3. Infectious causes of infertility in the male

Male infertility has been reported following severe systemic or local infections [4,36,37]. In male camels, trypanosomiasis (*Trypanosoma evansi*) has been asso-

ciated with severe testicular degeneration following bouts of fever and development of immune complexes that compromise Sertoli cell function [38,39]. Trypanosomiasis has also been associated with impairment of pituitary function, that may contribute to testicular degeneration and poor semen quality [39,40]. Effects of trypanosomiasis on testicular function disappear within 75–90 d after recovery of the male [39]. However, infertility may persist beyond this period or become permanent in severe cases.

Orchitis and epididymitis have also been associated with brucellosis caused by *Brucella abortus* and *Brucella melitensis* [41,42]. Orchitis and epididymitis due to filarial (*Dipetalonema evansi*) infestation have been reported in camels, with an incidence of 2.3–10% in wet regions [43–47]. In alpaca and llamas, most testicular infections are ascendant from scrotal wounds. However, a case of severe orchitis and epididymitis has been described in an alpaca following hematogenous spread of *S. equi zooepidemicus*, the cause of "alpaca fever" [48]. Attempts to treat orchitis or epididymitis with systemic antimicrobials are often unrewarding. The affected testicle will always undergo degeneration; therefore, if the infection is unilateral, the best option is orchidectomy. The effect of other infectious agents such as BVD virus, blue tongue, chlamydia, mycoplasma and Q-fever, on testicular function and or venereal transmission is largely unknown. These agents may directly or indirectly be involved in the high rate of testicular degeneration seen in camelids [4].

There are no reported infectious diseases of the accessory sex glands (prostate or bulbourethral glands). However a case of infectious prostatitis has been suspected in one camel [4].

4. Infectious causes of fetal loss

Studies on the incidence and etiology of abortion in camelidae are scarce [2,12]. The abortion rate in llamas and alpacas has been described as low [9,10]. In the dromedary, abortion rates range from 2% to 25% [31,49–54]. Various infectious, traumatic or toxic factors have been associated with abortion in camelidae. However, to date there are no studies on the role that each of these factors play in the incidence of abortion. This gap is probably due to the lack of routine laboratory investigations in the case of abortion in this species.

4.1. Etiology

In dromedary camels, reported infectious causes of abortion include brucellosis [12,55,56], placentitis or

uterine infections [12,27,31,57,58], toxoplasmosis [59], trypanosomiasis [12,60,61] and camel pox [62]. A case of abortion due to *Bacillus cereus*, an organism suspected to cause hemorrhagic disease, has been described in a 9-year-old dromedary female [58]. In this case, the fetal membranes had severe hemorrhagic necrotising placentitis accompanied by edema. *Bacillus cereus* was isolated from the placenta and different organs of the fetus (spleen, liver and intestines) [58]. Blue tongue abortion has been reported in camels in a disease called “da-chonou” in Africa [63]. Although leptospirosis is relatively rare in nomadic herds of camels [64], it may become important as more intensive dairy herds develop.

Brucellosis (*Brucella abortus* and *Brucella melitensis*) is endemic in almost all camel-producing countries, particularly in herds that commingle with other domestic ruminants [61,65–70]. With the development of commercial camel dairies in several countries, this disease should be seriously considered because of its impact on human health. There are no studies on vaccination or eradication strategies of camel brucellosis.

The role of trypanosomiasis in reproductive loss in camels has been known for decades [71]; severe outbreaks of abortion and premature births have been reported in acutely infected herds [72]. Early and late fetal loss in these situations can reach 80% in affected animals (A. Anouassi, personal observation). The pathogenesis of trypanosome-associated abortions or premature births is not well understood. Severe compromise of the health of the female and severe anemia may be involved. Trypanosoma infection may also directly affect pituitary function and steroidogenesis (decreased progesterone production or increased cortisol production) leading to abortion [39]. Placentitis and placental edema have been diagnosed in our laboratory following outbreaks of trypanosomiasis (A. Tibary and A. Anouassi, clinical observations). Prevention of this disease can be effectively accomplished by regular preventive treatment with the pregnancy-safe trypanocide, melarsomine, a melaminophenylarsine compound (Cymelarsan[®], Rhone Merieux) during the high-risk season [1,73].

The rickettsial disease, Q-fever, caused by *Coxiella burnetii*, is suspected as a cause of abortion in camelids and is a cause of concern because of its zoonotic characteristics [74,75]. In one African country, Chad, the sero-prevalence of Q-fever was 80% in tested camels and 100% of herds had seropositive animals [76].

In SAC, reported infectious causes of abortion in North America include leptospirosis, toxoplasmosis,

and chlamydiosis and other non-specific uterine infections [10,26,77]. Abortion has been induced in llamas experimentally infected with *Brucella abortus* [78]. Placental and fetal lesions observed were similar to those reported in ruminant brucellosis [78]. Other reported causes of abortions in SAC include listeriosis [79], sarcocytosis-induced eosinophilic myositis (Dalmeny disease) due to *Sarcocystis aucheniae* [80], *Streptococcus sanguis* type-1 [81], and *Arcanobacter pyogenes* abortion in an alpaca [82]. Ascendant and hematogenous placentitis has been diagnosed by one of the authors (A. Tibary, clinical observation) using ultrasonographic fetal and placental evaluation approaches similar to those described in equine practice. Ascending placentitis was diagnosed in three cases of recurrent vaginal prolapse.

Leptospirosis is endemic in many countries, including the USA [83–85]. In Argentina, seroprevalence of the disease varies between 47.3% and 96.2% in llamas, 0% and 13% in guanacos, and 9% and 62.8% in vicunas. The presence of seropositive wild camelids (guanacos and vicuna) is particularly important from an epidemiological perspective. The most frequently encountered serovars were *copenhageni* (serogroup *Icterohaemorrhagiae*) and *castellonis* (serogroup *Ballum*) [84]. Alpacas were shown to react to *L. interrogans* serovar after vaccination. However this reaction is variable and immunity may be of short duration [83]. In North America, polyvalent vaccines commercialized for the bovine are used heavily in SAC in areas where the disease is endemic. Here, animals are vaccinated three or four times annually.

The prevalence of abortion due to chlamydiosis in camelidae has not been thoroughly evaluated. Sheep vaccines are used in alpaca operations. However, there are no scientific reports regarding their efficacy.

Neospora-species associated abortion has been reported recently in alpacas and llamas in Peru. Neospora species DNA was detected by PCR in three of 15 fetuses examined, whereas Neospora antigens were detected in two fetuses by immunohistochemistry [86].

The role of pestiviral infections and particularly bovine viral diarrhoea virus (BVDV) infection in reproductive loss in camelids has been reviewed recently [87]. Early virological studies have shown that llamas may be infected without developing clinical signs [88]. Dromedary camels were also shown to be susceptible to BVDV infections, although the role of such infection in reproductive loss and neonatal disease is not yet clear [89,90]. More recent studies reported early fetal losses, abortions, and stillbirths in BVDV-

infected llamas and camels [87,91–95]. Birth of persistently-infected (PI) crias has been reported [93–95]. Congenital infection and birth of PI crias is being investigated by several laboratories (J. Evermann, personal communication).

4.2. Diagnostic approach for abortion

A precise diagnosis should be pursued in any case of abortion, premature birth or birth of a compromised or septicemic neonate. Diagnosis of the cause of abortion or in utero infection can be reached in the majority of cases with proper history, clinical observations and collection and submission of all required samples. A complete history regarding the whereabouts of the animal, its health and history just prior to abortion, should be taken. Samples from the dam should include acute and convalescent serum samples for serological testing of the most common infectious agents, and a uterine swab for microbiological studies.

The importance of placental examination in the diagnosis of abortion, stillbirths or premature births cannot be over-emphasized. Clients should be instructed to keep the placenta for proper examination. Detailed description of proper examination of the camelid placenta and its pathology is beyond the scope of this paper [12,17]. Placental anatomy is very similar to that of the equine, with the exception that the left horn is always the pregnant-horn (largest) [12,96]. The placenta should be examined as soon as possible following parturition. Weight of the placenta should be

determined. The placenta is usually expelled inside-out, with the allantoic surface exposed. It should be gently cleaned with cold water (to remove bedding, grass and soil), laid out flat and all surfaces examined. The umbilical cord should be of normal length. The amniotic sac and chorionic surface (red velvety surface) examined from the cervical “star” to the tips of the horns on all sides. The fourth membrane (epithelion or epidermal membrane) should be translucent. Fixed and unfixed placental samples should be taken from different normal and abnormal sites and submitted for histopathology, histochemistry, and culture. Several morphological characteristics of the placenta may allow ruling out infectious causes (such as umbilical cord torsion, body pregnancy and twin pregnancies). Chronic infectious inflammations are generally easy to detect, as they will cause the placenta to be thick and leathery. Cytological evaluation of a contact smear may reveal inflammatory changes and pathologic organisms.

Fetuses and neonates should be sent whole to a diagnostic lab. If fetal necropsy is performed in the field, proper precautions should be taken to document lesions or observations, prevent contamination and take proper samples. Fetal blood samples, as well as pleural and peritoneal fluids should be taken. Tissue samples from any grossly abnormal areas and from all major organs (i.e. liver, lung, kidney, adrenal gland, placenta, heart, thymus, brain, spleen and small intestine) should be taken for histopathology. If the breeding date is not known, the stage of pregnancy may be estimated from fetal weights or fetal body measurements based on

Table 3
Formulas for the estimation of gestational age (GA) in days from fetal measurements in camelids

Species	Formula	Correlation <i>r</i>	Reference
<i>Camelus dromedarius</i>	GA = (CRV + 23.99)/0.336	0.90	[98,151]
	GA = (TCL + 36.80)/0.501	0.90	[98,151]
<i>Llama glama</i>	GA = 44.77BPD – 51.713	0.88	[100]
	GA = 67.462 + 11.163W + 0.297BPD	0.88	
	GA = 16.66W + 169.448	0.99	
	GA = 9.94F + 102.029	0.91	
	GA = 8.23T + 91.276	0.86	
	GA = (BPD – 0.002399)43.02293	0.98	[101]
<i>Vicugna alpaca</i>	GA = (TH – 0.07137)46.94485	0.95	
	GA = (BPD – 0.11376)47.23287	0.98	[101]
	GA = (TH – 0.36436)52.87663	0.96	
	GA = 76.6 + 5.1W – 0.06W ²	0.98	[102]
	GA = 1.3 + 0.09 TL + 0.002TL ²	0.97	
	GA = 2.8 – 0.08CR + 0.002CR ²	0.96	
	GA = 3.9 + 0.1 CCR + 0.002CCR ²	0.98	

W = body weight (kg); CVR = Crown-vertebral rump length; TCL = total conceptus length; BPD = biparietal diameter; TH = thoracic height; F = femur length; T = tibia length; TL = total length of the fetus; CR = crown-rump length; CCR = Curved crown-rump length (all measurements are in cm).

correlation equations established for camels [97–99] and SAC [100–102] (Table 3). These measurements may also be helpful in determining intrauterine fetal growth retardation.

5. Mastitis

Clinical mastitis is relatively uncommon in camelids when compared with cattle. However, the incidence of mastitis may increase in dairy camels due to hand milking and teat malformation [103]. Acute mastitis has been reported to occur during the first few days following parturition, dystocia or cesarean section in the dromedary [104,105].

In acute mastitis, mammary secretions are generally watery, yellowish or blood-tinged clear. Clinical signs may include anorexia, fever, general depression, swelling, and pain of the udder, which may cause rejection of the newborn by the female. Bacteria isolated from acute mastitis in the dromedary include *Klebsiella pneumoniae*, *E. coli*, *Pasteurella haemolytica* and *Streptococcus agalactiae* [104,106]. Acute clinical mastitis is rarely diagnosed in llamas and alpacas. *Streptococcus zooepidemicus* has been isolated in a case of mastitis in a llama with anorexia and fever 3 d after parturition. The llama was treated successfully with intramammary cephalosporin and systemic antibiotics (ampicillin 11 mg/kg BW SC every 24 h for 7 d) [107]. Treatment of acute mastitis in camelids is based on systemic antibacterial and anti-inflammatory therapy and regular mammary gland stripping. Supportive treatment may be necessary in toxic mastitis.

A prevalence of 5–70% subclinical mastitis has been reported in camels from various countries [108–113]. Subclinical or chronic mastitis is suspected when the young fail to grow normally or when an anomaly of conformation of the udder is observed such as atrophy of one or more quarters, asymmetry, or presence of pustules on the surface. Pus and high cell counts (CMT) may be observed in milk [114,115].

The most common isolates from camel mastitis are *Streptococcus agalactiae* and *Staphylococcus aureus* [113]. However other isolates such as *Streptococcus uberis*, *Streptococcus dysgalactiae*, *Streptococcus pyogenes*, *Diplococcus pneumoniae*, *Staphylococcus aureus*, *E. coli*, *Bacillus cereus*, *Corynebacterium bovis*, *Pseudomonas aeruginosa*, *Pasteurella* spp., *Pasteurella haemolytica* (chronic suppurative mastitis), *Klebsiella* spp., *Corynebacterium pseudotuberculosis*, *Corynebacterium equi* and *Corynebacterium pyogenes*, *Candida albicans* have also been reported [103,105,110,114–125]. Bacteriological findings and correlation with the

California mastitis test (CMT) and somatic cell count (SCC) in bactrian camels are similar to those reported in dromedary camels [126–128]. Treatment of subclinical mastitis with antimicrobials has been suggested by several authors. However overuse of these drugs raises serious public health concerns of the development of antibiotic resistance [122,129–131].

The correlation between udder infections and the results of the CMT results is debated. The percentage of milk samples from CMT positive quarters yielding a positive bacteriological result can vary from 10% to 75% [103,110,117,120,126,132,133]. *N*-acetyl- β -D-glucosaminidase (NAGase) activity in milk was found to be a good indicator of subclinical mastitis in the camel [134,135]. However another report indicated that SCC was a better indicator of the presence of pathogenic microorganisms than NAGase [136].

In llamas, evidence of intramammary infection was found in up to 57% of the animals sampled [137,138]. A variety of organisms have been isolated from llama milk samples, including several minor pathogens (*Staphylococcus* sp., *Micrococcus* sp. and *Corynebacterium* sp.) as well as major pathogens (*Streptococcus uberis*, *Streptococcus equinus* and *Enterococcus* sp.). Although bacteria were isolated from 21% of milk samples in llamas, these infections did not seem to cause an inflammation of the gland, as suggested by the low rate of positive reactions on CMT and low somatic cell counts [137].

6. Neonatal losses

In addition to poor fertility, neonatal mortality has been reported as one of the leading causes of reduced performance of camelid herds [139]. The average pre-weaning mortality rate in the dromedary reared in traditional systems varies from 10% to 30% and may reach 60% in some situations [2,3,52,139,140]. In SAC, neonatal mortality can reach alarming proportions in some conditions [141].

In the llama and alpaca, neonatal infections represent a major cause of neonatal deaths [142]. Enterotoxemia caused by *Clostridium perfringens* types A and C, and pneumonia may lead to severe neonatal mortality outbreaks [142]. Potential pathogens in cria diarrhea include *E. coli*, rotavirus, coronavirus, *Cryptosporidium* spp., *Giardia*, *Coccidia* and *Salmonella* [143,144]. Neonatal losses due to diarrhea in camels follow a similar distribution [145]. The major risk factors for neonatal infection are failure of passive transfer, exposure of neonates during transport to breeding farms and high animal density within a farm [141,146].

7. Conclusions: strategies to reduce losses due to infectious diseases

Prevention of infectious causes of reproductive losses in camelids should be based on sound biosecurity measures designed to prevent the introduction and spread of disease in a population, herd, or group of animals [146]. Vaccination programs should be adapted to each individual farm condition (Table 4). It is important to realize that no vaccination program is 100% efficacious in disease prevention. Therefore minimizing the risk of introducing diseases and their spread is particularly important for contagious diseases or diseases that present as an explosive manifestation in a naïve herd.

Pre-breeding reproductive examinations including uterine culture and cytology should be required for all females coming onto the farm, especially those with a history of infertility or pregnancy loss. Males should also undergo a physical examination to reduce the chance of infectious disease. In camels, campylobacter and trichomonas testing may be relevant in some areas. In SAC, testing for chlamydiosis may become important in areas where the disease is prevalent. Vaccination status as well as previous exposure to specific disease agents should be determined for all animals.

Quarantine of recently introduced animals should be considered, particularly in regions with a high risk of contagious diseases. Animals returning from events should be placed in quarantine for a minimum of 3 weeks, where they should be monitored on a daily basis. Personnel attending quarantined animals should always wear protective clothing (coveralls, etc.) and boots or shoe covers that are devoted solely to the quarantine facility. All other equipment and supplies used in a

quarantine facility (halters, ropes, blankets, feeders, buckets, etc.) must be solely devoted to the facility.

Breeding hygiene should be strictly observed to avoid transmission of contaminants to females. The most important factor in establishment of uterine infection is over-breeding, which should be avoided by proper veterinary evaluation of the female. The risk of transmission of diseases by reproductive technologies has not been thoroughly investigated in camelid, due to lack of widespread use of these techniques in camelid production. However, pathogens present in semen or on the surface of embryos may become relevant in biosecurity of elite herds. The peculiarity of the uterine stage of embryos (hatched from the zona pellucida) used in embryo transfer makes it very difficult to remove bacteria and viruses using traditional methods of embryo washing.

In large herds, females should be grouped by pregnancy status and stage of gestation. Pre-parturient females should be monitored daily for rapid mammary development, premature lactation or abnormal vaginal discharge. Pastures should be checked regularly for evidence of abortions. A contingency plan should be formulated for actions to take in the case of abortion. This plan should include proper handling (prompt submission to laboratory) of biological tissues (placenta and abortus) and measures to isolate aborting females from the rest of the herd. In large herds, personnel working with pregnant and parturient animals should be “specialized” in this activity and have no contact with the rest of the herd. As with cattle, neosporosis is emerging as a possible cause of abortion in camelids. Measures should be taken to prevent direct contact of pregnant females with dogs or feed contaminated by feces.

Personnel attending parturient females should be educated to recognize abnormal pre-, intra- and

Table 4
Vaccines used in pregnant camelids for the prevention of reproductive and neonatal losses

Diseases	Species	Remark
Clostridial diseases	All camelids	<i>Cl. Perfringens</i> C, D and <i>Cl. Tetani</i> toxoid, 4–6 weeks before due date. Multivalent (seven- or eight-way) clostridial vaccines are not recommended in pregnant animals
Leptospirosis	All camelids	Endemic area, frequent vaccination required every 3–4 months with proper strains
<i>E. coli</i> diarrhea	All camelids	Autovaccine, 6 and 2 weeks before due date
Salmonellosis	All camelids	Autovaccine, 6 and 2 weeks before due date
Neonatal diarrhea (rotovirus and coronavirus)	All camelids	Inactivated vaccine, 4 and 2 weeks before parturition
Camelpox	Camels	Vaccination of young stock with attenuated virus may provide life-long immunity

postpartum situations requiring urgent veterinary attention. Hygiene should be emphasized for all personnel attending or assisting in birthing. Administration of adequate quantity and quality colostrum within the first 12 h of life should be emphasized. Parturient females with agalactia, insufficient colostrum quality or mastitis should be recognized early and their neonates given colostrum replacement (frozen stored camelid colostrum or goat colostrum). Early recognition of newborns with failure of passive transfer by IgG quantification or total protein determination should be emphasized. Transportation of females with their crias for early postpartum breeding is an important risk factor for SAC crias.

The potential for disease transmission by visitors should not be underestimated. Visitor contact with animals should be limited or discouraged, particularly for high-risk animals (pregnant females and breeding males). Access to a herd by the general public should be disallowed. Vector animal population (insects, birds and rodents) should be controlled. Proximity with other species (i.e. other ruminants and swine) should be avoided.

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