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Can *Chlamydia abortus* be transmitted by embryo transfer in goats?

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ABSTRACT

The objectives of this study were to determine (i) whether *Chlamydia abortus* would adhere to or penetrate the intact zona pellucida (ZP-intact) of early *in vivo*-derived caprine embryos, after *in vitro* infection; and (ii) the efficacy of the International Embryo Transfer Society (IETS) washing protocol for bovine embryos. Fifty-two ZP-intact embryos (8–16 cells), obtained from 14 donors were used in this experiment. The embryos were randomly divided into 12 batches. Nine batches (ZP-intact) of five embryos were incubated in a medium containing 4×10^7 *Chlamydia*/mL of AB7 strain. After incubation for 18 hours at 37 °C in an atmosphere of 5% CO_2, the embryos were washed in batches in 10 successive baths of a phosphate buffer saline and 5% fetal calf serum solution in accordance with IETS guidelines. In parallel, three batches of ZP-intact embryos were used as controls by being subjected to similar procedures but without exposure to *C. abortus*. The 10 wash baths were collected separately and centrifuged for 1 hour at 13,000 × g. The washed embryos and the pellets of the 10 centrifuged wash baths were frozen at −20 °C before examination for evidence of *C. abortus* using polymerase chain reaction. *C. abortus* DNA was found in all of the infected batches of ZP-intact embryos (9/9) after 10 successive washes. It was also detected in the 10th wash fluid for seven batches of embryos, whereas for the two other batches, the last positive wash bath was the eighth and the ninth, respectively. In contrast, none of the embryos or their washing fluids in the control batches were DNA positive. These results report that *C. abortus* adheres to and/or penetrates the ZP of *in vivo* caprine embryos after *in vitro* infection, and that the standard washing protocol recommended by the IETS for bovine embryos, failed to remove it. The persistence of these bacteria after washing makes the embryo a potential means of transmission of the bacterium during embryo transfer from infected donor goats to healthy recipients and/or their offspring. Nevertheless, the detection of *C. abortus* DNA by polymerase chain reaction does not prove that the bacteria found was infectious. Further studies are required to investigate whether enzymatic and/or antibiotic treatment of caprine embryos infected by *C. abortus* would eliminate the bacteria from the ZP.

1. Introduction

The classification of *Chlamydiaceae* has recently changed; there is now only one genus, *Chlamydia*, in the *Chlamydiaceae* family. *Chlamydia abortus* is one of 11 species in this genus [1].

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C. abortus is a gram-negative bacterium, which grows in the cytoplasm of eukaryotic cells; its unique life cycle includes a resistant infectious form, the elementary bodies (EBs), which alternate with a metabolically active noninfectious form, the reticulate bodies (RBs). The EB attach to the membrane of the host cell and promote endocytosis in a membrane limited vacuole called the inclusion, which does not fuse with lysosomes. The EB then transform into RB, which replicate by binary fission. After several divisions, the inclusion is filled with RB, which then transform back into infectious EB. These EB are released through host cell lysis or extrusion of the inclusion out of the host cell [2].

It is the causal agent of enzootic abortion of ewes [3] and is the most common infectious cause of abortion in many small ruminant-rearing countries [4,5], with the exception of Australia and New Zealand [6].

In addition to significant economic losses [7,8], *C. abortus* presents a zoonotic risk; exposure of pregnant women to infected sheep can lead to severe septicemia in the mother resulting in spontaneous abortion or stillbirth [9,10].

In sheep and goats, *C. abortus* infection typically causes abortion during the last two or 3 weeks of gestation or the birth of stillborn or weak lambs that die in the first days of life [8], although infected goats may abort at any time during pregnancy [11–13]. Other than reproductive failure, sheep and goats rarely display clinical signs of *C. abortus* infection, other than vulval discharge 2–3 days before abortion. In some cases, goats may shed *C. abortus* in vaginal fluids for up to 2 weeks before and after abortion; other signs, such as respiratory tract disease, polyarthritis, conjunctivitis, and retained placentas have been reported [11].

C. abortus can induce a persistent, subclinical infection in nonpregnant sheep and goats [3,14]. After abortion, ewes are considered to be immune to further lamb loss [3,15,16]. However, ewes can be chronically or persistently infected, continuing to shed organisms at estrus or at subsequent lambings [16–19]. It has also been reported that some lambs can be born healthy and survive infection, although they may go on to abort during their first pregnancy [15,20–22].

Sexual transmission of *C. abortus* is possible [9]; the bacteria have been found in fresh and cryopreserved semen, preputial washing fluid, in the male genital tracts of rams and bucks [23–26], and in the vaginal mucosa in sheep [17,27]. Experimental studies have shown that *C. abortus* can be excreted in semen of inoculated rams [28] and transmitted by experimentally infected semen to ewes [29]. Male fetuses can be contaminated *in utero* and adult males by mating with infected females [30]. These results reveal the main source of *in utero* infection and indicate a risk factor for the transmission of *C. abortus* during embryo transfer (ET).

Embryo transfer is used, nationally and internationally, for the introduction, improvement, and preservation of livestock genetics. Embryos are generally considered to present a lower risk of infectious disease transmission than live animals, on the basis of the results of extensive research using zona pellucida (ZP)-intact, *in vivo*-produced, embryos [31]. To reduce the risk of pathogen transmission, the International Embryo Transfer Society (IETS) has established guidelines for bovine ET. Specific conditions, including donor sanitary status, rejection of embryos without an intact ZP, specific washing procedures (minimum of 10 washes in the culture medium so that each wash represents an 100-fold dilution of the previous wash, the use of a new sterile micropipette for each wash) are required to avoid pathogen transmission by ET [32]. In addition, treatment of the ZP with several enzymes, such as trypsin, has been shown to be effective for removal or inactivation of certain pathogenic agents [32], with no deleterious effects on embryonic development [33,34]. However, these protocols must be tested for each pathogen and for embryos from each different species [32].

To our knowledge, there are no reported studies into the interaction of caprine or bovine embryos and *C. abortus*. To investigate the risk of *C. abortus* transmission *via* caprine ET, our study aimed to determine whether *C. abortus* adheres to or infects ZP-intact early caprine embryos *in vivo* after *in vitro* infection. We also evaluated the efficacy of the IETS washing procedure recommended to decontaminate bovine embryos exposed to *C. abortus in vitro*.

2. Materials and methods

2.1. Production of embryos

2.1.1. Goats

Fourteen healthy 3- to 6-year-old Saanen or Alpine goats from flocks in the Deux-Sèvres region of France were used as embryo donors. These goats were certified *C. abortus* free using ELISA for blood serum and conventional polymerase chain reaction (C-PCR) for vaginal swabs.

2.1.2. Synchronization and superovulation

The donor goats were synchronized by inserting intravaginal sponges impregnated with 45 mg of fluorogestone acetate (Chronogest, Intervet, Angers, France) for 11 days, combined with the intramuscular injection of 125 μg of prostaglandin analog (Estrumate, Shering-Plough Veterinaire, France) 48 hours before sponge removal (Day 9). Superovulation was induced by intramuscular injection of porcine FSH (pFSH; Merial, University of Liege, Belgium) given twice daily at 12-hour intervals for 3 days (Days 9, 10, and 11). The total dose of pFSH was 16 Armor units per goat, administered in decreasing amounts (4–4, 2–2, and 2–2). A 132-μg dose of porcine LH was added to the pFSH preparation on the last two injections (66 μg per injection) [35].

2.1.3. Fertilization

The donor goats were mated by 3- to 6-year-old Saanen or Alpine bucks from the Deux-Sèvres region of France, 24–36 hours after sponge removal. These bucks of proven fertility were certified *C abortus* negative, by ELISA and PCR and regularly controlled.

2.1.4. Collection of embryos

Embryos were collected by laparotomy 4 to 5 days after the onset of estrus. The goats were anesthetized with 5 mL of Zoletil 100 (Virbac, Nice, France) and were prepared for