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ORIGINAL PAPER

Campylobacter fetus is Internalized by Bovine Endometrial Epithelial Cells

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Abstract

Campylobacter fetus is an important venereal pathogen of cattle that causes infertility and abortions. It is transmitted during mating, and it travels from the vagina to the uterus; therefore, an important cell type that interacts with *C. fetus* are endometrial epithelial cells. Several virulence factors have been identified in the genome of *C. fetus*, such as adhesins, secretion systems, and antiphagocytic layers, but their expression is unknown. The ability of *C. fetus* to invade human epithelial cells has been demonstrated, but the ability of this microorganism to infect bovine endometrial epithelial cells has not been demonstrated. Bovine endometrial epithelial cells were isolated and challenged with *C. fetus*. The presence of *C. fetus* inside the endometrial epithelial cells was confirmed by the confocal immunofluorescence. *C. fetus* was not internalized when actin polymerization was disturbed, suggesting cytoskeleton participation in an internalization mechanism. To evaluate the intracellular survival of *C. fetus*, a gentamicin protection assay was performed. Although *C. fetus* was able to invade epithelial cells, the results showed that it did not have the capacity to survive in the intracellular environment. This study reports for the first time, the ability of *C. fetus* to invade bovine endometrial epithelial cells, and actin participation in this phenomenon.

K e y w o r d s: bacterial infection, pathogenicity, virulence, pathogen-host interaction, infectivity

Introduction

Campylobacter fetus is a microaerophilic, Gram-negative bacterium that causes embryonic mortality, abnormal estrus cycles, reduced fertility and abortions in 5–10% of cases in cattle and sheep. *C. fetus* is divided into three subspecies: *C. fetus* subsp. *fetus*, *C. fetus* subsp. *venerealis*, and *C. fetus* subsp. *testudinum*. *C. fetus* subsp. *venerealis* is the etiologic agent of bovine genital campylobacteriosis, which causes infertility, abortions and embryonic death, and is mainly isolated from the genital tract (Nachamkin et al. 2008). *C. fetus* subsp. *venerealis* resides in the epithelial crypts of the prepuce and is transmitted to the cow by copulation or artificial insemination with the contaminated semen. On the other hand, *C. fetus* subsp. *fetus* is a commensal bacterium of the gastrointestinal tract of cattle and sheep. It can be associated with an infertility syndrome in cattle and abortions in sheep (Irons et al. 2004).

Although *C. fetus* is an animal health problem, little has been studied regarding its pathogenicity mechanisms. However, considering the pathogenesis of infection by this organism, it must possess characteristics that allow it to colonize or invade tissues and evade the immune system response. Several virulence factors, such as adhesins, secretion systems, and antiphagocytic layers, have been identified in the genome of *C. fetus* (Kienesberger et al. 2014). Nonetheless, it is still necessary to investigate *C. fetus* interactions with animal hosts.

When *C. fetus* reaches the genital tract of the cow, epithelial cells are the first cell type that it interacts with. These epithelial cells play important roles in innate immunity, such as acting as physical and immunological barriers, signaling the activation of the immune system through the production of cytokines and chemokines and inducing death in the infected cells (Farage et al. 2011).

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In previous works, it has been shown that *C. fetus* is able to adhere to and invade human epithelial cells; for example, Graham (2002) determined that different strains of *C. fetus* subsp. *fetus* isolated from human, and the *C. fetus* ATCC 27374 strain isolated from cattle, adhered to 41.3–87.3% and were internalized within 25.2–34.6% of INT 407 cells. Additionally, Baker and Graham (2010) demonstrated that *C. fetus* subsp. *fetus* can invade and translocate into Caco-2 cells. Chiapparrone et al. (2014; 2016) demonstrated the adhesion mediated by flagella to MDBK cells and adhesion to different parts of the sperm cells in *C. fetus* subsp. *venerealis*. Even though it has been described the ability of *C. fetus* to adhere to bovine cells, the ability of invasion to bovine endometrial epithelial cells of the bovine-adapted *C. fetus* strains has not yet been described. In this work, the ability of *C. fetus* to adhere and invade into bovine endometrial epithelial cells is evaluated.

Experimental

Materials and Methods

Bacterial strains and culture conditions. *C. fetus* ATCC 27374 has been described previously (Salama et al. 1995). *C. fetus* was grown on the *Campylobacter* selective agar supplemented with 5% sheep blood at 37°C for 48 h in an anaerobic chamber under the microaerophilic conditions. *Salmonella enterica* subsp. *enterica* serovar Typhimurium ATCC 14028 has been previously described (Trüper et al. 2005). *S. enterica* Typhimurium was grown on the Luria Bertani agar and later inoculated in the hyperosmolar Luria Bertani Broth at 37°C for 12 h. *Escherichia coli* EPEC was donated by Dr. Jose Luis Puente from the Instituto de Biotecnología, Universidad Nacional Autónoma de México.

Primary endometrium epithelial cell cultures. Epithelial cells from the endometrium were recovered using the protocol by Skarzynski et al. (2000) with some modifications. The uterus was removed from five sacrificed cows 15 min after exsanguination. A piece of the uterus, 5 cm^2, was removed and washed three times in Hank's solution supplemented with 1.6 mg/ml gentamicin and transported to the laboratory in the same solution on ice. Serosa was removed from the tissue, and the rest was cut into small pieces (approximately 3 mm^2) and washed 3 times with pH 7.2 phosphate buffered saline (PBS) (NaH_2PO_4 1.9 mM, Na_2HPO_4 8.1 mM, NaCl 154 mM). Then, digestion solution (0.5 mg/ml collagenase Type I from *Clostridium histolyticum* (Sigma-Aldrich); 0.1 mg/ml DNAse (Thermo Fisher); 100 µg/ml gentamycin (Sigma Aldrich); in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (DMEM-S) was added and incubated at 37°C with oscillation for 2 h. The supernatant was recovered and centrifuged at 4000 × g for 10 min. The pellet was washed three times with DMEM:PBS (1:1). The pellets were resuspended in 5 ml of DMEM-S and filtered with a 40 µm strainer. The recovered cells were placed on the cell culture dishes with DMEM-S with 50 mM HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) and an antibiotic/antifungal (penicillin G 10 000 U, streptomycin 5 000 µg, and amphotericin B 12.5 µg). For fibroblast depuration, one-minute trypsinisation was performed every day for three days (Munson et al. 1988). The cell cultures were evaluated by RT-PCR and immunofluorescence to confirm that they were endometrial epithelial cells. First, the total RNA was extracted from cells using TRIzol following the manufacturer's methodology. Then, cDNA was synthesized using an AMV First Strand cDNA Synthesis Kit (New England Biolabs Inc.) following the manufacturer's instructions. The primers for Keratin 8 were as follows: forward 5'-CGTGTCAGAAATCTGAGACTGC-3' and reverse 5'-TGGTGGAGGACTTYAAGACC-3'. The PCR mixture was prepared with primers (40 nM), Master Mix (Fermentas) and 100 ng cDNA. The PCR conditions were as follows: pre-heat at 95°C for 5 min; 30 cycles of denaturalization at 94°C for 30 s, annealing at 60°C for 30 s and extension at 72°C for 20 s. The PCR products were evaluated by electrophoresis in 2% agarose gels stained with ethidium bromide. For immunofluorescence, a polyclonal antibody against bovine cytokeratin 18 (Santa Cruz Biotechnology) was used in the cell cultures fixed in plates.

C. fetus PCR confirmation. Three pairs of primers for the *asp*A, *gln*A, *glt*A and 16sRNA genes were designed. Before each assay, PCR was performed to confirm the purity of *C. fetus* cultures. The primer sequences and product sizes were as follows: *aspA*: F-5'-CCTATGACTTTAGGTCAAGAG-3', R-5'-TGTAG CTAGAGTACGGCAAG-3' (575 bp); *gltA*: F-5'-CGA-TATAGCGTGGCTAGCTG-3', F-5'-AGCGTGAGTAG ATCCTACG-3' (520 bp); *glnA*: F-5'-CTTCCGTTATC TCCATAAAGC-3', R-5'-GATGGTAGTTCTATAGA GGC-3' (649 bp); 16sRNA: F-5'-GAGATCACCAGGA ATACCC-3', R-5'-CACCTGTCTCAACTTTCTAGC-3' (351 bp). For these primers, the PCR conditions were a pre-heat of 95°C for 1 min, then 30 cycles denaturation at 94°C for 30 s, annealing at 50°C for 30 s and extension at 72°C for 30 s.

Cell adhesion assays. The adherence was evaluated by quantitative and qualitative methods. For quantitative evaluation, three independent adherence assays with three replicates were performed following the method described by Bacon et al. (2001). First, *C. fetus* was grown on the *Campylobacter* selective agar supplemented with 5% sheep blood for 48 h and harvested in PBS. *Escherichia coli* EPEC was used as an adhesion

control and was grown in hyperosmolar LB for 12 h. The bacterial inoculum was determined by spectrophotometry and plating. The multiplicity of infection (MOI) was 1000:1. The infected cell cultures were centrifuged at 165 × g for 3 min to maximize bacteria-cell contact and incubated for 1 h at 37°C. The percentage of adhered bacteria was calculated by the formula [(CFU intracellular / CFU inoculum) × 100]. For visual interpretation, cells were fixed with methanol and stained with 100% Giemsa for 40 min.

Intracellular survival assays. Three independent assays with three replicates per time were performed as described by Elsinghorst (1994) with some modifications. First, *C. fetus* was grown on the *Campylobacter* selective agar supplemented with 5% sheep blood for 48 h and harvested in PBS. To prepare the inoculum, bacteria were quantified by spectrophotometry and diluted in DMEM to an adjusted MOI of 1000:1. Epithelial cells of the endometrium were seeded at 40 000 cells per well in a 48-well dish. After washing three times with PBS, the epithelial cells were infected. The plates were centrifuged at 165 × g for 3 min to maximize bacterial-cell contact and incubated for 2 h at 37°C. Following incubation, the monolayers were washed three times with PBS and incubated with DMEM supplemented with 10% fetal bovine serum, 50 mM HEPES and 30 µg/ml gentamicin. The plating of the samples of the medium confirmed the complete killing of the extracellular *C. fetus.* Bacteria were recovered at 0, 2, 4, 10 and 24 h post-infection (p.i.); supernatant medium was removed, and the intracellular bacteria were recovered by adding 1 ml of Triton X100 1% for 5 min, and the solution was homogenized and added to a microcentrifuge tube. Fifty microliters of each well were plated on the *Campylobacter* selective agar supplemented with 5% sheep blood at 37°C for 72 h in an anaerobic chamber under low oxygen conditions (Oxoid Campy Gen, catalogue number CN0025A). *S. enterica* Typhimurium was used as a positive control of infection because they can invade the bovine reproductive tract (Hall and Jones 1977).

Cytoskeleton inhibition assays. To establish the possible mechanism involved in *C. fetus* invasion in these epithelial cells, cytoskeleton inhibition assays were performed. Cytochalasin D (Sigma-Aldrich) was prepared as a 1 mM stock in dimethyl sulfoxide, and nocodazole was prepared as a 10 mM stock in dimethyl sulfoxide. Gentamicin protection assays were performed as described above, with some modifications. Forty thousand cells per well were plated in a 48-well dish. After washing three times with PBS, DMEM with cytochalasin D (3 mM) or nocodazole (30 mM) was added to the wells, and the cells were incubated for 30 min at 37°C. Then, bacteria were added, and the inhibitor concentration was kept at the half during the infection. The plates were centrifuged at 165 × g for 3 min to maximize the bacteria-cell contact and incubated at 37°C. *C. fetus* infection lasted for 2 h with an MOI of 1000:1 and *Salmonella* infection for 15 min with an MOI of 50:1. After infection, the inoculum was removed, and cells were washed three times with PBS and DMEM supplemented with 10% fetal bovine serum, Hepes 50 mM, and 30 µg/ml gentamicin. Cells were lysed with 50 µl of Triton X100 1% in each well and plated on the *Campylobacter* selective agar supplemented with 5% sheep blood at 37°C for 72 h in an anaerobic chamber under low oxygen conditions (Oxoid Campy Gen, catalogue number CN0025A).

Immunofluorescence Microscopy. Epithelial cells from bovine endometrium were infected as described above. At 0 and 2 h p.i., cells were washed three times with PBS and fixed with methanol for 4 min at 4°C. The fixed cells were washed three times with PBS and permeabilized by incubation in PBS containing 0.1% saponin for one min. Then, coverslips were incubated with 50 µg/ml phalloidin-FITC diluted with 1% dimethyl sulfoxide (DMSO) for 1 h at room temperature. The coverslips were washed three times with saponin 0.05% and PBS. After that, coverslips were incubated with primary antibodies (rabbit anti-*C. fetus*) diluted 1:1000 (saponin 0.05%, 5% horse serum, PBS) for an hour at 37°C. Afterward, the coverslips were washed three times with saponin 0.05% and incubated with secondary antibodies (goat anti-rabbit (US Biological) and diluted with Alexa Fluor 594 to 1:1000 for 30 min at room temperature. After three washes with 0.05% saponin, coverslips were mounted onto glass slides. Images were acquired with a Zen Zeiss LSM800 fluorescence microscope.

Statistical analysis. Each assay was subjected to a Shapiro-Wilks test and a Welch's test. Then, to determine the time at which the intracellular bacterial count was different in each treatment, Student's T-test was used.

Results

***C. fetus* adheres to epithelial cells of bovine endometrium.** To confirm that *C. fetus* could adhere to epithelial cells from the endometrium, an adherence assay was performed. First, endometrial epithelial cell cultures were established from cells isolated from uterus tissue obtained from slaughtered cows. The cells established a monolayer until the second week of incubation when these cells presented a polygonal epithelial-like form (Fig. 1A). These cells were used until the seventh passage since later splits presented different morphologies and diminished cell proliferation rates. For cell type confirmation, cytokeratin 18 from epithelial cells was immunostained with Alexa 488 (Fig. 1B), and RT-PCR for keratin 8 was performed (Fig. 1C). The results obtained

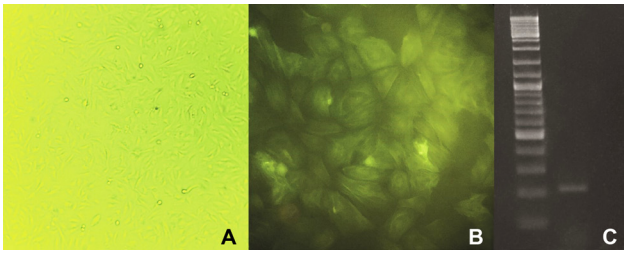

Fig. 1. Epithelial cells from bovine endometrium maintained in DMEM supplemented with 10% fetal bovine serum.

A) Normal appearance of the epithelial-like cells (20×). B) Immunofluorescence. Cytokeratin 18 of epithelial cells of bovine endometrium stained with Alexa 488 (green) (40×). C) The results of RT-PCR for keratin 8 on 2% agarose gel after staining with ethidium bromide. In left lane: DNA ladder (Thermo Fisher Scientific); right lane: the amplicon of keratin 8 (215 pb).

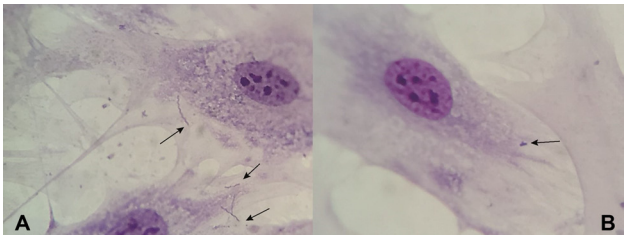

Fig. 2. The adherence assay. Epithelial cells from bovine endometrium were challenged with *C. fetus* (A) or *E. coli* (B) for 1 h, fixed with methanol and stained with Giemsa (100×). The arrows show the adhered bacteria.

show that cells in culture corresponded to endometrial epithelial cells. In the adherence assays, the average percentage of epithelial cell-adhered bacteria was 0.13%. Adhered *C. fetus* was observed by microscopy (Fig. 2A). *E. coli* EPEC showed a typically localized adherence pattern (Fig. 2B). These results confirm that *C. fetus* can adhere to endometrial epithelial cells.

***C. fetus* invades bovine endometrial epithelial cells.** After the adherence capacity of *C. fetus* was confirmed, a gentamicin protection assay was carried out to determine the intracellular survival of *C. fetus* in epithelial cells from bovine endometrium. The results obtained with MOI of 1000:1 showed a decrease in the number of *C. fetus* viable cells. At 0 h p.i there were an average (~) of 3795 CFU, at 2 h p.i they decreased to ~ 163 CFU, at 4 h there were ~ 36 CFU and 10 h p.i the organisms didn't grow. No viable cells were recovered at 24 h p.i., suggesting that the bacteria have the capacity to enter the cells, but it might not survive inside epithelial cells of the endometrium (Fig. 3). *Salmonella* invaded the bovine endometrial epithelial cells used in this study. At 0 h, the bacteria were present inside the cells ~ 8430 CFU, and at 4 h they started to replicate, increasing to ~ 14 585 CFU showing normal intracellular infection behavior in bovine epithelial cells (data not show). The presence of *C. fetus* inside the cells was confirmed by confocal microscopy. Before immuno-

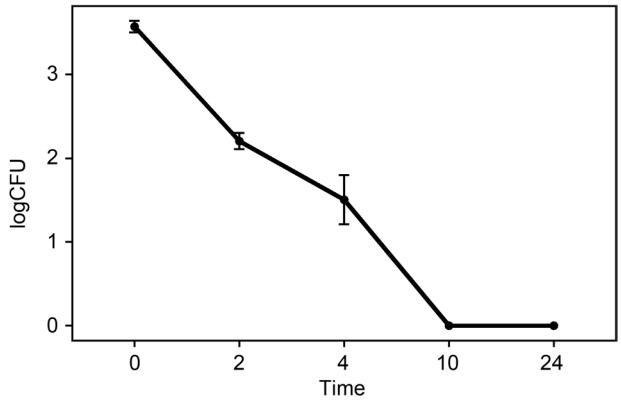

Fig. 3. The intracellular survival assay. Epithelial cells of endometrium of bovine were infected with *C. fetus* ATCC 27374 (a MOI of 1000:1). The intracellular bacteria were recovered and plated on the Campylobacter selective agar supplemented with 5% of blood. Average log CFU are shown at 0, 2, 4, 10 and 24 h p.i.

fluorescence, rabbit anti-*C. fetus* serum was adsorbed to avoid unspecific labeling and tested by Western blotting (Fig. S1, supplemental material). Additionally, the secondary antibody specificity and anti-rabbit HRP antibodies were tested by Western blotting. The results obtained in the Western blots, with a total bacterial protein extract and total cell protein extract, showed that adsorbed anti-*C. fetus* antisera recognized only antigens in *C. fetus* and not in epithelial cells, and the anti-rabbit antibodies did not recognize antigens in bacteria or epithelial cells (Fig. S1, supplemental material). The confocal fluorescence at 2 h p.i. showed the

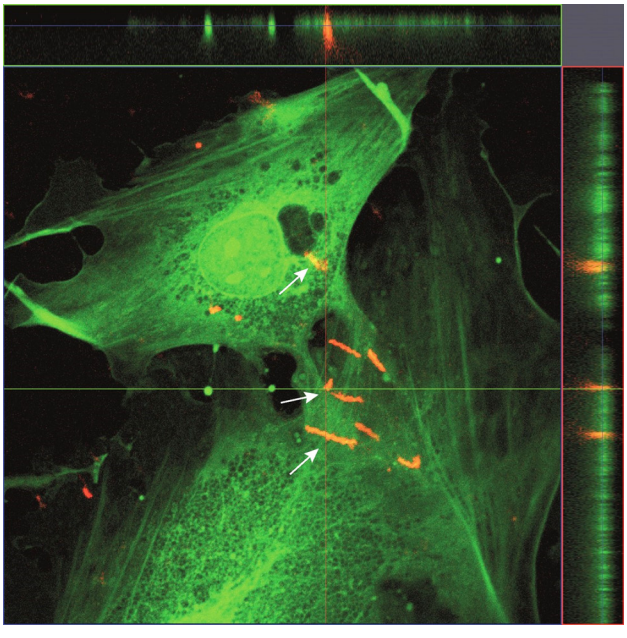

Fig. 4. Confocal differential fluorescent staining of internal *C. fetus* ATCC 27374 on the infected epithelial cells of bovine endometrium. Epithelial cells were grown on coverslips and infected with *C. fetus* ATCC 27374 at a MOI of 1000:1. Cytoskeleton was stained with phalloidin-FITC (green), and the bacteria with Alexa 594 (red) 2 h p.i. White arrows show intracellular *C. fetus* (70×).

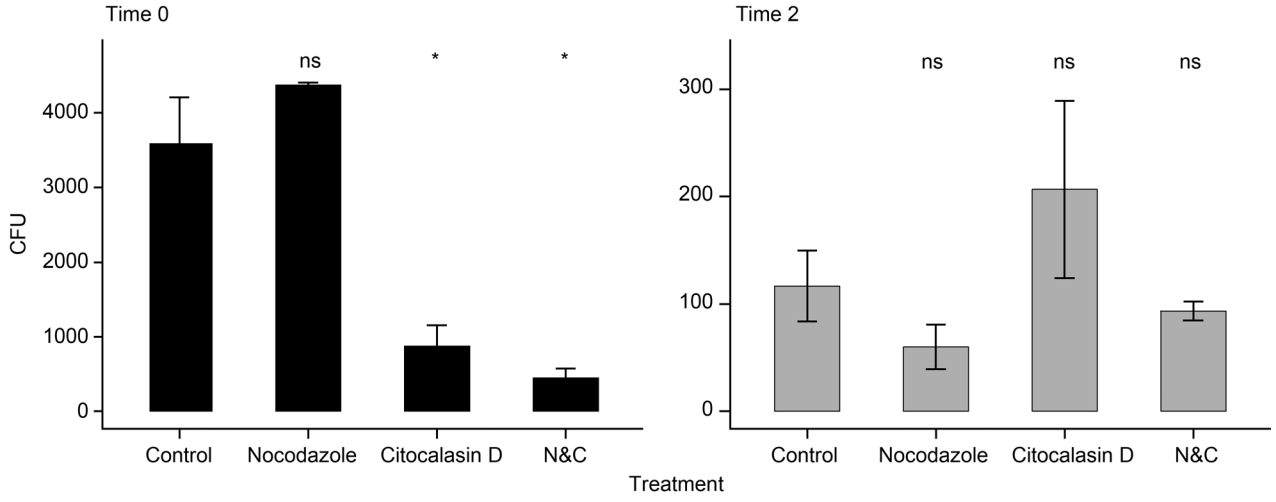

Fig. 5. The cytoskeleton inhibition assay. Epithelial cells of endometrium of bovine were treated with cytocalasin D or nocodazole before and during infection. The cells were infected with *C. fetus* ATCC 27374 (a MOI of 1000:1). The intracellular bacteria were recovered and plated on the Campylobacter selective agar supplemented with 5% of blood. Average log CFU are shown at 0 and 2 p.i. T-test was performed. All treatments were compared to the not-treatment control, *($p < 0.001$).

intracellular bacteria, demonstrating that *C. fetus* can invade epithelial cells from bovine endometrium but is not able to proliferate inside of them (Fig. 4).

***C. fetus* uses actin to invade bovine endometrial epithelial cells.** Internalization of *C. fetus* was modified when the cytoskeleton of the endometrium epithelial cells was altered. Treatment with cytochalasin D decreased the capability of internalization of *C. fetus* (~ 873 CFU). There was no change in *C. fetus* internalization when using nocodazole (~ 4330 CFU) suggesting that actin plays an important role in this process (Fig. 5).

Discussion

The invasion of *C. fetus* in some human cell lines, such as Hep-2 and Caco-2 has already been described (Konkel and Joens 1989; Baker and Graham 2010). Although these results suggest the ability of this microorganism to invade cells, the interaction with endometrial epithelial cells, the first type of cell that interacts with *C. fetus*, has not been described. In this work, an intracellular survival assay including gentamicin protection and immunofluorescence showed that *C. fetus* can invade the bovine endometrial epithelial cells, this result relates with the ones reported in the literature, which shows that *Campylobacter* spp. is able to invade cells (Konkel and Joens 1989; Graham 2002; Baker and Graham 2010).

The use of primary cultures for the evaluation of virulence of different pathogens has been widely reported. Most studies investigating the relationship between the host and bacteria, tend to focus on the cell types that comprise the biological barriers, signaling events within the host and the virulence factors of the pathogen, which are involved in the initial phase of the disease (Benjamin et al. 2011). In this work, a primary culture of bovine endometrial epithelial cells was established, considering that these cells could be the first in contact with *C. fetus*. The primary cultures showed a positive reaction against cytokeratin 18. The expression of this protein has been described in bovine endometrial epithelial cells (Haeger et al. 2015).

Cell culture models can compartmentalize and define the broad range of molecular mechanisms that underlie strategies of microbial virulence such as host receptor ligand binding and invasion mechanism. These mechanisms have not been identified in *C. fetus*; however, in this work, its ability to adhere to bovine cells was shown. The pathogenicity factors that could mediate this adhesion could be diverse. McSweegan and Walker (1986) described the role of lipopolysaccharide as a molecule that allows the adhesion of *C. jejuni* to INT407 cells. Monteville et al. (2003) identified a *C. jejuni cad*F homologous gene in *C. fetus*, which encodes for an outer membrane fibronectin binding protein (Accession No. Nz_CP00880). This protein in *C. jejuni* binds to fibronectin (Konkel et al. 1997), which is expressed on the epithelial cells of the endometrium (Mularoni et al. 1992). In *C. fetus* Moolhuijzen et al. (2009) have identified the *PEB1* gene, which participates in adhesion in *C. jejuni*. It has been described that *C. fetus* has a protein coat that may interfere in the contact with other cells (Yang et al. 1992). However, in this study, *C. fetus* was able to invade epithelial cells, so it is possible that the protein layer does not interfere, or it may exist some other mechanism, not yet described, for this bacterium that allows it to adhere the epithelial cells.

Intact epithelial surfaces are a highly effective barrier to evade invasion by pathogens. A capability to

disrupt intact epithelial surfaces is an important characteristic for many specialized bacterial pathogens. In this work *C. fetus* showed the capability to invade cells; however, internalization mechanisms have not been described for these species. *C. fetus* possesses a type IV secretion system (Kienesberger et al. 2014), which is used by many pathogens for substrate translocation, for example in *Bartonella henselae* this system translocates BepC and BepF, the proteins factors that trigger invasome-mediated internalization (Truttmann et al. 2011). In *C. fetus,* Ali et al. (2012) identified a CiaB homologous, which in *C. jejuni* is translocated through flagellum and is required for internalization (Konkel et al. 1999). More research is required in order to identify the virulence mechanisms that mediate cellular invasion.

The previous reports have demonstrated that *C. jejuni* make use of microtubules to invade epithelial cells (Oelschlaeger et al. 1993; Hu and Kopecko 1999). In this work, it was observed that when actin polymerization was inhibited, *C. fetus* could not be internalized in the same way as the control, suggesting that actin plays an important role in the internalization mechanism, too. Moreover, Baker and Graham (2010) showed that cytochalasin D treatment on Caco-2 epithelial cells was not able to inhibit *C. fetus* internalization. Those results differ from the ones obtained in this work, the treatment with cytochalasin D of our primary culture did not inhibit totally the invasion of *C. fetus*, it only reduced it. This difference could be explained by the treatment conditions with cytochalasin D or by the incubation time of the bacteria in the cellular infection assays.

Although in this work was shown that *C. fetus* had the ability to invade cells, its intracellular survival was minimal (at 10 hours post infection the number of the intracellular bacteria had decreased significantly). Treatment of the cells with nocodazole, a drug that inhibits endosome-lysosome fusion (Funato et al. 1997), did not help *C. fetus* to survive inside the cell. This suggests that their inability to persist within cells could be explained by metabolic adaptations for the intracellular environment and not necessarily by lysosomal degradation. The intracellular bacteria require the metabolic adaptations to remain alive in the harsh intracellular environment. For example, *S. enetrica subsp.* Typhimurium shows upregulation of glycolysis and the Entner-Doudoroff pathway during the vacuolar stage (Eisenreich et al. 2015). *Campylobacter* spp. does not ferment carbohydrates because it lacks phosphofructokinase and essential enzymes for the Entner-Doudoroff pathway, and its principal source of carbon are amino acids (Kelly 2008). *Legionella pneumophila* also lacks the glycolysis pathway and uses amino acids as a carbon source, but unlike *Campylobacter*, it uses the Entner-Doudoroff pathways as an important carbon source (Eisenreich et al. 2015).

Another explanation that could help understanding why in the intracellular survival assay *C. fetus* was internalized and eliminated from the cells, is the metabolic reprogramming, which does not allow *C. fetus* to grow on agar plates after having gone through the intracellular stage. In *C. jejuni*, during its intracellular stay, the respiration is reprogrammed, favoring the use of fumarate and reducing the expression of enzymes of aerobic respiration (Liu et al. 2012). In this way, when bacteria are recovered in artificial media, their growth is reduced. This explanation could be the least likely, however, in the work done by Watson and Galán (2008) this phenomenon was found.

The invasive nature of *C. fetus* has been well established *in vivo* in clinical veterinary situations and *in vitro* with human intestinal epithelial cells assays. Baker and Graham (2010) showed the translocation of *C. fetus* through the barriers of intestinal epithelial cells with the culture of Caco-2 cells. Louwen et al. (2012) also showed a similar phenomenon with *C. jejuni* in Caco-2 cells. Therefore, the short permanence of viable *C. fetus* in the endometrial culture cells could be interpreted as normal, considering that the only objective of *C. fetus* is to be translocated.

There are many studies on *Campylobacter* spp. invasion capability (Mooney et al. 2003; Watson and Galán 2008), however, this report presents the ability of *C. fetus* to invade bovine endometrium epithelial cells. This could be used in future work as a bovine infection model and be an important element for understanding the pathogenicity mechanisms of *C. fetus*.

C. fetus could have been originated as a pathobiont in humans and jump to bovine as their host, generating adapted strains. The bovine strains contain in their genome the unique accessory genes (virulence factors) not seen in human strains (Iraola et al. 2017). This would explain why *C. fetus* can persist in the intestine of humans (Lastovica and Skirrow 2000) and may be associated with infertility and sporadic abortions in cattle and sheep (Irons et al. 2004). The *C. fetus* strain used in this work was isolated from a clinical case of an aborted calve, and it is known to cause abortions in cattle (Smith and Taylor 1919; Véron and Chatelain 1973). Graham et al. (2002; 2010) used this strain in some invasion assays using human cell cultures.

In conclusion, this work showed that *C. fetus* adheres and invades bovine endometrial epithelial cells. Gentamicin protection assays and fluorescence microscopy suggest that *C. fetus* can survive inside cells for only a few hours (4 h). Additionally, *C. fetus* is internalized using an actin-dependent mechanism in this cell type. All knowledge generated in this area will serve to propose and develop new strategies for the control of pathogens.

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Conflict of interest

The authors do not report any financial or personal connections with other persons or organizations, which might negatively affect the contents of this publication and/or claim authorship rights to this publication.

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