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Comparative *ex vivo* infection with *Trypanosoma cruzi* and *Toxoplasma gondii* of human, canine and ovine placenta: Analysis of tissue damage and infection efficiency

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ABSTRACT

Trypanosoma cruzi, the causative agent of Chagas disease, and *Toxoplasma gondii*, which is responsible for Toxoplasmosis, are two parasites that cause significant protozoan zoonoses and consequently important economic losses in human, companion animals and livestock. For the congenital transmission to occur, both parasites must cross the barrier present in the mammalian placenta, which differs between species. Particularly, hemochorial, endotheliochorial and epitheliochorial placental barriers are present, respectively, in human, dog and sheep. The type of placental barrier has been associated with the probability of transmission of pathogens. In this study, we used experimental placental *ex vivo* infection models of *T. cruzi* and *T. gondii* in the above-mentioned mammals in order to study tissue alterations and to compare infection efficiency. Here, we infected placental term explants from human, dog and sheep and analyzed tissue damage by standard histological and histochemical methods. Comparative infection efficiency was determined by quantitative PCR. Both parasites are able to infect the different placental explants; however, more *T. gondii* parasites were detected, and *T. gondii* causes a more severe tissue damage in human and canine explants than *T. cruzi*. The histopathological changes observed in ovine placenta explants were similar in presence of both parasites. We conclude that the infection efficiency of *T. gondii* is higher, compared to *T. cruzi*, during the *ex vivo* infection of human, canine and ovine placental explants.

In addition, the *ex vivo* infection of mammalian placental explants constitutes an interesting experimental approach to study part of the infection mechanisms as well as host responses during congenital infection of both parasites.

1. Introduction

Trypanosoma cruzi (*T. cruzi*), the causative agent of Chagas disease, and *Toxoplasma gondii* (*T. gondii*), which is responsible for Toxoplasmosis, are two parasites that cause significant protozoan zoonoses [1,2] and consequently critical economic losses in human, companion animals and livestock [3–6]. Chagas disease is a devastating but neglected health problem in Latin America, but it is present in other non-endemic countries due to extensive global migration and congenital transmission [7]. On the other hand, *T. gondii* is one of the most successful parasites on Earth that is responsible for the infection of more than one billion people worldwide [8,9].

For the congenital transmission to occur, both parasites must cross the barrier present in the mammalian placenta [7,10,11]. In eutherian mammals, the placenta is a transitory organ that invades the maternal uterus to provide nutrition and gas exchange for the developing fetus and ensures adequate growth and development of the conceptus while supporting pregnancy-related changes in maternal physiological systems. The chorioallantoic is the main placenta in mammals during middle to late gestation and develops from the endometrium of the uterus and the trophoblast of the embryo. According to the extent of trophoblast invasion into the uterus, placentation is classified into hemochorial (highly invasive), endotheliochorial (moderate invasive) or epitheliochorial (low invasive) [10,12]. The human placenta is

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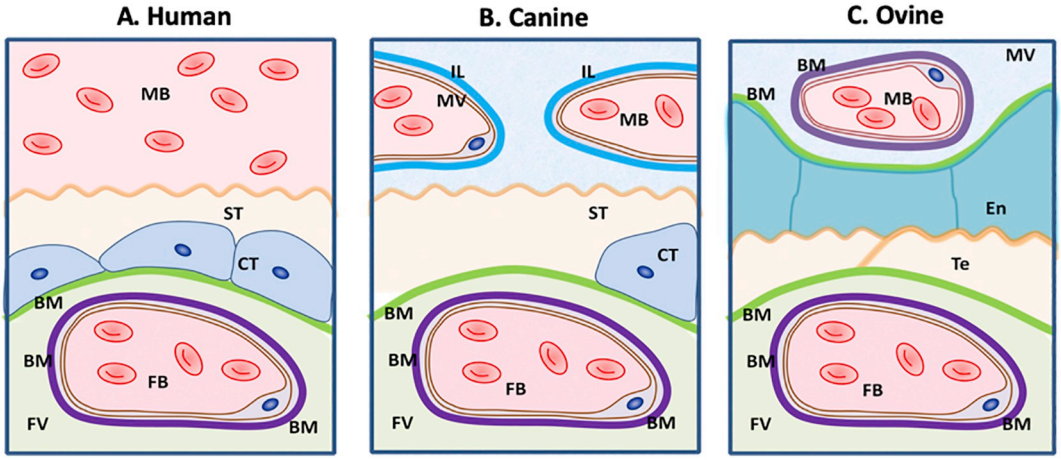

Fig. 1. Human, canine and ovine placental barriers: (A) The human placental barrier is formed by the trophoblast (a two layered epithelium: cytotrophoblast and syncytiotrophoblast), basal membranes, villous stroma and fetal capillary endothelium. (B) The canine placental barrier is located in the labyrinth zone and consists of the maternal endothelium, interstitial lamina, trophoblast, basal membranes and endothelium of the fetal capillaries. (C) The ovine placental barrier is composed of six tissue layers: maternal capillary endothelium, maternal endometrial connective tissue, maternal endometrial epithelium, trophoblast, chorionic connective tissue and fetal endothelium. MB: Maternal blood; ST: Syncytiotrophoblast; CT: cytotrophoblast; BM: basal membrane; Te: trophectoderm; Cy, cytotrophoblast; FB: fetal blood; FV: fetal vessel; En: endometrium; IL, interstitial lamina; MV: maternal vessel. The tissues that form the different placental barriers are summarized in Table 1.

hemochorial and is formed by a fetal portion, which originates from the *chorion frondosum*, and a maternal portion, or *basal decidua*, which develops from the endometrium. The functional units are the floating chorionic villi, that are formed by the trophoblast (a bi-stratified epithelium composed by syncytiotrophoblast and cytotrophoblast) and the villous stroma. The trophoblast comes into contact with maternal blood in the intervillous space and is delimited by a basal membrane from the villous stroma, which is the fetal connective tissue containing the fetal capillaries.

Therefore, the trophoblast, basal membranes, villous stroma and fetal capillary endothelium form the human placental barrier (Fig. 1A) [7,10]. The canine placenta is endotheliochorial, and three zones can be identified: labyrinth, junctional and glandular. The placental barrier is located in the labyrinth zone and consists of maternal endothelium, interstitial lamina, trophoblast, individual or conjoint basal membrane as well as the endothelium of the fetal vessels, which deeply indent the trophoblast (Fig. 1B) [10,12,13]. The interstitial lamina is a noncellular layer that surrounds the maternal vessels and is equivalent in ultrastructure and molecular composition to the basal membrane of the maternal endothelium [13]. The ovine placenta is epitheliochorial, and the fetal component is formed by the fusion of the avascular chorion and the vascular allantois. The placental barrier is composed by six tissue layers: maternal capillary endothelium, maternal endometrial connective tissue, maternal endometrial epithelium, trophoblast, chorionic connective tissue and fetal endothelium (Fig. 1C) [13,14]. The different tissues that form the different placental barriers are summarized in Table 1.

The type of placental barrier has been associated with the probability of transmission of pathogens [15]. Interestingly, the congenital transmission rate of *T. cruzi* in human (≈ 1–12%) and dog (10%) is low [7,16]. There is no information available about the congenital transmission rate in sheep. In contrast, the transmission rate for *T. gondii* is high in humans (≈50–70%) [17], and this form of infection is a significant cause of reproductive failure in sheep [18]. There are very scarce data in the literature about the congenital transmission rate for *T. gondii* in dogs [19]. However, the seroprevalence is high (≈ 30%) [20], suggesting that there could be a significant probability of congenital transmission as well as in human and sheep.

Placental tissue explants, particularly those from human, have been widely used in biomedical studies, including infection with both parasites [21–23]. However, there are no comparative studies regarding the infection of both parasites in different types of placenta. In spite that the *ex vivo* infection condition are not the same than *in vivo*, i. e. during congenital infection the parasites reach the placental barriers through the maternal circulatory system, it constitutes an interesting tool to study some of the infection mechanisms parasites as well as local placental responses [23,24]. Here we show histopathological analysis, collagen histochemistry and parasite DNA quantification in *ex vivo* infected human, canine and ovine placental explants with *T. cruzi* and *T. gondii*. Both parasites are able to infect the different placental explants and cause severe tissue damage, but the *ex vivo* infection efficiency of *T. gondii* is higher.

Table 1
Tissues that form the different anatomical barriers in human, canine and ovine placenta.

Tissues	Type of placental barrier		
Maternal tissues	Hemochorial (Human)	Endotheliochorial (Canine)	Epitheliochorial (Ovine)
Endothelium	–	+	+
Connective tissue	–	–	+
Epithelium	–	–	+
Fetal tissues			
Trophoblast/ Trophoectoderm	+	+	+
Connective tissue	+	+	+
Endothelium	+	+	+

2. Materials and methods

2.1. Cell cultures

VERO cells (ATCC® CCL-81) were grown in RPMI medium supplemented with 5% fetal bovine serum (FBS) and antibiotics (penicillin-streptomycin). Human foreskin fibroblasts (HFF) (ATCC® SCRC-1041) were grown in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% FBS and antibiotics. Both cell cultures were maintained at 37 °C in a humidified atmosphere with 5% CO_2 and with a replacement of the culture medium every 24–48 h [21].

2.2. Parasite culture and harvesting

T. cruzi: Semi-confluent VERO cells were incubated with epimastigotes of *T. cruzi* (Ypsilon strain) harvested in the late stationary phase. Trypomastigotes also present in that culture invade VERO cells and replicate intracellularly as amastigotes. After 72 h, amastigotes transform back into trypomastigotes which lyse the host cells. Infective trypomastigotes were recovered in the supernatant by low-speed centrifugation (500 xg). *T. gondii:* Semi-confluent HFF cells were infected with *T. gondii* RH tachyzoites (kindly provided by Dr. Sergio Angel, IIB-INTECH, CONICET-UNSAM, Chascomús, Buenos Aires, Argentina) at a multiplicity of infection of 3 to 5 parasites per cell. After 40 h of infection, infected cells were washed and monolayers were scraped from the flasks and passed through 20-, 23-, and 25-gauge needles. Infective tachyzoites were purified from host cell debris with a 3.0-μm Isopore filter (Merck Millipore) [21,25].

2.3. Placental explant cultures and parasite infection

Human, canine and ovine term placentas were obtained from pregnancies with normal fetuses. Exclusion criteria were maternal, fetal or placental pathologies. Maternal blood from the placentas was analyzed for the presence of anti-*T. cruzi* and anti-*T. gondii* antibodies by rapid IgG/IgM ELISA detection kits according to the manufacturer's instructions. Human, canine and ovine placentas were obtained from "Hospital San José, Servicio de Salud Metropolitano Norte", Santiago; "Red de Atención Veterinaria, Universidad de Chile (RAV)" and "Estación Experimental Germán Greve Silva de la Facultad de Ciencias Agronómicas, Universidad de Chile", respectively. The respective ethics committees gave their approval (N° 041–2011, human placenta; N° 16–2016, animal placentas). The organs were collected in cold, sterile, saline-buffered solution (PBS) and processed no more than 30–60 min after delivery. Human placenta explants (HPE) were obtained from the central part of the placental cotyledon, and maternal and fetal surfaces of each placenta were discarded in order to obtain explants that contain the free floating chorionic villi [23]. Canine placenta explants (CPE) were obtained from the labyrinth zone of the canine placenta. Regarding the ovine placenta explants (OPE), it is important to point out that the explants were obtained from the fetal cotyledon of the sheep placentome, which therefore lacked the maternal caruncle. The isolated tissues were washed with PBS to remove blood, cut in approximately 0.5 cm^3 pieces and co-cultured with *T. cruzi* trypomastigotes or *T. gondii* tachyzoites (1×10^5/ml) in 24-well plates of RPMI culture media supplemented with inactivated FBS (10%) and antibiotics for 24 h. Additionally, CPE and OPE were co-cultured for 2, 6 and 12 h (Supplementary Fig. 1), and reproductible tissue alterations were evident at 24 h of incubation. All experiments were performed in triplicate in at least three different placentas of each specie.

2.4. DNA amplification by real-time PCR

Genomic DNA was extracted from the placental tissue with the Wizard Genomic DNA Purification Kit (Promega®) according to the manufacturer's instructions and quantified by QUBIT Fluorometric System (Invitrogen®). Before DNA extraction, the different explants were washed, at least ten times in order to assure the elimination of parasites in the culture media or attached to the placentas. For amplification parasite DNA, specific pairs of primers were used. Thus, for *T. cruzi* DNA detection, a 182 bp sequence of satellite DNA was amplified by using TCZ primers: TCZ-F 5'-GCTCTTGCCCACAMGGGTGC-3′ and TCZ-R 5'-CAAGCAGCGGATAGTTCAGG-3′ [26]; for *T. gondii* DNA detection, a 98 bp sequence of the *T. gondii* B1 gene was amplified by using TOXO-F:5´-AGCGTTCGTCCTCAACTATCGATTG-3′ and TOXO-R:5´-TCCCCTCTGCTCGCGAAAAGT-3 primers [27]. Each reaction mix contained 200 nM of each primer (forward and reverse), 1 ng of DNA from samples, 12.5 μL of SensiMix® SYBR Green Master Mix (Bioline®) and H_20 for a total of 25 μL. Amplification was performed in an ABI Prism 7300 sequence detector (Applied Biosystems®). The cycling programs were as follows: *T. cruzi*: This consisted of a first step at 20 °C for 2 min, a denaturation step at 95 °C for 10 min and 40 amplification cycles of 95 °C (15 s), 60 °C (15 s) and 72 °C (30 s). Finally, a dissociation stage was added ranging from 60 to 95 °C [28]. *T. gondii:* This consisted of a first step at 50 °C for 2 min, a denaturation step at 95 °C for 10 min and 40 amplification cycles of 90 °C (15 s), 60 °C (15 s) and 72 °C (30 s) following a dissociation stage ranging from 60 to 95 °C [27]. Standard calibration curves for determining the number of *T. cruzi* and *T. gondii* parasites per DNA detection were performed (Supplementary Fig. 2). Data was expressed as number of parasites/100 ng of tissue DNA [21,28–30].

2.5. Histological and histochemical techniques

The different placental explants were fixed in 10% formaldehyde in 0.1 M of phosphate buffer (pH 7.3) for 24 h, then dehydrated in alcohol, clarified in xylene, embedded in paraffin and sectioned at 5 μm. Paraffin histological sections were stained with hematoxylin-eosin for routine histological analysis and with picrosirius red–hematoxylin for collagen histochemistry. Then, ten fields were selected randomly from each sample. The histopathological damage and collagen histochemistry was scored [31] as described in Table 2. .

2.6. Statistics

All experiments were performed in triplicate in at least 3 placentas from each specie. Results are expressed as means ± S.D. The significance of differences was evaluated using Student's *t*-test for paired data or by ANOVA followed by Dunnett's post-test.

3. Results

3.1. Trypanosoma cruzi and Toxoplasma gondii infect human, canine and ovine placental explants

HPE, CPE and OPE were each co-incubated with 10^5 *T. cruzi* trypomastigotes or *T. gondii* tachyzoites for 24 h. *T. cruzi* (Fig. 2A) and *T. gondii* (Fig. 2B) parasite DNA can be detected in each of the different placental explants. Regarding *T. cruzi*, in HPE, an average of 68.4 ± 19.3 parasites/tissue were detected, which is significantly higher than the number of parasites identified in canine [15.3 ± 5.6 ($p \leq .0001$)] or ovine [52.4 ± 9.9 ($p \leq .05$)] placentas (Fig. 2A). The number of *T. gondii* parasites detected in the different placental explants were higher (over 100 parasites/tissue) than in those with *T. cruzi* (less than 100 parasites/tissue). In HPE, we detected an average of 346.6 ± 30.2 *T. gondii* parasites/tissue, which is significantly lower

Table 2
Scores for the analysis of the histopathological damage and collagen disorganization.

Score	Histopathological damage	Collagen disorganization
1	Attached trophoblast/trophoectoderm Intact fetal connective tissue	Strong collagen birefringence
2	Slight trophoblast/trophoectoderm detachment and/or fetal connective tissue disorganization	Moderate collagen birefringence
3	Almost complete detachment of trophoblast/trophoectoderm detachment and/or fetal connective tissue disorganization	Low collagen birefringence
4	Complete detachment of the trophoblast/trophoectoderm and disorganization or destruction of the fetal connective tissue	Absence of collagen birefringence

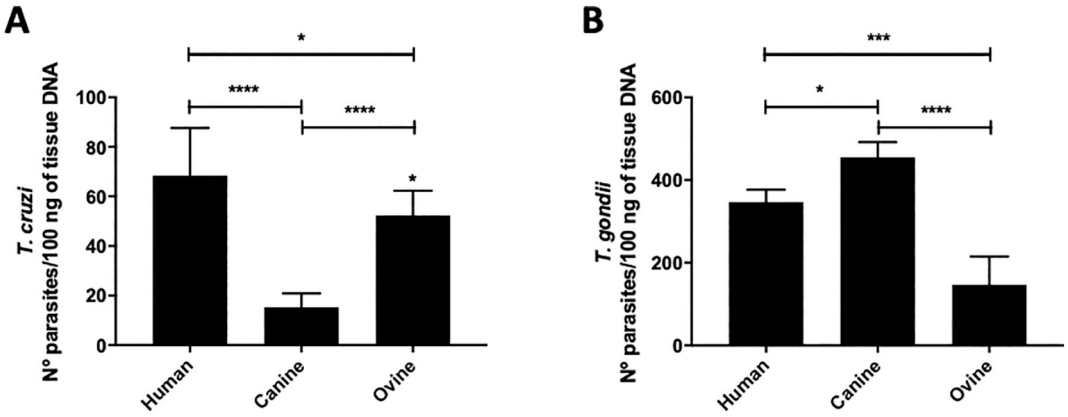

Fig. 2. *Trypanosoma cruzi* and *Toxoplasma gondii* infected human, canine and ovine placental explants: HPE, CPE and OPE were each co-incubated with 10^5 *T. cruzi* trypomastigotes (A) or *T. gondii* tachyzoites (B) for 24 h. Data was expressed as number parasites/100 ng of tissue DNA and analyzed analyzed by ANOVA followed by Dunnett's post-test. All values, given as the means ± S.D., correspond to at least 3 independent experiments performed in triplicate.* $p < .05$, *** $p < .001$, ****$p < .0001$.

than the parasites detected in canine [455.0 ± 36.9 ($p \leq .05$)] and higher than in ovine [146.8 ± 68.5 ($p \leq .001$)] (Fig. 2B).

The difference between parasites detected in canine and ovine placentas were also significant for *T. cruzi* and *T. gondii* (both, $p \leq .0001$). Fig. 3 shows the analysis of the amount of parasite equivalences between *T. cruzi* and *T. gondii*. Thus, the differences were statistically significant for HPE ($p \leq .0001$) (Fig. 3A), CPE ($p \leq .0001$) (Fig. 3B) and OPE ($p \leq .01$) (Fig. 3C).

3.2. *Toxoplasma gondii caused more severe tissue damage in human placental explants than Trypanosoma cruzi*

HPE were co-incubated with 10^5 *T. cruzi* trypomastigotes or with *T. gondii* tachyzoites for 24 h. Both parasites showed detachment and destruction of the trophoblast (Fig. 4 B–C, arrows) compared to control non-infected explants (Fig. 4 A). However, only *T. gondii*-incubated explants showed chorionic villi with complete detachment of the trophoblast (Fig. 4 C). The score of the histopathological damage increased, respectively, from 1.24 ± 0.25 (non-infected samples) to 2.89 ± 0.64 and 3.41 ± 0.35 ($p \leq .0001$) in *T. cruzi* and *T. gondii*-infected samples (Fig. 4D). The tissue damage was significantly higher in *T. gondii* infected HPE ($p \leq .001$). Collagen histochemistry (Fig. 4 *E*-H) showed severe disorganization of collagen I () in parasite incubated explants (Fig. 4 *E*-F asterisk) compared to the control samples (Fig. 4 D). Thus, the score of collagen disorganization increases from 1.23 ± 0.05 to 2.98 ± 0.49 (*T. cruzi* infected samples) and 3.80 ± 0.16 (*T. gondii* infected explants) ($p \leq .0001$). Similarly, to the observed tissue damage, the collagen I disorganization was significantly higher in *T. gondii* infected samples ($p \leq .0001$) (Fig. 4 H).

Graphs show the quantification of the histopathological damage (D) and collagen I disintegration (H). All values, which are presented as mean ± S.D., correspond to at least 3 independent experiments performed in triplicate. *** $p < .001$, ****$p < .0001$.

3.3. *Toxoplasma gondii caused more severe tissue damage in canine placental explants than Trypanosoma cruzi*

CPE were co-incubated with 10^5 *T. cruzi* trypomastigotes or *T. gondii* tachyzoites for 24 h. CPE incubated with both parasites showed a significant tissue damage (scores: *T. cruzi*: 2.25 ± 0.42; *T. gondii*: 3.40 ± 0.45, $p \leq .0001$) as evidenced by the destruction of the trophoblast (Fig. 5 B–C, arrows) compared to control non-infected explants (Fig. 5 A, score for histopathological damage: 1.25 ± 0.04). However, the *T. gondii*-induced damage was significantly higher (Fig. 5 D, $p \leq .0001$) than the induced by *T. cruzi*. In *T. gondii* infected samples, areas with complete detachment of the trophoblast (Fig. 5 C, arrows) as well as disorganization of the fetal connective tissue was evident (Fig. 5 C, asterisk). Collagen histochemistry (Fig. 5 *E*-H) showed a similar significant damage in samples incubated each of both parasites (scores: *T. cruzi*: 2.37 ± 0.41; *T. gondii*: 2.48 ± 0.58, $p \leq .0001$) as evidenced by a partial loss of collagen I birefringence (Fig. 5 E-F asterisk). .

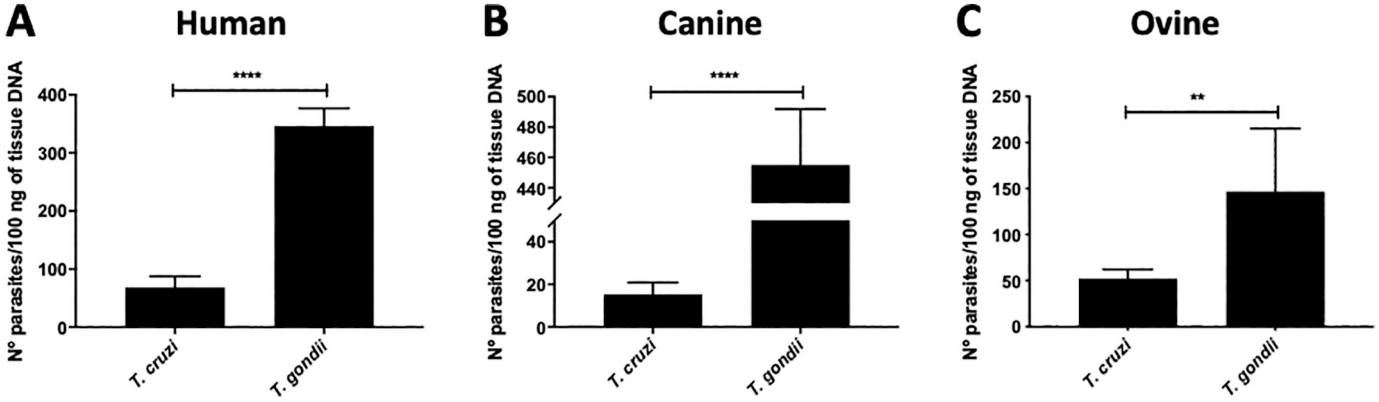

Fig. 3. More *Toxoplasma gondii* parasites were detected in human, canine and ovine placental explants than *Trypanosoma cruzi*: HPE (A), CPE (B) and OPE (C) were each co-incubated with 10^5 *T. cruzi* trypomastigotes or *T. gondii* tachyzoites for 24 h. Data was expressed as the as number parasites/100 ng of tissue DNA and analyzed by Student's t-test for paired data. All values, which are presented as mean ± S.D., correspond to at least 3 independent experiments performed in triplicate. *** $p < .001$, ****$p < .0001$.

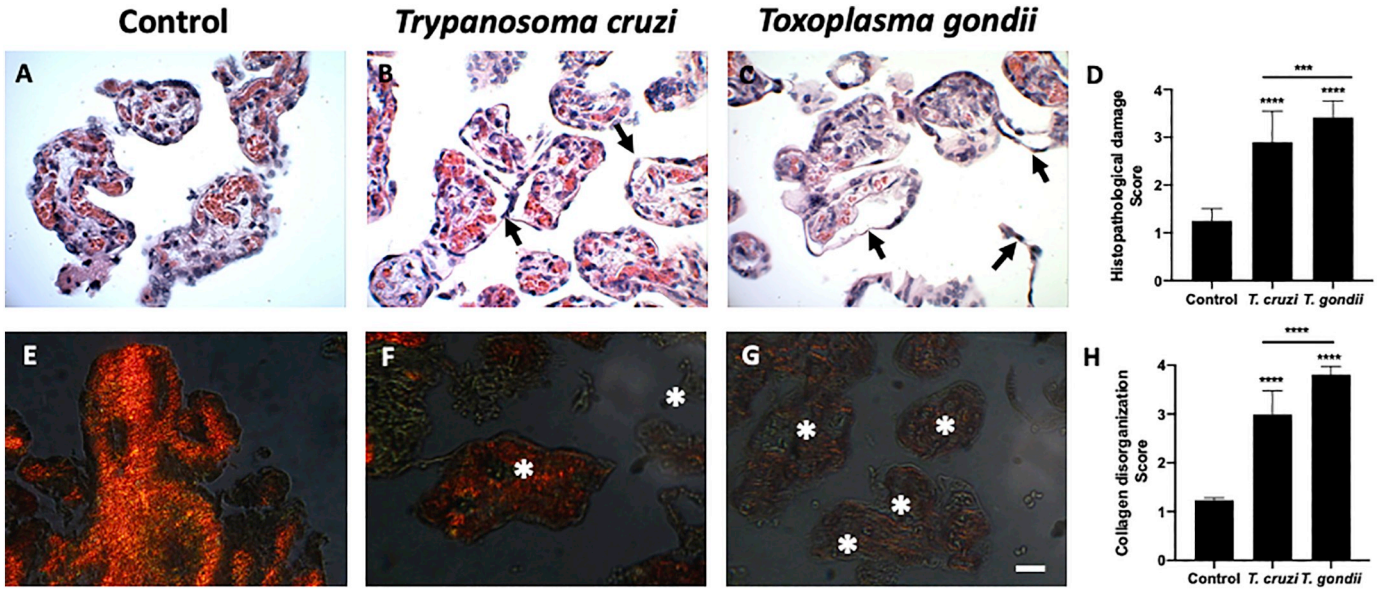

Fig. 4. *Toxoplasma gondii* caused more severe tissue damage in human placental explants than *Trypanosoma cruzi*: HPE were co-incubated with 10^5 *T. cruzi* trypomastigotes (B, E) or *T. gondii* tachyzoites (C, F) for 24 h. HPE incubated with both parasites showed destruction of the trophoblast (B—C, arrows) compared to control non-infected explants (A). *T. gondii*-induced damage was more evident, showing a complete detachment of the trophoblast (C, arrows) as well as disorganization of the fetal connective tissue (C, asterisk). Collagen histochemistry (*E*-G) showed a partial loss of collagen I birefringence in CPE incubated presence of each parasite (F-G asterisk) compared to the control samples (E), indicating disorganization of collagen I. Explants were processed for routine histological techniques and stained with hematoxylin-eosin (A-C) or picro-sirius red (E-G). Bar scale: 25 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Graphs show the quantification of the histopathological damage (D) and collagen I disintegration (H). All values, which are presented as mean ± S.D., correspond to at least 3 independent experiments performed in triplicate. ****$p < .0001$.

3.4. *Toxoplasma gondii and Trypanosoma cruzi induce similar tissue damage in ovine placental explants*

OPE were co-incubated with 10^5 *T. cruzi* trypomastigotes or *T. gondii* tachyzoites for 24 h. OPE incubated with both parasites showed severe destruction and detachment of the trophectoderm (Fig. 6 B—C, arrows)

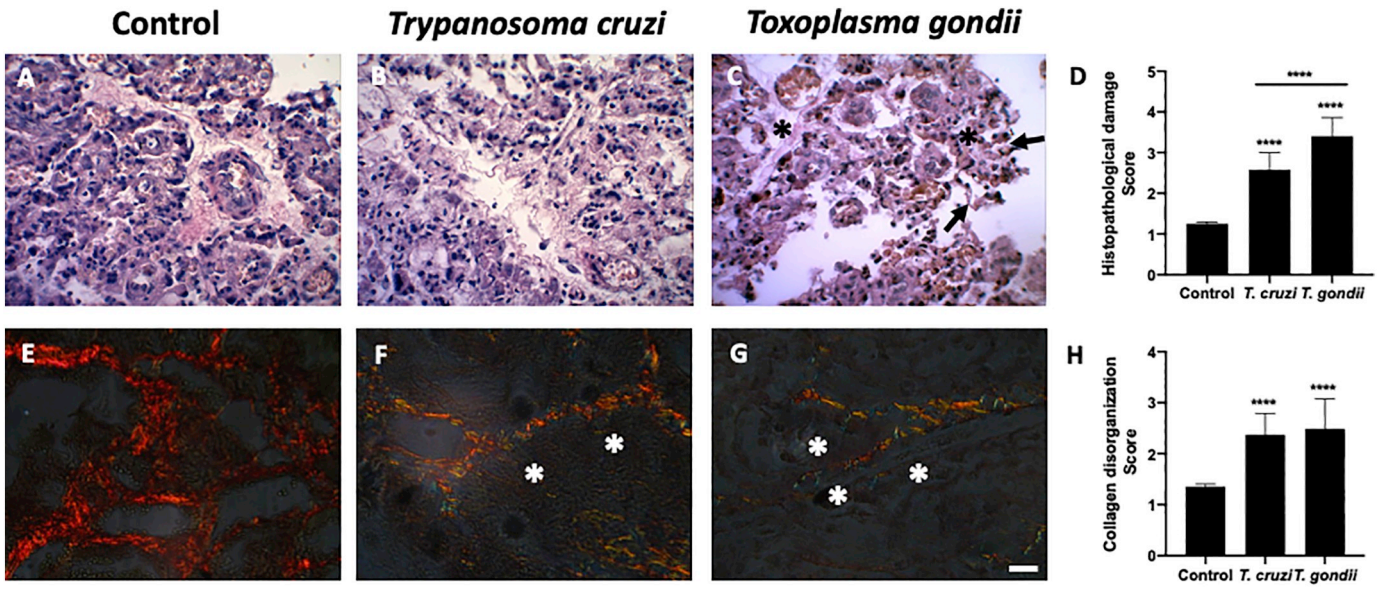

Fig. 5. *Toxoplasma gondii* caused more severe tissue damage in canine placental explants than *Trypanosoma cruzi*: CPE were co-incubated with 10^5 *T. cruzi* trypomastigotes (B, E) or *T. gondii* tachyzoites (C, F) for 24 h. CPE incubated with both parasites showed destruction of the trophoblast (B—C, arrows) compared to control non-infected explants (A). *T. gondii*-induced damage was more evident, showing a complete detachment of the trophoblast (C, arrows) as well as disorganization of the fetal connective tissue (C, asterisk). Collagen histochemistry (E-G) showed a partial loss of collagen I birefringence in CPE incubated presence of each parasite (F-G asterisk) compared to the control samples (E), indicating disorganization of collagen I. Explants were processed for routine histological techniques and stained with hematoxylin-eosin (A-C) or picro-sirius red (*E*-G). Bar scale: 25 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

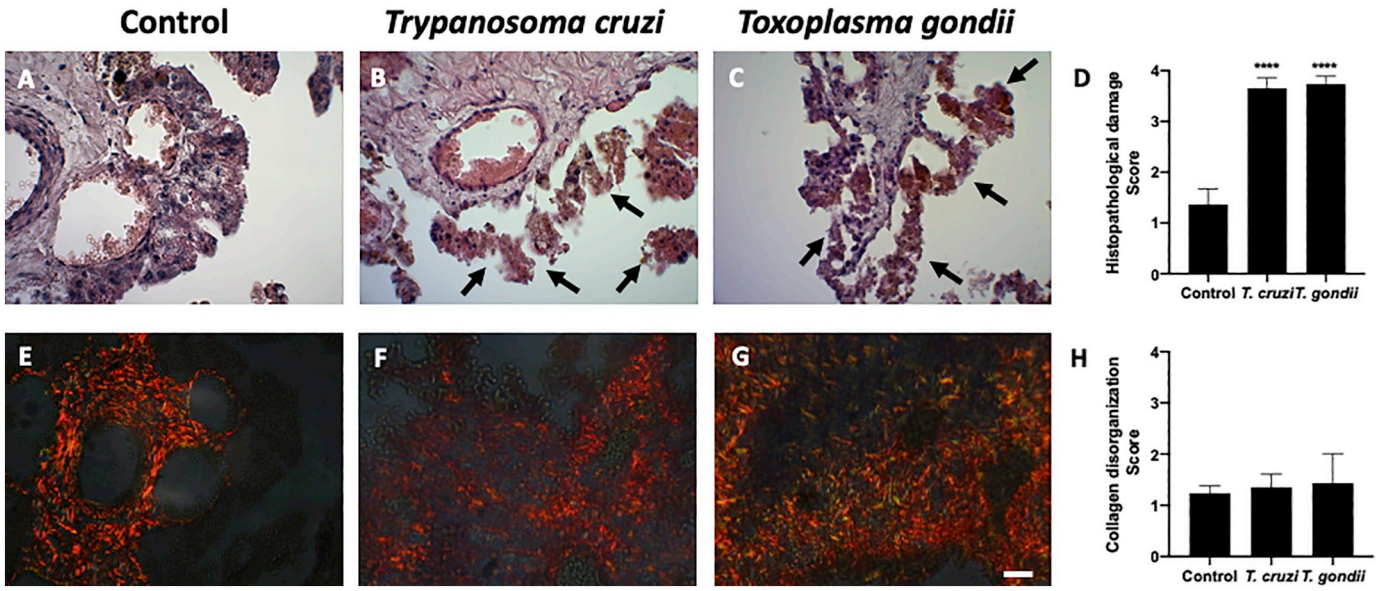

Fig. 6. *Toxoplasma gondii* and *Trypanosoma cruzi* induced similar tissue damage in ovine placental explants: OPE were co-incubated with 10^5 *T. cruzi* trypomastigotes (B, E) or *T. gondii* tachyzoites (C, F) for 24 h. OPE incubated with both parasites showed severe destruction and detachment of the trophectoderm (B—C, arrows) compared to control non-infected explants (A). However, collagen histochemistry did not show significant alterations in collagen I organization (E-H). Placental tissue samples were processed for routine histological techniques and stained with hematoxylin-eosin (A-C) or picro-sirius red (E-G). Bar scale: 25 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

compared to control non-infected explants (Fig. 6 A). The score of histopathological damage increased, respectively, from 1.36 ± 0.31 (non-infected samples) to 3.65 ± 0.21and 3.73 ± 0.15 ($p \leq .0001$) in *T. cruzi* and *T. gondii*-infected samples (Fig. 6D). However, collagen histochemistry did not show significant alterations in collagen I organization; thus, control as well as parasite-incubated explants showed similar birefringence (Fig. 6 *E*-H).

Graphs show the quantification of the histopathological damage (D) and collagen I disintegration (H). All values, which are presented as mean ± S.D., correspond to at least 3 independent experiments performed in triplicate., $****p < .0001$.

4. Discussion

The interaction between hosts and pathogens, including parasites, is the most critical factor in determining the success of an infection. Host-parasite interaction includes invasion of the host through primary barriers, such as the placental barrier, evasion of host defenses, pathogen replication in the host and immunological capacity of the host to control or eliminate the pathogen [32].

The primary function of the placenta is to act as an interface between the mother and the developing embryo/fetus. The placenta performs many important functions throughout gestation including anchoring the developing fetus to the uterine wall, mediating maternal immune tolerance, metabolic exchange, hormone synthesis and forming a barrier against xenobiotics and pathogens [7,12]. Different animal species present a diversity of placental invasiveness, and therefore there is also variation in the morphology of these organs [12,15]. Considering that the complexity of the placental barriers increases from human (hemochorial) to ovine (epitheliochorial), it could be assumed that the parasites cross the hemochorial barrier more easily than the others. Moreover, it has been proposed that in the hemochorial placenta, where the trophoblast is in direct contact with maternal blood, the placental infection and therefore the transmission to the fetus is facilitated. However, the same hemochorial barrier favors the transfer of maternal antibodies to the fetus, and in less invasive placentas, a greater variety of pathogens is observed [15]. Our results show that both *T. cruzi* and *T. gondii* are able to infect human, canine and ovine placental explants and that the amount parasite/tissue in the tissue is not related to the complexity of the placental barrier. Thus, there are fewer parasites/tissue present in OPE than in HPE in spite of the fact that our OPE lacks the maternal caruncle; contrarily, more *T. gondii* DNA can be found in CPE than in HPE and OPE (Fig. 2). However, comparing the amount of each parasites/tissue, *T. gondii* is always more infective than *T. cruzi*, since the amount of parasites/tissue is higher in the three different placental explants (Fig. 3). Considering that a lower amount of parasite DNA was found in the OPE, in spite of lacking the maternal part of the placentome, some specific and not yet identified defense mechanisms of the fetal part might be present. Both parasites damage the placental barrier, and in HPE, CPE and OPE, the trophoblast/trophectoderm is destroyed (Figs. 4-6). We have previously shown that *T. cruzi* induces trophoblast destruction and detachment in HPE in a parasite-concentration dependent manner [23] and that host matrix metalloproteases (MMPs) are involved in this process [28]. Besides, both parasites secrete proteases (*e.g.*, cysteine proteases) that contribute to the cell and tissue invasion [33]. Interestingly, in OPE, the parasite-induced collagen I destruction was less severe than in HPE or CPE (Fig. 6). Collagen I constitute a basic component of the tri-dimensional network of the extracellular matrix (ECM) and is formed by different types of collagen and elastic fibers, proteoglycans and glycoproteins. If the “basic skeleton” of the ECM is destroyed, the normal conformation of the ECM is disorganized, a condition which may facilitate the mobilization of the parasite inside the tissue to its target. Additionally, it has been proposed that ECM alterations produced by pathogens not only promote its motility in tissues and its entrance into cells but also alter the presence of cytokines and chemokines, which in turn permits to modulate and escape both the inflammatory response and the immune response [23,34]. The individual components of the ECM probably vary in the connective tissue of different species, explaining at least partially the differences in collagen I disorganization in HPE, CPE and OPE.

On the other hand, several studies have shown that the placenta elicits effective defense mechanisms against a great variety of pathogens [7,35–37]. For instance, we have previously proposed that in the human placenta, the trophoblast epithelial turnover can be considered as a local defense mechanism against *T. cruzi* [7,38]. Moreover, in

human placenta, *T. cruzi* induces a specific pro-inflammatory cytokine profile in HPE in comparison to *T. gondii* that only increases the immunomodulatory interleukin-8 secretion in the explants [21]. Other studies show that human placental cells present different susceptibility to *T. gondii* [39] and *T. cruzi* [40], being syncytiotrophoblasts (superficial trophoblast cell layer) that are more resistant to infection than cytotrophoblasts (trophoblast germinal layer) [39–41]. The parasite-induced destruction of the trophoblast eliminates the parasite-resistant cells and might therefore allow the invasion of parasite-susceptible cells and ECM. The trophoblast expresses small cationic antimicrobial peptides (AMP), such as defensins [42], which have been described to have parasiticidal activity against both parasites in other tissues [43,44]. The ovine trophoblast also expresses small cationic AMP, particularly cathelicidins antimicrobial peptides that present activity against other abortifacient organisms, such as *Waddlia chondrophila* [45].

The systemic response of the different animal species should also be considered. *T. cruzi* can be transmitted congenitally in the acute as well as in the chronic phase of the disease [46]. Interestingly, in human and dog, *T. gondii* is mainly transmitted in the acute phase of infection [17,47], but in sheep, the parasite is also transmitted in chronically infected animals [48]. In our experimental models, the systemic response cannot be studied and the parasites does not reach the placental barrier through the maternal circulatory system. However, the *ex vivo* infection of placental explants constitutes excellent tools, not only for studying the mechanism of congenital infection but also for understanding the invasion process in placental tissues.

5. Conclusion

We conclude that the infection efficiency of *T. gondii* is higher, compared to *T. cruzi*, during the *ex vivo* infection of human, canine and ovine placental explants.

Declaration of Competing Interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.parint.2020.102065.

References

[1] N. Akritidis, Parasitic, fungal and prion zoonoses: an expanding universe of candidates for human disease, Clin. Microbiol. Infect. 17 (2011) 331–335 Internet. cited 2018 Dec 13. Available from https://linkinghub.elsevier.com/retrieve/pii/S1198743X14638647.

[2] WHO, Chagas disease in Latin America: an epidemiological update based on 2010 estimates, Relev Epidemiol Hebd [Internet] 90 (2015) 33–43 cited 2018 Dec 11. (Available from) http://www.ncbi.nlm.nih.gov/pubmed/25671846.

[3] K.J. Esch, C.A. Petersen, Transmission and epidemiology of zoonotic protozoal diseases of companion animals, Clin. Microbiol. Rev. 26 (2013) 58–85.

[4] O. Rodríguez-Morales, M.A. Ballinas-Verdugo, R. Alejandre-Aguilar, P.A. Reyes, M. Arce-Fonseca, *Trypanosoma cruzi* connatal transmission in dogs with Chagas disease: experimental case report, Vector-Borne Zoonotic Dis [Internet]. 11 (2011) 1365–1370. Available from: https://doi.org/10.1089/vbz.2010.0231.

[5] S.A. López-Cancino, E. Tun-Ku, H.K. De la Cruz-Felix, C.N. Ibarra-Cerdeña, A. Izeta-Alberdi, A. Pech-May, et al., Landscape ecology of Trypanosoma cruzi in the southern Yucatan Peninsula, Acta Trop [Internet] 151 (2015) 58–72 cited 2018 Dec 13. (Available from) https://linkinghub.elsevier.com/retrieve/pii/S0001706X15300693.

[6] J. Gutierrez, J. O'Donovan, A. Proctor, C. Brady, P.X. Marques, S. Worrall, et al., Application of quantitative real-time polymerase chain reaction for the diagnosis of toxoplasmosis and enzootic abortion of ewes, J. Vet. Diagn. Investig. 24 (2012) 846–854 Internet. cited 2018 Dec 13. Available from https://doi.org/10.1177/1040638712452730.

[7] A. Liempi, C. Castillo, I. Carrillo, L. Muñoz, D. Droguett, N. Galanti, et al., A local innate immune response against Trypanosoma cruzi in the human placenta: the epithelial turnover of the trophoblast, Microb. Pathog. 99 (2016) 123–129.

[8] T.P. Arantes, W.D.Z. Lopes, R.M. Ferreira, J.S.P. Pieroni, V.M.R. Pinto, C.A. Sakamoto, et al., Toxoplasma gondii: Evidence for the transmission by semen in dogs, Exp Parasitol [Internet] 123 (2009) 190–194 Elsevier Inc.. (Available from) https://doi.org/10.1016/j.exppara.2009.07.003.

[9] M.B. Melo, K.D.C. Jensen, J.P.J. Saeij, Toxoplasma gondii effectors are master regulators of the inflammatory response, Trends Parasitol. 27 (2011) 487–495 Internet. cited 2018 Dec 13. Available from https://linkinghub.elsevier.com/retrieve/pii/S1471492211001504.

[10] K. Benirschke, P. Kaufmann, P. Baergen, Pathology of the Human Placenta [Internet]. 6 Th, SpringerLink, New York, 2012 Available from: http://www.springer.com/medicine/pathology/book/978-3-642-23940-3.

[11] U. Kemmerling, A. Osuna, A.G. Schijman, C. Truyens, Congenital transmission of Trypanosoma cruzi: a review about the interactions between the parasite, the placenta, the maternal and the Fetal/neonatal immune responses [internet], Front. Microbiol. 2019 (1854), https://doi.org/10.3389/fmicb.2019.01854 Available from:.

[12] S. Furukawa, Y. Kuroda, A. Sugiyama, A comparison of the histological structure of the placenta in experimental animals, J. Toxicol. Pathol. 27 (2014) 11–18 Internet. Available from https://www.jstage.jst.go.jp/article/tox/27/1/27_2013-0060/_article.

[13] F.B.P.B.G. Wooding, Comparative Placentation: Structures, Functions and Evolution, (2018).

[14] D. Sammin, B. Markey, H. Bassett, D. Buxton, The ovine placenta and placentitis-A review, Vet. Microbiol. 135 (2009) 90–97 Internet. cited 2018 Dec 13. (Available from) http://linkinghub.elsevier.com/retrieve/pii/S0378113508003945.

[15] I. Capellini, C.L. Nunn, R.A. Barton, Microparasites and Placental Invasiveness in Eutherian Mammals, in: M.L. Baker (Ed.), PLoS One [Internet], 10 2015, p. e0132563 cited 2018 Dec 13. (Available from) https://doi.org/10.1371/journal.pone.0132563.

[16] M.C. Fabrizio, N.J. Schweigmann, N.J. Bartoloni, Modelling American trypanosomiasis in an endemic zone: application to the initial spread of household infection in the argentine Chaco, Zoonoses Public Health 61 (2014) 545–559.

[17] Y. Carlier, C. Truyens, P. Deloron, F. Peyron, Congenital parasitic infections: a review, Acta Trop. 121 (2012) 55–70 Internet. cited 2018 Dec 11. Available from https://linkinghub.elsevier.com/retrieve/pii/S0001706X11003147.

[18] P. Castaño, M. Fuertes, I. Ferre, M. Fernández, C. Ferreras M del, J. Moreno-Gonzalo, et al., Placental thrombosis in acute phase abortions during experimental toxoplasma gondii infection in sheep, Vet. Res. 45 (2014) 9 Internet. cited 2018 Dec 13. Available from http://www.veterinaryresearch.org/content/45/1/9.

[19] R. Calero-Bernal, S.M. Gennari, Clinical toxoplasmosis in dogs and cats: an update, Front. Vet. Sci. 6 (2019) 54 Internet. cited 2019 Mar 22. Available from https://doi.org/10.3389/fvets.2019.00054/full.

[20] H. Langoni, F. Fornazari, R.C. da Silva, E.T. Monti, F.B. Villa, Prevalence of antibodies against toxoplasma gondii and Neospora caninum in dogs, Braz. J. Microbiol. 44 (2013) 1327–1330 Internet. cited 2018 Dec 13. Available from http://www.ncbi.nlm.nih.gov/pubmed/24688530.

[21] C. Castillo, L. Muñoz, I. Carrillo, A. Liempi, C. Gallardo, N. Galanti, et al., Ex vivo infection of human placental chorionic villi explants with Trypanosoma cruzi and toxoplasma gondii induces different toll-like receptor expression and cytokine/chemokine profiles, Am. J. Reprod. Immunol. 78 (2017) 1–8.

[22] U. Kemmerling, C. Bosco, N. Galanti, Infection and invasion mechanisms of Trypanosoma cruzi in the, (2010), pp. 307–316.

[23] J. Duaso, G. Rojo, G. Cabrera, N. Galanti, C. Bosco, J.D.J.D. Maya, et al., Trypanosoma cruzi induces tissue disorganization and destruction of chorionic villi in an ex vivo infection model of human placenta, Placenta [Internet], 31 Elsevier Ltd, 2010, pp. 705–711 (Available from), https://doi.org/10.1016/j.placenta.2010.05.007.

[24] R.E.R. Fretes, U. Kemmerling, Mechanism of Trypanosoma cruzi placenta invasion and infection: the use of human chorionic villi explants, J. Trop. Med. 2012 (2012).

[25] S.C. Nardelli, F.-Y. Che, N.C. Silmon de Monerri, H. Xiao, E. Nieves, C. Madrid-Aliste, et al., The histone code of Toxoplasma gondii comprises conserved and unique posttranslational modifications, MBio 4 (2013), https://doi.org/10.1128/mBio.00922-13 Internet. (e00922–13. Available from). cited 2018 Dec 13.

[26] K.L. Cummings, R.L. Tarleton, Rapid quantitation of Trypanosoma cruzi in host tissue by real-time PCR, Mol. Biochem. Parasitol. 129 (2003) 53–59 Elsevier.

[27] M.H. Lin, T.C. Chen, T.T. Kuo, C.P.C. Tseng, C.P.C. Tseng, Real-time PCR for quantitative detection of toxoplasma gondii, J. Clin. Microbiol. 38 (2000) 4121–4125 Internet. cited 2018 Dec 13. Available from http://www.ncbi.nlm.nih.gov/pubmed/11060078.

[28] C. Castillo, R.A. López-Muñoz, J. Duaso, N. Galanti, F. Jaña, J. Ferreira, et al., Role of matrix metalloproteinases 2 and 9 in ex vivo Trypanosoma cruzi infection of human placental chorionic villi, Placenta [Internet], 33 Elsevier Ltd, 2012, pp. 991–997 (Available from), https://doi.org/10.1016/j.placenta.2012.10.004.

[29] M.W. Pfaffl, A new mathematical model for relative quantification in real-time RT-PCR, Nucleic Acids Res. 29 (2001) e45 Internet. cited 2018 Dec 13. (Available from) http://www.ncbi.nlm.nih.gov/pubmed/11328886.

[30] T. Duffy, C.I. Cura, J.C. Ramirez, T. Abate, N.M. Cayo, R. Parrado, et al., Analytical performance of a multiplex real-time PCR assay using TaqMan probes for quantification of Trypanosoma cruzi satellite DNA in blood samples, in: A. Debrabant

(Ed.), PLoS Negl. Trop Dis. [Internet], 7 2013, p. e2000 cited 2019 Mar 22. (Available from) http://www.ncbi.nlm.nih.gov/pubmed/23350002.
[31] K.N. Gibson-Corley, A.K. Olivier, D.K. Meyerholz, Principles for valid histopathologic scoring in research, Vet. Pathol. 50 (2013) 1007–1015.
[32] R. Sen, L. Nayak, R.K. De, A review on host–pathogen interactions: classification and prediction, Eur. J. Clin. Microbiol. Infect. Dis. 35 (2016) 1581–1599 Internet. cited 2018 Dec 20. Available from http://www.ncbi.nlm.nih.gov/pubmed/27470504.
[33] J.L. Siqueira-Neto, A. Debnath, L.-I. McCall, J.A. Bernatchez, M. Ndao, S.L. Reed, et al., Cysteine proteases in protozoan parasites, in: P. Sinnis (Ed.), PLoS Negl Trop Dis [Internet], 12 2018, p. e0006512 cited 2018 Dec 27. (Available from) http://www.ncbi.nlm.nih.gov/pubmed/30138453.
[34] A.P.M.P. Marino, A.A. Silva, R.T. Pinho, J. Lannes-Vieira, Trypanosoma cruzi infection: a continuous invader-host cell cross talk with participation of extracellular matrix and adhesion and chemoattractant molecules, Br. J. Med. Biol. Res. = Rev Bras Pesqui Medicas E Biol [Internet] 36 (2003) 1121–1133 cited 2018 Dec 12. (Available from) http://www.ncbi.nlm.nih.gov/pubmed/12886468.
[35] N. Arora, Y. Sadovsky, T.S. Dermody, C.B. Coyne, Microbial vertical transmission during human pregnancy, Cell Host Microbe [Internet], 21 Elsevier Inc., 2017, pp. 561–567 cited 2018 Dec 11. (Available from) http://www.ncbi.nlm.nih.gov/pubmed/28494237.
[36] A. Heerema-McKenney, Defense and infection of the human placenta, APMIS 126 (2018) 570–588 Internet. cited 2018 Dec 27. Available from http://www.ncbi.nlm.nih.gov/pubmed/30129129.
[37] J.R. Robbins, A.I. Bakardjiev, Pathogens and the placental fortress, Curr. Opin. Microbiol. 15 (2012) 36–43 Internet. cited 2018 Dec 27. Available from https://linkinghub.elsevier.com/retrieve/pii/S1369527411002025.
[38] A. Liempi, C. Castillo, J. Duaso, D. Droguett, A. Sandoval, K. Barahona, et al., Trypanosoma cruzi induces trophoblast differentiation: A potential local antiparasitic mechanism of the human placenta? Placenta 35 (2014) 1035–1042.
[39] S.E. Ander, E.N. Rudzki, N. Arora, Y. Sadovsky, C.B. Coyne, J.P. Boyle, Human placental syncytiotrophoblasts restrict *Toxoplasma gondii* attachment and replication and respond to infection by producing immunomodulatory chemokines, in: J.C. Boothroyd (Ed.), MBio [Internet], 9 2018 cited 2019 Jan 7. (Available from) http://www.ncbi.nlm.nih.gov/pubmed/29317509.
[40] C. Díaz-Luján, M.F. Triquell, A. Schijman, P. Paglini, R.E. Fretes, Differential susceptibility of isolated human trophoblasts to infection by Trypanosoma cruzi, Placenta 33 (2012) 264–270 Internet. cited 2019 Jan 7. Available from https://linkinghub.elsevier.com/retrieve/pii/S0143400412000082.
[41] C. Díaz-Luján, M.F.F. Triquell, C. Castillo, D. Hardisson, U. Kemmerling, R.E.E. Fretes, Role of placental barrier integrity in infection by Trypanosoma cruzi, Acta Trop [Internet]. 164 Elsevier B.V, 2016, pp. 360–368 (Available from), https://doi.org/10.1016/j.actatropica.2016.09.021.
[42] D. Klaffenbach, D. Friedrich, R. Strick, P.L. Strissel, M.W. Beckmann, W. Rascher, et al., Contribution of different placental cells to the expression and stimulation of antimicrobial proteins (AMPs), Placenta 32 (2011) 830–837 Internet. cited 2019 Jan 7. Available from https://linkinghub.elsevier.com/retrieve/pii/S0143400411004164.
[43] T. Tanaka, M.M. Rahman, B. Battur, D. Boldbaatar, M. Liao, R. Umemiya-Shirafuji, et al., Parasiticidal activity of human alpha-defensin-5 against toxoplasma gondii, In Vitro Cell Dev Biol Anim, 46 2010, pp. 560–565 Internet. cited 2019 Jan 7. Available from https://doi.org/10.1007/s11626-009-9271-9.
[44] C.A. Johnson, G. Rachakonda, Y.Y. Kleshchenko, P.N. Nde, M.N. Madison, S. Pratap, et al., Cellular response to Trypanosoma cruzi infection induces secretion of defensin α-1, which damages the flagellum, neutralizes trypanosome motility, and inhibits infection, in: J.F. Urban (Ed.), Infect Immun [Internet], 81 2013, pp. 4139–4148 cited 2019 Jan 7. (Available from) https://doi.org/10.1128/IAI.01459-12.
[45] C. Coyle, N. Wheelhouse, M. Jacques, D. Longbottom, P. Svoboda, J. Pohl, et al., Ovine trophoblasts express cathelicidin host defence peptide in response to infection, J. Reprod. Immunol. 117 (2016) 10–16 Internet. cited 2019 Jan 7. Available from https://linkinghub.elsevier.com/retrieve/pii/S0165037816300134.
[46] Y. Carlier, S. Sosa-Estani, A.O. Luquetti, P. Buekens, Congenital Chagas disease: an update, Mem Inst Oswaldo Cruz 110 (2015) 363–368 Internet. cited 2018 Dec 11. (Available from) http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0074-02762015000300363&lng=en&tlng=en.
[47] I.I.G.G. Taques, T.R. Barbosa, C. Martini A de, L.C. Pitchenin, Í. Braga, A.L.T. de Melo, et al., Molecular assessment of the transplacental transmission of toxoplasma gondii , Neospora caninum , Brucella canis and Ehrlichia canis in dogs, Comp. Immunol. Microbiol. Infect. Dis. 49 (2016) 47–50 Internet. cited 2019 Jan 8. Available from https://linkinghub.elsevier.com/retrieve/pii/S0147957116300935.
[48] J.L. Reis-Cunha, R.P. Baptista, G.F. Rodrigues-Luiz, A. Coqueiro-dos-Santos, H.O. Valdivia, L.V. de Almeida, et al., Whole genome sequencing of Trypanosoma cruzi field isolates reveals extensive genomic variability and complex aneuploidy patterns within TcII DTU, BMC Genomics 19 (2018) 816 Internet. cited 2018 Dec 19. Available from http://www.ncbi.nlm.nih.gov/pubmed/30424726.