OMICS International

Global Veterinary Summit

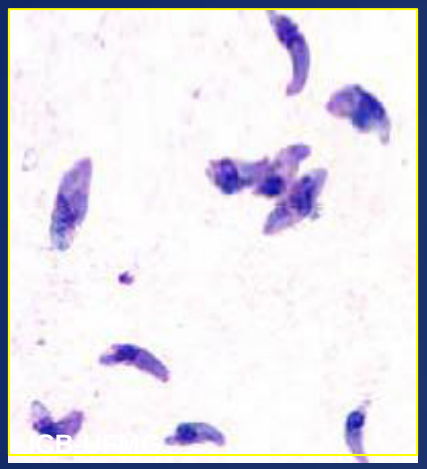
August 31-September 2, 2015 Florida, USA

Venereal transmission of *Toxoplasma gondii* in goats after a buck was experimentally infected

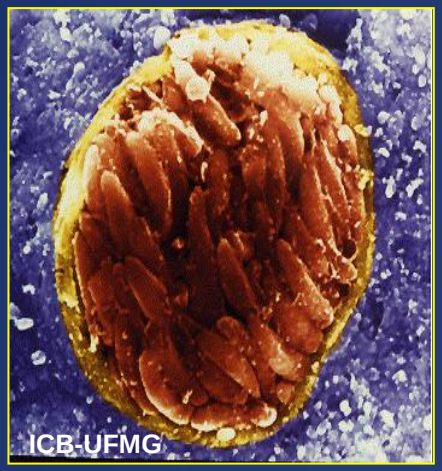
Dra. FLAVIANA SANTOS WANDERLEY
Universidade Estadual de Ciências da Saúde de Alagoas
Brazil

Toxoplasma gondii

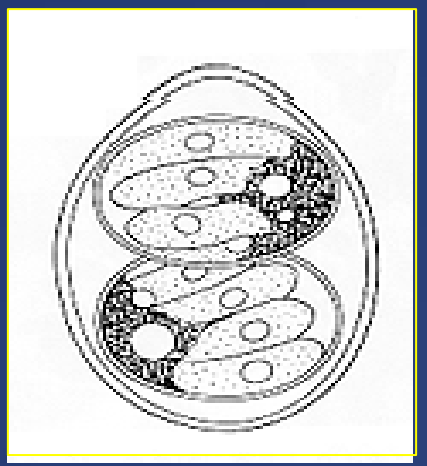
- *Toxoplasma gondii:* obligate intracellular affecting humans, domestic and wild animals (Beaman et al., 1995);
- Most common transmission forms: carnivorism in felines and fecal-oral route (oocysts) for herbivores hosts (Dubey et al., 1996)



Tachyzoite



Cyst



Oocyst

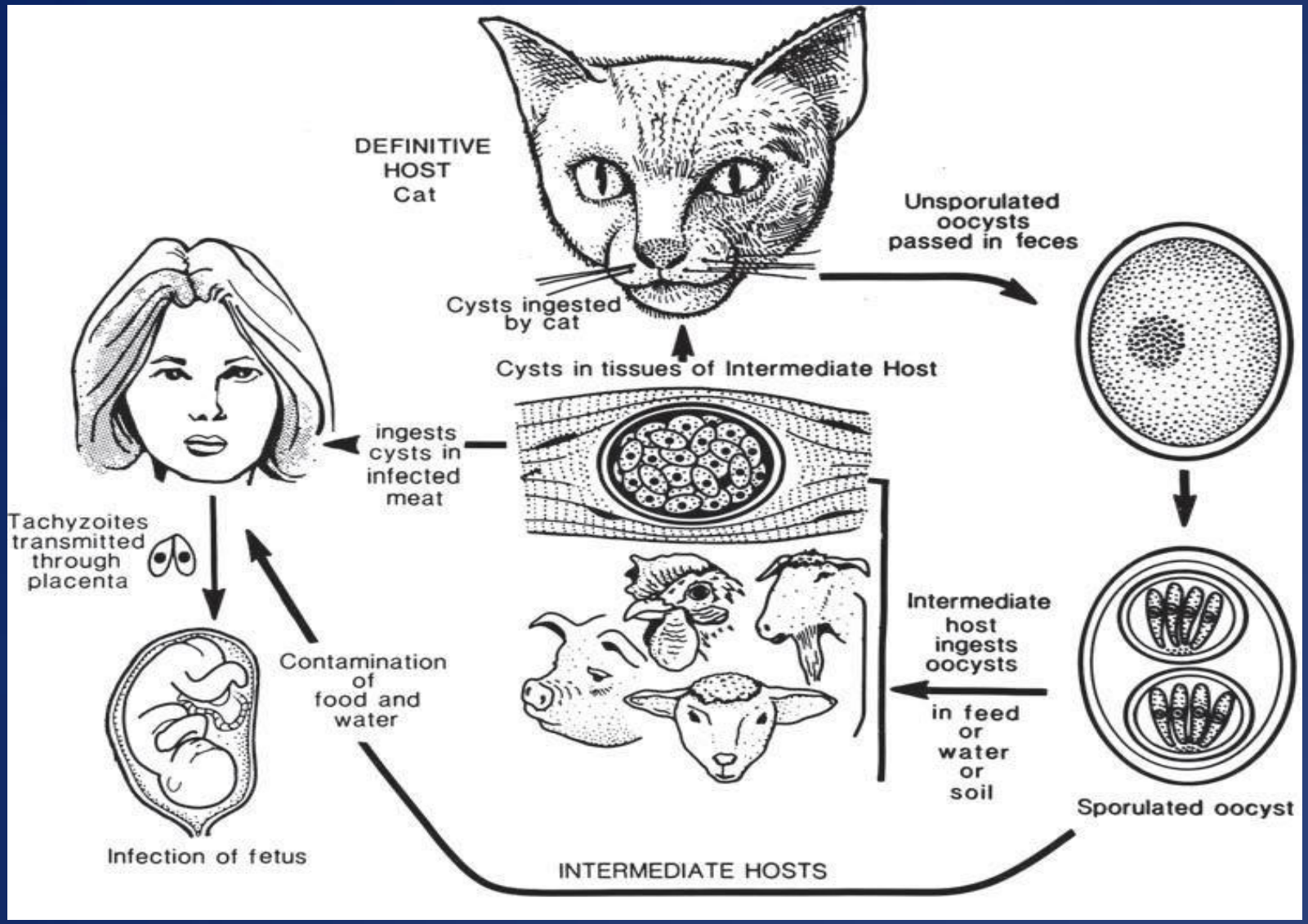


Figure - Life cycle of *Toxoplasma gondii* (Dubey et al.,1998)

- First evidence in goats: Feldman and Miller (1956) in New York;

- Munday & Mason (1979): Toxoplasmosis an important cause of reproductive losses in goats (Dubey, 1987);

- Abortion and repeated neonatal loss in subsequent pregnancies (Dubey, 1982);

- *T. gondii*: semen from goats infected orally (oocysts) from day 7 post infection by bioassay (DUBEY; Sharma, 1980);
- Moraes et al. (2010a): confirmed infection in sheep through semen experimentally contaminated .



- Wanderley et al. (2013): vaginal insemination with semen infected with tachyzoites of *Toxoplasma gondii* is able to infect goats.

J. Parasitol., 99(4), 2013, pp. 610–613
© American Society of Parasitologists 2013

EXPERIMENTAL VAGINAL INFECTION OF GOATS WITH SEMEN CONTAMINATED WITH THE "CPG" STRAIN OF *TOXOPLASMA GONDII*

Flaviana Santos Wanderley, Wagnner José Nascimento Porto*, Diogo Ribeiro Câmara*, Nadine Louise Nicolau da Cruz*, Bruna Catarina de Oliveira Feitosa*, Roberta Lemos Freire†, Érica Paes Barreto Xavier de Moraes‡, and Rinaldo Aparecido Mota‡

Programa de Pós, graduação em Biociência Animal, Universidade Federal Rural de Pernambuco Rua Dom Manoel de Medeiros, s/n, Dois Irmãos, 52171-900, Recife, PE, Brazil. Correspondence should be sent to: *rinaldo.mota@hotmail.com*

ABSTRACT: The objective was to characterize the transmission of *Toxoplasma gondii* in goats experimentally infected vaginally with semen contaminated with the CPG strain (genotype III). Ten female goats were randomly allocated into 2 groups (G1 and G2), each with 5 animals, and inseminated during estrus. Goats in G1 were inseminated with semen containing 1×10^5 tachyzoites, whereas those in G2 (control) were inseminated with semen free from tachyzoites (insemination = day 0). In G1, seroconversion (indirect immunofluorescence reaction) and DNA (polymerase chain reaction) in the blood was present in 4/5 and 3/5, respectively, from the 7th day. In G2, all goats were negative in all tests. Embryonic reabsorption occurred in 4 of 5 goats from G1 between days 21 and 49. In conclusion, artificial vaginal insemination with semen containing tachyzoites of *T. gondii*–infected goats and is a potential transmission route of this parasite through semen.

Small Ruminant Research 123 (2015) 301–305

Contents lists available at ScienceDirect

Small Ruminant Research

journal homepage: www.elsevier.com/locate/smallrumres



Venereal transmission of *Toxoplasma gondii* in goats after a buck was experimentally infected

Flaviana Santos Wanderley[a,*], Wagnner José Nascimento Porto[b], Diogo Ribeiro Câmara[b], Vinicius Vasconcelos Gomes de Oliveira[c], João Luiz Garcia[d], Pedro Paulo Feitosa de Albuquerque[e], Andréa Alice da Fonseca Oliveira[e], Rinaldo Aparecido Mota[e]

[a] Universidade de Ciências da Saúde de Alagoas, Rua Dr. Jorge de Lima, 113, Trapiche da Barra, CEP 57010-382 Maceió, Alagoas, Brazil
[b] Unidade Educacional Viçosa, Campus Arapiraca, Universidade Federal de Alagoas, CEP 57700-000 Viçosa, AL, Brazil
[c] Laboratório de Doenças Parasitárias dos Animais Domésticos, Departamento de Medicina Veterinária, Universidade Federal Rural de Pernambuco, Rua Dom Manoel de Medeiros, s/n Dois Irmãos, CEP 52171-900 Recife, PE, Brazil
[d] Departamento de Medicina Veterinária preventiva, Universidade Estadual de Londrina, Rodovia Celso Garcia Cid/PR 445, KM 380, CEP 86051-990 Londrina, PR, Brazil
[e] Laboratório de Doenças Infecciosas dos Animais Domésticos, Departamento de Medicina Veterinária, Universidade Federal Rural de Pernambuco, Rua Dom Manoel de Medeiros, s/n Dois Irmãos, CEP 52171-900 Recife, PE, Brazil

OBJECTIVE

The objective was to prove the venereal transmission of *Toxoplasma gondii* in goats

METHODS

Development of the experiment

São Luiz farm,
UFAL, Viçosa, Al, Brazil.

Course in Veterinary Medicine



- License: CEUA-UFRPE – Protocol 007/2010

Animals

- Two Saanen bucks with history of fertility (CBRA, 1998).

Andrologycal examination

- History, general examination and genital system;
- Behavior (libido);
- Spermiogram (morphology, motility, vigor) (CBRA, 1998).



Composition of the experimental groups

- Goats: 10 multiparous does,

  G1 = 5 animals (infected group)

  G2 = 5 animals (control group)



- Vaccinated (Rabies and Clostridial diseases
- Dewormed



- Negative Serology for:
  T. gondii (RIFI)
  N. caninum (RIFI)
  B. abortus (BAA)
  Chlamydophila abortus (CF)



- Maintained in a intensive screened bay system.

Strain

- ME-49 strain;
- Oocysts were provided by the research group at the Universidade Estadual de Londrina;
- Kept under refrigeration.

Experimental Infection

- Orally: syringe (5 ml) coupled to a catheter;
- Dosage: $2x10^5$ oocysts.

Induction of estrus

.**G1/G2:** Sodium Cloprostenol (Ciosin® Shering Plough, Brazil)- 0,3 ml intramuscular route.

Synchronization

- 01 female → synchronized 04 days post-infection (d.p.i.) of the buck;
- 03 females → synchronized 06 d.p.i.;
- 01 female → synchronized 10 d.p.i.

Natural mating

G1	G2
02 females → 08 d.p.i.	01 female → 08 d.p.i.
01 female → 09 d.p.i.	02 females→ 09 d.p.i.
01 female → 10 d.p.i.	01 female →10 d.p.i.
01 female → 13 d.p.i.	01 female →12 d.p.i

- 2 a 3 times during estrus
- **G1:** infected buck
- **G2:** uninfected buck

Serological (RIFI) and molecular examinations (PCR)

Blood

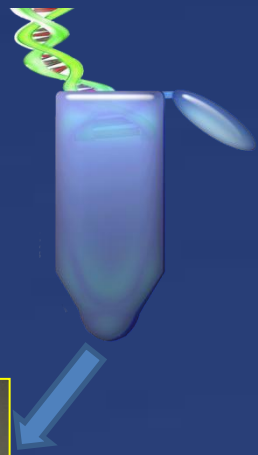
- Females: collected in 0, 7, 14, 21, 28, 49, 63, 90 and 123 days after natural mating and on the day of birth;
- Kids: after the birth and before ingestion of colostrum;

Blood and semen

- Bucks: 0, 3, 5, 7, 11, 14, 21, 28, 35, 42, 49, 56, 63 e 70 d.p.i.

Polymerase Chain Reaction - PCR

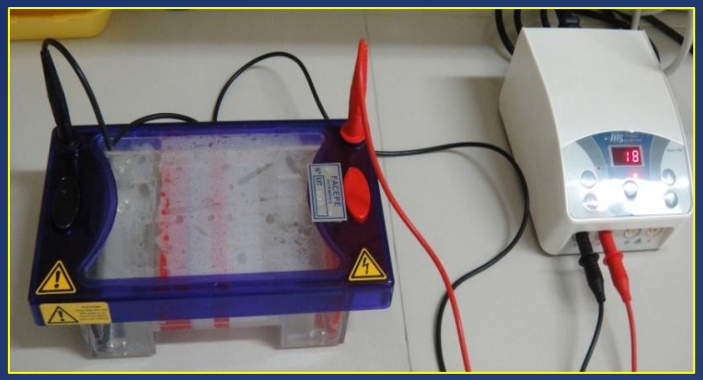
- DNA extraction: “Qiagen DNA Easy Blood and Tissues Kit” (Qiagen®, Hilden, Germany);
- Primers: TOX4 e TOX5 (Homan et al., 2000);
- Amplifying a repeat region of 529 bp.

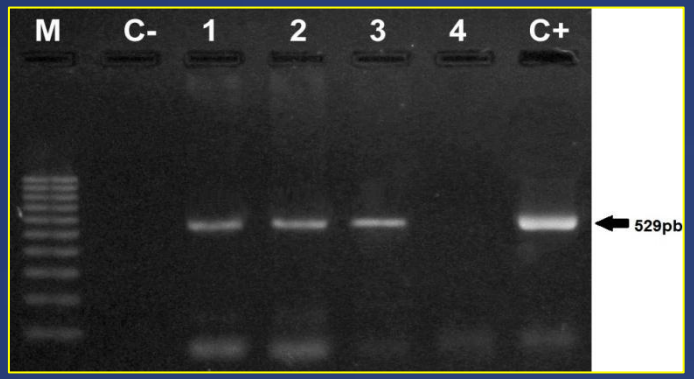




Polymerase Chain Reaction – PCR

- Detected by eletrophoresis
- Agarose gel (2%)
- Visualized under ultraviolet light
- Photodocumented







Genetic sequencing

- Reactions: both strands of primers (TOX4 e TOX5) de based on the Big Dye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems), using ABI PRISM 3100 (Applied Biosystems);

- Sequence analisis: BioEdit e MEGA 5 software; compared with the NCBI database using BLASTn.

Clinical monitoring

- Daily
- Measured parameters: respiratory rate, heart rate, temperature, general condition.



Diagnosis and monitoring of pregnancy

Ultrasound examinations

- Between 15 and 60 days of pregnancy, weekly, trans-rectal;

- After 60 days of pregnancy, at intervals of 15 days, trans-abdominally;

- Birth followed with collecting placentas (PCR, histopathology).

Histopathological examination

At the end of the experiment, euthanasia of all animals (CFMV, 2012)

- Does: 240 days after mating;
- Kids: 90 days after birth;
- Buck: 240 d.p.i.
- Organs: liver, spleen, kidneys, medulla, brain, lung, heart, uterus, ovaries and testicles.

RESULTS

Serological diagnosis

Infected buck

Soroconversão

Day 7: titer 64;

Day 11: maximal titer of 1024 , mantained for 70 days;

G1: 2/5 females on the 123 days after mating (titer 32);

Buck uninfected, G2 : seronegatives.

Molecular diagnosis

Toxoplasma gondii DNA

Infected buck

- Blood and semen (day 3 to day 70 post-infecction)
- Kidney and spleen

G1

- Blood: 2/5 females,
  1/5 7^0 day after mating and
  1/5 day of the birth;
- Organs: 2/5 females e 4/5 kids positives in at least one

Buck uninfected,G2: *T. gondii* DNA was not found.

Table 1 – Results of PCR in tissues from bucks, does, and kids in G1, infected with *Toxoplasma gondii.*

Animal	Liver	Spleen	Kidney	Medulla	Brain	Lung	Heart	Placenta	Ovary
1									
1 *			+						
2									
3					+				+
4	+	+	+						
4 *					+		+		
4 *			+						
5									
5 *							+		
5*									
Buck		+	+						

*** kids**

Genetic sequencing

Molecular amplicon identities were confirmed: 99% similarity with the *T. gondii* DNA sequences (GenBank).

Clinical signs

Infected buck

- Between the 3rd and 8th d.p.i.
- Apathy, hyporexia, coughing, hyperthermia (maximal temperature of 41.3◦C), tachycardia, and tachypnea;

Females G1

- Dry cough, with rales beginning on the 9th day after mating;

- Buck and does (G2): no clinical alterations.

Monitoring of pregnancy

G1: 3 out of 5 of the females gave birth at full term with kids clinically healthy ;

Embryonic reabsorption in 1 out of 5 (34 days);

Abortion in 1 out of 5 (42 days).

G2: all females gave birth to full-term clinically healthy kids.



Figure 1 – abortion in goats at 42 days of pregnancy

Histopathological examination

- Lung: hemorrhage; thickening of the alveolar septa; perivascular mononuclear and interstitial infiltration; compatible with interstitial pneumonia;

- **Liver:** multifocal necrosis and the presence of predominantly neutrophilic polymorphonuclear infiltration;

- Brain: perivascular gliosis with focal hemorrhage.

Table 2 –Results of laboratory examinations of does and viable newborn goats when does were mated with a buck infected with Toxoplasma gondii

Animal/ Group	Serology	PCR blood	PCR organs	Pregnancy Diagnosis	Reabsorption	Abortion
G1						
1	+	+		x		
1*			+			
2	+			x	x	
3			+	x		x
4		+	+	x		
4 *		+	+			
4*			+			
5				x		
5*			+			
5 *						
G2						
6				x		
6*						
7				x		
7*						
7*						
8				x		
8*						
8*						
9				x		
9 *						
10				x		
10*						

+, positive; serology (titration = 32); * viable newborns.

CONCLUSION

The results obtained in the present study allowed the authors to conclude that *T. gondii* was transmitted via goat semen.

Acknowledgements













Thank you!

E-mail: flavianasw@hotmail.com