Detection of *Toxoplasma gondii* on Multiple Goat Farms in Delaware

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Abstract

Toxoplasmosis is considered one of the most important reproductive diseases as it is a zoonotic disease that causes abortion, mummification, weak kids, and stillbirths. Goats are highly vulnerable to toxoplasmosis, which is caused by the parasite, *Toxoplasma gondii*. This parasite plays a major role in the transmission of toxoplasmosis to animals and humans. Therefore, the objective of this project is to characterize the level of *T. gondii* infection on multiple goat farms in Delaware. To complete this project, blood samples were collected individually from a total of 161 goats from ten producer farms (Farm1 = 11; Farm2 = 20; Farm3 = 15; Farm4 = 19; Farm5 = 12; Farm6 = 11; Farm7 = 20; Farm8 = 11; Farm9 = 22; Farm10 = 20) in the state. White blood cells were isolated from whole blood and used for the isolation of genomic deoxyribonuclease acid (DNA). The isolated DNA were then used in a polymerase chain reaction (PCR) with forward and reverse primers designed for *T. gondii* surface antigen-1 (a *T. gondii* specific surface antigen). After PCR, the amplicons were fractionated on agarose/ethidium bromide (EtBr) gel for DNA separation based on size and visualized by using a Gel Doc Imager. Data indicated that sixteen of the 161 animals tested, were positive for *T. gondii* (Farm2 = 8, Farm3 = 1, Farm4 = 4, and Farm5 = 3). Under the conditions of this study, it can be concluded that four farms were positive for toxoplasmosis.

Keywords: *Toxoplasma gondii*, Blood sampling, Polymerase chain reaction, Small Ruminant, Surface antigen-1

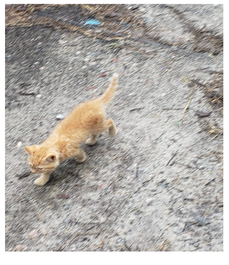
Introduction

Small ruminant production is widely dispersed throughout the world and serves as a source of food for many (FAO, 2014). Small ruminants make a very precious contribution and provide tangible benefits such as cash income, meat, manure, fiber, and skins (Jaitner et al., 2001). Currently, this industry is considered one of the fastest developing animal production systems in the United States (U.S.; Liu et al., 2013). Presently, the demand for goat meat has increased in the U.S. due to recent immigration with individuals coming from countries that uses small ruminant meat for food, religious customs and rituals slaughters (Ibrahim, 2017). Additionally, goat meat is in demand because it is considered to be a healthy red meat alternative where three ounces of goat meat only has 122 calories, 2.9 grams (g) fats, 23 g protein and 63.8 mg cholesterol (Patel, 2018), which is lower than that of cattle. Therefore, goat production is considered a significant and economically viable sector.

Unfortunately, goats are susceptible to a variety of parasitic infections caused by helminths (nematodes, trematodes, cestodes) and protozoa (Benavidez, 2009; Siddiki et al., 2010; Gebremedhin et al., 2014) that leads to the diminution of animal productivity (Terfassa et al., 2018). These parasitic infections can become very detrimental to the sustainability of livestock production. One such infection is toxoplasmosis that is caused by the zoonotic apicomplexan protozoan parasite known as *Toxoplasma gondii*. Toxoplasmosis has a significant impact on small ruminant production worldwide (Jones et al., 2014), causing a variety of reproductive complications, blindness, and death in younger animals (Dubey and Lindsay, 2006; Edwards and Dubey, 2013). *Toxoplasma gondii* is an intracellular protozoan that is dispersed throughout the world and efficient at infecting all warm-blooded animals through many routes (Tenter et al., 2000; Guo et al., 2016).

Objective

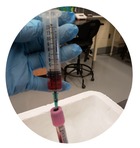
To characterize the level of *Toxoplasma gondii* infection on multiple goat farms in Delaware.

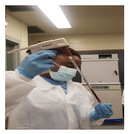


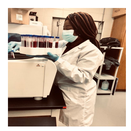
Materials and Methods

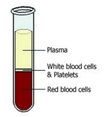
- **Blood samples were collected from animals and white blood cells (peripheral blood mononuclear cells; PBMC) were isolated.**

1. Blood samples were collected from the jugular veins of 10 to 20 goats in New Castle, Sussex, and Kent counties in Delaware.
2. Deposited into Ethylenediaminetetraacetic acid (EDTA) tubes and stored on ice.
3. Placed a 1:1 ratio of Histopaque to whole blood in 15mL centrifuge tubes and centrifuge to separate blood in to plasma, white blood cells, and red blood cells.
4. Aspirated white blood cells (WBCs) and washed twice in centrifuge using 1x phosphate buffered saline (PBS).
5. Decanted WBC pellet into 2mL tube and store for later use.



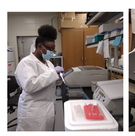


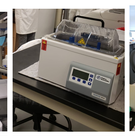


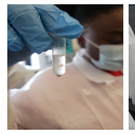


- **Deoxyribonuclease acid (DNA) Extraction**

6. White blood cells were suspended in TSK buffer and incubated at 50 ^{O}C for one hour.
7. Samples were incubated with cetyltrimethyl-ammonium bromide (CTAB) at 65 ^{O}C for 15 minutes
8. Phenol-chloroform-isoamyl alcohol (PCI) was added and samples vortexed, centrifuged and supernatant decanted.
9. PCI treatment repeated and samples centrifuged
10. Aqueous layer was transferred into new tube and centrifuged.
11. Supernatant was decanted and DNA allowed to air-dry, then store for further use.
12. Agarose gel was created for gel electrophoresis, which was done using water as a negative control, pure *T. gondii* DNA as a positive control, DNA from farm animals as sample of interest.
13. Analyze results.





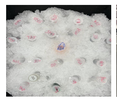


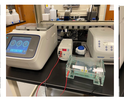
- **Polymerase chain reaction (PCR)**

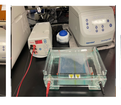
13. PCR was conducted using surface antigen one (**SAG-1**) specific forward and reverse primers to amplify the SAG-1 gene
14. Gel electrophoresis was conducted using a 1% agarose gel to separate the genes by size and allow for visualization of the SAG-1 gene.

PCR sample set up 25µl

- 12.5 µl master mix of GoTaq
- 0.5 µl- forward primer
- 0.5 µl-Reverse primer
- 1 µl – DNA template
- 10.5 µl-Nuclease water







Results and Discussion

Molecular detection of *Toxoplasma gondii* using PCR

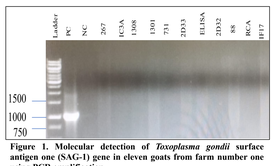


Figure 1. Molecular detection of *Toxoplasma gondii* surface antigen one (SAG-1) gene in eleven goats from farm number one using PCR amplification.

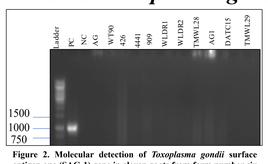


Figure 2. Molecular detection of *Toxoplasma gondii* surface antigen one (SAG-1) gene in eleven goats from farm number six using PCR amplification.

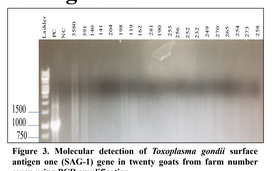


Figure 3. Molecular detection of *Toxoplasma gondii* surface antigen one (SAG-1) gene in twenty goats from farm number seven using PCR amplification.

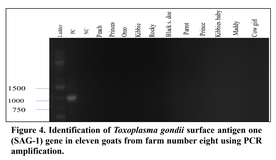


Figure 4. Identification of *Toxoplasma gondii* surface antigen one (SAG-1) gene in eleven goats from farm number eight using PCR amplification.

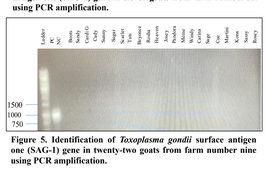


Figure 5. Identification of *Toxoplasma gondii* surface antigen one (SAG-1) gene in twenty-two goats from farm number nine using PCR amplification.

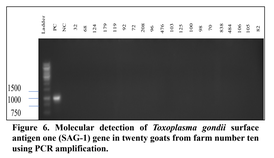


Figure 6. Molecular detection of *Toxoplasma gondii* surface antigen one (SAG-1) gene in twenty goats from farm number ten using PCR amplification.

Data indicated that the SAG-1 gene was not present on farms 1, 6, 7, 8, 9, and 10. Therefore, there was no economic impact of toxoplasmosis on these six goat farms. In addition, *Toxoplasma gondii* was not present in 145 of the goats that were sampled.

Results and Discussion Continued

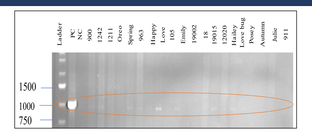


Figure 7. Gel image of *T. gondii* PCR amplicons in twenty goats White Blood Cell using PCR technique.

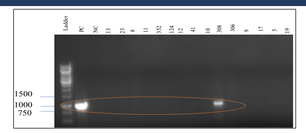


Figure 8. Molecular confirmation of *T. gondii* SAG-1 gene in fifteen goats White Blood Cell using PCR technique.

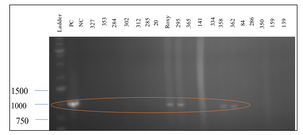


Figure 9. Molecular detection of *T. gondii* SAG-1 gene in nineteen goats using PCR technique.

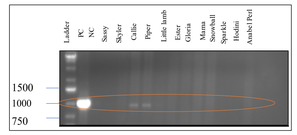


Figure 10. Gel image of *T. gondii* PCR amplicons in twelve goats using PCR technique.

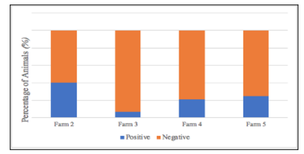


Figure 11. Total percentage of animals on four goat farms that tested positive for *Toxoplasma gondii* surface antigen one (SAG-1) gene.

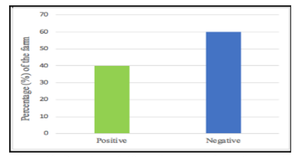


Figure 12. Percentage of 10 small ruminant farms throughout Delaware that tested positive or negative for *Toxoplasma gondii* using the SAG-1 gene as a molecular marker.

There was a prevalence of *Toxoplasma gondii* in sixteen goats found on farms 2 (n =8), 3 (n = 1), 4 (n = 4), and 5 (n = 3). Similar research was conducted on Delaware State University (DSU) small ruminant farm and the data indicated that 18 out of 36 (50%) meat goat does were positive for *T. gondii* infection when they utilize the same PCR protocol with the SAG-1 gene (Unpublished data, 2017). A study done in U.S. by Dubey and colleagues (2011) reported that 53.4% (125/234) of goat hearts acquired from slaughter facilities tested positive for *T. gondii.* Additionally, study conducted in Italy found *T. gondii* in 60.6% (/127) of goats tested on six farms (Mancianti et al., 2013).

Overall, *T. gondii* was present in 9.9% (16/161) of animals tested and on 40.0% (4/10) of the farms. However, there was no difference ($P > 0.05$) observed between farms that were positive for *T. gondii* compared to the farms that were negative. A significantly lower ($P \leq 0.011$) number of animals were positive for *T. gondii* infection than those that were negative.

Conclusion

Under the conditions of this study, there was a presence of *Toxoplasma gondii* on four goats farms out of ten farms in three counties of Delaware. This presence of *T. gondii* infection on small ruminant farms in Delaware can be linked to the high number of cats and kittens that have free access to barns, feed, and feed rooms on these farms.

References

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