Preliminary Detection of *Toxoplasma gondii* on Multiple Small Ruminant Farms in Delaware

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Abstract

The daily production of meat and dairy products of small ruminants have become an important source of revenue for limit resource, beginning and small farmers in the United States. This is due to recent immigration and a push for a healthier red meat product. However, sheep and goats are highly vulnerable to infections from *Toxoplasma gondii* that plays a major role in the transmission of Toxoplasmosis to animals and humans. Toxoplasmosis is considered one of the most important reproductive diseases as it is a zoonotic disease that causes abortion, mummification, weak kids, and stillbirths in sheep and goats. Therefore, the objective of this project is to characterize the level of *T. gondii* infection on small ruminant farms in Delaware. To complete this project, blood samples were collected individually from total of 46 goats from three producer farms (Farm 1 = 11; Farm 2 = 20; Farm 3 = 15) 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 (SAG-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 one of the 46 animals tested was positive for *T. gondii* (Farm 3). Under the conditions of this study, it can be concluded that only one farm was positive for toxoplasmosis. Data from 17 more farms are being analyzed and will give a clearer understanding of the prevalence of toxoplasmosis in the state of Delaware.

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 small ruminant 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; Getz,1998). Therefore, small animal production is considered a significant and economically viable sector.

Unfortunately, small ruminants are susceptible to a variety of parasitic infections caused by helminths (nematodes, trematodes, cestodes) and protozoa (Benavidez, 2009, Gebremedhin et al., 2014; Siddiki et al., 2010) 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 (Guo et al., 2016; Tenter et al., 2000).

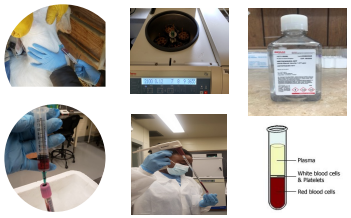
Objective

To characterize the level of *Toxoplasma gondii* infection on small ruminant farms in Delaware

Materials and Methods

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

1. The blood samples were collected from the jugular veins of 10 to 20 goats or sheep.
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 tube and commence centrifugation.
4. Aspirated white blood cells (WBCs) and washed in centrifuge using 1x Phosphate buffered saline (PBS).
5. Decanted WBC pellet into 2mL tube and store for later use.



- **Deoxyribonuclease acid (DNA) Extraction**

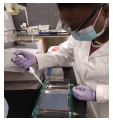
6. Suspended cells in TSK buffer and incubated the samples.
7. Incubated with cetyltrimethylammonium bromide (CTAB) and vortex with phenol-chloroform-isoamyl alcohol (PCI).
8. Centrifuged and repeated PCI treatment.
9. Centrifuged and transferred aqueous layer into new tube.
10. Centrifuged and air-dry DNA and store for further use.
11. Created an agarose gel for gel electrophoresis.
12. Ran gel using water as a negative control, pure *T. gondii* DNA as a positive control, DNA from animals on Delaware farms and 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

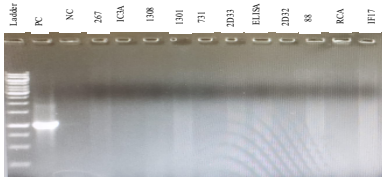


Figure 1. Gel image of *Toxoplasma gondii* PCR amplicons for farm number one in Delaware

Data indicated that the SAG-1 gene was not present in any of the 11 animals tested. It can be concluded that *Toxoplasma gondii* is not present in the goat farm. Therefore, there was no economic impact of toxoplasmosis in small ruminants on this farm.

Results and Discussion Continued

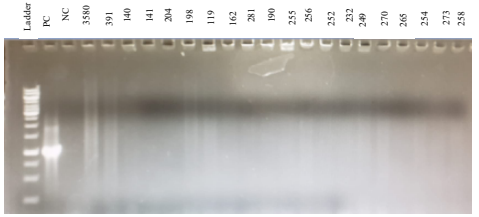


Figure 2. Gel image of Toxoplasma gondii PCR amplicons for farm number two in Delaware

Data indicated that none of the 20 goats tested were positive for *T. gondii* as the SAG-1 gene was not present.

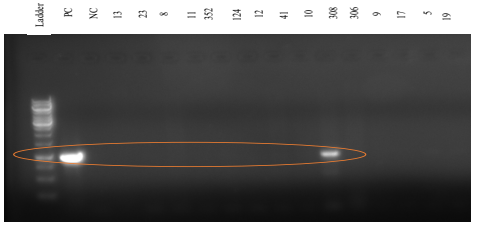


Figure 3. Gel image of Toxoplasma gondii PCR amplicons for farm number three in Delaware

We found that there is a prevalence of *Toxoplasma gondii* in one doe found on farm three out of the 15 does tested. Similarly, a study conducted by Dubley and colleagues (2008) using blood samples collected from the heart of 383 lambs showed that 27.2% of lambs were infected by *T. gondii*. Even though the prevalence in low on this farm, it will be good to pay special attention to this herd as the disease can be spread since it is zoonotic.

Conclusion

Under the conditions of this study, we found that there is a prevalence of *Toxoplasma gondii* on only one farm. We intend to educate farmers about *Toxoplasma gondii*. Additionally, more farms will be tested to determine how prevalent *T. gondii* infection is, in Delaware.

References

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