2014

The Prevalence of Encysted Toxoplasma & Sarcocystis in Consumer-grade Pork, Beef, and Lamb in Michiana

Ross Trecartin

J. N. Andrews Honors Program
Andrews University

HONS 497
Honors Thesis

The Prevalence of Encysted Toxoplasma & Sarcocystis in Consumer-Grade Pork, Beef, and Lamb in Michiana

Ross Trecartin

March 31, 2014

Advisor: Bill Chobotar

Primary Advisor Signature: Bill Chobotar

Department: Biology

Abstract

Commonly present in the muscle of animals are the tissue cysts of *Toxoplasma* and *Sarcocystis*. The aim of this study was to determine the presence and levels of *Toxoplasma/Sarcocystis* tissue cysts in retail pork, beef, and mutton throughout the Michiana area. A total of 36 samples from the three species of interest were exposed to a digestive solution mimicking stomach conditions. The solution consisted of 0.75% pepsin, 0.86% NaCl, and HCl adjusted to a pH of 1-2. The digested samples were then strained through several layers of cheesecloth, centrifuged, and examined for the presence of parasites. *Toxoplasma/Sarcocystis* were detected in all species of meat with an overall prevalence of 55 %. Although not ubiquitous in every meat product, improper preparation could leave the consumer at risk of infection.

Introduction

Animal flesh is a source of a many of parasites that also infect humans. Some of these include *Taenia saginata*, the beef tapeworm, and *Taenia solium*, the pork tapeworm. Also commonly present in the muscle of animals are the tissue cysts of *Toxoplasma* and *Sarcocystis* (Schmidt & Roberts 2009). Humans can acquire these parasites by ingesting meat that has been improperly prepared. For some dishes, cookbooks will instruct the reader to sear the meat but not completely cook it though, or that a steak should be cooked so that the outside is brown, but the interior is still pink and juicy. Sufficient preparation will destroy encysted *Toxoplasma* and *Sarcocystis* (Tenter et al. 2000). Although the risk of acquiring an infection of *Toxoplasma* or *Sarcocystis* is eliminated if the meat is cooked well enough, being able to determine the amount of consumer grade meat has the potential to cause an infection if improperly prepared is useful. The aim of this research project was to determine the prevalence of *Toxoplasma*/*Sarcocystis* in retail meat in the Michiana area.

Materials and Methods

Sample Selection

A total of 36 samples from three species (pork, beef, and lamb) were purchased from nine retail stores in the Michiana area. The samples were boneless cuts of fresh muscle tissue that had not been treated by temperature extremes. Care was taken to temporally separate the purchases in order to decrease the probability of two samples being from the same animal. As this study is concerned with general

prevalence of *Toxoplasma & Sarcocystis,* no attempt to compare the various retailers to each other was made. Each retailer was assigned a letter of the alphabet to ensure anonymity.

Digestion[1]

The samples were processed *in vitro,* in conditions similar to those that occur *in vivo*. Each 50 g sample was ground in a meat grinder, placed in a flask, and immersed in a digest solution consisting of 0.75 % pepsin, 0.86% NaCl, and adjusted to a pH between 1 and 2. The flask containing the digesting sample was then maintained at 37° C for 2 hours in an agitating water bath (Jacobs et al. 1960, Box et al. 1978, Potts 1992).

Concentration & Analysis

When digestion was complete the residual artifacts were strained out with several layers of cheesecloth, and the digestion mixture concentrated via two rounds of centrifugation (10 min at 1000 rpm). The Supernatant was poured off and the sample was re-suspended in physiological saline (0.85 % NaCl). A wet mount was prepared and the concentrated digest solution examined with light microscopy. In order to be accepted as positive, numerous parasites were confirmed by both members of the research team. Before any sample was accepted as negative at least six slides were systematically searched without any *Toxoplasma/Sarcocystis* observed.

---

[1] The digestion process utilized in this project was described by Jacobs and colleagues in 1960. In 1978 Box and McGuinness used the same digestion process to break down the diaphragms of various animals. In 1992 Robert Potts used the methodology of Jacobs et al. and Box et al. to design the digestive portion of his experiment.

Results

Toxoplasma/Sarcocystis zoites were confirmed to be present in 20 of the 36 samples examined (Table 1, Figure 1). Of the 16 pork samples examined two were confirmed to be infected by *Toxoplasma/Sarcocystis*. Zoites were confirmed to be present in all 16 of the beef samples examined. The four lamb samples included two that were infected with *Toxoplasma/Sarcocystis* species. Thus the overall prevalence of infection observed in the samples examined was 55 %: pork 12.5 %, lamb 50.0 %, and beef 100.0 % respectively (Figure 2).

Discussion

While an overall 55% infection rate was found in our samples, there was a marked difference between the types of meat. Based on the work of Dubey and colleagues (2005), we expected to find more *Toxoplasma/Sarcocystis* in pork than in beef. Indeed the relative importance of cattle in transmission of *T. gondii* through meat has been reported to be relatively small (Tenter et al. 2000). The results obtained in the current study showed the prevalence of *Toxoplasma/Sarcocystis* to be 12.5 % in pork, 50 % in lamb, and 100 % in beef (Figure 2). The difference between the results we expected from a review of the literature, and the values we determined during the study may be explained in the way our experiment was constructed. In the literature most studies either selected for *Toxoplasma* or *Sarcocystis* but not both concurrently. Thus the higher prevalence in beef may be a result of high *Sarcocystis* infection and not *Toxoplasma*. In order to differentiate between these two parasites,

techniques that are beyond the scope of this study such as nucleic acid identification and or immunological assays are required.

The prevalence of *Toxoplasma/Sarcocystis* found in retail markets throughout Michiana has implications for meat preparation. If measures are not taken to treat the meat with sufficient temperature to destroy the parasites, a horizontal infection in humans may occur. Proper preparation of meat may include freezing fresh meat at temperatures below 20 C and or cooking until meat no longer has a pink interior. If fresh meat is prepared properly *Toxoplasma* and *Sarcocystis* will no longer be infective and their presence in a sample will not have an adverse effect.

Literature Cited

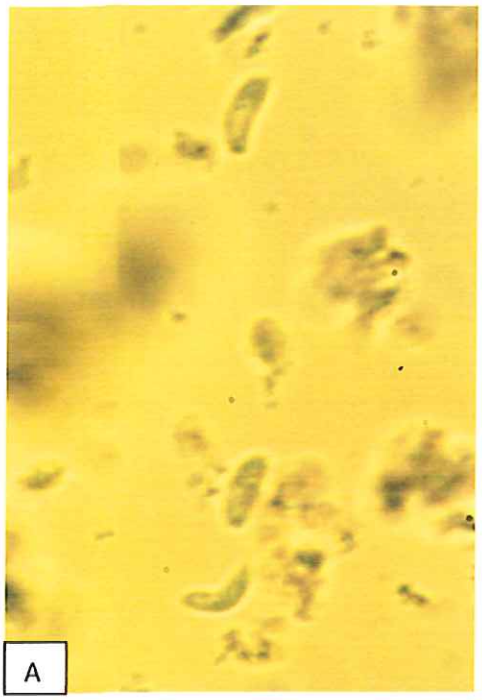
Schmidt, G., & Roberts, L. (2009). *Foundations of Parasitology* (9th ed.). New York: McGraw-Hill.

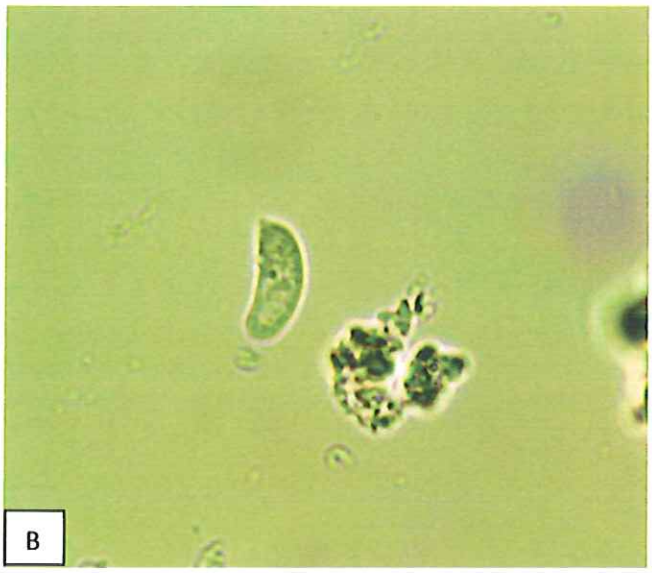
Tenter, A. M., Heckeroth, A. R., Weiss, L. M. (2000). *Toxoplasma gondii: from animals to humans. International Journal for Parasitology, 30*(12-13), 1217-1258.

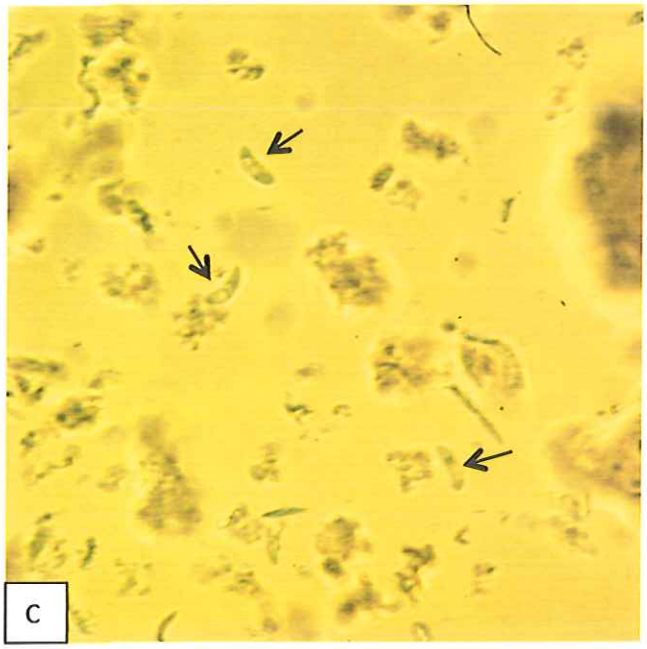
Box, E. D., & McGinness, T. B. (1978). *Sarcocystis* in Beef from Retail Outlets Demonstrated by Digestion Technique. *The Journal of Parasitology, 64*(1), 161-162.

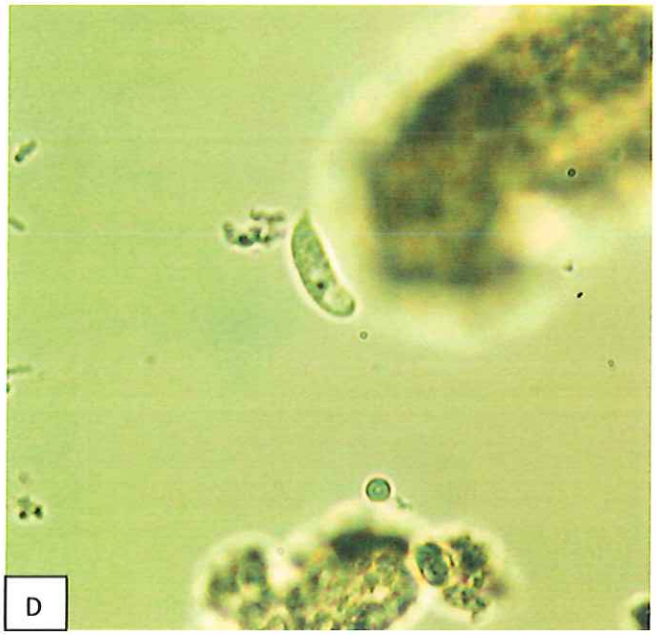
Robert, P. (1992). The Prevalence of *Toxoplasma* and *Sarcocystis* Cysts in Meat Purchased From Retail Food Stores in the Southwest Michigan Area. Andrews University.

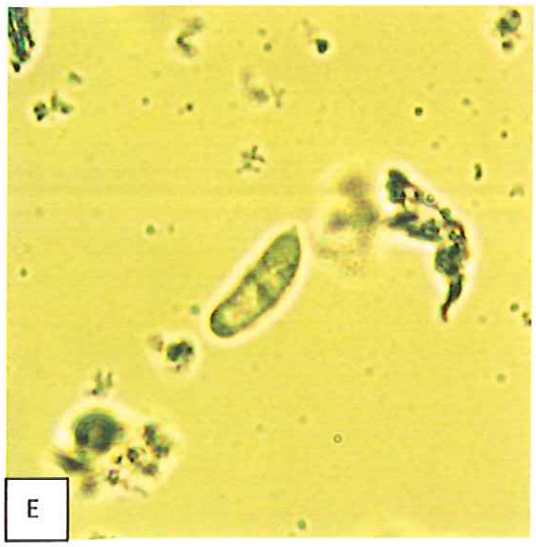
Dubey, J. P., Hill, D. E., Jones, J. L., Hightower, A. W., Kirkland, E., Roberts, J. M., Marcet, P.L., Lehmann, T., Vianna, M.C.B., Miska, K., Sreekumar, C., Kwok, O.C.H., Shen, S.K., Gamble, H.R.(2005). Prevalence of viable *Toxoplasma gondii* in beef, chicken, and pork from retail meat stores in the United States: risk assessment to consumers. *The Journal of Parasitology, 91*(5), 1082-1093.











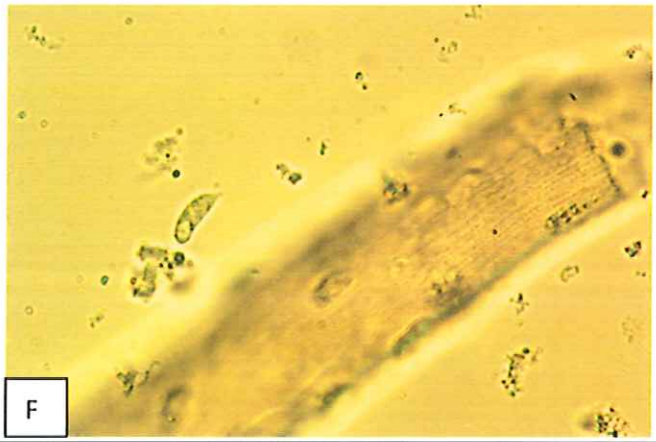


*Figure 1.*Zoites of *Toxoplasma/Sarcocystis* appear as banana-shaped cyst merozoites. Photographs were taken with an oil immersion lens 1000X magnification. A: free range lamb, Australia. B: beef heart. C: three zoites (arrows) free range lamb, Australia. D: beef heart. E: beef chuck shoulder. F. beef chuck shoulder.

Pork Samples	Parasites Present	Beef Samples	Parasites Present	Lamb Samples	Parasites Present
1A	+	2A	+	4A	(-)
1B	+	2B	+	6A	+
3A	(-)	5A	+	16A	(-)
8A	(-)	7A	+	28A	+
8B	(-)	9A	+		
12A	(-)	10A	+		
13A	(-)	11A	+		
13B	(-)	14A	+		
15A	(-)	19A	+		
17A	(-)	20A	+		
18A	(-)	21A	+		
22A	(-)	23A	+		
26A	(-)	24A	+		
30A	(-)	25A	+		
31A	(-)	27A	+		
32A	(-)	29A	+		

Table 1 records the presence or absence of *Toxoplasma/Sarcocystis* in each sample. Samples that are confirmed positive or negative are demarked "+", and "(-)" respectively.

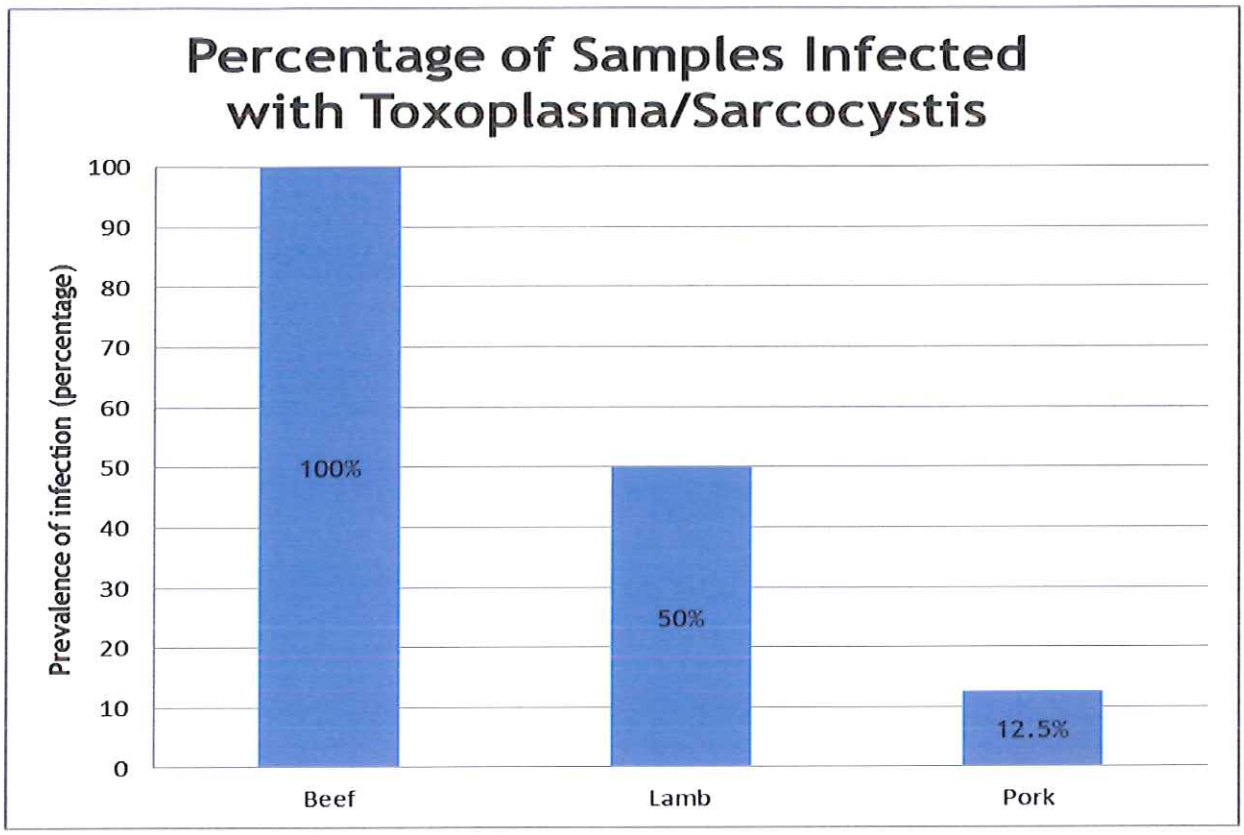


Figure 2 illustrates the overall prevalence of *Toxoplasma/Sarcocystis* in the three species of meat studied.