Tick-Transmitted *Babesia* in Cattle on the Island of Dominica

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Introduction

Ticks are an important vector for arboviruses and transmit diseases that affect animals and humans alike such as Lyme disease in humans and Babesiosis in livestock. They are considered ectoparasitic arthropods that feed on the blood of humans, livestock, and fowl. Females are much larger than their counterparts due to how big their idiosoma can expand to supply nutrients to their hundreds of young. There are some species that spend their entire lives on one host, while there are others that spend their entire lives on many hosts. These organisms are primitive apterous and ametabolous arthropods. the ticks are split up into three different families. The first being ixodidae which are the hard ticks, the second being Argasidae which are the soft ticks, and the last family is Nuttallielladae which has no medical importance at this time.

Basic anatomy includes a capitulum and idiosoma; the capitulum includes the chelicera, hypostome, and the palps and the idiosoma includes the eight legs, the eyes (can be absent) between the first coxae and second coxae. Ticks have an extra leg segment found in arachnids called the patella and on their first pair of legs they have what what is called a hallers orgen whcih acts as a chemosensor. Ticks feed by cutting an opening in the host, followed by inserting their hypostome while secreting anticoagulants to keep the blood flowing and glue to anchor themselves down. The soft ticks have all these characteristics and their capitulums are are on the underside on their bodies. This family practices a certrain a certain behavior called neosomy in which they can slow down their metabolic system allowing them to go without food for longs periods of time. They are commonly found in places such as cabins in the woods.

Hard ticks take it a step further; they have a hard plate called a scutum or dorsal shield located

right above their capitulums. Females have a reduced scutum while males have one that extends all the way over their idiosomas. The capitulum extends forward in front of the body and not on the under-side; it's very distinct looking down on a tick. One way being able to tell the difference between the species are the spines that emerge out of their coxae. Hard ticks are found almost anywhere; they wait on the edges of plants with their front legs in the air waiting for potential prey walking by.

The purpose of this experiment was to survey the population of cattle for Babesia to the veterinarians' discretion on what they believed to be sick and infected animals. Our experiment is a continuation of a previous experiment conducted 4 years ago; this experiment was conducted using information from Dr. Holman and the previous groups methods and materials. This report is going to be used as a reference for future projects and the results shall be used to assess health risks and level of infection within regions.

Materials and Methods:

In order to obtain the tick and blood samples utilized in this study, visits were made to varying locations on the island of Dominica where the protozoan parasite *Babesia* was thought to be present.

Dr. Lennox St. Aimee obtained the anti-coagulant blood by means of the jugular vein in the cattle and placed the samples in Vacutainers. Immediately after they were labeled accordingly and placed within a cooler on ice. Cattle restraint methods used are depicted in *Figure 1.1 and 1.2*.

The ticks on the other hand were cautiously and slowly uplifted by the head with forceps so as to not twist the body and cause further transmission. Gloves were worn. Upon collection they were placed in 20 ml glass scintillation vials and kept out of direct sunlight in a well shaded area. All ticks collected from one animal were placed within the same vial and labeled with that animal's number. Field collection data for both blood and tick samples can be seen in *Table 1*.

In total 13 of 17 cattle provided tick samples and 16 of 17 provided blood samples. Sample quantities are not the same for each animal due to unexpected absence in ticks or muscle thickness that made blood withdrawal difficult and not possible.

Samples from cattle numbered one through five were obtained on May 30, 2012 from the Southeast, the Livestock Facility near Melville Hall, and Douglas Bay. Samples from cattle numbered six through sixteen were collected on June 1, 2012 in Douglas Bay, at several locations in Grand Bay, and Grand Coulibille. When samples were collected the following data was recorded in a notebook and translated into Table 1: date of collection, animal #, tick count per animal, blood vial #, cattle breed, sex, collection location, and the name of the producer/farmer whom owned the animal. Sample vials and Vacutainers were labeled with cattle #, sex, location, and producer/farmer name. All labeling was completed with a 0.5 mm Pigma Pen.

All of the blood and tick samples were taken to the Ministry of Agriculture's Molecular Diagnosis Lab to be processed onto glass slides. To preserve samples the blood was kept in a refrigerator and the ticks were left in the air-conditioned lab. Slides were made there on June 1, June 4, and June 8, 2012. Here following is a description of how each sample was prepared to make permanent mount slides.

First the stain was created; the ratio of stain to water was 1:20. Five ml of Giesma (Sigma Accustain GS-500) was added to a graduated cylinder with 95 ml of water and poured into a 250mL Erlenmeyer flask for use. The stain had to be made fresh daily.

Then, 16 inch rulers were placed along the length of the sink so as to provide a slide stand to stain on.

Slide Staining Method for Anti-Coagulant Blood Samples

The slides were first cleansed with 95% ethanol and dried clean with a Kimwipe on both sides. After this, with latex gloves being worn, a blood droplet was taken from a sample Vacutainer by means of a BD 5mL syringe and Terumo 18G x 1" needle. Although this was all that was available, the needle gauge was quite large and it is recommended that in future experiments a 23 gauge needle be used instead to minimize the size of the blood droplet. Otherwise the smears may appear too thick and cause the clumping of red erythrocytes which in turn make the slides difficult to view under microscopy.

After the application of a single drop to a glass slide it was smeared along the entire length of the slide to about a ½ inch of where the label would go. This was done by using a 24 x 50 mm VMR Cover Glass to gently drag out the blood. See Figure 2 for a visual of the slide set-up. After this, it was time to affix the red blood cells to the slide; methanol (methyl alcohol) was applied to the length of the blood smear by use of a plastic Nalgene bottle so as to cover it in its entirety. This was allowed to dry for approximately 15 minutes. Then the methanol was applied a second time and allowed to dry for an additional 15 minutes. After ensuring that the Methanol was completely dry the fresh stain was applied over the entire smear by means of a glass Pasteur pipette. Then after allowing 30 minutes to pass and checking that the slide was dry it was

cautiously rinsed with water and allowed to air dry. Upon completion a ½ inch wide label was created with VMR tape and adhered directly to the left side of the slide with the word "Blood" and the animal # it came from (i.e. a blood sample from animal #1 was labeled "Blood #1"). Labels were made using a 0.5mm Pigma Pen. The slide was then ready for viewing.

This was the procedure for all of the blood sample slides. However, each time a new slide was prepared a new needle and syringe set had to be used to prevent cross-contamination between blood samples. Pasteur pipettes could be reused in the application of the Giesma stain so long as they didn't come into contact with the slides.

Slide Staining Method for Tick Hemolymph

First, slides were cleansed with 95 % ethanol and dried with KimWipes. Then a tick was taken with forceps and popped; the body of the tick was squeezed gently to allow the hemolymph within to come out onto the slide (in the same area where the blood droplet would have been placed had this been a blood slide; refer again to *Figure 2).* The tick hemolymph was then spread across the slide. To be sure that the hemolymph layer was thin and not clumpy any present clumps were picked off gently with forceps. Next, methanol was applied by a Nalgene bottle so as to cover the hemolymph area and allowed to air dry for 15 minutes. Finally the stain was added to the hemolymph area and allowed to dry for 15 minutes. After, the slide was rinsed gently with water and allowed to air dry. Upon completion a ½ inch wide label was created with VMR tape and adhered directly to the left side of the slide with the word "Tick" and the animal # it came from (i.e. a tick sample from animal #1 was labeled "Tick #1"). Labels were made using a 0.5mm Pigma Pen. The slide was then ready for viewing.

Upon completion of all of the slides, they were viewed one by one under a Nikon Eclipse E800 Microscope to scan for signs of Babesia. In particular we were looking for things that were stained blue and purple that looked similar to reference slides/photographs given to us by Dr. Patricia Holman and Dr. Tom Craig from Texas A&M University, some of which can be viewed in Figure__. When using the microscope we utilized Nikon Immersion Oil for viewing under the 60x/1.4 objective. On occasion, however, we did utilize the 40x/1.4 and 100x/1.4 objectives when needed.

When items of interest were found we photographed them using the Nikon Digital Camera Dxm1200F attached to the microscope. The camera was hooked up to an Hp Workstation XW4100 computer and the photographs were manipulated and saved through the Lucia G program.

After viewing, the slides were put away in a slide box lined with KimWipes so that any leftover immersion oil would drop off. **It was vital that since Xylene was not available to cleanse oil immersed slides that they were not wiped or cleaned with anything for this could damage the smear.** The microscope lenses that touched oil were then cleaned off with Kodak Lens Cleaner and dried off by the gentle dragging of a piece of lens paper across the top.

Chemical/Material Disposal:

Needles were capped and placed within an incineration bin (Cin-Bin) immediately after use. Any broken slides and used cover glasses were placed in as well. Used Syringes were rinsed down the sink and then thrown into a hazardous materials garbage bag. The Giesma stain mixture was also poured down the sink. As for the ticks and blood, they were placed in their own Ziploc baggies and sealed. Dr. Lennox St. Aimee disposed of them for us.

Figure 1.1: Cattle Restraint

Figure 1.2: Cattle Head Restraint

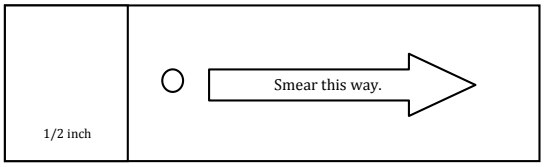

Figure 2: Slide Set-up

Data:

Table 1: Field Data Collection for Ticks and Anti-Coagulant Blood Samples from Cattle on the Island of Dominica (2012)

Date of Collection:	Cattle #	Tick Vial #-count	Blood Vial # (anti-coagulant)	Breed	Sex	Collection Location:	Producer:	Collected From:
May 30, 2012	#1	no ticks	#1	Brahman	F	South East?	Brentanol	no ticks
May 30, 2012	#2	17	#2	Holstein	M	Livestock Facility	Not Applicable	hind lower legs, flank, neck
May 30, 2012	#3	13	#3	Holstein	F	Livestock Facility	Not Applicable	lower legs, teats, flank
May 30, 2012	#4	3	#4	Brahman	F	Livestock Facility	Not Applicable	hip bone area
May 30, 2012	#5	13	#5	Brahman	M	Douglas Bay	Not Applicable	lower legs, flanks, hip bone area
June 1, 2012	#6	no ticks	#6		F	Grand Bay	Jullian John	no ticks
June 1, 2012	#7	no ticks	#7		M	Grand Bay	Jullian John	no ticks
June 1, 2012	#8	3	#8		M	Grand Bay	Jullian John	flanks
June 1, 2012	#9	6	#9		M	Grand Bay (l2)	Alexander	hip bone, flanks
June 1, 2012	#10	no ticks	#10		M	Grand Bay (l2)	Alexander	no ticks
June 1, 2012	#11	1	#11	Brahman	F	Grand Bay (l2)	Alexander	flank
June 1, 2012	#12	9	#12		F	Grand Bay (l2)	Alexander	flanks, lower legs
June 1, 2012	#13	1	#13		F	Grand Bay (l3)	Alexander	flank
June 1, 2012	#14	3	#14		F	Grand Bay (l4)	Alexander	flank
June 1, 2012	#15	7			F	Grand Coulibllle	Arthur Leuleerent	flank, shoulder blade
June 1, 2012	#16	2	#16		F	Grand Coulibille	Anthony Creek	shoulder blade, flank
June 8, 2012	#17	no ticks	#17	unknown	F	Sultan	Mann Mitchell	no ticks
L=location								

Results;

Table 2: Slide Scanning Results for Blood Samples. Coordinates given are based on the Vernier scale of the Nikon Eclipse E800 Microscope.

Sample #	Magnification	Slide Coordinates for areas of interest:		Photo #	Incidence of Babesia
		Vertically	Horizontally		
1	60x	----------	----------	None taken	Unknown
2	60x	----------	----------	None taken	Unknown
3	60x	----------	----------	None taken	Unknown
4	60x	----------	----------	None taken	Unknown
5	60x	103	24.5	None taken	Negative
6	60x	100	28.1	None taken	Negative
7	60x	99.6	27	Blood_07_01	Negative
8	60x	----------	----------	None taken	Unknown
9	60x	----------	----------	None taken	Unknown
10	60x	----------	----------	None taken	Unknown
11	----------	Slide not viewed		None taken	Unknown
12	----------	Slide not viewed		None taken	Unknown
13	60x	----------	----------	None taken	Unknown
14	----------	Slide not viewed		None taken	Unknown
15	----------	No blood collected to view		None taken	Unknown
16	----------	Slide not viewed		None taken	Unknown
17	60x	----------	----------	None taken	Negative

Table 3: Slide Scanning Results for Tick Samples. Coordinates given are based on the Vernier scale of the Nikon Eclipse E800 Microscope.

Sample #	Magnification	Slide Coordinates for areas of interest:		Photo #	Incidence of Babesia
		Vertically	Horizontally		
1	----------	No ticks collected to view		None taken	Unknown
2	60x	----------	----------	None taken	Negative
3	60x	96.6	12.2	None taken	?
		97.0	12.1		
4	60x	----------	----------	None taken	Negative
5.1	40x	98.0	49.9	?	+ ?
	60x	102.5	49	?	
		103.0	48.5	?	
		103.0	48	?	
5.2	60x	99.1	18.9	?	
6	----------	No ticks collected to view		None taken	Unknowm
7	----------	No ticks collected to view		None taken	Unknown
8	----------	Slide not viewed		None taken	Unknown
9	----------	Slide not viewed		None taken	Unknown
10	----------	No ticks collected to view		None taken	Unknown
11	----------	Slide not viewed		None taken	Unknown
12	----------	Slide not viewed		None taken	Unknown
13	----------	Slide not viewed		None taken	Unknown
14	----------	Slide not viewed		None taken	Unknown
15	60x	108.0	41.5	Tick_15_01	?
		108.2	36.5	Tick_15_02	
		110.0	44.0	Tick_15_03	
16	----------	Slide not viewed		None taken	Unknown
17	60x	?	?	None taken	?

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