A Study of Babesia in Cattle on the Island of Dominica

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Abstract

The objective of this study was to take a random survey of cows on the island of Dominica and test for *Babesia* species. Areas on the island of Dominica include Cottage, Soufiere, Grand Bay, Castle Bruce, Mahaut River, Bataca and Hatton Garden. Ticks and blood were collected from the cows and samples were brought to the lab for slide preparation in order to determine if the cow tested positive for *Babesia*. Less than 20% of cattle showed positive signs of *Babesia*.

Introduction

In cattle, Bovine babesiosis is a major disease transmitted by ticks. This disease is caused by a protozoan parasite, *Babesia* species. Physical signs of infected cattle include fever, anorexia, depression, increased respiratory rate, muscle tremor, reluctance to move, and behavioral changes such as circling, head pressing, mania and convulsions. (1) The disease is more likely to affect older animals and is not often seen in cattle less than six months. The post-mortem signs include different degrees of congestion, pallor or jaundice, blood that is watery, red urine, sub-serosal haemorrhages, enlarged spleen with a soft red pulp, enlarged brown or yellow liver, and a gall bladder that is filled with thick, granular bile.(1) For this study, blood samples were taken and fixed onto slides to detect the presence of babesiosis. Ticks were collected on cows and hemolymph was examined

for the presence of *Babesia*. To control the disease, a combination of a tickicide and a babesiosis vaccine are most effective.

In Dominica, the ticks that are most prevalent on cattle are *Boophilus microplus,* 'Tropical Cattle Tick'*, and Amblyomma variegatum* 'Tropical Bont Tick.' *B. microplus* is a one host tick found in the family Ixodidae. It is characterized as being a hard tick that has mouthparts that protrude forward, a hexagonal basis capitulum, an oval spiracular plate, and palps that are short and ridged dorsally and laterally. No festoons are present in *B. microplus*. In females, the anal groove is absent and in males adanal plate an accessory shields are present (4) *Ambloymma variegatum* is a three-host tick found on small animals, sheep, goats, and cattle. It is found in the family Ixodidae and is characterized by long and strong mouthparts. The palps are long and the second segment is twice as long as it is wide. Even thought the tick does not have an adanal shield present, the festoons are well developed.

The four stages of the cattle tick are egg, larvae, nymph, and adult. An adult female will first lay up to 3,000 eggs on the ground. After 2-6 months the eggs will hatch into larvae, also known as 'seed ticks,' and move up onto blades of grass, for up to 8½ months, in search of a host. After they find a host, they will feed for up to a week, molt, and become the nymph stage. As a nymph, they will feed for another week, molt, and turn into adults. At the adult stage, the male will feed, but will not fully engorge with blood as his main purpose is to mate with the females. On the contrary, the adult female will fully engorge with blood and feed for about a week. At the end of the week, the female will mate and release herself from the host and lay eggs on the ground and the cycle continues. (3)

Babesia has also affected humans. Babesiosis a disease caused by *Babesia* can produce flu like symptoms including fever, chills, headaches, and pain. Severe cases can result in shortness of breath, hemolytic anemia, and hemoglobinuria jaundice. Treatment for babesiosis includes both oral atovaquone with oral azithromycin.

Materials and Methods

In this study, the materials used consisted of a data sheet, Magellan GPS tracker, 20 mm dram vials, orange centrifuge tubes, gloves, an identification key, Leica compound microscope, VMR Frosted 75x25x1mm slides, VMR cover glass 22x22mm No 1 1/2, 1:20 solution of Giemsa Stain, purified (deionized) water, small bucket, mesh screen, syringe, needles, labels, Pigma pens, methanol, and ethanol .

In the field, ticks were collected off of cattle on May 27, 28 and June 2. Throughout this study, Dr. Lennox St. Aimee aided in the process of sampling from cattle in several locations on the island. He would drive us out to random locations and inform the owners that we were conducting a random sample on cattle for *Babesia*. The cattle were restrained with a leash and a nose lead to prevent any damages to people. The doctors that were helping that day would collect two blood samples, one with anti-coagulant and one without any anti-coagulant. These samples were kept in a cooler with ice packs until we returned to the lab later in the afternoon. Following blood collection, students would then pick off the ticks by hand while they were wearing latex gloves and put them in separate vials. Each vial was assigned to one cow and labels were placed in each vial to prevent confusion. On May 27, ticks were collected on the north side, at

Cottage from Creole cattle. On May 28, ticks were collected on the south side, at Soufriere and Grand Bay from Holstein and Creole mixed cattle. On June 2, ticks were collected on the east side, at Castle Bruce, and in Carib Territory. At each collecting site, a GPS record was taken in addition to noting the sex, breed, and producer of the cattle. (A chart is provided below.)

When collecting ticks from cattle Latex gloves should be worn at all times. The head of the tick was grasped and pulled straight back in order to preserve the mouth parts for identification. Ticks were most commonly found on the hindquarters, neck and head area of the cattle. The ticks were collected live and stored in a vial for hemolymph extraction which would be performed in the lab.

Slides of hemolymph and blood were prepared in the lab on May 29, June 3, and June 5 to detect for *Babesia*. On May 29, the 1:10 (Giemsa Stain to water) and 1:20 of Giemsa Stain was tested to see which had the best result. It was determined that the 1:20 solution had the best result. (The stain must be remade each day.) The live tick leg was cut with a blade and the body was gently squeezed with forceps in order to extract the hemolymph. The hemolymph was fixed once with Methanol, allowing the methanol to dry completely before staining the slide for 15 minutes, and rinsing it with purified water.

At each location, blood samples were taken from the cattle and stored in an anti-coagulant tube inside the lab's refrigerator. In the lab, the blood samples were drawn out using a syringe and a single drop was placed on the slide. (For each blood sample a different syringe was used in order to prevent cross contamination.) The blood was fixed with methanol twice, allowing the methanol to dry each time, stained for 30 minutes, and

rinsed with purified water. A small bucket was placed in the sink and a wire screen was placed over it to lay the slides for staining and rinsing.

Results

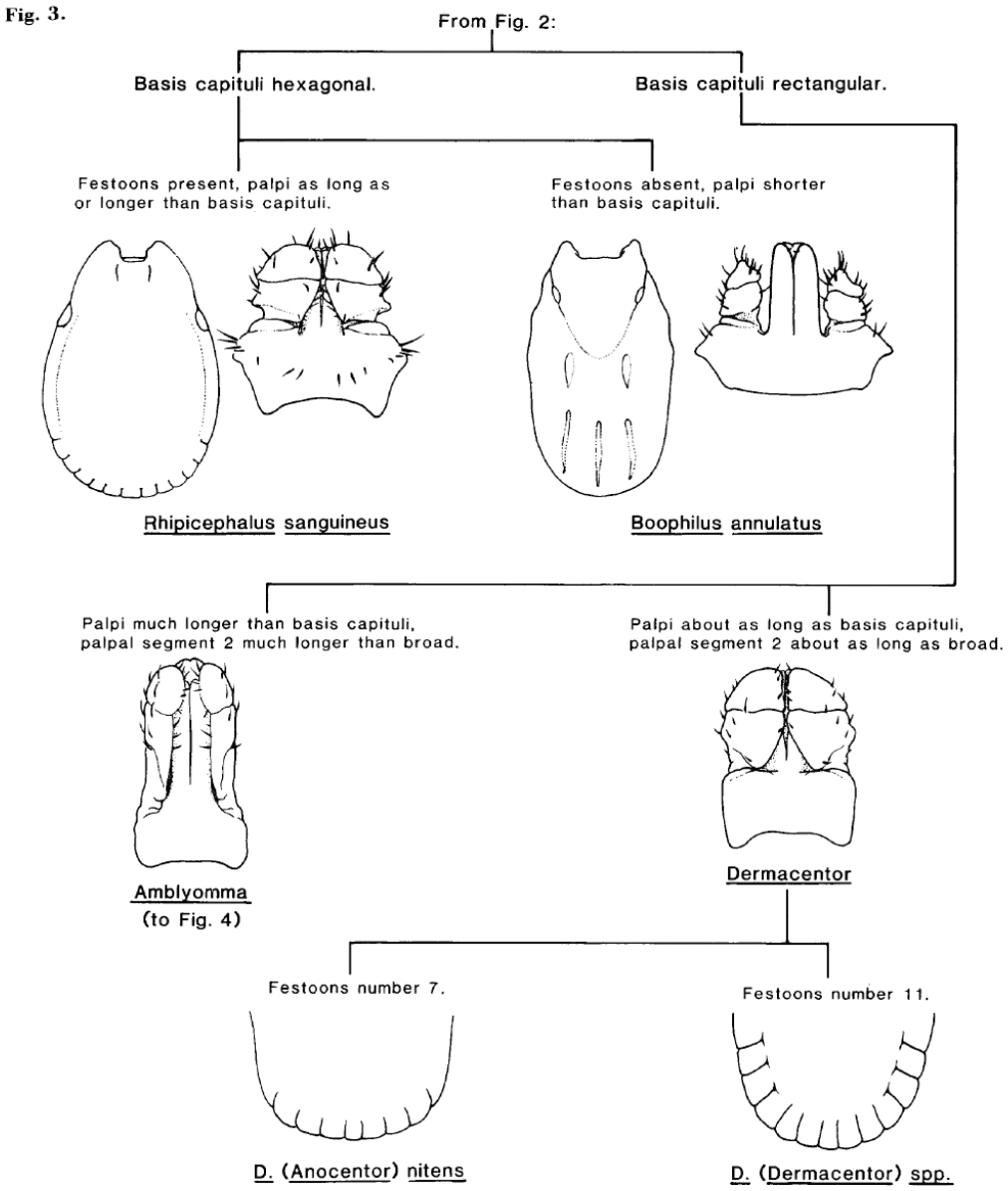
Out of the 26 cattle tested for *Babesia*, only five tested positive after their blood smears were reviewed through the microscope. On May 27th, the first day ticks were collected, no samples came back positive for *Babesia*. Places that were sampled were Cottage, Fedrick Roberts Ranch, Serran Cognet Ranch, and Soufiere (coordinates 15°20.93N, 61°21.93W; 15°37.20N, 61°27.77W; and 15°34.47, 61°26.24 respectively). On May 28th, the second day ticks were collected, a female Holstein cow that Andre Charles owned tested positive for *Babesia* (detected in tick hemolymph). A female Creole Mix cow that was in Grand Bay at Julian John's Ranch also tested positive for *Babesia* (detected in cow's blood). Areas collected on May 28th were in regions in and around Soufiere and Grand Bay (the following are coordinates of where we visited starting with the first area we went to and ending with the last: 15°34.47N, 61°26.54W; 15°13.81N, 61°20.98W; and 15°14.58N, 61°18.64. The last day of tick collection occurred on June 2nd. On this day, a Creole mix cow tested positive for *Babesia* (found in tick hemolymph). The same day, a Creole Mix cow from Dorime Mason at Bataca tested positive for *Babesia* (detected in tick hemolymph). A Creole Mix cow from Hatton Garden also tested positive for babesia (detected in cow's blood). Places collected on June 2nd were Castle Bruce, Mahaut River, Bataca and Hatton Garden (the following are coordinates of where we visited starting with the first area and ending with

the last 15º25.78N, 61º15.68W; 15º25.81N, 61º15.90W; 15º 25.24N, 61º15.17W; 15º30.26N, 61º15.98W; and 15º31.08N, 61º16.58W).

Date	Blood Label	Animal #	Location Name	Location #	GPS Reading	Producer Name	Location Ticks Collected	Breed	Sex	Babesia found
5-27-09	Nor 1	1	Cottage	1-1	15° 20.93 N 61° 21.93 W	Paul	Face, underbelly, hind leg	Creole	Female	No
5-27-09	Nor 2	2	Cottage	1-1	15° 20.93 N 61° 21.93 W	Paul	Neck, side	Creole	Female	No
5-27-09	No blood	3	Cottage	1-1	15° 20.93 N 61° 21.93 W	Paul	Neck, testicles, sides, ears	Creole	Male	No
5-27-09	Nor 3	4	N/A	2-1	15° 37.20 N 61° 27.77 W	Frederick Roberts	No ticks, pesticide applied	Creole	Male	No
5-27-09	No blood	5	N/A	3-1	15° 34.47 N 61° 26.24 W	Serran Cognet	shoulder	Creole	Male	No
5-27-09	Nor 4	6	N/A	3-1	15° 34.47 N 61° 26.24 W	Serran Cognet	Neck, stomach, back, udder	Creole	Female	No
5-28-09	Sou 1	7	Soufriere	1-2	15° 34.47 N 61° 26.54 W	Andre Charles	No ticks collected	Holstein	Female	Yes
5-28-09	Sou 2	8	Soufriere	1-2	15° 34.47 N 61° 26.54 W	Andre Charles	Udder, left hindleg	Holstein	Female	No
5-28-09	Sou 3	9	Soufriere	1-2	15° 34.47 N 61° 26.54 W	Andre Charles	Flank, chest, hind leg	Holstein	Female	No
5-28-09	No blood	10	Soufriere	2-2	15° 13.81 N 61° 20.98 W	Isodore Bellot	Flank, neck, udder, shoulder	Holstein - Creole mix	Female	No
5-28-09	Sou 4	11	Soufriere	1-2	15° 34.47 N 61° 26.54 W	Andre Charles	No ticks collected	Holstein	Female	No
5-28-09	Sou 5	12	Soufriere	2-2	15° 13.81 N 61° 20.98 W	Isodore Bellot	Flank, hindleg, neck	Holstein	Female	No
5-28-09	Sou 6	13	Soufriere	2-2	15° 13.81 N 61° 20.98 W	Isodore Bellot	Shoulder, flank, under arms, hind quarter	Holstein	Female	No

5-28-09	Sou 7	14	Grand bay	3-2	15° 14.58 N 61° 18.64 W	Julian John	Hind leg, hind	Creole mix	Female	Yes
5-28-09	Sou 8	15	Grand bay	3-2	15° 14.58 N 61° 18.64 W	Julian John	hindquarter	Holstein	Female	No
5-28-09	Sou 9	16	Grand bay	3-2	15° 14.58 N 61° 18.64 W	Sammy Pacqette	Hindquarter, tail head, flank	Creole mix	Female	No
5-28-09	Sou 10	17	Grand bay	3-2	15° 14.58 N 61° 18.64 W	Leroy Bastein	Neck, flank, hindquarter	Creole mix	Female	No
5/28/09	No blood	18	Grand bay	3-2	15° 14.58 N 61° 18.64 W	Leroy Bastein	hindquarter	Creole mix	Male	No
6/2/09	East 1	19	Castle Bruce	1-3	15° 25.78N 61° 15.68W	Isol Mingo	Tail head	Creole mix	Female	No
6/2/09	East 2	20	Castle Bruce	1-3	15° 25.78N 61° 15.68W	Isol Mingo	Hind leg, inner thigh	Creole mix	Female	No
6/2/09	East 3	21	Castle Bruce	2-3	15° 25.81N 61° 15.90W	Unknown	Neck, left hindquarter	Creole mix	Female	Yes
6/2/09	East 4	22	Mahaut River	3-3	15° 25.54N 61° 15.17W	Madeline Valond	None	Creole mix	Female	No
6/2/09	East 5	23	Mahaut River	3-3	15° 25.54N 61° 15.17W	Madeline Valond	None	Creole mix	Male	No
6/2/09	East 6	24	Bataca	4-3	15° 30.26N 61° 15.98W	Dorime Mason	Hinquarters, head, tail head	Creole mix	Female	No
6/2/09	East 7	25	Bataca	4-3	15° 30.26N 61° 15.98W	Dorime Mason	Hindquarters	Creole mix	Female	Yes
6/2/09	East 8	26	Hatton Garden	5-3	15° 31.08N 61° 16.58W	Unknown	neck	Creole mix	Female	Yes

Charts and Diagrams

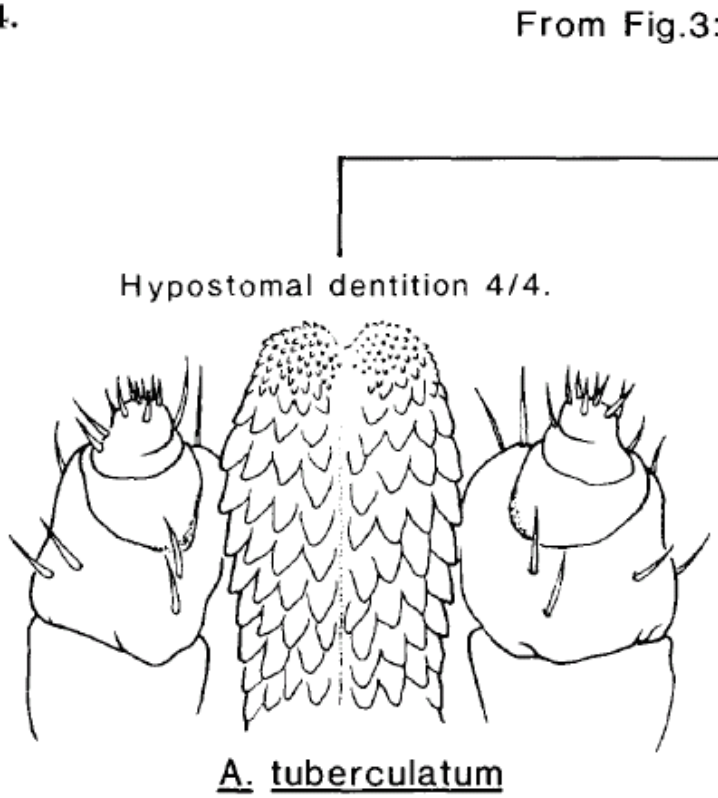


Pictorial Key of Boophilus

Source: (Keirans, 438)

Note: Key was used to determine Boophilus microplus, which has very similar structures to Boophilus annulatus.

Fig. 4.



Pictorial Key of Amblyomma

Source: (Keirans, 439)

Amblyomma varigatum
Source: (www.nhc.ed.ac.uk/index.php?page=24.25)

Boophilus microplus
Source: (www.ento.csiro.au/aicn/name_s/b_690.htm)

Discussion

In conclusion our study of *Babesia* worked well. Five cows showed positive signs for the protozoan *Babesia*. Further study of the blood smears will be conducted back at Texas A&M University. In order to determine whether the cow has anaplasmosis, PCR testing will be conducted on the blood on the slides as well as hemolymph from the ticks. Dr. Patricia Holman and the staff at the Texas A&M University Veterinary Sciences Department will be taking the thick blood smears that we have made to determine whether the cows have anaplasmosis.

Some difficulties occurred when trying to distinguish *Babesia* infected platelets from healthy ones in the slides that we made. It proved very difficult to distinguish the protozoan babesia from other structures in the blood; in particular platelets that had been smeared during fixing of the slides. Although Dr. Holman's protocol was followed for the staining of slides, some of the slides did not appear clear. Often some of the structures of the hemolymph and blood were not completely distinguishable when viewing through the microscope. Our group did

our best to distinguish what was *Babesia* and what was not, based on the pictures and diagrams provided by Dr. Holman and Dr. Craig. In further studying of the protozoan *Babesia* on the Island of Dominica, it may be best to practice detection of *Babesia* on the slides before arriving to conduct further studies on this matter. This will reduce the amount of error in diagnosing whether the cow has *Babesia*.

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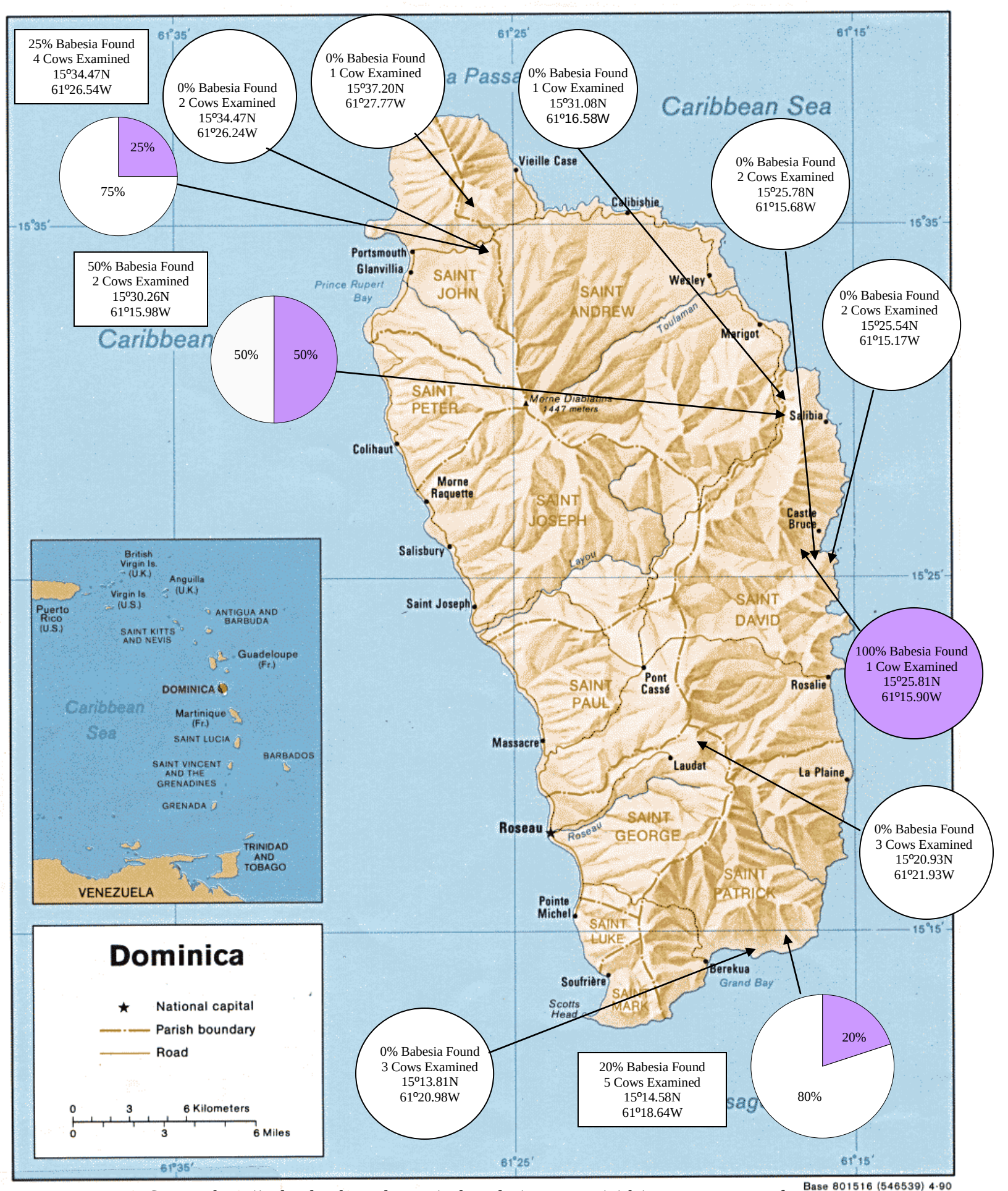

Source: http://upload.wikimedia.org/wikipedia/commons/e/eb/DominicaMap.gif