Geographic distribution of the invasive cattle tick *Rhipicephalus microplus*, a country-wide survey in Benin

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Abstract

The cattle tick *Rhipicephalus microplus* is currently invading the West African region, and little information is available on the spread of this exotic tick in this region. We set out a country-wide field survey to determine its current distribution in Benin. Ticks were collected on cattle from 106 farms selected by random sampling covering all regions of the country. *R. annulatus* was found in 70 % of all farms, *R. decoloratus* was found in 42 %, *R. geigyi* was found on 58 %, and *R. microplus* was found on 49 % of all farms. There is a clear geographic separation between the indigenous *R.* species and *R. microplus*. *R. annulatus* occurs mainly in the northern departments, but it was also observed in smaller amounts in locations in the south. The presence of *R. decoloratus* is limited to the northern region, and in most locations, this tick makes up a small proportion of the collected ticks. The tick *R. geigyi* tends to be dominant, but occurs only in the four northern departments. The observations concerning *R. microplus* are entirely different, this species occurs in the southern and central region. The results of this survey confirm the invasive character and displacement properties of *R. microplus*, since in less than a decade it has colonized more than half of the country and has displaced indigenous ticks of the same genus in a large number of the sampled locations.

Keywords

Rhipicephalus microplus; Babesia bovis; geographic tick distribution; country-wide tick survey; cattle

Introduction

Global costs of tick-borne diseases in cattle can be estimated between 13.9 and 18.7 billion US$ per year (DeCastro, 1997). The major pest of cattle worldwide is the cattle tick *Rhipicephalus microplus*, transmitting *Babesia bigemina, B. bovis* and *Anaplasma marginale*. The related economic losses were deemed so high that in the USA this tick has been eradicated at huge expenses by long-term acaricide use and strict control of animal movement (Corson et al., 2004). Despite their efforts, other countries have not been able to eradicate *R. microplus* (De Castro, 1997Pegram et al., 2000). The cattle tick is well established in Latin America (Evans et al., 2000) and Australasia (Estrada-Peña and Venzal, 2006) and is currently invading the West African region where it was first reported in 2007(Madder et al., 2007), . It has managed to establish itself and has replaced the indigenous *Rhipicephalus (Boophilus)* species (Madder et al., 2011). This situation has also been observed in other West-African countries. *R. microplus* was found in massive numbers during a cross-sectional survey in the department of Mono, in the south of Benin (Madder et al., 2012a).

The spread of *R. microplus* threatens rural populations, which often depend on livestock for their survival. Little to no information is available on the climatic limits of this tick in the West-African region. To fill this gap, we set out a country-wide field survey in order to determine the current distribution of *R. microplus* in Benin.

Material and Methods

Study area

Benin, officially the Republic of Benin, borders Togo to the west, Nigeria to the east and Burkina Faso and Niger to the north. Its geographic extent is situated between latitudes 6° and 13°N, and longitudes 0° and 4°E. Administratively, Benin is divided into 12 departments (Fig. 1). Benin's climate is hot and humid. Although the total yearly precipitation is similar throughout the country, varying between 1000 and 1300 mm, three different rainfall patterns can be observed (Fig. 2). The north of the country has one short rainy season, with a high precipitation peak in August. This peak rapidly declines after September. The central region

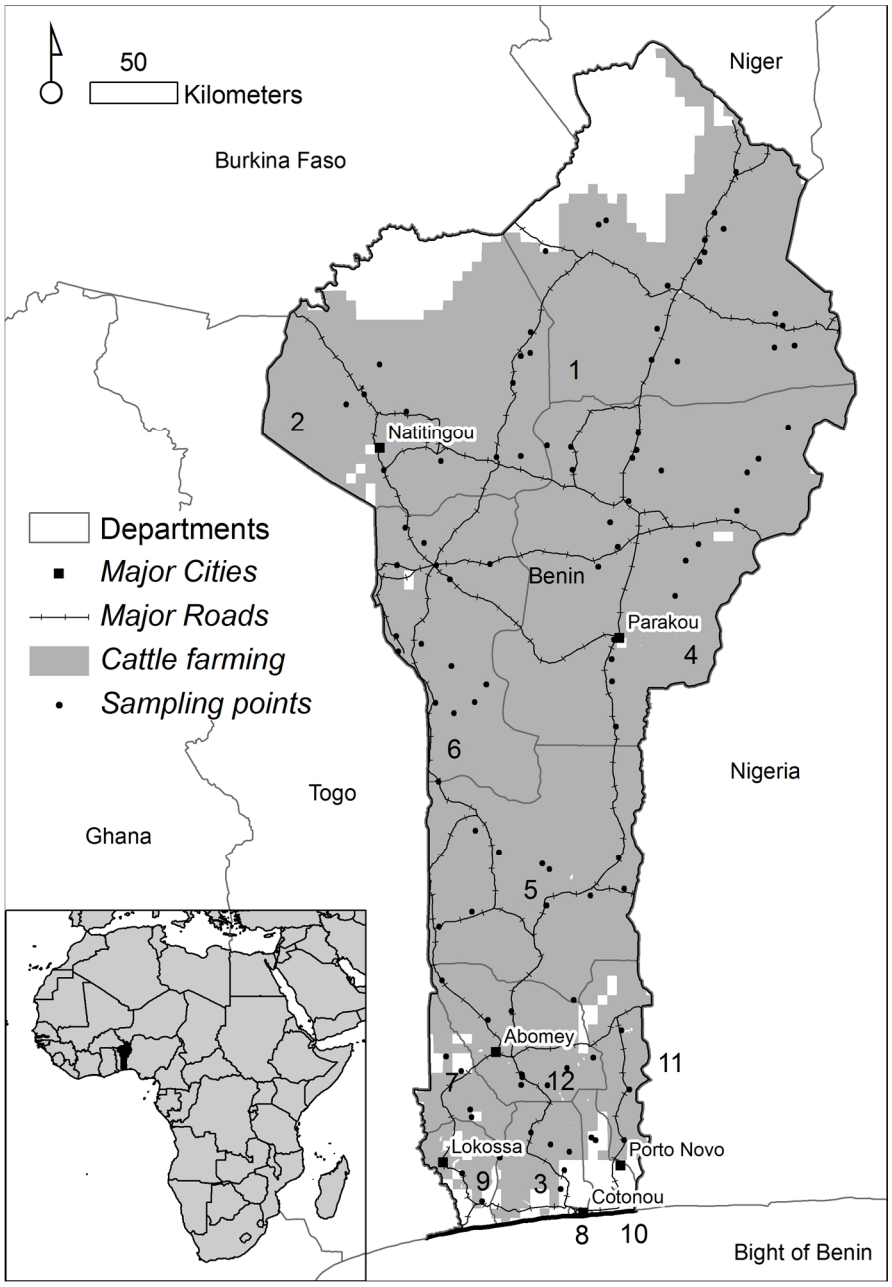

Fig. 1. Locations of the farms that were sampled for the country-wide survey of Benin. The numbers on the map refer to the ID number of the departments; their names are listed in Table 1.

features a long rainy season from the beginning of March to the end of October. Monthly rainfall values are not higher than 160 mm. There is a slight dip in rainfall in August, but both peaks are of the same height. The southern region has two rainy and two dry seasons per year. The principal rainy season is from April to late July, with a shorter, less intense rainy period from late September to November. The main dry season is from December to April, with a short cooler dry season from late July to early September (Hijmans et al., 2005).

In total, 106 farms were selected by random sampling (Fig. 1). The entire country was considered suitable for cattle raising, with the exception of a small region in the north and in the south of the country (Wint and Robinson, 2007).

In warm regions, tick survival is to a large degree dependent on the available humidity, and tick abundance starts to decline at the end of the rainy season. As the rainfall pattern features a north-south gradient (Fig. 2), the field missions were organized accordingly (Table 1). Sampling took place from 12-19 September 2011 in the northern region, from 7-16 November 2011 in the central region, and between 4 and 13 December 2011 in the south. Data from three additional points in the department of Atakora in the northwest were collected on October 5th 2011. The number of sampling points per department was proportional to the area of each department. In the northern part of the country, the departments are more extensive, therefore more sample points were taken in Alibori, Atakora, Borgou, Donga and Collines. In smaller departments in the southern part of the country (e.g. Ouémé, Plateau and Kouffo), a lower number of farms were sampled. In the Littoral no ticks were collected, since this department coincides with the country's capital Cotonou and contains no land suitable for cattle.

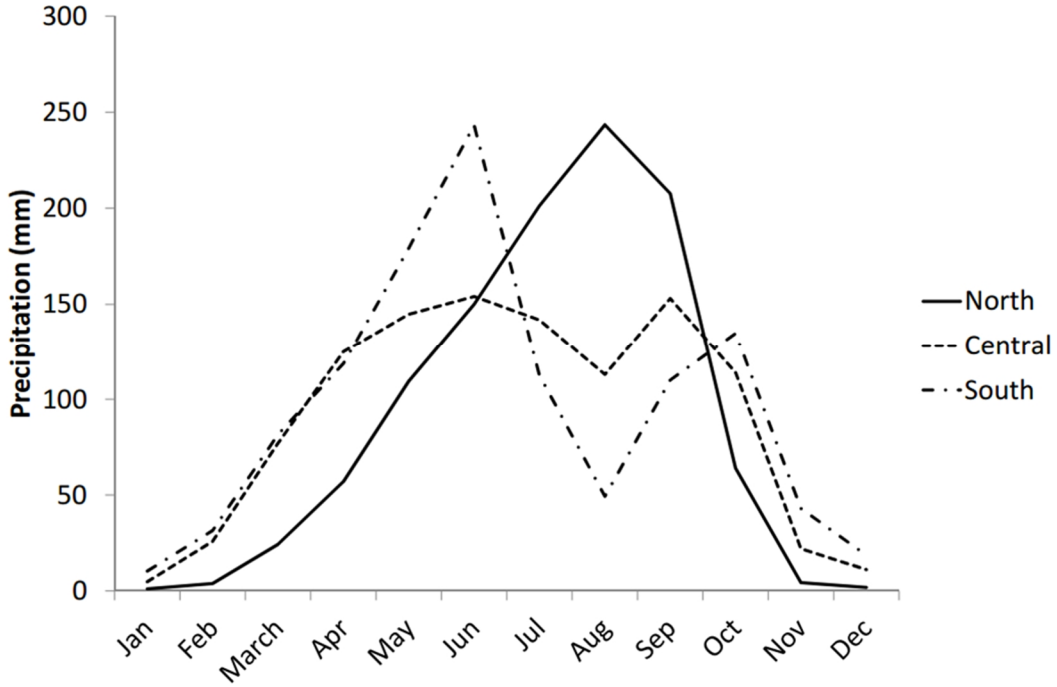

Fig. 2. Rainfall pattern in the three regions in Benin (Hijmans et al., 2005)

Table 1. Sample points in the different departments. The ID numbers in column 1 refer to the numbers on the map in Fig. 1.

ID	Department	Region	Field mission	Nr. of farms
1	Alibori	North	September	18
2	Atakora	North	September & October	13
3	Atlantique	South	December	5
4	Borgou	North	November	22
5	Collines	Centre	December	11
6	Donga	North	November	15
7	Kouffo	Centre	December	4
8	Littoral	South	-	0
9	Mono	South	December	3
10	Ouémé	South	December	2
11	Plateau	Centre	December	3
12	Zou	Centre	December	10

Tick collection

On each of the 106 farms visited, ticks were collected from at least two domestic bovine hosts (ICTTD, 2007; Pérez De Leán et al., 2010). The full body was sampled using forceps, with the exception of the flank lying on the ground. Where possible, all ticks on the inspected body were removed. On highly tick-infested animals, a sub-sample of the ticks present was collected, but care was taken to collect ticks from the different predilection sites: the base of tail and perianal region, perineum, legs, axillae, hooves, udder, scrotum, belly, dewlap, head and ears (Peter et al., 1998; ICTTD, 2007). The ticks were stored in plastic vials containing 70 % alcohol, which were labelled using the sampling date and the village name. Smartphones were used for the recording of geographic coordinates of each farm. For data management, two software applications were used simultaneously: (i) EpiCollect, a generic freeware developed at the Imperial College of Londen (Aanensen et al., 2009; Madder et al., 2012b), and (ii) VECMAP, currently under development by an ESA-funded European Consortium (ESA, 2012). Both applications allow the collection of geo-localized field data, including geographic coordinates and forms for an in-situ questionnaire. Both applications use a wireless internet connection to transfer the data to a centralized database on the worldwide web.

Tick identification

Tick identification was done based on morphology using a Zeiss stereoscope (80-fold magnification) and a Zeiss microscope (100 to 200-fold magnification). Only adult specimens were identified up to species level using both taxonomic descriptions (Walker et al., 2003) and morphological keys (Madder, 2012a, 2012b). The identification of a subset of 10 individuals of each of the four *Rhipicephalus (Boophilus)* tick species was confirmed molecularly using a PCR–RFLP test (Lempereur et al., 2010). Although species of other genera were collected during the different surveys (mainly *Amblyomma*, *Hyalomma* and *Rhipicephalus*), they are not discussed in this article, partly because they made up a minority of the collected ticks and partly because no changes have been observed in their distribution since the last country-wide survey (Vercruysse et al., 1982).

For each sample point, the total number of identifiable *R. (Boophilus)* spp. specimens was counted, and the proportion for each of the four encountered species was calculated.

Results

In total, close to 14.000 ticks were collected and identified, of which 12.378 belonged to the species *R. Boophillus*. *R. annulatus* was found in 70 % of the farms, *R. decoloratus* was found on 42 % of all farms, *R. geigyi* was found on 58 % of all farms, and *R. microplus* was found on 49 % of all farms. The morphological identification of the four *Rhipicephalus (Boophilus)* tick species was confirmed by a PCR-RFLP test. A number of ticks however, were found with hybrid morphology, featuring most characteristics of *R. microplus*.except that the 1st segment of the palps showed a ventro-internal protuberance bearing a pectinate seta. These individuals will be analysed further using molecular tools.

We supply maps of the geographic distribution of the four *Rhipicephalus (Boophilus)* species in Fig. 3. There is a clear geographic separation between the indigenous *R. (Boophilus)* species and *R. microplus*. *R. annulatus* occurs mainly in the northern departments (Alibori, Atacora, Borgou and Donga) where it reaches high proportions, but it was also observed in some locations in the south

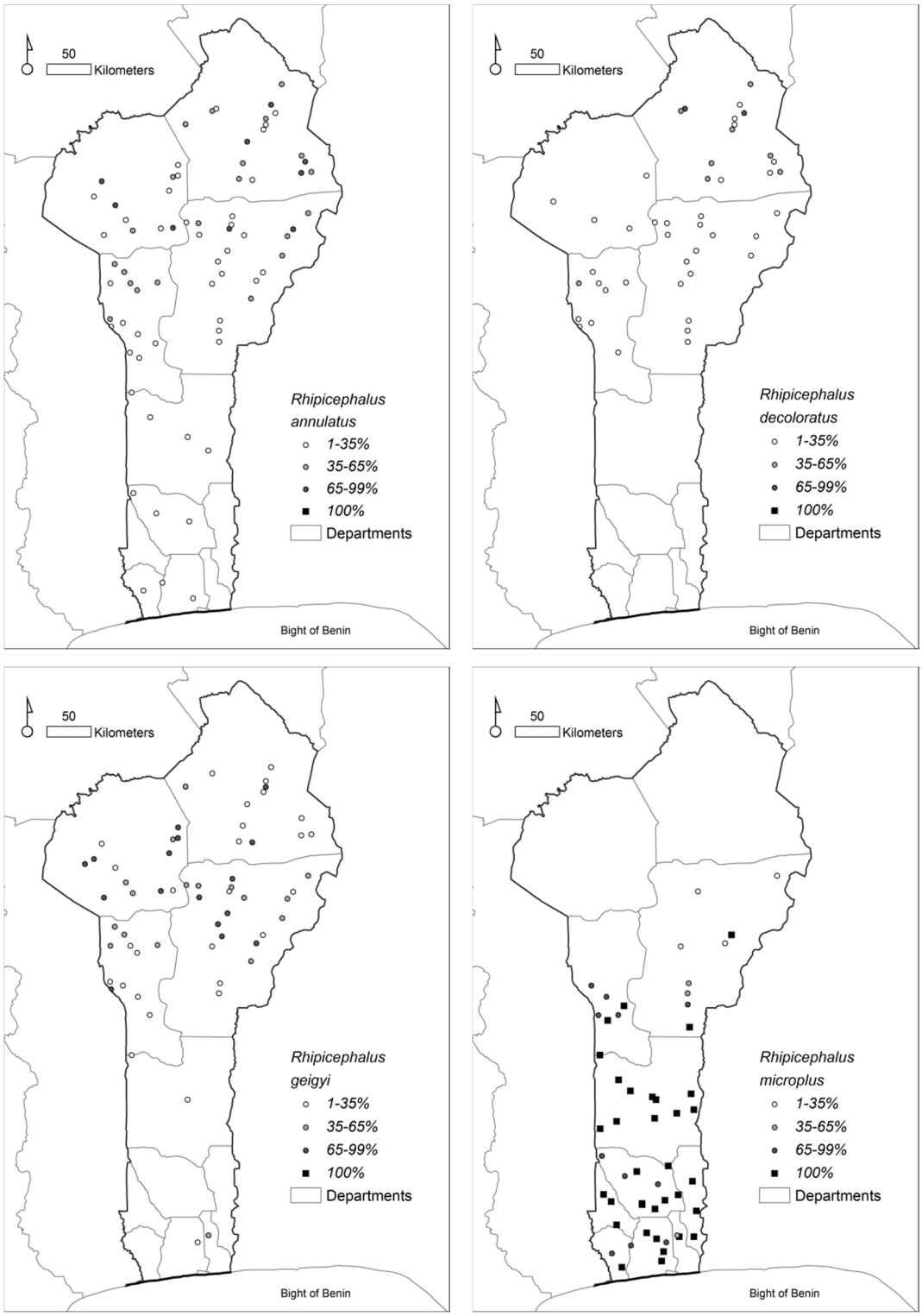

Fig. 3. Observed distribution of four *Rhipicephalus (Boophilus)* species in Benin

(Collines, Zou, Mono and Atlantique), here it constitutes a minority of the collected *R. (Boophilus)* spp. ticks. The presence of *R. decoloratus* is limited to the northern region, and in most locations, this tick makes up a feeble proportion of the collected ticks. The highest proportions are situated in the northeast of the

country (Alibori). In contrast, the species *R. geigyi* tends to be dominant, but occurs only in the four northern departments. This species was equally found in one location in the central region (Collines), and two locations in the south (Atlantique and Ouémé), where it constitutes a feeble fraction of the total number of collected ticks. The observations concerning *R. microplus* are entirely different, this species occurs mainly in the southern and central region, and in the most southern of the northern departments (Borgou and Donga). In sites where it is found, this tick makes up the larger part of the sample and also occurs in high numbers. On average, 63 *R. microplus* specimens were collected per animal sampled. For the indigenous *R. spp* species, the number of tick individuals per sampled animal was respectively 7 for *R. annulatus*, 9 for *R. geigyi* and 5 for *R. annulatus*. In the south, nearly all collected ticks were identified as *R. microplus*. It is also noteworthy that *R. microplus* is the only species that made up 100 % of a *R. (Boophilus)* spp. sample. The three other *R. (Boophilus)* spp. ticks co-exist in their environment.

These results can also be consulted at the project website:
http://epicollectserver.appspot.com/project.html?name=TickRisk_3.

Discussion & Conclusion

This survey of more than 100 locations provides a first and comprehensive country-wide survey of the distribution of the invasive species *R. microplus* in Benin. Although the presence of this vector of cattle fever was confirmed in West Africa, there was no information on the extent of its current distribution in the country (Madder et al., 2012a).

This survey is an invaluable dataset both for local and regional decision makers to estimate the economic burden and to design an efficient control strategy for this cattle pest, as well as for scholars studying the spread of this invasive species.

The most recent publications about the presence of *R. (Boophilus)* ticks in Benin only report *R. annulatus*, *R. geigyi* and *R. decoloratus*. The first two being present in the entire country and *R. decoloratus* present in the extreme north (Vercruysse et al., 1982). According to the authors *R. decoloratus* replaces *R. geigyi*, the most common species, in the more arid regions of the country although a small number of this species was also observed on ovines in the south of the country (Farougou

et al., 2007a). Later studies performed by Farougou and colleagues in the northern departments (Farougou et al., 2006, 2007b) do not mention the presence of *R. decoloratus* any more although this species was collected in the present study in the northern departments, although in low numbers.

This survey also reveals the presence of hybrid *R. spp.* specimens with morphological characteristics of more than one species. It was assumed that these are sterile hybrids resulting from the mating of an indigenous *R. spp*. female with an invasive *R. microplus* male. These individuals will be the subject of further research in the coming months.

As *R. microplus* is considered to be a coastal tick, and requires a relatively high level of humidity, it was alarming to observe the presence of *R. microplus* in the northern departments of Borgou and Donga. The results of this survey confirm the invasive character and displacement properties of *R. microplus*, since in less than a decade it has colonized more than half of the country and has displaced indigenous ticks of the same genus in a large number of the sampled locations. So far *R. microplus* only remains absent from the two most northern departments, known to harbour a large quantity of cattle. Moreover, it is quite possible that this tick has not yet reached its full climatic range and it might spread further northwards into more arid regions during the following years. Since cattle are extremely mobile in Benin, and both small and large (international) transhumance is a widespread practice, the spread of *R. microplus* could be quite rapid and is already causing regional concern. As a result of the frequent and seasonal cattle migration, mainly north-south, more research is necessary to determine the difference between established and temporal *R. microplus* populations, the latter being re-introduced by migrating animals on a yearly basis. Furthermore, the displacement of *R. annulatus, R. decoloratus* and *R. geigyi* in the central and southern departments could have altered the epidemiology of *Babesia bovis* and *B. bigemina*. The main concern however is the observed acaricide resistance of *R. microplus*, leading to inappropriate use of acaricides and other chemicals (Madder et al., 2011).

Although the sampling was performed according to a standardized and repeatable method, the resulting data are poorly suited for the study of tick abundance. This is because the abundance of ticks is highly dependent on human factors, such as general health condition of the herd and the (good) use of acaricides by the

herdsmen. Also, it was not possible to sample all 106 farms during the same period in time. A part of the farms, situated in the northern departments, was visited during the rainy season to increase the likelihood of finding a sufficient number of ticks, while the farms situated in the central and southern region were visited at the end of the rainy season. Especially the southern departments are suitable for ticks throughout the year because of the tropical rainforest climate. Given the fact that ticks were found in sufficient number at all locations, the data provide a good baseline for presence/absence-studies.

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Annex 1 – Collected data

Table 2. Geographic coordinates of the visited farms, collection data and collected ticks. Four *Rhipicephalus* species were encountered during identification. A value '0' indicates absence, '1' indicates presence of a given species in the sample.

			Rhipicephalus			
Latitude	Longitude	Date	*annulatus*	*decoloratus*	*geigyi*	*microplus*
10.763	2.926	12/09/2011	1	1	1	0
10.933	2.829	13/09/2011	1	1	1	0
11.277	3.040	13/09/2011	1	1	1	0
11.390	3.068	13/09/2011	1	1	1	0
10.772	2.791	13/09/2011	1	1	1	0
10.835	3.428	14/09/2011	1	1	1	0
10.845	3.531	14/09/2011	1	1	1	0
10.949	3.469	14/09/2011	1	1	0	0
11.010	3.431	14/09/2011	1	1	1	0
11.154	2.875	15/09/2011	1	0	1	0
11.447	3.164	15/09/2011	1	1	0	0
11.327	3.065	15/09/2011	1	1	1	0
11.741	3.228	15/09/2011	1	1	0	0
11.532	3.118	15/09/2011	1	1	1	0
11.332	2.244	16/09/2011	1	0	1	0
11.491	2.558	16/09/2011	1	1	0	0
11.469	2.518	16/09/2011	1	1	1	0
11.333	2.245	16/09/2011	1	0	1	0
10.807	2.166	17/09/2011	1	1	1	0
10.652	2.075	17/09/2011	1	0	1	0
10.792	2.117	17/09/2011	1	0	1	0
10.915	2.167	17/09/2011	1	0	1	0
10.265	1.991	17/09/2011	1	1	1	0
10.352	1.631	18/09/2011	1	1	1	0
10.244	1.703	18/09/2011	1	0	1	0
10.271	2.116	18/09/2011	1	0	1	0
10.504	1.526	18/09/2011	1	0	1	0
10.198	1.409	19/09/2011	1	0	1	0
10.593	1.307	5/10/2011	1	0	1	0
10.541	1.213	5/10/2011	0	1	1	0
10.748	1.386	5/10/2011	1	0	1	0
6.731	2.651	11/10/2011	0	0	0	1
9.321	2.597	7/11/2011	1	1	1	1
9.219	2.584	7/11/2011	1	1	1	1
8.871	2.607	7/11/2011	0	0	0	1
9.104	2.588	7/11/2011	1	1	0	1
9.802	2.617	8/11/2011	1	1	1	0
9.927	2.576	8/11/2011	1	1	1	0
9.548	2.914	8/11/2011	1	0	1	0

Latitude	Longitude	Date	*Rhipicephalus*			
			annulatus	*decoloratus*	*geigyi*	*microplus*
9.699	2.515	8/11/2011	1	1	1	1
10.258	3.344	9/11/2011	1	0	1	0
9.730	2.968	9/11/2011	1	0	1	1
10.185	3.287	9/11/2011	1	1	1	0
9.815	3.036	9/11/2011	1	0	1	1
9.987	3.234	9/11/2011	1	1	1	0
10.261	2.693	10/11/2011	1	0	1	1
10.421	3.500	10/11/2011	1	1	1	1
10.195	2.842	10/11/2011	1	1	1	0
10.200	2.383	11/11/2011	1	1	1	0
10.391	2.725	11/11/2011	1	1	1	0
10.037	2.673	11/11/2011	1	1	1	0
10.302	2.714	11/11/2011	1	1	1	0
10.327	2.253	12/11/2011	1	1	1	0
10.319	2.376	12/11/2011	1	1	1	0
9.820	1.617	13/11/2011	1	1	1	0
9.705	1.679	13/11/2011	1	1	1	0
9.900	1.518	13/11/2011	1	0	1	0
9.633	1.749	14/11/2011	1	1	1	0
9.705	1.476	14/11/2011	1	1	1	0
9.712	1.957	14/11/2011	1	1	1	0
9.260	1.484	15/11/2011	1	1	1	0
9.184	1.757	15/11/2011	1	0	1	1
9.339	1.471	15/11/2011	1	1	1	0
9.298	1.602	15/11/2011	1	1	1	1
9.090	1.937	16/11/2011	1	0	0	1
8.941	1.771	16/11/2011	1	0	0	1
8.994	1.677	16/11/2011	1	0	0	1
8.997	1.879	16/11/2011	0	1	1	1
6.560	1.813	4/12/2011	1	0	0	1
6.417	1.915	4/12/2011	0	0	0	1
6.889	1.854	5/12/2011	0	0	0	0
7.163	1.730	5/12/2011	0	0	0	1
6.850	1.863	5/12/2011	0	0	0	1
7.088	1.808	5/12/2011	0	0	0	1
8.029	2.651	6/12/2011	0	0	0	1
7.941	2.251	6/12/2011	0	0	0	1
8.191	2.623	6/12/2011	0	0	0	1
7.993	2.476	6/12/2011	1	0	0	1
8.130	2.265	7/12/2011	1	0	1	1
8.219	2.004	7/12/2011	0	0	0	1
8.159	2.227	7/12/2011	0	0	0	1
8.587	1.691	8/12/2011	1	0	1	1
7.833	1.694	8/12/2011	0	0	0	1
7.909	1.863	8/12/2011	0	0	0	1

Latitude	Longitude	Date	*Rhipicephalus* *annulatus*	*decoloratus*	*geigyi*	*microplus*
8.332	1.882	8/12/2011	1	0	0	1
7.454	2.389	9/12/2011	0	0	0	1
7.351	1.944	9/12/2011	1	0	0	1
7.396	2.069	9/12/2011	0	0	0	1
7.556	1.709	9/12/2011	1	0	0	1
7.267	2.281	10/12/2011	1	0	0	1
7.158	2.491	10/12/2011	0	0	0	1
7.014	2.254	10/12/2011	0	0	0	1
7.103	2.356	10/12/2011	0	0	0	1
7.057	2.123	11/12/2011	0	0	0	1
7.017	2.117	11/12/2011	0	0	0	0
6.480	2.321	11/12/2011	1	0	0	1
6.672	2.368	11/12/2011	0	0	1	1
7.069	2.122	11/12/2011	0	0	0	1
6.709	2.270	11/12/2011	0	0	0	1
6.577	2.342	11/12/2011	0	0	0	1
6.770	2.170	12/12/2011	0	0	0	1
6.641	2.007	12/12/2011	1	0	0	1
6.730	2.502	13/12/2011	0	0	0	1
6.744	2.479	13/12/2011	0	0	1	1
6.993	2.677	13/12/2011	0	0	0	1
7.297	2.636	13/12/2011	0	0	0	1