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Epidemiology of bovine anaplasmosis and babesiosis in Costa Rica

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Abstract

A serum bank created by the National Brucellosis Control Program during 1991 consisted of approximately 4000 sera collected from farms in each of the seven provinces in Costa Rica. Sera were used to determine the prevalence of antibodies directed against *Anaplasma marginale*, *Babesia bigemina* and *Babesia bovis* in Costa Rica and to study some geographical, ecological and management factors which could influence the epidemiology of the infection. The overall seroprevalence in bovines was 72.4%, 55.4% and 54.1% for *A. marginale*, *B. bigemina* and *B. bovis*, respectively. The Moran's index or spatial autocorrelation coefficient, which tests the significance of geographical patterns in disease distribution, indicated two foci of seropositivity in the country for *B. bovis*: one located mainly in the dry tropical forest and the other located in the tropical moist forest. Suggested foci were corroborated by a risk assessment using the random effects models.

1. Introduction

Bovine anaplasmosis and babesiosis were studied in Costa Rica by the Ministry of Agriculture and Livestock in collaboration with the Food and Agriculture

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Organization from 1977 to 1980. Annual economic losses due to mortality of adult cattle were calculated to be US$64 000 (McCauley and Perez, 1980). Livestock production systems in the country vary according to ecologic zones; these are natural sets of landscapes ranging from swamps to ridge tops which vary according to altitude, precipitation and temperature (Holdrige, 1967).

To study patterns of disease occurrence and relating those patterns to spatial, temporal or management characteristics is how epidemiologists look for causal factors of observed outcomes. Quantitative methods for the analysis of geographic disease patterns have been only recently used by epidemiologists (Hungerford, 1991).

Ecological comparison of geo-political areas can be useful in the investigation of potential factors influencing the serological status of tick-borne diseases in cattle. Environmental factors could have a considerable influence on the incidence of diseases in animals (Gettinby and Byrom, 1991). In vector-borne diseases humidity, rainfall and temperature are all factors which can modify the transmission rate because environmental characteristics influence the survival of potential vectors.

The aim of this study was (1) to determine the prevalence of antibodies against *Anaplasma marginale*, *Babesia bigemina* and *Babesia bovis* in Costa Rica, and (2) to study the geographic distribution within the country of antibody levels against the three hemoparasites.

2. Materials and methods

2.1. Study population

Costa Rica has an area of 51 260 km^2, and is divided politically into seven provinces, each subdivided into cantons and districts. The country has several mountain systems that divide the country into five geographical areas.

In the central valley where soils are derived from volcanic sediments, rainfall is approximately 2500 mm over an 8 month season and temperature is moderated by altitude. In Holdridge's (1967) life zone classification, this area includes premontane moist forest and lower montane rain forest. The predominant livestock production system is intensive dairying, mainly based on year round grazing on improved pastures, e.g. Kikuyu grass (*Pennisetum clandestinum*) or Star grass (*Cynodon plectostachyus*), with supplementary feeding of concentrates. Average herd size is approximately 50 cows. Pure-bred Holstein and Jersey dairy cows are used. Only heifer calves are reared. Milk average production levels on these farms are approximately 6000 l per lactation.

Humid tropical lowlands on the Atlantic coast have a flat topography with leached colluvial and alluvial soils, with rainfall of 2000–4000 mm over an 8 month season. Conditions are hot and humid. Tropical moist–wet forest is the predominant life zone (Holdridge, 1967). Production systems vary with the settlement pattern and three types of farms occur: small farms with about 20 cows

each, mainly kept for dairying, medium sized farms with some 100–200 animals (mainly beef and dairy-beef purpose farms), and a few larger individual holdings for beef production.

The medium altitude zone on the foothills on the Atlantic side of the central highlands is transitional between the two above mentioned areas. Premontane rain forest is the principal life zone. Dual purpose and dairy production systems are rapidly expanding, mainly on medium-scale farms.

The dry Pacific coast areas in the northwest still experience more that 1200 mm rainfall, distributed over about 7 months. Life zones vary from tropical dry forest to premontane moist forest. Extensive medium-sized cow–calf beef farms are the predominant enterprise in this area.

The wet Pacific lowlands and foothills in the southwest are the wettest areas in the country (tropical wet forest). As far as livestock is concerned they are mainly used for extensive cow–calf operations for beef cattle production.

A serum bank created by the National Brucellosis Control Program, executed during 1991 by the Ministry of Agriculture, was used for this study. It consisted of approximately 4000 sera collected from farms (in each of the seven provinces in Costa Rica) by a proportional design that used the farms listed in the 1982 national livestock survey (Consejo Nacional de la Produccion, 1982) as a sampling list frame. Each serum sample was classified according to ecological life zone (Holdrige, 1967). A proportion of cows was allocated to each ecological area according to its percentage of total number of cows (number of cows by ecological area/total number of cows). Finally, a proportional quota of sera by ecological life zone area was obtained, using an expected prevalence of 50% (to obtain a conservative sample, i.e. one that is larger than required) an error level of 5% and a confidence level of 99.5%. A minimum sample size of 689 sera was calculated. Seven hundred and seventeen sera were selected (random selection of the quota of each ecological area using a random number table) to allow for loss or contamination of sera (see Table 1). The following information was available for the farms: (1) origin of each serum sample, (2) herd size, (3) farm size, (4) farm type (dairy, dual purpose, cow–calf), and (5) location of the farm.

2.2. Serological assay

Sera were stored at $-20°C$ for less than 1 month until tested for antibodies against *A. marginale* using the rapid-card agglutination test as prescribed by the manufacturer (Brewer Diagnostic kit, Wescott and Dunning, Inc.). The indirect fluorescent antibody test (IFAT) as described by Payne and Scott (1982) was used to detect antibodies to *B. bigemina* and *B. bovis*. Results of both serological tests were recorded as positive or negative. The IFAT was performed at a 1:80 dilution for the sera and a 1:200 dilution for the conjugate.

2.3. Statistical analysis

Means, standard errors, and medians of the farm characteristics were calculated using PROC UNIVARIATE in Statistical Analysis Systems Institute Inc.

(SAS Institute Inc., 1989). Continuous variables were categorized using quartiles. Crude odds ratios were calculated for risk factors using PROC FREQ in SAS (SAS Institute Inc., 1989).

Spatial statistical analysis was used to test the significance of geographic patterns in disease distribution (describing them as either clustered, random or dispersed). In theory a random distribution is one in which the prevalence in an area is in no way influenced by other areas. A cluster will be one in which some pattern in the location of the prevalence exists and a dispersed pattern would be one in which the prevalence is evenly and regularly distributed throughout the study areas. With non-binary data, such as continuous or ordinal values, the method developed by Moran (1950) can be employed. The Moran's index (the spatial autocorrelation coefficient) tests two possible null hypotheses: (1) normality and (2) randomization. The normality hypothesis assumes sampling with replacement and is employed when a priori probability is considered, i.e. based on information inferred from a larger area. The randomization hypothesis assumes a sampling without replacement. No reference is made to outside factors. In this study, the spatial autocorrelation index (using the Moran's coefficient) was used to test the null hypothesis of randomization (Ebdon, 1985):

$$I=\frac{n\sum_{(c)}(x_i-\bar{x})(x_j-\bar{x})}{J\sum(x-\bar{x})^2}$$

where I is the spatial autocorrelation coefficient (the calculated value of I will define a population as either dispersed, random or clustered), n is the number of areas in the study, J is the number of joins (borders between neighbors), x is the prevalence of an area, $\bar{x}$ is the mean of all the values of x, and x_i and x_j are the values of contiguous areas (areas on either side of a join or border). The expected value of I is

$$E_I=-\frac{1}{n-1}$$

The standard deviation of I for testing the hypothesis of randomness is (Ebdon, 1985):

$$\sigma_I=\sqrt{\frac{n[J(n^2+3-3n)+3J^2-n\sum L^2]-k[J(n^2-n)+6J^2-2n\sum L^2]}{J^2(n-1)(n-2)(n-3)}}$$

where L is the number of areas to which an area is joined and k is the level of kurtosis of the distribution of the variable x and is calculated as:

$$k=\frac{\sum(x-\bar{x})^4}{n\sigma^4}$$

where

$$\sigma = \sqrt{\frac{\sum (x - \bar{x})^2}{n}}$$

Regression models

Since the sampling unit was ecological area, while the unit of interest was the individual animal, and because of possible lateral transmission and clustering of cases within herds, a logistic binomial for distinguishable data model was performed using EGRET (Statistics and Epidemiology Research Corporation, 1990). In the multivariate analysis, all potential confounding variables (farm size, herd size, type of production system) were used, along with the ecological areas to provide as complete control of confounding as possible. For variables with three or more levels, each level of the variable was compared with all other levels combined as a reference level. Ecological areas with low sampling frequency, less than 10, (lower montane moist forest, lower montane wet forest, lower montane rain

Table 1
Seroprevalence (%) of 717 cattle, Costa Rica 1990–1991

Ecological zone	No. of cattle	%	*Anaplasma*	*B. bigemina*	*B. bovis*
Tropical dry forest, moist transition	74	10.3	72	51	55
Tropical moist forest	180	25.1	71	56	53
Tropical moist forest, perhumid transition	10	1.4	70	70	20
Tropical moist forest, premontane transition	43	6.0	76	35	44
Tropical wet forest	37	5.2	81	67	62
Tropical wet forest, premontane transition	43	6.0	76	67	51
Premontane moist forest	20	2.8	65	55	90
Premontane moist forest, basal transition	24	3.3	66	37	83
Premontane wet forest	70	9.8	74	53	57
Premontane wet forest, basal transition	191	26.6	68	56	50
Premontane rain forest	2	0.3	100	0	0
Lower montane moist forest	3	0.4	66	0	33
Lower montane wet forest	8	1.1	87	87	87
Lower montane rain forest	9	1.3	77	78	11
Montane wet forest	1	0.1	0	100	0
Montane rain forest	2	0.3	100	100	100
Farm size: 0–50 ha	217	30	22	15	18
Farm size: 51–115 ha	174	24	17	14	12
Farm size: 116–264 ha	171	24	18	13	11
Farm size: > 264 ha	155	22	15	12	14
Herd size: 0–40 head	210	30	21	15	15
Herd size: 41–75 head	171	24	17	13	13
Herd size: 76–158 head	181	25	18	16	14
Herd size: > 159	155	22	16	12	13
Farm type: beef	302	42	30	23	25
Farm type: dual purpose	303	42	30	22	20
Farm type: milking	112	16	12	9	10

Table 2
Logistic binomial regression for seroprevalence of anaplasmosis, by ecological area, Costa Rica, 1990–1991

Term	β	SE (β)	P value	OR	95% CI (OR)
Intercept	1.00	0.37	0.01	2.37	1.31–5.69
Tropical moist forest (TMF)[a]	−0.03	0.35	0.93	0.97	0.49–1.91
TMF transition perhumid	0.02	0.80	0.98	1.01	0.21–4.50
TMF transition premontane	0.31	0.49	0.54	1.35	0.51–3.56
Tropical wet forest (TWF)	0.51	0.53	0.34	1.65	0.58–4.69
TWF transition premontane	0.04	0.54	0.94	1.03	0.35–3.00
Premontane moist forest (PMF)	−0.57	0.68	0.40	0.56	0.14–2.15
PMF transition basal	−0.35	0.54	0.52	0.70	0.24–2.04
Premontane wet forest (PWF)	−0.02	0.42	0.95	0.97	0.42–2.24
PWF transition basal	−0.12	0.34	0.73	0.89	0.45–1.75
PRF–LMMF–LMWF–LMWF–MWF–MRF[b]	0.17	0.65	0.79	1.18	0.32–4.22
Farm size: 51–115 ha[c]	−0.37	0.29	0.21	0.69	0.38–1.23
Farm size: 116–264 ha	0.36	0.38	0.34	1.43	0.67–3.03
Farm size: >264 ha	0.11	0.42	0.80	1.11	0.48–2.56
Herd size: 41–75 head[d]	0.03	0.28	0.91	1.03	0.59–1.80
Herd size: 76–158 head	−0.15	0.35	0.67	0.86	0.43–1.70
Herd size: >159 head	−0.17	0.40	0.67	0.84	0.38–1.84
Farm type: dual purpose[e]	0.01	0.20	0.98	1.00	0.66–1.50
Farm type: milking	0.40	0.38	0.30	1.49	0.69–3.18
Random term	0	0.06			

[a]Tropical dry forest moist transition used as reference level.
[b]Premontane rain forest–lower montane moist forest–lower montane rain forest–montane wet forest–montane rain forest.
[c]Lower level of 0–50 ha used as reference level.
[d]Lower level of 0–40 animals used as reference level.
[e]Beef farms used as reference level.

forest, montane wet forest and montane rain forest) were collapsed into one category.

3. Results and discussion

A. marginale, *B. bovis* and *B. bigemina* were widespread in the country (Table 1). Nevertheless, the geographic distribution varied according to hemoparasite. The results of the spatial analysis for *A. marginale* ($I=0.069$, z value$=0.838$, $P=0.42$), and for *B. bigemina* ($I=0.083$, z value$=0.947$, $P=0.94$) failed to reject the null hypothesis of randomness, implying a random distribution for seropositivity against *A. marginale* and *B. bigemina*. On the other hand, $I=0.235$, z value$=2.314$, $P=0.03$, for *B. bovis*, indicated a clustered distribution for seroprevalence against *B. bovis*. Possibly, bordering cantons shared similar ecological and management factors favorable to transmission of *B. bovis*. Transmission of the parasites studied was principally by ticks of the genus *Boophilus* (Young, 1988;

Table 3
Logistic binomial regression for seroprevalence of *B. bigemina*, by ecological area, Costa Rica, 1990–1991

Term	β	SE (β)	*P* value	OR	95% CI (OR)
Intercept	−0.06	0.45	0.89	0.94	0.38–2.30
Tropical moist forest (TMF)[a]	0.12	0.43	0.78	1.12	0.48–2.61
TMF transition perhumid	0.48	1.02	0.63	1.61	0.21–11.84
TMF transition premontane	−0.97	0.59	0.10	0.37	0.11–1.21
Tropical wet forest (TWF)	0.67	0.62	0.27	1.96	0.57–6.62
TWF transition premontane	0.37	0.66	0.56	1.45	0.40–5.29
Premontane moist forest (PMF)	−0.19	0.85	0.82	0.82	0.15–4.42
PMF transition basal	−0.78	0.67	0.24	0.45	0.12–1.70
Premontane wet forest (PWF)	0.06	0.51	0.90	1.06	0.38–2.89
PWF transition basal	0.11	0.43	0.80	1.11	0.48–2.56
PRF–LMMF–LMWF–LMWF–MWF–MRF[b]	0.55	0.72	0.44	1.74	0.42–7.14
Farm size: 51–115 ha[c]	0.01	0.36	0.97	1.01	0.49–2.05
Farm size: 116–264 ha	−0.52	0.44	0.23	0.59	0.24–1.41
Farm size: > 264 ha	−0.01	0.51	0.85	0.90	0.33–2.46
Herd size: 41–75 head[d]	0.49	0.33	0.14	1.64	0.85–3.17
Herd size: 76–158 head	0.94	0.41	0.02	2.56	1.13–5.80
Herd size: > 159 head	0.61	0.48	0.21	1.84	0.70–4.87
Farm type: dual purpose[e]	−0.17	0.26	0.51	0.84	0.50–1.40
Farm type: milking	0.12	0.48	0.78	1.12	0.46–2.70
Random term	0	0.10			

[a]Tropical dry forest moist transition used as reference level.
[b]Premontane rain forest–lower montane moist forest–lower montane rain forest–montaine wet forest–montane rain forest.
[c]Lower level of 0–50 ha used as reference level.
[d]Lower level of 0–40 animals used as reference level.
[e]Beef farms used as reference level.

Lawrence and de Vos, 1990). Anaplasmosis can occur in the absence of babesiosis with transmission also accomplished by other genera of ticks, by insect vectors, and mechanical agents (Callow, 1984). Consequently, differences between seroprevalence figures could be explained by the suitability of certain geographical areas for ticks and other vectors, from direct effects of the climate and vegetation on the free-living stages of the potential vectors, and by indirect effects of the climate on the resistance of the cattle (Sutherst et al., 1988).

The results of the spatial analysis were corroborated by regression analysis using the random effects models. No statistically significant association was found between any geographical area and the seroprevalence of *A. marginale* (Table 2) and *B. bigemina* (Table 3). Only medium size farms (76–150 heads) had an increased odds of seropositivity of *B. bigemina*. These results suggest that Costa Rica is a homogeneous, endemic environment with enough ticks present to maintain transmission in cattle and tick populations (Mahoney and Ross, 1972).

However, for *B. bovis* two ecological areas, premontane moist forest transition to basal and premontane moist forest were associated with higher seroprevalence

Table 4
Logistic binomial regression for seroprevalence of *B. bovis*, by ecological area, Costa Rica, 1990–1991

Term	β	SE (β)	P value	OR	95% CI (OR)
Intercept	0.22	0.53	0.68	1.24	0.43–3.50
Tropical moist forest (TMF)[a]	0.204	0.86	0.67	1.22	0.47–3.18
TMF transition perhumid	−2.35	1.31	0.07	0.95	0.07–1.24
TMF transition premontane	−0.13	0.72	0.85	0.87	0.21–3.61
Tropical wet forest (TWF)	0.78	0.74	0.29	2.17	0.50–9.34
TWF transition premontane	0.11	0.89	0.90	1.11	0.19–6.42
Premontane moist forest (PMF)	2.46	1.29	0.05	11.8	0.93–149.0
PMF transition basal	2.73	0.93	0.01	15.3	2.45–95.5
Premontane wet forest (PWF)	0.69	0.58	0.24	1.98	0.62–6.27
PWF transition basal	0.27	0.49	0.58	1.30	0.49–3.40
PRF–LMMF–LMWF–LMWF–MWF–MRF[b]	−0.70	0.88	0.43	0.49	0.08–2.83
Farm size: 51–115 ha	−0.61	0.46	0.18	0.54	0.21–1.34
Farm size: 116–264 ha	−1.83	0.58	0.01	0.16	0.05–0.50
Farm size: >264 ha	−1.31	0.67	0.05	0.26	0.07–1.00
Herd size: 41–75 head[d]	0.79	0.44	0.07	2.19	0.92–5.18
Herd size: 76–158 head	1.27	0.55	0.02	3.56	1.22–10.3
Herd size: >159 head	1.46	0.62	0.02	4.33	1.27–14.7
Farm type: dual purpose[e]	−0.44	0.34	0.19	0.64	0.33–1.25
Farm type: milking	−0.18	0.59	0.77	0.84	0.26–2.68
Random term	0.60	0.13			

[a]Tropical dry forest moist transition used as reference level.
[b]Premontane rain forest–lower montane moist forest–lower montane rain forest–montane wet forest–montane rain forest.
[c]Lower level of 0–50 ha used as reference level.
[d]Lower level of 0–40 animals used as reference level.
[e]Beef farms used as reference level.

(Table 4). Tropical moist forest showed a tendency toward lower seroprevalence. These three ecological life zones covered 24 cantons of the 52 cantons of the country. There are differences of maintenance transmission thresholds between species of *Babesia*, determined by the bites per tick per day, infection rates in cattle, and inoculation rates from differences in transovarian transmission rate. *B. bovis* is transmitted to cattle by infected larvae (Riek, 1964), and *B. bigemina* is transmitted by nymphs and adults (Riek, 1964; Dalgliesh et al., 1978). Inoculation thresholds for *B. bovis* vary from one geographical location to another, depending on tick activity; on the other hand, inoculation thresholds in *B. bigemina* varied less than for *B. bovis* from one location to another (Haile et al., 1992). These differences in thresholds can create, as in this case, zones of differential risk (Smith, 1983). Smaller farms (0–40 ha) showed an increase odds of infection, as did farms with more than 40 head, apparently indicating that farms with larger stocking rate (head per hectare) had an increased odds of seropositivity.

4. Conclusion

Two areas of non-random, seroprevalence of *B. bovis* were detected in Costa Rica; one is located mainly in the province of Guanacaste (northwestern and dry

pacific region). The annual precipitation is from 1000 to 2000 mm, and the average temperature is 24°C. The other focus is located in the lowlands of the provinces of Alajuela, Heredia and Limon (tropical moist forest, southern Caribbean coast) with an annual precipitation of 2000–4000 mm and an average temperature higher than 24°C.

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