EPIDEMIOLOGICAL DETECTION OF ANAPLASMA INFECTION IN CATTLE IN NORTHERN SAMAR USING CARD AGGLUTINATION TEST AND BLOOD FILM TECHNIQUE

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ABSTRACT

Vector-borne, protozoan blood parasitic infection caused by pathogenic *Anaplasma species* is considered a significant yet underreported disease of cattle and water buffaloes. This study was conducted to detect Anaplasma infection in cattle in Northern Samar. The study examined 200 heads of cattle irrespective of age and sex from three geographical areas of the province (Central, Pacific and Balicuatro areas) using serological card agglutination test (CAT), and blood film technique (BFT). Both tests were respectively done for serologic screening and morphological identification of the organism. *Anaplasmacentrale*, existing alone or together with *A. marginale* in red blood cell (RBC) on stained blood smears were identified morphologically. Other contributory factors such as age, sex and breed in assessment of its epidemiological presence have been considered.CAT revealed a 37% seropositive Anaplasma infection rate in cattle with the three geographical areas of the province showing varying degrees of positivity (Central Area, 19.00%; Pacific Area, 11.00%; and Balicuatro Area, 7.50%). The prevalence of anaplasmosis was found to be independent of age and sex ($p > 0.05$). No relationship of the breed of cattle to the prevalence of the disease was ascertained since all the experimental animals in the province were crosses of native and imported breeds. CAT as screening test was found to be effective in Anaplasma detection complementing that of BFT. This study reported the presence of cattle anaplasmosis for the first time in the province of Northern Samar. Further epidemiological study covering other areas in Eastern Visayan region is encouraged.

Key words: *anaplasmosis, BFT, CAT, cattle, Northern Samar, serology*

INTRODUCTION

The fattening of feeder cattle is an important industry for backyard farmers. However, anaplasmosis has the potential to cause significant losses in animals that survive the acute phase of the infection (Bundza and Samagh, 1982; Lincoln, 1990; Ybañez et al, 2013). The cost of the disease, control measures and drugs to treat affected cattle together with quarantine laws that restrict the movement of infected cattle are burdensome to the cattle raisers, farmers and entrepreneur.

Anaplasmosis is a tick-borne, protozoan blood parasitic infection caused by pathogenic *Anaplasma species* (*A. marginal* and *A. centrale*). The parasite infects red blood cells and cause severe anemia. Pathogenic *Anaplasma species*infection is considered a significant yet underreported disease of large ruminants particularly those affecting cattle and water buffaloes. The disease can cause considerable economic loss due to severe anemia with eventual deaths, weight reduction, decline in milk production, and lowered reproductive capacity (Rodríguez-Vivas et al, 2004;Stoltsz, 1993; Souslby, 1982;Ybañez et al, 2013).

In the absence of scientific confirmatory evidence of anaplasmosis in Northern Samar, astudy on the detection of Anaplasma infection in cattle in Northern Samar using card agglutination test (CAT) and blood film technique (BFT) was done. Card agglutination test was used considering its low cost and ease of testing procedure whereby comparatively reliable result with other serological test such as enzyme-linked immunosorbent assay (ELISA) has been reported (Molloy et al, 1999). Likewise, this was conducted to assess the relationship of age, sex and breed on the prevalence of the disease in cattle. Overall, this was conceptualized to contribute on the development of cattle farming and industry in the province.

MATERIALS AND METHODS

Animal and Geographical Sampling

Two hundred (n=200) heads of sexually mature cattle, irrespective of age and sex were selected through simple random sampling and were subjected to blood examination to detect the presence of the parasitic disease under study. The samples were collected in three (3) geographical areas of the province representing Balicuatro, Central and Pacific areas.These pre-selected samples represent 9.96% (200/2009) of the total cattle population in the area.

Blood Sampling and Examination

Blood sampling and film preparation utilized the clinical pathology protocol as described (Stockham and Scott, 2013).Using a 9 ml plain vacutainer with attached needle and special holder, the external jugular vein was anchored by placing the thumb of the left hand in the jugular furrow to occlude the vein, while the right hand manipulated the vacuum tube.Pressure was relieved on the vein while the tube was filling. Each of the tube with the blood sample was labeled with the animal identification (age, sex, and identification number).

Using an applicator stick, drops of blood from the tube were obtained for blood smear and the prepared slide was labeled with same animal identification as that of the tube where the blood sample was taken for CAT.It was air dried, fixed with 10% methanol for 10 seconds, and then wrapped with a clean sheet of paper. The prepared slides were brought to the CVM Diagnostic Laboratory for staining and for the blood smear examination.

While blood films were prepared in the field, the blood collected in the vacutainers was allowed to stand at room temperature in a leaning position until after the blood clotted. The clotted blood was stored in a container with cracked ice and transported to the CVM Diagnostic Laboratory where the serum in each tube was pipetted and transferred to serum vials to be stored at -20^{o}C temperature.Smears were examined under oil immersion objective (OIO) light microscopy for morphological identification of the blood parasite (Ristic, 1981).

Card Agglutination Test

Immediately after completing the 200 blood samples, the sera were shipped tothe PhilippineAnimal Health Center Parasitology Laboratory, Visayas Avenue, Quezon City, Philippines, for serological test using the Card Agglutination Test (PAHC, 2001).

Data Analysis

Seropositivity rate was determined as the proportion of animals positive for Anaplasma antibody using the formula:

$$\text{Seropositivity Rate} = \frac{\text{Number of positive sera}}{\text{Total number of serum samples examined}} \times 100$$

Prevalence of Anaplasmosis determined by blood film technique (BFT) was computed as:

$$\text{Prevalence Rate} = \frac{\text{Number of positive smears}}{\text{Total number of blood smears examined}} \times 100$$

Further, the data were analyzed using averages and percentages and presented in graphs and tabular forms. The relationship of seroprevalence of *Anaplasma spp.* to age, sex and breed of cattle was determined using the chi-square (x^2) analysis with the formula

$$x^2 = \frac{\sum(/fo - fe/ - .5)^2}{fe}$$

Where: x^2 = chi-square value

fo = observed frequency

fe = expected frequency

RESULTS AND DISCUSSION

Serologic screening using card agglutination test (CAT) yielded 37.5% (75/200) seropositive Anaplasma infection rate in cattle in Northern Samar. Table 1 shows the profile of Anaplasma detection using CAT according to sex and age.

Table 1. Profile of Anaplasmosis Detected by CAT in Cattle in Northern Samar According to Sex and Age Groups

AGE BRACKET (years)	CAT RESULT						
	NO. OF SAMPLES	POSITIVE			NEGATIVE		
		f	% (of the age bracket)	% (of the total sample)	f	% (of the age bracket)	% (of the total sample)
1 – 5	140	53	37.86	26.50	87	62.14	73.50
6 – above	60	22	36.67	11.00	38	66.67	90.50
TOTAL	200	75		37.50	125		62.50

SEX	MALE (17.5% of the total sample)			FEMALE (82.5% of the total sample)		
	CATPOSITIVE			CATPOSITIVE		
GEOGRAPHIC AREAS	NO. OF SAMPLE	f	% (of the total M sample)	NO. OF SAMPLE	f	% (of the total F sample)
Balicuatro	6	3	8.57	26	12	7.27
Central	17	10	28.57	60	28	16.97
Pacific	12	2	5.71	79	20	12.12
TOTAL	35	15	42.85	165	60	36.36

Legend:
CAT positive – presence of *Anaplasmaantibodies.*
CAT negative – absence of *Anaplasmaantibodies.*

The efficacy of CAT as a diagnostic tool for *Anaplasma spp*. infection on the blood samples collected from cattle has indicated a 37.50% positive result of *Anaplasma spp*.In a study done in Australia on comparing card agglutination test to that of sophisticated enzyme-linked immunosorbent assay, it was found that CAT has similar sensitivity and specificity of 98% and 100% respectively (Molloy et al, 1999). Thus, the current test used in this study affirmed the reliable presence of this infectious disease.

In this result, age bracket was limited only to two (2) pooled groups (1-5 years old and 6 years old and above) to identify the experimental animals based on production performance. The 1-5 year old grouping was considered the cattle's prime, and productive years while the 6 and above years old was considered as the age of declining sexual productivity and animals that are about to be culled.Cattle of ages 1-5 years old had the highest number of samples tested (140) where 53 or 37.86% of the samples were found to be positive of Anaplasma antibodies. The age bracket 6 years old and above, with 60 samples had 22 or 36.67% positive samples. This result indicates that cattle of all ages are prone to *Anaplasma spp*. infection.

Furthermore, the result of the serologic examination on cattle according to sex wherein 15 positive reactors to CAT were recorded in the male group and 60 positive reactors in the female group. While there were more female (165) than male (35) subjects tested, the CAT result showed that, percentage-wise, more male (42.85%) subjects were found to be seropositive forAnaplasmosis than the female (36.36%) subjects were. It can be deduced that such result might be due to the claim of cattle owners in dispersal programs; it is common practice that bulls or male cattle are loaned to other dispersal beneficiaries in adjacent municipalities during breeding season. Thus, increasing the chance for more bulls to be exposed to tick-infestation on transit and in other farms thereby become infected with the disease. This was supported by statistical analysis using chi-square test, whichrevealed that the prevalence of Anaplasmosis infection was found to be independent of the age and sex of cattle.Correlating infectivity according to breed was no longer processed according to breed predilection since almostall of the sampled cattle were crosses of native and imported breeds.

In a research conducted by Derrota*et al*. (2002) in Batangas, they found that Anaplasmosis is present throughout the province but contend that there was little evidence of overt clinical disease suggesting agreement to early 50's literature findings thatit may be

attributed to a high degree of innate resistance that probably exist to Anaplasmosis in cattle on said locale.

Table 2. Cattle Anaplasmosis Based on Blood Film Technique (BFT)

AREA	No. of SAMPLES	BFT POSITIVE		AGE						SEX			
				1-5		6-10		11-above		M		F	
		f	%	f	%	f	%	f	%	f	%	f	%
Balicuatro	32	15	7.50	11	16.93	2	3.08	2	3.08	3	4.62	12	18.46
Central	77	32	16.00	27	41.54	5	7.70	0	0	9	13.85	23	35.38
Pacific	91	18	9.00	12	18.47	6	9.33	0	0	2	3.08	16	24.62
TOTAL	200	65	32.50	50	76.94	13	20.01	2	3.08	14	21.54	51	78.46

Blood Film Technique

The blood film technique was used as a means to confirm and validate CAT result and to identify morphologically the parasites (Stoltsz, 1993; Souslby, 1982; Ristic, 1981).

Table 2 shows the number of microslides that were positively identified to contain *Anaplasma spp.* using the oil immersion objective of conventional microscopy.

The table indicated that there were 65 or 32.50% of the samples that tested positive to the BFT. The age distribution of these sampled cattle revealed 50 or 76.94% belonging to the 1-5 year old bracket and 15 or 23.19% in the 6-year old and above bracket. As regards sex, 51 or 78.46% of the positive samples were female and only 14 or 21.54% were male.The trend in the result of the BFT paralleled the trend in the result of the CAT, but fewer in number, for there were CAT positive samples that did not yield parasites in blood films.

The morphological identification of the parasite in the microslide films supported the concept that Northern Samar is not Anaplasmosis-free.Thus, measures should be constituted by concerned agencies considering that this infectious disease categorized as "a disease of farm concern" may lead to untoward health and economic losses. The same alarming concern was likewise noted on studies done in select areas in Cebu, and in Luzon, Philippines (Ochirkhuu et al, 2015; Ybañez et al, 2013).

Generally, Anaplasma spp. infection in seropositive cattle has documented for the first time the presence of said infectious disease

in Northern Samar. It is therefore recommended that further epidemiological or molecular-based approach study on *Anaplasmosis* be conducted in the province including other provinces in Eastern Visayan region in order to come up with effective control measures against further spread of the disease.

CONCLUSIONS

Anaplasmosis is present in about 33% of the cattle in the province based on the CAT result of the blood samples taken from the experimental animals in the area during the study period. The age and sex of the animals are not considered factors in the prevalence of the disease since cattle of all ages and sexes are susceptible to the disease.

Combined with morphological BFT, the results have indicated the efficacy of CAT as a reliable screening diagnostic tool for Anaplasma spp. infection detection on the blood samples collected from cattle in Northern Samar.

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REFERENCES

Bundza, A.andSamagh, B. S. (1982). Acute anaplasmosis in imported cattle. *The Canadian Veterinary Journal*, 23(11), 337-9.

Derrota, G. R., J. B. Molloy, R. C. Abila, A. B. Mateo, R. V. Cacho, J. A. Batolos, and R. E. Bock.(2002). A survey of the prevalence of bovine babesiosis and anaplasmosis in Batangas province.Completed Research Report, BAI, PAHC, Quezon City.

Lincoln, SD (1990). "Anaplasmosis." IN: Large Animal Internal Medicine. Smith, B. P. (ed.), St. Louis:CV Mosby Co., pp. 742-763.
K. 2015.

Molloy, J.B., Bowles, P.M., Knowles, D.P., McElwain, T.F., Bock, R.E., Kingston, T.G., Blight, G.W. and Dalgliesh, R.J. (1999). Comparison of a competitive inhibition ELISA and the card agglutination test for detection of antibodies to Anaplasmamarginale and Anaplasmacentrale in cattle. *Australian Veterinary Journal*, 77(4), 245-249.

Ochirkhuu, N., Konnai, S., Mingala, C.N., Okagawa, T., Villanueva, M., Pilapil, F.M.I.R., Murata, S. and Ohashi, K. (2015). Molecular epidemiological survey and genetic analysis of vector-borne infections of cattle in Luzon Island, the Philippines. *Veterinary Parasitology*, 212(3-4), 161-167

PAHC (2001). Laboratory Manual on Tick Fever Diagnosis. Bureau of Animal Industry, Diliman, Quezon City, pp. 4-6.

Ristic, M. (1981). Anaplasmosis. In Diseases of Cattle in the Tropics (pp. 327-344). Springer, Dordrecht.

Stockham, S. L., & Scott, M. A. (2013). Fundamentals Of Veterinary Clinical Pathology. John Wiley & Sons.

Stoltsz, W. H. (1993). "Bovine Anaplasmosis" IN: Howard, J. L. (author) 1993. Current Veterinary Therapy and Food Animal Practice. W. B. Saunders, Co., Philadelphia, p. 558.

Souslby, EJL. (1982) Helminths, arthropods and protozoa of domestic animals. 7th Ed. London: Baillierie Tindall.

Ybañez, A.P., Sivakumar, T., Ybañez, R.H.D., Vincoy, M.R.B., Tingson, J.A., Perez, Z.O., Gabotero, S.R., Buchorno, L.P., Inoue, N., Matsumoto, K. and Inokuma, H. (2013). Molecular survey of bovine vector-borne pathogens in Cebu, Philippines. Veterinary parasitology, 196(1-2), 13-20.