

COMPARATIVE PREVALENCE OF SUBCLINICAL BOVINE ANAPLASMOSIS UNDER DIFFERENT CATTLE MANAGEMENT SYSTEMS IN HARYANA

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ABSTRACT

A total of 452 blood smears were prepared from cattle kept under four types of dairy production systems (three large organized dairy farms, six commercial dairy farms, nine small holder dairy production units and twelve Gaushalas) for detecting *Anaplasma marginale* infection during 2007. During the study period, none of the cattle sampled had clinical symptoms of anaplasmosis. Overall prevalence of *A. marginale* in cattle was 46.9%. Large organized dairy farms had significantly higher prevalence (59.2%) than commercial dairy farms (46.07%), Gaushalas (44.1%) and small holder dairy units (35%). Overall parasitaemia of *A. marginale* in cattle was 0.14%. Large organized dairy farms had significantly higher parasitaemia (0.16%) than commercial dairy farms (0.11%), Gaushalas (0.13%) and small holder dairy units (0.14%). High prevalence of *A. marginale* and absence of clinical cases in cattle highlights the endemic stability of anaplasmosis in Haryana state.

Key words: *Anaplasma marginale*, prevalence, cattle management systems, Haryana

Bovine anaplasmosis has been described in domestic and wild animals, i.e. in cattle, buffalo, bison, African antelopes and the mule deer (Bram, 1975; Kuttler, 1984; Kocan *et al.*, 2000; Dumler *et al.*, 2001; Kocan *et al.*, 2003). Principal vectors are ixodid ticks, mainly *Boophilus microplus* (*Rhipicephalus B. microplus*) but other species of the genera *Rhipicephalus*, *Dermacentor*, *Haemaphysalis*, *Hyalomma* and *Ixodes* can also transmit *Anaplasma* spp. In addition, iatrogenic and mechanical transmission by insects (i.a. *Tabanus* spp. *Stomoxys* spp.) is possible. There are many *Anaplasma* species but *A. marginale* and *A. centrale* are the most important ones (Anon., 1996). While *A. marginale* causes a clinical disease, comparatively much less pathogenic *A. centrale* can induce only a moderate degree of anaemia, with clinical outbreaks in the field being extremely rare (Kreier and Ristic, 1963). Microscopically, *A. marginale* looks like solid dots on the margins of RBC. As the infection progresses, more and more RBCs contain parasites and are destroyed (Stewart *et al.*, 1981). It usually causes sub-acute or chronic disease but sometimes acute disease is observed in older animals especially in dairy cows of non-indigenous breeds. The disease is characterized by fever, severe anemia, jaundice, brownish urine, loss of appetite, dullness or depression, rapid deterioration of physical condition, muscular tremors,

constipation, pale mucous membrane and labored breathing (Bram, 1983). The diagnosis is based on the clinical symptoms and must be confirmed by direct microscopy or by immunodiagnosis. In acute cases, 10 to 50 % of the RBCs can be infected. These are easy to observe in smears stained with Giemsa unless at the peak of phagocytosis.

Various cattle production or management systems commonly practiced in Haryana state include small holder dairy units, commercial dairy farms, large organized farms and Gaushalas. Small holder dairy production system is a traditional farming system and consists of a few milking animals along with calves and heifers. The commercial dairy farms generally maintain 10-50 milking animals mostly cross bred cattle with or without buffaloes. Large organized dairy farms maintain large number of animals with better management practices with scientific approach. Gaushalas are charitable institutions run by trusts to house the unproductive cattle particularly indigenous, non-lactating, weak, aged and disabled stray cattle. However, healthy cattle, lactating cows and calves are also often sheltered.

Cattle management systems are known to influence the prevalence of diseases as the cattle production approaches are different. Though various epidemiological aspects of bovine anaplasmosis have been investigated in detail, yet the epidemiology of the infection in the different cattle management systems

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has not been studied. The study was aimed at investigating and comparing the prevalence of *A. marginale* in cattle of four different dairy production systems in Haryana state, India.

MATERIALS AND METHODS

Place of study: The present study was conducted in different cattle management systems (Table 1) in Hisar district (29°10' N, 75°46' E) and some adjoining areas of Bhiwani district in the state. Cattle blood samples were collected from small holder dairy units of nine villages, six commercial dairy farms, three large organized dairy farms and nine Gaushalas as detailed in Table 1. Chaudhary Charan Singh Haryana Agricultural University (CCS HAU) Animal Breeding Farm maintains native and cross bred cattle. Government Livestock Farm sector-1 (GLF-1) maintains native breeds of cattle like Hariana, Sahiwal and Tharparkar while GLF-3 maintains cross bred cattle.

Preparation and staining of thin blood smears from animals: A drop of peripheral blood was taken from ear tip of the animal. Before collection of blood,

the area of the ear tip was thoroughly clipped and wiped with methanol. The blood smears were prepared on clean, grease free glass slides, air dried and labeled with a lead pencil. Smears were stained with the standard procedure of Giemsa staining. A total of 452 thin blood smears were prepared for the prevalence studies of anaplasmosis.

Interpretation of stained blood smears: Blood smears were examined microscopically. Approximately 10,000 RBCs (40 fields) per slide were observed. Morphologically, *A. marginale* were observed as solid dots on the periphery of the RBCs.

Statistical Analysis: For comparing the prevalence of anaplasmosis in blood smears of different cattle population Duncan multiple range test was applied.

RESULTS AND DISCUSSION

Prevalence of *A. marginale* in different cattle management systems: Per cent prevalence of *A. marginale* organisms in cattle using blood smear examination in different cattle management systems is shown in Table 2. Of the 452 cattle examined, *A.*

Table 1
Different cattle management systems screened for *Anaplasma marginale* infection in cattle

Sr. no.	Small holder dairy units (village name)	Commercial dairy farms	Large organized farms	Gaushalas
1	Raipur	Mahinder Singh Sardar Dairy Farm Multani Chowk	CCS HAU Animal Breeding Farm	Shri Haryana Kurukshetra Gaushala, Hisar
2	Arya Nagar	Prem Dairy Farm Multani Chowk	Government Livestock Farm Sector-1	Devi Bhavan Gaushala, Hisar
3	Mirzapur	Raghubir Dairy Farm, Satrod	Government Livestock Farm Sector-3	Shri Balaji Gaushala, Mangali
4	Dhansu	Davender Sardar Dairy Farm, Thandi Sadak	-	Shri Agroha Trust Gaushala, Agroha
5	Ladwa	Billu Dairy Farm, Gujran Mohalla	-	Krishna Pranami Gaushala, Kaimari
6	Dabra	Khatana Dairy Farm, Padav Chowk	-	Mahajan Gaushala, Satrod Khurd
7	Satrod Kalan	-	-	Niana Gaushala, Niana
8	-	-	-	Shri Radha Krishnan Gaushala, Siwani
9	-	-	-	Shri Krishna Gaushala, Siwani
10	-	-	-	Gomatha Shakti Peeth, Badwa
11	-	-	-	Shahpur Gaushala, Shahpur
12	-	-	-	Data Gaushala, Data

Table 2

Prevalence and per cent parasitaemia of *Anaplasma marginale* under different cattle management systems

Cattle management system	No. of blood smears examined	Per cent positive for <i>A. marginale</i>	Per cent parasitaemia of <i>A. marginale</i>
Large organized dairy farms	130	59.2 ^a	0.16 ^a
Gaushalas	120	44.1 ^b	0.13 ^b
Commercial farms	102	46.07 ^b	0.11 ^b
Small holder dairy units	100	35 ^b	0.14 ^b
Over all	452	46.9	0.14

Values bearing common superscripts for a parameter do not differ significantly at 5% level of significance.

marginale organisms were detected in 212 (46.9%) animals. Average parasitaemia of the four cattle management systems for *A. marginale* varied from 0.11 to 0.16%. The parasitaemia across the management systems ranged from 0.01 to 0.61%. The data were analysed by grouping the four cattle management systems separately. The results of blood smear examination (Table 2) revealed that both the prevalence and parasitaemia were significantly higher in the large organized dairy farms as compared to cattle of small holder dairy production units, Gaushalas and commercial dairy farms.

Prevalence of *A. marginale* in large organized dairy farms: Out of 130 cattle examined, *A. marginale* were detected in erythrocytes of 75 (57.69%) animals (Table 3). The parasitaemia on an average was 0.16%. The parasitaemia across the large organized dairy farms ranged between 0.01 to 0.57%. The data were also analysed by grouping the animals of the three large organized dairy farms separately. Significantly higher number of animals (68%) were carriers at the GLF-3 and CCS HAU Animal Breeding Farm (70%) as compared to cattle at GLF-1 (30%). However, the parasitaemia was significantly higher (0.18%) in the GLF-1 as compared to the CCS HAU Animal Breeding Farm (0.13%).

Prevalence of *A. marginale* in small holder dairy production units: Of the 100 cattle examined, *A. marginale* was detected in erythrocytes of 40 (40%) animals (Table 4). The parasitaemia ranged between 0.1 to 0.3% (average 0.14%). The parasitaemia across the small holder dairy production units ranged between 0.01 to 0.61%. The data were also analysed by grouping together the small holder dairy units of a village (Table 4).

Table 3

Prevalence and per cent parasitaemia of *Anaplasma marginale* in large organized dairy farms

Name of the large organized system farm	No. of blood smears examined	Per cent positive for <i>A. marginale</i>	Per cent parasitaemia of <i>A. marginale</i>
CCS HAU Animal Breeding Farm	50	70 ^a	0.13 ^b
GLF-1	30	30 ^b	0.18 ^a
GLF -3	50	68 ^a	0.17 ^a
Over all	130	59.2	0.16

Values bearing common superscripts for a parameter do not differ significantly at 5% level of significance

Prevalence of *A. marginale* in Gaushalas: Comparison of per cent prevalence and per cent parasitaemia of *A. marginale* in Gaushalas is presented in Table 5. Out of 120 cattle examined, *A. marginale* were detected in erythrocytes of 52 (44.1%) animals. The parasitaemia ranged between 0.1 to 0.2% (average 0.1%). The parasitaemia across the Gaushalas ranged between 0.01 to 0.32%. The data were also analysed by grouping the animals of the ten Gaushalas separately. The results of blood smears revealed that per cent prevalence was significantly higher in Devi Bhavan Gaushala and Mahajan Gaushala while per cent parasitaemia was not significantly different between Gaushalas (Table 5).

Prevalence of *A. marginale* in commercial dairy farms: Comparison of per cent prevalence and per cent parasitaemia of *A. marginale* in commercial dairy farms is presented in Table 6. Of the 102 cattle examined, *A. marginale* were detected in erythrocytes of 46 (46.07%) animals. Parasitaemia of *A. marginale* ranged between 0.1 to 0.2% (average 0.1%). The data were analysed by separately grouping the six commercial dairy farms as shown in Table 6.

This is probably the first epidemiological study to assess and compare prevalence of *A. marginale* in different cattle management systems in India. Subclinical infection of *A. marginale* in cattle has been reported frequently as a concurrent infection with other blood protista parasites like *Theileria*, *Babesia* and *Trypanosoma* (Afridi *et al.*, 1985; Rao *et al.*, 1986; Mallick *et al.*, 1987; Jithendran, 1997; Julie *et al.*, 2005; Raina *et al.*, 2005; Soundararajan and Rajavelu, 2006). Concurrent infection of *T. annulata* was also observed in many blood smears in the present study.

Surprisingly, prevalence of *A. marginale* was

Table 4
Prevalence and per cent parasitaemia of *Anaplasma marginale* in small holder dairy production units

Name of the village	No. of blood smears examined	Per cent positive for <i>A. marginale</i>	Per cent parasitaemia of <i>A. marginale</i>
Raipur	13	38.4 ^b	0.1 ^b
Arya Nagar	17	70.5 ^a	0.1 ^b
Mirzapur	10	60 ^a	0.2 ^{ab}
Dhansu	20	15 ^b	0.3 ^a
Ladwa	20	30 ^b	0.1 ^b
Dabra	10	30 ^b	0.1 ^b
Satrod Kalan	10	60 ^a	0.2 ^{ab}
Over all	100	35	0.14

Values bearing common superscripts for a parameter do not differ significantly at 5% level of significance

significantly higher in large organized dairy farms as compared to cattle of small holder dairy production units Gaushalas and commercial dairy farms. The parasitaemia of *A. marginale* was also significantly higher in large organized dairy farms as compared to small holder dairy production units, Gaushalas and commercial dairy farms. Because of more availability of water in large organized farms' humidity inside the animal house increases which favors survival of *Boophilus* tick population in these farms thereby facilitating the transmission. Moreover, higher transmission rates between cattle and ticks result once the infection is maintained in a farm over several generations of *Boophilus* ticks. In large farms (Gaushalas, organized dairy farms and commercial dairy farms), within herd transmission of anaplasmosis becomes easier than scattered small holder dairy units as the probability of transmission by the tick adapted to complete their life cycle in the animal houses increases due to high density of animals. However, only large organized dairy farms had significantly higher infection while Gaushalas and commercial dairy farms were not significantly different from the small holder dairy production system. It seems better care and tick control practices in commercial dairy farms and the presence of mostly indigenous cattle in Gaushalas may be responsible for this difference. Lower prevalence of the parasite in the indigenous breeds is demonstrated clearly by the difference in the prevalence in the GLF-1 (30%) where only indigenous breeds are maintained and GLF-3 (68%) where only crossbred cattle are maintained.

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Table 5
Prevalence and per cent parasitaemia of *Anaplasma marginale* in Gaushalas

Name of the Gaushala	No. of blood smears examined	Per cent positive for <i>A. marginale</i>	Per cent parasitaemia of <i>A. marginale</i>
Shri Haryana Kurukshetra			
Gaushala, Hisar	20	55 ^{ab}	0.2 ^a
Devi Bhavan			
Gaushala, Hisar	10	60 ^a	0.1 ^a
Shri Balaji			
Gaushala, Mangali	20	55 ^{ab}	0.1 ^a
Shri Agroha Trust			
Gaushala, Agroha	10	30 ^{ab}	0.1 ^a
Krishna Pranami			
Gaushala, Kaimari	10	30 ^{ab}	0.1 ^a
Mahajan Gaushala,			
Satrod Khurd	10	60 ^a	0.1 ^a
Niana Gaushala, Niana	10	20 ^b	0.1 ^a
Shri Radha Krishnan			
Gaushala, Siwani	10	50 ^{ab}	0.1 ^a
Shri Krishna			
Gaushala, Siwani	10	20 ^{ab}	0.1 ^a
Gomatha Shakti			
Peeth, Badwa	10	30 ^{ab}	0.2 ^a
Total	120	44.1	0.1

Values bearing common superscripts for a parameter do not differ significantly at 5% level of significance

Research Institute (ILRI) that small holder dairy production system is the most vulnerable system for the ticks and tick-borne diseases (<http://www.ilri.org/InfoServ/Webpub/fulldocs/InvestAnim/Book1/media/Appendex/7/7.2.1.htm>) in India. However, the present study revealed that this group is the least vulnerable amongst the various dairy production systems to *Anaplasma* infection in the state. However, none of the 452 animals sampled across production systems showed clinical anaplasmosis. Absence of clinical cases points towards the establishment of endemic stability within the dairy systems. A similarly high prevalence of anaplasmosis in cattle and buffaloes highlighting endemic stability has been reported by Rajput *et al.* (2005) in Sindh, Pakistan.

The prevalence and parasitaemia of *A. marginale* varied among different cattle management systems as well as within various locations of the same management system. Epidemiological variables (breeds, nutritional status, population size, feeding practices, herd system and use of acaricides) related to host, vector and environment in the different cattle management systems

Table 6
Prevalence and per cent parasitaemia of
***Anaplasma marginale* in commercial dairy farms**

Name of the commercial dairy farm	No. of blood smears examined	Per cent positive for <i>A. marginale</i>	Per cent parasitaemia of <i>A. marginale</i>
Mahinder Singh Sardar Dairy Farm,			
Multani Chowk	15	40 ^b	0.1 ^b
Prem Dairy Farm,			
Multani Chowk	15	73.3 ^a	0.2 ^a
Raghubir Dairy Farm,			
Satrod	15	53.3 ^b	0.1 ^b
Davender Sardar Dairy Farm,			
Thandi Sadak Billu Dairy Farm, Gujaran Mohalla	20	50 ^b	0.1 ^b
Khatana Dairy Farm,			
Padav Chowk	22	31.8 ^b	0.1 ^b
Total	102	46.07	0.1

Values bearing common superscripts for a parameter do not differ significantly at 5% level of significance

might have exerted various influences resulting into variations in the prevalence and parasitaemia in the different cattle management systems as well as within the same management system at different locations. It seems *A. marginale* is commonly present in the cattle of the area across all management systems despite significant differences in prevalence and per cent parasitaemia while the clinical cases are rare.

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