# ANAPLASMA AND ANAPLASMOSIS IN LIVESTOCK OF KARNATAKA – A RETROSPECTIVE

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*Anaplasma* is an obligate intraerythrocytic bacteria of family Anaplasmataceae of the order Rickettsiales which causes the dreadful disease, anaplasmosis among livestock and this organism is always dealt along with other blood protozoans, *Theileria* and *Babesia*. Though anaplasmosis caused by *A. marginale* was known to be prevalent in native cattle of Karnataka, its occurrence in cross-bred dairy cattle of Bengaluru was reported by Jagadish Kumar *et al*. (1977). This disease along with other haemoprotozoans gained more attention in late sixties in Karnataka state after the impact of import of foreign breeds of cattle for upgrading indigenous stock to improve their milk yield. The cross-bred progenies from these cattle are more susceptible to these disease compare to indigenous stock which acted as carriers of infection to the susceptible animals through ticks and other haematophagous insects. The carrier status of infection existed in indigenous animals as well as the newly recovered ones from this disease. By considering the importance of this infection in dairy cattle in terms of loss in milk reduction, cost of treatment, mortality and morbidity, an ICAR scheme of five years duration "On the biology and control of *Anaplasma*" was implemented at Veterinary College, Bengaluru during 1974 which contributed important information on different aspects of the disease. Another major source of information on this disease was from the accumulated data for about seven years period provided by the four Veterinary Diagnostic Laboratories (VDL) located at strategic places like Bengaluru, Mysuru, Tumukuru and Hassan, established by the Karnataka Dairy Development Project with the financial assistance of World Bank and the technical support from the University of Agricultural Sciences, Bengaluru. These laboratories helped for the prompt recognition disease problems especially of the bovine population of dairy co-operative societies (DCS) of the eight southern districts of Karnataka such as Bengaluru, Kolar, Mysuru, Mandya, Kodagu, Tumukuru, Hassan and Chikkamagaluru. Incidences of anaplasmosis in livestock in various parts of Karnataka, its pathogenesis, diagnostic methods adopted, advanced serological investigations, surveillance, recognition of vectors and treatment followed were enlightened in this review. Further, attempts were made to know its occurrence in certain captive animals. Success achieved in growing *A. marginale* organisms in tissue-culture and in attenuating them widened the scope for developing suitable vaccine.

## SPECIES OF ORGANISMS

Two species of *Anaplasma* occur in

Karnataka, *A.marginale* in cattle and buffaloes and *A. ovis* in sheep.

## MORPHOLOGY

The Giemsa-stained smears prepared out of the blood of positive cattle showed typical *A. marginale* bodies in about 60.0% of affected erythrocytes, one or two bodies per cell (Jagadish Kumar *et al*., 1977; Ravindranath *et al*., 1982). When the fresh positive blood was mixed with equal quantity of 0.5% new methylene blue stain and examined as wet smear preparation, the *Anaplasma* bodies appeared as small bright dots or tiny blue particles of 0.5µ size inside the erythrocytes exhibiting a floating type of movement with a tendency to be at the periphery (Setty, 1983; 2002).

## INCIDENCE

The available data on the incidence of *A. marginale* infection in cattle and buffaloes based on stained blood smear examinations have been presented in Table I. It could be inferred from the Table that the overall incidence of infection in affected animals was within the range of 0.37%-46.45% with one exception of 60.87% infection of an epizootic proportion in cross-bred heifers. The data on incidence have been analyzed seasonal, breed as well as age-wise and noted below.

### Seasonal incidence

Analysis of data of positive cases of *A. marginale* infection for the six years (1978-1984) period of VDL, Bengaluru and Mysuru indicated higher seasonal infection was observed in south-west (SW) monsoon followed by north-east (NE) monsoon seasons. Data from VDL, Hassan revealed that summer season was more favorable for the spread of infection while VDL, Tumukuru recorded higher prevalence during cold weather followed by SW monsoon and no infection was noticed in NE monsoon (Seshadri *et al*., 1985). The seasonal studies indicated that *A. marginale* infections occurred throughout the year, initiated from the pre-monsoon months (Muraleedharan *et al*., 2005). But Ravindranth *et al*. (1982) reported this disease in September-October as an outbreak-form.

### Breed incidence

Among exotic pure breeds Holstein-Friesian had shown 42.8% and Jersey had 26.5% infection whereas cross-bred cattle had higher rate of 46.4% infection compared with 16.7% in non-descript indigenous cattle (Jagannath and Krishna Murthy, 1980). Ravindranath *et al*. (1982) reported very high percentage (60.86%) of infection in cross-bred heifers at a farm in suburban Bengaluru. In the jurisdiction of VDL, Mysuru, Muraleedharan *et al*. (2005) noticed that the cross-bred cattle showed more infection (1.51%) than indigenous cattle (0.86%) while pure-bred exotic cattle did not show infection. Among the cross (x) between exotic breeds and local cattle, Red Dane x (2.67%) followed by Jersey x (2.08%) showed more susceptibility to infection than by Holstein-Friesian x (1.24%).

### Age incidence

As per the data of VDL, Mysuru, the cattle below six month of age were not susceptible to *A. marginale* infection. The minimum age at which infection was observed was one year. Other age group-wise incidences was as follows: 6 month to 1 year-1.33%; 1 to 4 year-1.38%; 4 to 8 year-1.48% and <8years-1.06% indicating a declined trend. The animals of one year of age alone showed higher rate of infection indicating more susceptibility in comparison with other age groups with four years class-intervals (Muraleedharan *et al*., 2005).

## CONCURRENT INFECTION WITH HAEMOPROTOZOANS

A combined infection of *A. marginale* and *T. annulata* was seen in four cattle (Anon,

1979). Of the 60 positive cattle (n=4521) identified at VDL, Mysuru (Muraleedharan *et al*., 2005), 10 had concurrent infection of *A. marginale* and *T. annulata* with predominance of *A. marginale* in three cases, theilerial infection in two cases and the remaining five cases shared almost equal number of these parasites.

## PATHOGENESIS

Cattle positive for *A. marginale* infection had pyrexia of 40-41$^{o}$C marked anaemia, dyspnoea, increased pulse rate, lachrymation, salivation, shivering, pale bulbar conjunctiva, visible mucus membrane icteric including vulvar, swelling of sub-maxillary region enlarged lymph nodes, increased pulse rate and respirations were shallow and labored. The udder, teats and muzzle of one of the affected cows were distinctly yellow giving the appearance of "yellow cow" (Jagadish Kumar *et al*., 1977; Ravindranath *et al*., 1982; Muraleedharan *et al*., 2008).

### Haematology

Jagadish Kumar *et al*. (1977) and Ravindranath *et al*. (1982) reported that most of the clinical anaplasmosis about 60% of the erythrocytes (60.0%) had infected with one or two organisms per cell. The infected erythrocytes had a punched out appearance with marked anisocytosis, polychromasia, basophilic stippling with presence of many megaloblasts and normoblasts. The haematologial values of acute anaplasmsis were: Hb: 3-8g/dl, TEC: 2 m/cmm, TLC: 10700/cmm, ESR: nil/hr and PCV: 9% (Jagadish Kumar *et al*., 1977 and Ravindranath *et al*., 1982). Scanty to high parasitaemia (2.0-60.0%) was recorded by Muraleedharan *et al*. (2005) in cattle of Mysuru-Mandya area.

### Experimental infection of calves

Experimental infections of *A. marginale* were induced to 25 splenectomized and 5 intact calves maintained in tick proof sheds, by injecting varying quantities of blood with 80% parasitaemia and the blood collected from them were stored for 52 days at +4$^{0}$C. The calves on recovery from *Anaplasma* infection remained carriers. Infected erythrocytes were stored in liquid nitrogen at –196$^{0}$C as per procedures after adding 10% dimethyl sulphoxide (DMSO) to minimize haemolysis. The recovered calves from clinical form remained as carriers. For rapid multiplication of *A. marginale*, cortisone (decadron) was administered to *Anaplasma* carrier experimental calves for four consecutive days, but it did not flare up the infection (Anon, 1979; Krishna Murthy and Jagannath, 1980a).

### Experimental infection of laboratory animals

Inoculation of 0.2 ml of 60% *Anaplasma* infected blood intravenously (i/v) to rabbits, guinea-pigs, mice, hamsters and to four a day-old embryonated eggs by intra-yolk-sac route failed to set up infection. Capillary agglutination test conducted in these laboratory animals also proved negative showing their non-susceptibility to infection. No visible lesions were observed in chicks-embryos except for haemorrhages and no sign of disease in experimental animals. The administration of decadron (2mg) to these animals did not promote the establishment of this organism (Krishna Murthy and Jagannath, 1980b).

## SEROLOGICAL DIAGNOSIS

Various serological tests using indig enous antigens had been tried to detect *Anaplasma* infection, especially in the carrier animals whose did not always exhibit the organisms by GBS examinations (Anon, 1979).

### 1. Capillary-tube agglutination test

Krishna Murthy *et al*. (1978; 1994) conducted capillary-tube agglutination (CA) test using antigen prepared using local strain of *A. marginale* as per the method of Ristic

with minor modifications such as the blood samples were frozen at –20°C instead of –65°C and antigen was stored both at –20°C as well as at +4°C. Sera collected from clinical cases and experimentally infected calves were found 100% positive to CA test whereas as high as 80.95% of the 21 suspected cattle and 3.08% of 130 apparently healthy cows were shown positive instead of 4.76% and 2.31% corresponding to GBS examination. The CA test further indicated a higher prevalence of infection in exotic and cross-bred cattle (20.72%) compared with that of local cattle (6.07%). The clinically cured exotic cows following oxytetracycline therapy remained positive for CA test up to 100 days, but they were found negative when tested after 300 days. Inoculation of 20ml blood of these animals to two splenectomized calves failed to establish infection in them for an observation period of 30 days which indicated the complete clearing of organisms as well as antibodies. The efficacy of locally prepared CA test antigen was found equivalent to that of imported antigen supplied by Miodrag Ristic of the University of Illinois, USA. It was also noted that the antigen did show any cross or non-specific reaction with the sera of normal sheep, goats, dogs, rabbits as well as man and those of cattle recently suffered from theileriosis, babesiosis, brucellosis and foot and mouth diseases.

**2. Card agglutination test**

The coloured card agglutination antigen was prepared with local strain of *A. marginale* following the method of Todorovic and Kuttler for *Babesia bigemina* antigen. Out of 251 sera samples tested, 31.07% showed positive reaction to card test as against 27.89% to CA test and 25.90% by GBS examination. The test was found to be sensitive and dilutions 1:4 and 1:6 of antigen gave better results than the higher dilutions. The blood smears and sera collected from indigenous cattle were all negative for *Anaplasma* while those of cross-bred cattle from Bengaluru and its suburbs were, however, found to be carriers of infection by card agglutination tests and CA tests. In detection of carrier condition the card test appeared to be more efficient than CA test (Anon, 1979).

**3. Gel diffusion test**

Gel diffusion test was done as per Ouchterlony method using CA antigen. Positive reactions were noted with precipitation band formation in the agar in between the wells containing corresponding antigen and sera. Out of 15 sera samples including four known positives, only one (6.67%) was found positive indicating that the method was not satisfactory (Anon, 1979).

**4. Complement fixation test**

The antigen for complement fixation test (CFT) was prepared as per Gates *et al*. Out of 30 sera samples tested, only six (20.0%) were found positive to CFT. Sera positive for *Theleria*, *Babesia*, Foot and Mouth disease and *Brucella* when tested against known antigen by CFT showed negative results. It was observed that the CFT appeared to be more sensitive in detecting the *Anaplasma* positive cases than CA test and Card test (Anon, 1979).

**5. Fluorescent antibody technique**

Two methods of fluorescent antibody technique (FAT) were tried. Direct and indirect FAT were applied on unknown and known blood smears using fluorescein isothiocynate tagged antibodies. In the indirect method, tagging was effected to antibovine globulins.

**a) Direct method**

Serum containing antibodies against *A. marginale* was used for conjugation with fluorescein isothiocynate. The hyperimmune serum against *Anaplasma* in the rabbit was used for conjugation and test was conducted following standard procedure. When 20 known positive slides were examined, all gave

sufficient fluorescence to detect the organisms whereas known negative slides did not give any fluorescence. Another 30 blood smears were also examined, of which 10 (33.33%) found to be positive and the remaining showed negative results. In the direct method, non-specific reactions were observed. In the direct method, non-specific reactions were observed (Anon, 1979).

**b) Indirect method**

Thirteen known positive slides were also positive and five known negative cases did not show fluorescence**.** The sera samples of 30 more suspected cases for anaplasmosis from Bengaluru were tested by indirect FAT and proved to be negative. Indirect FAT was appeared to be more sensitive than direct FAT (Anon, 1979).

**6. Immuno-peroxidase test**

The immuno-peroxidase test (IPT) using *Anaplasma*-induced globulin was developed for detection of *A. marginale* in cattle. The test was performed for the first time in protozoan disease diagnosis. Antigen-antibody reaction was observed by the blue colour reaction under ordinary microscope. It was possible to detect more number of positive animals (53.0%) compared to that of conventional GBS examinations (40.9%) when 66 blood smears of field cases had been subjected to these two tests. The blood smears positive for *Theileria. annulata, Trypanosoma evansi* and *Babesia bigemina* failed to show a positive reaction with IPT and thereby confirmed the specificity of this test (Krishna Murthy and Jagannath, 1980a).

**7. Leucocyte migration inhibition test**

The cells involved in producing cellular immunity were mainly sensitized T. lymphocytes and macrophages which acted directly on *Anaplasma* organisms or attempted to phagocytose and digest them. The procedure of Ristic and Nyindo was adopted for conducting leucocyte migration inhibition test. The leucocyte migration was inhibited in two splenectomized infected calves as against one healthy control calf indicating that the test appeared to be sensitive (Anon, 1979).

**8. Delayed cutaneous hypersensitivity test**

Out of 6 recovered calves received injection of 0.1 ml of *Anaplasma* CA test antigen, 5 (88.33%) showed cutaneous swelling. By application of above two tests (Sl. No. 7 and 8) the status of cellular immunity of *A. marginale* among cattle could be assessed (Anon, 1979).

**Tissue culture**

**a) Bovine lymph node culture**

Pure lymph node cells (LNC) from a foetal bovine calf, negative for *A. marginale* were obtained and grown into a monolayer as well as inoculum of washed erythrocytes containing *A. marginale* organisms were prepared following standard procedures. Monolayer cells were serially passaged at least 8 times to obtain a well established cell line. Monolayer cell of each passage up to 8$^{th}$ passage was inoculated with infected blood from different sources. The multiplication of the organisms in the LNC was not rapid for the first 24-48 hr, but later it was hastened 3-4 times by repeated passages. The organisms were seen on the cytoplasm of the LNC and in certain places inclusion bodies were also observed inside cytoplasm of the cells. The treatment of LNC with diethylaminoethyl dextran (DEAE-D) solution and phytohaemagglutinin (PHA) did not improve the multiplication of the organisms. One ml of the inoculum of *A. marginale* organisms grown on 8$^{th}$ passage were inoculated into splenectomized calf. The calf showed 1.0 percent parasitaemia on the 48$^{th}$ day which reached 20.0 percent on 56$^{th}$ day. The parasitaemia came down to 2.0 percent and

later calf became negative for infection. But it reappeared on 90th day and subsequently calf died following extreme anaemia (Anon, 1979).

**b) Leucocyte cell culture**

The leucocyte cell culture was attempted following the standard procedure. Monocytic leucocyte cells (MLC) were collected for tissue culture from the blood of a calf below six months. Multiplication of leucocytes was fast during the 2nd and 3rd day. The leucocytic culture was obtained on 9th day and the MLC were sub-cultured till a good cell line was obtained. *Anaplasma* organisms were inoculated to cell line of leucocytes. The MLC was also treated with DEAE dextran and PHA at the rate of 30mg/ml respectively to encourage the multiplication of *Anaplasma* organisms. Repeated passage of *A. marginale* organisms in MLC tissue culture showed limited multiplication of organisms in spite of treatment with DEAE dextran and PHA. The multiplication of organisms in MLC was much less compared to the LNC monolayer tissue culture (Anon, 1979).

**Premunition trials**

Preimmunization with co-infectious immunization is an effective means of producing immunity to anaplasmosis. Non-splenectomized calf which did not contain any antibodies for *A. marginale*, was injected 20ml blood i/v from a carrier cattle having about 1 percent parasitaemia and revealed *A. marginale* on the 19th day of post-inoculation and clinical disease with lasted for a week. After 45 days of initial infection when CA test detected antibody titre 1:2, the calf was challenged with blood 15ml blood having 37 percent parasitaemia, resulted in the rise of serum titre to 1:16. On challenging with infected blood, the calf did not exhibit clinical disease, but with occasional *A. marginale*. Five more calves were preimmunized in the same way. Similarly a splenectomized calf with a low parasitaemia of 3 percent was inoculated with blood having 60 percent parasitaemia remained normal without any increase in parasitaemia (Anon, 1979).

**Irradiation of *Anaplasma* organisms**

Ten ml of *Anaplasma* infected blood with 60 percent parasitaemia after exposure to UV rays for 20 minutes was inoculated to a healthy calf while control calf was inoculated with the same quantity of positive blood without irradiation. Higher parasitaemia of 32 percent was observed early on the 19th day of infection in experimental calf whereas it was only 18 percent on the 22nd day in the case of control. The result was suggestive of enhancement of infection rather than attenuation of organisms by the effect of UV radiation (Anon, 1979).

**Vectors of *Anaplasma***

Common ixodid ticks of 1830 cattle of Bengaluru were identified and their seasonal incidence was studied along with the biology of ixodid tick, *Rhipicephalus* (*Boophilus*) *annulatus* (Jagannath *et al*. (1979; 1982). The engorged ticks collected soon after their dropping off from *Anaplasma* positive experimental calf showed many round bodies in the gut contents and the smears of gut contents and salivary gland was subjected to direct FAT and fluorescence was observed only in salivary gland smears after 72h confirming the presence of *Anaplasma*.

**Transmission trials in vectors**

The transmission of *A. marginale* by *R.* (*B.*) *annulatus* was investigated in splenectomized calves. The hatched out larvae from 2g eggs from the engorged females collected from splenectomized calf infected with *A. marginale* and they were utilized to infect another healthy uninfected splenectomized calf on which the ticks completed their life cycle. During the infection period, the blood smears of calf were examined

daily, but smears did not show the presence of *Anaplasma* organisms, and Hb as well as PCV values of the calf remained normal indicating that trans-ovarian transfer of infection did not occur. Further the experiments on stage to stage transmission also proved negative (Anon, 1979; Jagannath, 1988).

**Tick tissue culture**

Culturing of tick tissue of *R.* (*B.*) *annulatus* in the laboratory for growing *Anaplasma* organisms as per the methods of Yunker and Cory was attempted. The clumps of tissue and tissue debris were seen floating in the culture medium after 1-2 days of setting up of culture. Slowly the clumps began to attach to the surface and cell attachment was enhanced after a week. Large granular fibroblastic type of cells appeared in between the tissue clumps. Half of the medium was replaced every week with fresh medium. This process gradually eliminated the tissue debris and by about 4-5 weeks, the surface was covered with closely packed epithelial cells of *R.* (*B.*) *annulatus*. In total, success had been achieved in preparing 15 primary cultures and 9 sub-cultures of tick tissue (Anon, 1979).

**Infection in other animals**

**Sheep**

Concurrent infection of *A. ovis* with *Theileria hirsi* was noted among two (4.4%) out of 45 sheep examined (Muraleedharan *et al.*, 1994).

**Deer and elephants**

Four out five Sambar deer (80.0%) of Shimoga zoo acted as carriers of *Anaplasma* whereas 37 Indian elephants of Chikkamagaluru district did not show infection (Anon, 1979).

**Treatment with oxytetrcycline**

Jagadish Kumar *et al*. (1977) and Ravindranath *et al*. (1982) successfully treated cattle with oxytetracycline hydrochloride (OTC) 5mg/kg b. w. injections by i/m for 3-5 days and the same treatment was also followed in 72 clinical cases in cattle (Anon, 1979). Supplementary treatment with haematinics hastened the recovery. The same treatment was undertaken in 11 carrier cattle and 10 experimental calves. Treated cases showed marked clinical improvement in 2-3 days with normothermia and disappearance of causative organisms. The animals recovered to normal state within a fortnight. The blood picture of these animals became normal, i. e. normocytic and normochromic appearance and Hb concentration ranged between 7.5 and 9g/dl (Ravindranath *et al*., 1982). In clinical cases where Hb content reduced to 4g/dl and below, did not respond to OTC treatment and died due to anoxia and asphyxia (Anon, 1979). Ananda *et al*. (2014) also treated positive cases with OTC in 500ml normal saline by i/v.

**Treatment with hyperimmune sera**

Hyperimmune sera having high antibody titre were used in acute clinical cases for producing passive immunity. The immune sera were collected from the experimental calves, the recovered animals from acute cases and also from the convalescent animals. The serum was stored at $-20^0$C in deep freeze after adding 1: 1000 dilution of merthiolate solution. The inoculation of hyperimmune sera 50mg i/v for 2 days had relieved clinical symptoms and timely recovery was observed in severely affected cases (Anon, 1979).

**CONCLUSION**

Contributions on anaplasmosis from scientists of Karnataka are a very significant as they expressed the technical capabilities to carry out advanced researches on the diagnosis and to contain the highly spreading nature of this vector-borne disease. Now is the appropriate time for pursuing molecular aspects of diagnostic assays and proteomic studies to detect early and minute levels of *A. marginale*

**Table. I.**

| | | | | |
|---|---|---|---|---|
| Bengaluru dt. | *1375 | 5.24 | CB, local | Seshadri *et al.*(1985) |
| Kolar dt. | *1234 | 1.70 | CB, local | Seshadri *et al.*(1985) |
| Hassan dt. | *2726 | 0.37 | CB, local | Seshadri *et al.*(1985) |

**Table. I.** Incidence of *Anaplasma marginale* infection in cattle and buffaloes based on stained blood smear examinations

| District/locality | No. exam. | Percent | Breeds | Reference |
|---|---|---|---|---|
| **A. Cattle** | | | | |
| Bengaluru | | 8 cases | CB | Jagadish Kumar *et al.*(1977) |
| Karnataka | 413 | 24.70 | | Anon (1979) |
| Bengaluru | 134 | 2.31 | CB | Krishna Murthy *et al*. (1978; 1994) |
| Bengaluru | 42 | 42.85 | HF | Jagannath & Krishna Murthy (1980) |
| Bengaluru | 32 | 26.47 | JR | Jagannath & Krishna Murthy (1980) |
| Bengaluru, Haasan, Mysuru, Tumukuru Chikkamagaluru | 155 | 46.45 | CB | Jagannath & Krishna Murthy (1980) |
| Bengaluru | 18 | 16.67 | Local | Jagannath & Krishna Murthy (1980) |
| Bengaluru | 66 | 40.90 | Cattle | Krishna Murthy & Jagannath (1980) |
| Bengaluru | 24 | 60.87 | CB | Ravindranath *et al.*(1982) |
| Bengaluru dt. | *1375 | 5.24 | CB, local | Seshadri *et al.*(1985) |
| Hassan dt. | *2726 | 0.37 | CB, local | Seshadri *et al.*(1985) |
| Chikkamagaluru dt. | *145 | 0.69 | CB, local | Seshadri *et al.*(1985) |
| Tumukuru Dt. | *3154 | 0.73 | CB | Seshadri *et al.*(1985) |
| Kodagu dt. | 125 | 0.00 | CB, local | Seshadri *et al.*(1985) |
| Bengaluru, Mysuru & Tumukuru | 4081 | 2.30 | Dairy herd | Setty *et al.*(1985) |
| Mysuru dt. | 3318 | 1.51 | Exotic, CB, local | Muraleedharan *et al.*(1994; 2005) |
| Mandya dt. | 1174 | 0.85 | Exotic, CB, local | Muraleedharan *et al.*(1994; 2005) |
| Karnataka, different areas | 11,755 | 6.60 | | Harish *et al*.(2006) |
| Shivamogga, in and around | *566 | 2.83 | JRx, HFx, Hallikar, Amrith- mahal | Ananda *et al.*(2014) |
| Shivamogga dt. | 215 | 2.70 | | Krishna Murthy *et al.*(2014) |
| **B. Buffaloes** | | | | |
| Shivamogga | 93 | 0.00 | | Jagannath & Krishna Murthy (1980) |
| Shivamogga dt. | 85 | 2.35 | | Krishna Murthy *et al.*(2014) |
| Tumukuru dt. | *3154 | 0.00 | | Seshadri *et al.*(1985) |
| Mysuru dt. | 344 | 0.58 | | Muraleedharan *et al.*(1991) |
| Mandya dt. | 117 | 0.00 | | Muraleedharan *et al.*(1991) |

*Bovine (mainly cattle including a few buffaloes; HF= Holstein-Friesian; RD= Red Dane; JR= Jersey; CB or x = cross with exotic and indigenous breeds; ND= Non-descript.

carrier conditions in cattle, buffaloes and the wild-counter parts since success have already achieved in some of these aspects with respect to other haemoparasitic infections in Karnataka such as *Theileria*, *Babesia* and *Trypanosoma*. A highly sensitive and specific PCR-ELISA test developed abroad (Gale *et al*. 1996; Braz Junior *et al*., 2000) and the outcome of the studies on PCR conducted in Punjab (Singh *et al*., 2012; Sharma *et al*., 2015) could be adopted in Karnataka for detecting antibodies against *A. marginale* in sera of bovines in carrier status more efficaciously. Moreover the progress achieved in ICAR Scheme of the in the state in culturing the organisms could be utilized for developing an effective vaccine against anaplasmosis.

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