International Journal of Research Studies in Biosciences (IJRSB)
Volume 6, Issue 7, 2018, PP 13-21
ISSN No. (Online) 2349-0365
DOI: http://dx.doi.org/10.20431/2349-0365.0607003
www.arcjournals.org

Bovine Anaplasmosis and its Associated Risk Factors in and Around Wolaita Sodo Town, Southern Ethiopia

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Abstract: *Bovine anaplasmosis is one of a vector borne disease of cattle which cause a major problem on the health and production of cattle in tropical and sub-tropical regions. A cross-sectional study was carried out from November 2016 to April 2017 on cattle population to determine the overall prevalence of bovine anapalsmosis and its associated risk factors in and around Wolaita Sodo town. A total of 384 blood samples were collected from randomly selected cattle in the study area. Age, sex, origin, breed, body condition, tick infestation and previous treatment were considered as risk factors in the study. Giemsa stained thin blood smears were prepared and examined under oil immersion lens (100x) of microscope for the presence of anaplasma based on morphology and position within RBC. An overall prevalence of bovine anapalsmosis was 11.46% and A.marginale was relatively most prevalent species (7%), then A.centrale (2.6%) and co-infection of both species (1.8%). The variation of animal origin with the prevalence of disease was not statistically significant (P>0.05). In addition the association of breeds, sex, age and body condition with the prevalence of anaplasmosis was not significant (P>0.05). Animal infested with tick was more exposed to the infection of the disease than that of non infested animals. As a result tick infestation was significantly (p<0.05) associated with the occurrence of anaplasmosis. Similarly, the prevalence of anaplasmosis was significantly (p<0.05) related with animals that have history of previous injection within two months before sample collection. Anemia (PCV< 24) was also significantly correlated with incidence of bovine anaplasmosis compared with non anemic animals (PCV ≥ 24). In conclusion, bovine anaplasmosis is one of the important tick borne diseases of cattle that present in the study area. Therefore, appropriate disease control measures should be done to improve livestock production.*

Keywords: *Anaplasmosis, Cattle, Giemsa stain, tick, Wolaita sodo*

1. INTRODUCTION

Hemoparasites have great economic impact on livestock affecting 80% of the world cattle population and causes economic loss due to morbidity and mortality. They also impair the export and import trade of live animal and animal products by down grading their quality (Kasozi *et al*., 2014). The arthropod transmitted hemoparasitic diseases are important vector-borne diseases of tropical and subtropical parts of the world especially to the sub saharan Africa country including Ethiopia (Setotaw *et al*., 2014). In Ethiopia there are 47 species of ticks found on livestock and most of them have importance as vector and disease causing agents and also have damaging effect on skin and hide production as reviewed by (Tadesse and Sultan, 2014). Among tick-borne diseases anaplasmosis, babesiosis, cowdriosis and theileriosis (*T. mutan*) are major diseases in Ethiopia (Silesh *et al*., 1996).

From this bovine Anaplasmosis is one of the most important vector borne infectious diseases in cattle mainly caused by *Anaplasma marginale* and rarely by *A.centrale*. The organisms are Gram-negative obligate intracellular rickettsial bacteria (Rymaszewska & Grenda, 2008), classified in the genus Anaplasma, belonging to the family Anaplasmataceae of the order Rickettsiales (Kahn, 2005). The scientific name is based on its staining characteristics and location within the host cell, with "Anaplasma" referring to the lack of stained cytoplasm and "marginale" indicate the peripheral location of the organism in the host erythrocyte (de la Fuente *et al*., 2005).

Anaplasma species are transmitted either mechanically or biologically by arthropod vectors and transplacentally from cow to offspring across placenta (Kocan *et al*. 2003). Mechanically transferred from infected to susceptible cattle by biting flies or iatrogenically, by blood-contaminated fomites including needles or surgical instruments (Kocan *et al*., 2010). Biological transmission occur when

tick ingest infected erythrocytes then pathogen replicates within the ticks gut and salivary glands, and subsequently transmitted via tick saliva into uninfected animal during feeding(Aubry & Geale, 2011). More than 20 species of ticks are identified as vectors of disease. Among these genera of *Boophilus, Dermacentor, Rhipicephalus* and *Hyalomma* are important vectors. R. *decoloratus* and R. *evertsi evertsi* are the most abundant tick in Africa that can transmit *A. marginale* (Walker *et al*., 2003; Rubaire-Akiiki *et al*., 2004).

Once susceptible cattle are infected with *Anaplasma*, the organism multiplies in the bloodstream and attaches to the animal's red blood cells. Infected and even uninfected erythrocytes are subsequently phagocytized by bovine reticuloendothelial cells (Radwan *et al*., 2013). The phagocytosis of erythrocytes results in progressive haemolytic anaemia and icterus without hemoglobinemia or hemoglobinuria (Kocan *et al*., 2010; Aubry & Geale, 2011).

During acute phase about 70 % of all erythrocytes are infected and the number of blood cells destroyed exceeds the number of blood cells that the body can produce, then the animal develop characteristic clinical sign like anemia, fever, weight loss and icterus without hemoglobinuria (Kieser *et al*., 1990; Kocan *et al*., 2003). The severity of the disease increases with age. Mainly animals over two year of age develop acute disease associated with a high mortality, contrary to calves that are less susceptible. In endemic areas, indigenous or zebu-cross cattle have developed natural resistant to both ticks and anaplasmosis (Sergio, 2009). During the acute phase of infection bovine anaplasmosis can be diagnosed microscopically in Giemsa-stained blood smears by presence of intraerythrocytic initial bodies but in case of persistently infected animals serological test like cELISA and card agglutination test (CAT) more reliable (OIE, 2012).

Control measures of disease includes maintenance of Anaplasma-free herds, controlling movement of infected animal, testing and elimination of carrier cattle, vector control, prevention of iatrogenic transmission, administration of antibiotics and preimmunization with live vaccines and immunization with killed vaccines (Aubry and Geale, 2011). In general, bovine anaplasmosis is one of the most important diseases of ruminants worldwide causing significant economic losses in the livestock industries mainly due to the high morbidity and mortality (Kocan, *et al*., 2003). However there is little information on prevalence of bovine anaplasmosis in Ethiopia and no published information is available in the Walaita Sodo, Southern Ethiopia.

Therefore, this study was intended with the objectives

- To determine prevalence of bovine anaplasmosis in cattle and
- To identify risk factors associated with the prevalence of disease in the study area.

2. MATERIALS AND METHODS

2.1. Description of the Study Areas

The study was conducted in five selected village of Wolaita Sodo town and the surrounding area namely Wadu, Ofa Sere, Ofa Gandaba, Humbo Larena and Ziga Borikoshe kebele from November, 2016 to April, 2017. The study area is located in Wolaita zone within Southern Nation Nationalities People Regional (SNNPR) region, southern part of Ethiopia, which is located at 385kms from the Addis Ababa at south west direction. Geographically the area lies between 6 36' N to 7 18' N latitude and 37 12' E to 38 24' E longitude with an elevation of 1650-2500 meters above sea level. The highest mountain is Damota 2500m which is found in the zone. The area is characterized by bimodal rainfall pattern with the high rainy season extending from June to September and a small rainy season occurring from February to April, dry season extend from October - June including little rain season. The average temperature of the area is 20^{o}c with mean annual rainfall of 800-1400mm. The livestock population of Wolaita zone is estimated to be 762, 684 bovine, 182, 480 ovine; 129, 475 Caprine, 42303 equines, 886, 674 poultry and 47,527 beehives (CSA, 2013).

2.2. Study Population

The target population for study was bovine species of various sex and different age groups, body condition, locality and breed in the selected villages that kept under extensive management system by smallholders.

2.3. Study Design and Sampling Method

A cross-sectional study design was conducted from November 2016 to April 2017 within selected five villages to determine the prevalence of bovine anaplasmosis and associated risk factors by using microscopic examinations. The cattle for examination were selected from each village by using simple random sampling method. Information regarding age, breed, sex, origin, body condition of the cattle and previous injection for treatment or vaccination were recorded during sample collection. Animals were carefully checked for presence and absence of tick infestation. Age and body condition of animals determined according to De-lahunta and Habel (1986) and Morgan *et al*. (2006), respectively.

2.4. Sample Size Determination

The desire sample size was calculated by using the formula given in Thrusfield (2007). Since this study is the first, the expected prevalence of bovine anaplasmosis in the study area was assumed as 50%. The parameters that were used 95% confidence interval and 5% desired level of precision. By substituting these values in the formula, the sample size of study population was 384 cattle.

$$N = (1.96)^2 \frac{Pexp\,(1-pexp)}{d^2}$$

Where:

N=Sample size,

Pexp= Expected prevalence (50%)

d = desired level of precision (5%).

2.5. Sample Collection and Transportation

Blood samples were collected from animals after proper restraining of the sampling animal. Then about 3-4 ml blood sample was collected from jugular vein of each animal by using EDTA tubes through aseptic method. During sampling each sample was properly labeled about relevant information before transporting. Then samples were transported to the Sodo Regional Veterinary Laboratory at the same day for examination by using ice box with ice packs.

Laboratory Methodology

Giemsa staining procedures and microscopic examination of slides was conducted according to OIE (2010).The dried blood thin smear was flooded with methyl alcohol for two minutes. Fixed slides were stained in working dilutions of Giemsa's stain (1:10) in staining rack for 30minutes. The slides were washed with tap water and air dried. A drop of oil was placed on the smear and slide was examined under oil immersion lens (100x) of microscope for the presence of *Anaplasma* and morphology. *Anaplasma marginale* appear as dense, rounded and deeply stained intraerythrocytic bodies, approximately 0.3 – 1.0µm in diameter. Most of these bodies are located on or near the margin of the erythrocyte. This feature distinguishes *A. marginale* from *A. centrale*, as in the latter most of the organisms have a more central location in the erythrocyte. At least of 50 fields per slide were screened before declaring negative for presence of any rickettsial organisms (OIE, 2008).

2.6. Measuring of Packed Cell Volume

Blood samples were collected from jugular vein of animal by using EDTA tube for smear preparation and PCV measuring. Then blood sample taken from EDTA tube into a capillary tube. The capillary tubes were placed in micro haematocrit centrifuge with sealed end outer most. The tube was loaded symmetrically to ensure good balance. After screwing the rotary cover and closing the centrifuge lid, the specimens were allowed to centrifuge at 12,000 rpm for 5 min. Tubes were placed in haematocrit reader and the reading result was expressed as a percentage of packed red cells to the total volume of whole blood. Animals with PCV < 24% were considered to be anemic.

2.7. Data Management and Analysis

The data was recorded and entered into Microsoft excel database system and statistical analysis was done. The SPSS versions 20 software of computer program was applied for the statistical analysis.

Chi-square used to know the association between disease prevalence and risk factors like breed, age, sex, body condition, origin, tick infestation and previous injection. The prevalence of bovine anaplasmosis was calculated as the number of positive animals that examined microscopically by thin smear Giemsa stain divided by the total number of animals examined multiplied by100. A statistically significant association between variables was said to be exist if the calculated $p<0.05$ at 95% confidence level.

3. RESULTS

Totally 384 cattle examined for bovine anaplasmosis by using Giemsa-stained blood smear, the overall prevalence 11.46% [44/384] was recorded. Prevalence of disease within five selected villages namely Humbo larena, Ofa sere, Ofa ganidaba, Wadu and Ziga borikoshe were 17.8%, 10.3%, 10%, 7.4% and 12% respectively. The highest prevalence was recorded at Humbo larena 13[17.8%] and the lowest was recorded at Wadu 6[7.4%]. However, there was no statistically significant variation ($P>0.05$) observed as indicated in Table 1.

Table1. *The prevalence of bovine anaplasmosis based on locality*

Origin	Total examined	Positive	Prevalence (%)	χ^2	P- value
Humbo	73	13	17.8	4.471	0.346
Ofa Sere	78	8	10.3		
Ofa Ganidaba	60	6	10		
Wadu	81	6	7.4		
Ziga Borikoshe	92	11	12		
Total	384	44	11.46		

The prevalence of breed was 11.2% in local and 13% in crossbred and variation between breed was not significant ($P > 0.05$) as shown in table 2. According to the sex, age and body condition the highest record observed on female(11.9%), adult (12.8%) and poor(15.7%) respectively. whereas the lowest prevalence was recorded on male (10.9%), young (8.7%) and good(6.2%), but no statistically significant variation ($P>0.05$) was observed between age, sex and body condition of examined animals and infection.

The statistically significant variation ($P< 0.05$) was observed in the prevalence of anaplasmosis among those animal with tick infested and none infested. The highest prevalence (14.3%) was observed in animals with tick infested and the lowest prevalence (7.1%) was observed on non infested animal as indicated in the Table 2 below. According to the previous injection for any vaccination or treatment within two months and prevalence of bovine anaplasmosis, the highest prevalence were recorded on previously injected animals (16.3%) while lowest prevalence were recorded on non injected animals (7.8%). The observed variation was statistically significant ($P< 0.05$) as indicated in the table 2 below. The occurrence of anaplasmosis in cattle having PCV value ≤ 24% (anemic) was 20.4% while in the cattle having PCV value >24% (non-anemic) was 8% as indicated in Table 2. There was statistically significant variation ($p<0.05$) between PCV value and disease prevalence.

Table2. *The prevalence of bovine anaplasmosis based on different risk factors*

Risk factors	Total examined	positive	Prevalence (%)	χ^2	P- value
Breed				0.129	0.719
Local	338	38	11.2		
Cross	46	6	13		
Sex				0.086	0.769
Male	165	18	10.9		
Female	219	26	11.9		
Age				1.463	0.226
Young(<3 years)	127	11	8.7		
Adult(≥3 years)	257	33	12.8		
Body condition				4.405	0.111
Poor	134	21	15.7		
Medium	185	19	10.3		
Good	65	4	6.2		
Tick infestation				4.720	0.030

Present	230	33	14.3		
Absent	154	11	7.1		
Previous injection				6.659	0.010
Injected	166	27	16.3		
Non injected	218	17	7.8		
PCV				11.763	0.001
Anemic(<24)	108	22	20.4		
Non anemic(≥24)	276	22	8		

In the present study, totally 44 anaplasma species were identified by Giemsa stain examination and prevalence of observed species was *A. marginale* 27[7%], *A. centrale* 10[2.6%] and co- infection of *A. marginale* and *A. centrale* was 7[1.8%] as indicated in the Table 3. This indicates *A. marginale* was more prevalent than *A. centrale*.

Table3. *The prevalence of bovine anaplasmosis based on species of pathogen*

Total examined	Anaplasma species	Positive	Prevalence (%)
384	*A. marginale*	27	7
	A. centrale	10	2.6
	Co- infection	7	1.8
	Total positive	44	11.46

4. DISCUSSION

In the present study, the overall prevalence of bovine anaplasmosis by using Giemsa- stained blood smear examination was 11.46% and this result agree with the finding of Angwech *et al*., (2011), Kasozi *et al*., (2014), Zein *et al*., (2013) and Mushtaqa *et al*., (2015) who found 10.4% (Gulu district, Notheren Uganda), 14.4% (Central and Western Uganda), 8% (South Kordofan, Sudan) and 11.25% (Lahore district, Pakistan) respectively by using similar thin smear Giemsa stain method.

However, this study was lower than the result of Silesh (1996), Abou-Elnaga *et al*., (2009), Zein *et al*.,(2013) and Khan *et al*., (2017) which reported from different Ethiopia dairy cattle 99%(serological method), Matrouh district, Egypt 37% (Giemsa stain) and 67% (cELISA), Sudan 66%(ELISA) and Pakhtunkhwa-Pakistan 28% (Giemsa stain) respectively. This might be due to difference on management and farming system, sensitivity of the test used and variation of Argo-ecology and abundance of principal vector in the area.

Similarly the high result was recorded through Giemsa stained method in the following countries namely South-western Uganda by Palmfjord, (2015), Dinajpur district, Bangladesh by Kispotta *et al*., (2016) and Tamil district of Nadu(India) by Arunkuma and Nagarajan, (2013) with prevalence of 30.8%, 18.5% and 19.3 % respectively. The high prevalence in the Uganda suggested due to the endemic instability of disease in the Kiruhura district Palmfjord, (2015) and in case of Bangladesh, the climatic condition of Bangladesh is highly favorable for growth and multiplication of tick which enhance the occurrence of *Anaplasma marginale*, *Anaplasma centrale* in animals (Samad and Goutam, 1984). Additionally high report within the Tamil district of Nadu (India) may be due to the difference of sampling method. The sample was collected from suspected cattle showing a clinical sign of high fever, pale mucous membrane, lacrimation and lymph node enlargement (Arunkuma and Nagarajan, 2013).

The present study disagree with the finding of Setotaw *et al*., (2014), Kamani *et al*., (2010) and Sajid *et al*., (2014), they found 1.9% in Debre-Zeit, Central Ethiopia, 1.9% from Nigeria and 4.17% from Khanewal district, Pakistan respectively through similar Giemsa staining method. This may be due to difference on study area, use of acaricides during tick infestation and abundance principal vector population variation. Moreover, the prevalence varies from region to region, due to the changes in climatic condition, intensity of tick infestation, and also use of contaminated needles and instruments transmission is an efficient way of infection spreading (Bock, *et al*. 2003, Kivaria, 2006**).**

In the current study there was slight difference of disease prevalence observed within five selected villages namely Humbo larena (17.8%), Ofa sere (10.3%), Ofa ganidaba (10%), Wadu (7.4%) and Ziga borikoshe were (12%). The highest prevalence was recorded at Humbo larena 13(17.8%) and the lowest finding was recorded at Wadu 6(7.4%). This might be due to minor variation of agro-

ecological and grazing system of animal. However, the origin was not statistically significant (P>0.05) for the occurrence of disease. In the same way both local and cross breed had no significant association (P>0.05) with disease but relatively high prevalence observed within cross breed (13%) than the local breed (11.2%). Some literatures justify, in endemic areas indigenous cattle were relatively resistant while modified cattle, especially European breeds, are highly susceptible (Taylor *et al.*, 2007), constant exposure of infections and development of immunity against infection lower prevalence in indigenous cattle. On the contrary, more attention in the management of crossbred cattle gave less chance of pre exposure of vectors and develops no or less immunity, resulting frequent occurrence of such diseases (Siddiki *et al.*, 2010).

In the present study a little higher finding was recorded in female (11.9%) compared to male animals (10.9%) and the difference was not statistically significant, this finding is comparable with those of previous report of Kispotta *et al*., (2016) of Dinajpur district, Bangladesh male (13.48%) and female 22.52% and that of Atif *et al* (2012) from Pakistan male (7%) and female (10.8%). Higher prevalence in female population was due to hormonal disturbances due to its use in milk production, draught power and breeding system which cause it to weakened immune system (Kamani *et al.*, 2010).

Cattle of all ages are susceptible to anaplasmosis, whereas severity and mortality rate increases with increase of animal age (Aubry & Geale, 2011). In the present study the slightly high prevalence was recorded among adult (12.8%) then young (8.7%) although the difference not statistically significant (P>0.005). This result was in line with the finding of Khan *et al*. (2017) from Pakhtunkhwa-Pakistan and Zein *et al*., (2013) from south Kordofan state, Sudan who found high record in adult. Cattle of more than 4 years of age were at higher risk as compared to age below 4 years age groups (Atif *et al*., 2013).

The prevalence of the disease based on the body condition of the animals was 15.7%, 6.2% and 4.08% for poor, medium and good scoring respectively and no statistically significant association(P>0.05) observed. As the result indicates relatively high record observed on the animals with poor body condition then medium lastly on good. However, loss of body condition not always associated with this disease, it can be caused by other chronic disease of parasite, bacteria, viral, burden of ectoparasite and nutrition deficiency or poor management systems of the animals. In addition during this study period, the season was dry and there was scarcity of feed and drinking water which cause poor body condition of animals.

In the current study association of tick infestation with Bovine anaplasmosis resulted statistically significant variation (P<0.05) in the occurrence of disease. The presence of the two principal biological vectors *R. decoloratus* and *R. evertsi evertsi* for *A. marginale* matters the epidemiology of disease (kocan *et al*., 2004). According to Wolde and Mohammed (2014) report the prevalence of *Rhipicephalus (Boophilus) decolaratus* and *Rhipicephalus evertsi-evertsi* in the current study area was 41% and 17.14% respectively. Therefore the presence of this two principal ticks and other vectors may significantly associated with occurrence of anaplamosis in the study area.

An animals that were injected for vaccination within 2 months before examination were more affected than those not injected animals and there was statistical difference between treated and untreated groups (P<0.05). *Anaplasma marginale* also can be readily transmitted during vaccination against other diseases unless a fresh or sterilized needle is used for injecting each animal (Reinbold *et al*., 2010a). According to one report, a needle used on an infected animal leads to a 60% chance of the next animal being infected if the same needle is used (Soren and Christine, 2008). In case of our country, during vaccination most animals are injected with one non disposable needle without disinfecting. Therefore, this high finding with the association previous injection might be related with the reuse of contaminated needles and instruments during vaccination and treating the animals.

The result obtained by measuring of packed cell volume (PCV), 108 animals were found anemic (PCV < 24) from this 22(20.4%) samples positive for anaplasmosis and the rest 22(8%) positive sample were found from non anemic (PCV ≥ 24) animal. In the current finding prevalence of disease within anemic animal and non anemic was 20.4% and 8% respectively. The association of PCV with disease occurrence was statistically significant (P < 0.05). According to Kocan *et al*., (2004) report, infected erythrocytes are subsequently phagocytized by bovine reticuloendothelial cells, the phagocytosis of erythrocytes resulting in development of mild to severe hemolytic anemia (Kocan *et al*., 2010).

In the current finding the higher infection rate of *A.marginale* (7%) was recorded, followed by *A.centrale* (2.6%) then mixed co-infection (1.8%). These results slightly coincides with the results of Khan *et al*. (2004) from Pakistan, and differ from the report of Abou-Elnaga *et al*., (2009) who found *A.marginale* (11.5%), *A.centrale* (15.5%) and co-infection (10%) from Matrouh district, Egypt.

5. CONCLUSION AND RECOMMENDATIONS

Bovine anaplasmosis is one of the most important vector borne diseases of the cattle that examined in the current study area to determine prevalence and its associated risk factors. The overall prevalence of disease was 11.46% and *A. marginale* relatively more prevalent agent for the infection of animal than the *A.centrale*. Among risk factors origin, breed, sex and age were not significantly associated whereas tick infestation, previous treatment and PCV were significantly correlated with the occurrence of disease. Therefore, the current study reveals that presence of tick on the animal body and previous injection with needle influence the disease prevalence in the study area.

Based on the current finding the following recommendations were forwarded:

- Appropriate tick control measure should be carried out to minimize the risk of the vector.
- Disinfected or disposable needle should be used to avoid cross contamination during vaccination and treatment.
- Since Giemsa stain not sensitive test for carrier animal the further study should be conducted by using serological and molecular diagnostic method to know accurate status of disease.

ACKNOWLEDGEMENTS

First of all I would like to praise my Almighty God in heaven by the name of Jesus Christ for His uncountable grace, peace and mercy in my life and also enabling me to accomplish this study.

I would like to thank my advisor Dr. Mukarim Abdurrahman for his incalculable and unreserved advise, intellectual guidance, and devotion of time in correcting this paper. I am grateful to the Jimma University School of Veterinary Medicine.

I would also love to express my sincere and heartfelt gratitude to my beloved parents for Mark and Marta, also for my brothers and sisters for their financial and moral support during my study.

My thankfulness also extend to Sodo Regional Veterinary Laboratory Staffs

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Citation: *T. Markos & Mukarim Abdurahaman, "Bovine Anaplasmosis and its Associated Risk Factors in and Around Wolaita Sodo Town, Southern Ethiopia", International Journal of Research Studies in Biosciences (IJRSB), vol. 6, no. 7, pp. 13-21, 2018. http://dx.doi.org/10.20431/2349-0365.0607003*