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A Review on the Diagnostic and Control Challenges of Major Tick-Borne Haemoparasite Diseases of Cattle

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Abstract
The tick-borne diseases of livestock constitute a complex of several diseases; and their single common feature is that they can all be transmitted by different developmental stages of ticks. Piroplasms (*Babesia* and *Theileria* species) and *Anaplasma* species are the cause, in cattle, of high morbidity and mortality, decreased meat and milk production, and an impediment to the upgrading of indigenous breeds of cattle and to the introduction of more productive, exotic breeds in the tropics and subtropics. Despite these facts, not much is known about the epidemiology and phylogeny of cattle piroplasms and anaplasmosis for many years. In the past two decades, for diagnostic purpose, new techniques such as PCR and Reverse line blot/RLB hybridization as well as advanced serological techniques (CFT, c-ELISA, Dot ELISA, IFAT, CAT and I-ELISA) have been developed. Since then, although their limitations like due to lack of specifity and sensitivity, these diagnostic techniques relatively made surveys and typing of piroplasms, anaplasmosis and other haemoparasites easier and more reliable than the blood smears methods. The acute case of TBDs may be controlled by treatment, yet in most cases the controls of TBDs have been based primarily on intensive tick control using acaricides. Moreover, live vaccines for tick-borne diseases control have well-known limitations however for many countries they represent the only available means of disease control. Structural changes in the provision of veterinary services, associated with reduced budget allocations, economic and social changes in livestock production systems, increased costs of acaricides and labour, combined with the increasing incidence of acaricide resistance in ticks have led to a demand for more cost effective and sustainable approaches to the control of tick and of the disease they transmit. Thus, a federal regulation that pertain the interstate movement of TBDs carriers, effort invested in the research and development of high quality standardized diagnostic test, use of integrated control approaches and much greater emphasis to determine the economic importance of TBDs is needed.
Keywords: Anaplasmosis, Babesiosis, Control, Diagnostic challenges, Theileriosis

1. INTRODUCTION

The tick-borne diseases of livestock constitute a complex of several diseases whose etiological agents may be protozoal, rickettsial, bacterial or viral; their single common feature is that they can all be transmitted by different developmental stages of ticks (Pipano and Shkap, 2006). The families Ixodidae contain many bloodsucking species that are important pests of domestic mammals including cattle as well as humans, and probably exceed all other arthropods in the number and variety of disease agents they transmit (Silke, 2009 and Scott Moses, 2011). Tick-borne diseases are present throughout the world, but are most numerous and exert their greatest impact in the tropical and subtropical regions (Sathaporn *et al*., 2004). Many of the tick-borne diseases are haemo-parasitic (Scott Moses, 2011). During the past decades, many tick borne haemo-parasitic diseases have been identified in domestic animals. Thus, the major tick borne haemoparasites of cattle include *Babesia, Theileria, Anaplasma* and *Ehrlichia ruminantium*, the causative organisms of heart water (Zahid *et al.,* 2005 and Silke, 2009).

Babesia, theileria and anaplasma species are the cause, in cattle, of high morbidity and mortality, decreased meat and milk production, and loss of draught power and manure. They are also an impediment to the upgrading of indigenous breeds of cattle and to the introduction of more productive, exotic breeds in the tropics and subtropics of the world including the African livestock industries (Radostits *et al.,* 2006). The effects of haemoparasites often depends on the species and immunity of the host, and can vary from development of severe disease due to the infection with haemoparasites to a completely inapparent infection without any signs of disease (Zahid *et al.,* 2005 and Radostits *et al.,* 2006).

TBDs have also been implicated in losses amongst animals, some of which endangered species; and also hamper livestock export market (Penzhorn, 2006). Despite these facts, not much is known about the epidemiology and phylogeny of piroplasms and anaplasmosis for many years. But, during the past two decades, for diagnostic purpose new techniques, such as Polymerase chain reaction /PCR and Reverse line blot/RLB hybridization as well as advanced serological techniques (CFT, c-ELISA, Dot ELISA, IFAT, CAT and I-ELISA) have been developed. Since then, although their limitations, these diagnostic techniques relatively made surveys and typing of piroplasms, anaplasmosis and other haemoparasites easier and more reliable than the blood smears methods (Gale *et al.,* 1996 and Zahid *et al.,* 2005).

The controls of TBDs have been based primarily on intensive tick control using acaricides. However,

recent studies have shown that as this approach may not be cost-effective in terms of increasing the productivity of cattle (Figueroa *et al.,* 1999 and Sathaporn *et al*., 2004). Thus, the use of concealed tick antigens for vaccination of cattle has served as the basis of a commercial vaccine, but there are still potential drawbacks that should be addressed (Sathaporn *et al*., 2004). The acute case of TBDs may also be controlled by treatment. Because of the need for higher dosages, toxic effects with prolonged use and longer treatment regimens, the use of drugs is generally limited to control and it not to sterilize carriers (Kocan *et al*., 2003).

Generally, structural changes in the provision of veterinary services, associated with reduced budget allocations, economic and social changes in livestock production systems, increased costs of acaricides and labour, combined with the increasing incidence of acaricide resistance in ticks have led to a demand for more cost effective and sustainable approaches to the control of ticks and tick borne diseases. Therefore, the objective of this review is: to compile existing information on the challenges of diagnostic tests and the problems associated with the control of the major tick borne haemoparasitic diseases of cattle, and to provide insight on the most recent developments and current needs on diagnostic assays for these major diseases.

2. General Descriptions of Tick-Borne Haemoparasite Diseases

2.1 Babesiosis

Bovine babesiosis is caused by the intraerythrocytic protozoan parasites of the genus *Babesia*. There are four major species of Babesia which causes bovine babesiosis and include *Babesia bovis*, *B.bigemina*, *B.divergens* and *B.major* (Callow *et al*., 1997 and Zintl, 2003).

The vector of babesiosis is the tick (Callow *et al*., 1997). In general term, bovine babesiosis associated with *B.bigemina* and *B.bovis* is an important disease of tropical and subtropical regions between 40^0N and 32^0S which includes Africa (OIE, 2005). Both species are transmitted transovarially by *Boophilus* ticks, but only tick larvae transmit *B.bovis*, whereas nymphs and adults transmit *B.bigemina*. In temperate regions *B.major* and *B.divergens* occurs commonly. Other important vectors include *Haemaphysalis*, *Rhipicephalus* and other *Boophilus* species of ticks (Zintl, 2003 and Radostits *et al*., 2006).

Babesia bovis is more pathogenic than the other three species of *Babesia*. Babesiosis are characterised by high fever, haemoglobinuria, anorexia, increased heart and respiratory rate, jaundice mucous membrane, and sometimes in *B.bovis* infection nervous signs as a result of sequestration of infected erythrocytes in cerebral capillaries (Radostits *et al*., 2006).

There is an age-related immunity to primary infection of cattle. Young calves possess strong innate immunity against infection that lasts for approximately 6 months after birth and is abrogated with the removal of the spleen (Zintl, 2003). Infected animals develop a life-long immunity against reinfection with the same species. There is also evidence of a degree of cross-protection in *B.bigemina*-immune animals against subsequent *B.bovis* infections (Zahid *et al*., 2005). There is also evidence that in endemic region with high level of tick infestation, immunity remains at high level. Stress like parturition and concurrent diseases increases the risk of clinical diseases. Moreover, mild form of the disease is associated with less pathogenic species or with relatively resistant hosts (Radostits *et al*., 2006).

Bovine babesiosis is the most economically important of TBDs, because of direct losses of production and restriction of movement of cattle for trade by quarantine laws (Zintl, 2003). Many animals die or undergo a long period of convalescence entailing loss of meat and milk production. Incidental costs of immunization and treatment add to the economic burden (Zahid *et al*., 2005 and Radostits *et al.,* 2006).

2.2 Anaplasmosis

Anaplasmosis, formerly known as gall sickness, traditionally refers to a disease of ruminants caused by intraerythrocytic organisms of the order the genus Anaplasma (Aiello and Mays, 2007 and Silke, 2009). Two species of Anaplasma are known to infect cattle- *Anaplasma marginale* and *A.centrale*. *Anaplasma marginale* is the causative agent of clinical anaplasmosis in cattle, and *A.centrale* causes mild anaplasmosis in cattle (Kocan *et al.*, 2003).

The source of anaplasma infection is always the blood of an infected animal (Palmer, 2001 and Radostits *et al.,* 2006). Numerous, over 20 species of tick world-wide; including *Boophilus, Dermacentor, Rhipicephalus, Ixodes, Hyalomma* and *Ornithodoros,* can transmit Anaplasma species (Aiello and Mays, 2007 and Scott Moses, 2011). Infection of cattle is endemic in tropical and subtropical areas that support large populations of the vectors (Silke, 2009). It occurs more sporadically in temperate climate areas. Reservoirs of infection are usually carrier cattle, wild ruminants and sheep (Palmer, 2001 and Radostits *et al.,* 2006).

Transovarial infection is known to take place but there is little information on the development of the parasite in the tick. Mechanical transmission via dipterans biting flies and mosquitoes can also occur. Furthermore, transplacental transmission has been reported and is usually associated with acute infection of the dam in the second or third trimester of gestation. It may also be spread through the use of contaminated needles, castrating knives, dehorning and tattoo instruments (Gerald *et al.,* 2000 and Aiello and Mays, 2007).

Most researchers have been observed infection differences among breeds under field conditions. Bos indicus are not as commonly affected, probably because of their relative resistance to heavy tick infestation. Breeds with black or red coat color have a higher risk of infection than those with white coats in regions where biting flies are the insect vector. Dairy breeds may be at greater risk for iatrogenic transmission (Radostits *et al.,* 2006 and Aiello and Mays, 2007).

All cattle are susceptible to infection but age at infection is a major determinant for the severity of clinical disease. Young calves are less susceptible to infection than older cattle and, when infected, development of clinical disease is less (Rogers and Shiels, 1979 and Torioni *et al*., 1998). Splenectomized calves are fully susceptible to infection, which may be more severe than in the adult. Infection between six months and three years of age has increasing risk of clinical illness and animals infected after 3 years of age are commonly affected by a per acute fatal form of the disease. Adult cattle introduced to endemic area are very susceptible and susceptibility increases with stress or by concurrent occurrence with other diseases (Richey and Palmer, 1990).

Economic importance of anaplasmosis are from death and abortion in clinical cases, loss of production in sick and recovered animals, and costs associated with diagnosis and preventive measures such as tick control (Kocan *et al*., 2000). In developed countries with the disease, exports of cattle to countries that do not have it are constrained. A major cost in developing countries is the constraint to efficient production and the limit to the introduction of susceptible cattle breeds with superior genetics (Richey and Palmer, 1990 and Scott Moses, 2011).

2.3 Theileriosis

Theileriosis results from infection with protozoa in the genus Theileria of the suborder Piroplasmorina. Theileria species are obligate intracellular parasites. The two most pathogenic and economically important are *T.parva*, which causes East Coast fever, and *T.annulata*, which causes tropical theileriosis. *T.mutans, T.orientalis/buffeli, T.velifera,* and *T.taurotragi* can also infect domesticated ruminants (Radostits *et al*., 2006 and Brown, 2008).

They are transmitted by Ixodidae ticks, and have complex life cycles in both vertebrate and invertebrate hosts. Since ticks became active following the onset of rain an outbreak is seasonal (Brown, 2008). There are six identified *Theileria* species that infect cattle and is found in 13 countries in sub-Saharan Africa (Pipano and Shkap, 2004). *Theileria annulata* (tropical theileriosis) occurs from southern Europe and the Mediterranean coast through the Middle East and North Africa, and into parts of Asia. Endemic regions of *T.annulata* and *T.parva* do not overlap. *Theileria taurotragi* and *T.mutans* generally cause no disease or mild disease, and *T.velifera* is non-pathogenic. These latter three parasites are mainly found in Africa including Ethiopia, and overlap in their distribution; thus, complicating the epidemiology of theileriosis in cattle (Solomon *et al*., 1998; Pipano and Shkap, 2004 and Radostits *et al*., 2006).

Most *T.parva* stocks produce a carrier state in recovered cattle, and recent studies using DNA markers for parasite strains have shown that *T.parva* carrier animals are a source of infection and can be transmitted naturally by ticks in the field (Zintl, 2003). The virulence of the parasite strain, sporozoite infection rates in ticks and genetic background of infected animals may vary the severity of theileriosis. Indigenous cattle in theileriosis endemic areas are often observed to experience mild disease or subclinical infection, while introduced indigenous or exotic cattle usually develop severe diseases (Pipano and Shkap, 2004 and Brown, 2008).

The schizont stage initially causes marked hyperplasia and lympho- proliferative the regional lymph node at the site of tick bite. And later a lymphoid depletion and disorganization due to massive lymphocytolysis and depressed leucopoesis may be due to activation of natural killer cells like macrophages. The infected animal shows enlargement of the lymph nodes, fever, a gradually increasing respiratory rate, dyspnoea and/or diarrhoea (Brown, 2008). The most common post-mortem lesions of the schizont stage are atrophy of the cellular content of the lymph node and spleen, enlarged regional lymph nodes, pulmonary oedema, froth in the trachea, erosions and ulceration of the abomasum, and enteritis with necrosis of Payer's patches, while anaemia and jaundice are features of the piroplasms pathology (Radostits *et al*., 2006). A nervous syndrome called 'turning sickness' is sometimes seen observed in *T.parva*-endemic areas, and is considered to be associated with the presence of intravascular and extravascular aggregations of schizont-infected lymphocytes, thrombosis and ischaemic necrosis (Pipano and Shkap, 2004; Radostits *et al*., 2006 and Brown, 2008).

Theileriosis causes major constraints on livestock development in Africa, Asia and Middle East. Many theileria parasites cause diseases in cattle, of which one of the most economically important is East Coast fever (ECF), caused by *T. parva*. The parasite causes high morbidity and mortality in exotic cattle, thus inhibiting the introduction of improved cattle into endemic areas (Zintl, 2003 and Radostits *et al*., 2006).

3. The Common Diagnostic Techniques for Babesiosis and its Challenges

3.1 Identification of the agent

Wright-Giemsa stained blood smear

Wright-Giemsa a stained blood smear from cattle with clinical signs remains the most commonly used method to

confirm diagnosis of babesiosis (OIE, 2005). For *B. bigemina,* the onset of fever in acute infection is coincides with the presence of intraerythrocytic parasites, and easily microscopically detectable at very low levels from capillary bloods due to characteristic parasite morphology (Radostits *et al*., 2006). In contrast, *B. bovis* may cause clinical disease with severe neurological signs followed by death with few/no detectable parasitized erythrocytes in peripheral blood. The sequestration of *B. bovis* parasitized erythrocytes in cerebral capillaries is readily identifiable in histological sections but not currently easily diagnosed in stained peripheral blood smear (OIE, 2000; OIE, 2005 and Radostits *et al*., 2006).

There is no exact correlation between the percentage of erythrocytes containing protozoa and the severity of the clinical signs. The sensitivity of this technique is relatively poor and such that it can detect parasitaemias as low as 1 parasite in 10^7 red blood cells (RBCs). Species differentiation is good in thin films but poor in the more sensitive thick films. This technique is usually adequate for detection of acute infections, but not for detection of carriers where the parasitaemias are mostly very low. If fever is subsided, impossible to find the parasites since they are rapidly removed from the circulation (Bock *et al*., 2000 and OIE, 2000).

PCR detection and identification of agents

A number of PCR techniques have been described that can detect and differentiate species of *Babesia* in carrier infections (Holman *et al*., 2003). However, PCR assays generally do not lend themselves well to large-scale testing and are unlikely to supplant serological tests as the method of choice for epidemiological studies (Bock *et al*., 2000).

In-vitro culture methods of Babesia

In-vitro culture methods have also been used to demonstrate the presence of carrier infections of *Babesia* species (Jorgensen *et al*., 2004). The minimum parasitaemia detectable by this method depend, to a large extent, on the facilities available and the skills of the operator. In general, this method is cumbersome and expensive, and obviously not suitable for routine diagnostic use (Bock *et al*., 2000 and Holman *et al*., 2003).

3.2 Inoculation of suspect blood into susceptible (splenectomized) calves

Direct detection of persistently infected carrier cattle has been limited to either inoculation of suspect blood into susceptible (usually splenectomized) calves and test feeding of ticks with subsequent microscopic examination or tick transmission to susceptible cattle (Jorgensen *et al*., 2004). The inability to examine large numbers of carriers, the variation of incubation period and the lack of quantitation has severely limited the number of studies that can be done on the epidemiological significance of carrier cattle (OIE, 2000; OIE, 2005 and Radostits *et al*., 2006).

3.3 Serological Tests

Because of the difficulty in finding protozoa in smears in animals during the subclinical stages of the disease, especially in surveillance studies for the detection of the infection in herds or areas, much attention has been directed to serological tests. These serological tests are now well-established, but none of them enjoys a completely satisfactory reputation.

Complement fixation test (CFT)

The CFT has been the most used serological test for the detection of bovine Babesia species antibodies. There is evidence in the literature that suggesting the presence of antibodies is not necessarily an indication of immunity nor is absence of detectable antibodies necessarily an indication of a lack of immunity (Jorgensen *et al*., 2004 and Bose *et al*., 2005).

Indirect Fluorescent Antibody (IFA) test

The indirect fluorescent antibody (IFA) test is widely used to detect antibodies to *Babesia* species, but the *B.bigemina* test has poor specificity. Cross-reactions with antibodies to *B.bovis* in the *B.bigemina* IFA test are a particular problem in areas where the two parasites coexist. The IFA test has the disadvantages of low sample throughput and subjectivity (Bose *et al*., 2005).

Enzyme-Linked Immunosorbent assay (ELISA)

An internationally validated enzyme-linked immunosorbent assay (ELISA) for the diagnosis of *B. bovis* infection has been developed (De Echaide *et al*., 2007). But, despite the efforts of several investigators in different laboratories, there is still no similarly validated ELISA for *B. bigemina*. ELISAs for detection of antibodies to *B.bigemina* typically have poor specificity. In one study conducted by El-Gaysh *et al*., (2008), *B.bigemina* antiserum appeared to react non-specifically with fibrinogen. ELISAs have also been developed for *B.divergens* (Chauvin *et al*., 2009) using antigen derived from culture, *Meriones* or cattle, but there does not

appear to be one that has been validated internationally. The specificity of the ELISA has been estimated at 97.0% and the sensitivity for detection of antibodies in experimentally infected cattle is 95.7% (De Echaide *et al*., 2007).

4. The Common Diagnostic Techniques for Anaplasmosis and its Challenges

4.1 Identification of the Agent

Giemsa stained smear

Samples from live cattle should include thin blood smears and blood collected into an anticoagulant. Smears from live cattle should preferably be prepared from blood drawn from the marginal ear vein, tip of tail, as well as, because of in contrast to *Babesia bovis, Anaplasma* do not accumulate in capillaries, so blood drawn from the jugular or other large vessel is satisfactory (Johnston *et al.,* 2000). Because of the rather indistinctive morphology of *Anaplasma,* it is essential that smears be well prepared and free from foreign matter, as specks of debris can confuse diagnosis. Thick blood films as used for the diagnosis of babesiosis are not appropriate for the diagnosis of anaplasmosis, as *Anaplasma* are difficult to identify once they become dissociated from erythrocytes (Gerald *et al.,* 2000 and Aiello and Mays, 2007).

Microscopic examination of blood smears stained with Giemsa stain is the most commonly used method; it is not very rapid and accurate as other techniques of anaplasma identification, serological tests as well as some expensive commercial stains (Camco-Quik and Diff-Quik) of blood smears. And also, the infection becomes visible microscopically 2–6 weeks following transmission. Quite severe anaemia may persist for some weeks after the parasites have become virtually undetectable in blood smears (Johnston *et al.,* 2000 and Radostits *et al.,* 2006).

Nucleic-acid-based tests

The other available test is a DNA test that looks for Anaplasma DNA in the bloodstream to detect *A.marginale* infection in carrier cattle (Eriks *et al.,* 1989). For this purpose a radioactive RNA probe, which detected parasitaemia as low as 0.000025%, has been described by Figueroa *et al.* (1999). Infected ticks have also been identified using a cloned DNA probe (Gale *et al.,* 1996). The analytical sensitivity of polymerase chain reaction (PCR)-based methods has been estimated at 0.0001% infected erythrocytes, but at this level only a proportion of carrier cattle would be detected. A sensitive and potentially specific nested PCR has been used to identify *A.marginale* carrier cattle. This technique is capable of identifying as few as 30 infected erythrocytes per ml of blood, well below the lowest levels in carriers. However, nested PCR poses significant quality control problems for routine use. Laboratories running this assay should recognise problems in specificity due to nonspecific amplification (Ge *et al.,* 1997).

4.2 Inoculation of suspected blood into a Splenectomised Calf

An expensive procedure, but one that may occasionally be justified to confirm infection, particularly in latently infected cattle, is the inoculation of blood from the suspect animal into a splenectomised calf (Jorgensen *et al*., 2004). A quantity of up to 500 ml of the donor's blood in anticoagulant is inoculated intravenously into the splenectomised calf, which is then tested by blood smear examination at least every 2–3 days. If the donor is infected, Anaplasma observed in smears from the splenectomised calf generally within 4 weeks, but this period may extend up to 8 weeks. The inability to examine large numbers of carriers, large amount of blood taken from the donor and the lack of quantization has severely limited the application of this technique (Bradway *et al.,* 2001).

4.3 Serological Tests

Anaplasma infections usually persist for the life of the animal. However, except for occasional small recrudescences, *Anaplasma* cannot readily be detected in blood smears after an acute parasitaemic episode. Thus, a number of serological tests have been developed with the aim of detecting latently infected animals. Some of the common serological tests are listed below.

The Complement Fixation Test/CFT

It is satisfactory for use in cattle, goats and sheep but the antibody titer is highest during the active phase of the disease and sufficiently low in carrier animals to give a proportion of false negative results. False positive reactions can occur because of erythrocyte contamination of the *A.marginale* antigen and the presence of antibodies to erythrocytes in some cattle sera (Bradway *et al.,* 2001 and Radostits *et al*., 2006). Some data confirm that as it lacks sensitivity and fails to detect a significant proportion of carrier cattle. Therefore, the Complement Fixation Test can no longer be recommended as a reliable assay to detect infected animals (Bradway *et al.,* 2001).

Card Agglutination Test/CAT
The CAT is that; it is sensitive, may be undertaken either in the laboratory or in the field, and gives a result within a few minutes. However, because of the CAT antigen is a suspension of *A. marginale* particles, nonspecific reactions may be a problem (Kocan *et al*., 2000).

Competitive Enzyme-linked Immunosorbent assay/c-ELISA
A c-ELISA using a recombinant antigen termed rMSP-5 and bovine anti-major surface protein-5 (MSP-5) specific monoclonal antibody (MAb) has proven very sensitive and specific for detection of *Anaplasma*-infected animals (Ndung'u *et al.,* 1995 and Torioni *et al.,* 1998). Due to the establishment of carrier states in infected animals, the c-ELISA is regarded as a reliable screening test for identifying *A.marginale*-infected cattle. However, cross-reactivity among *Anaplasma* species has been reported when the c-ELISA is used to classify cattle infected with *A.marginale* (Dreher *et al*., 2005 and **James** *et al*., 2010). Additionally, the lag time between infection and the anti-MSP-5 antibody response may allow for the misclassification of cattle sub acutely infected with *Anaplasma* species (**James** *et al*., 2010).

Indirect enzyme-linked immunosorbent assay/I-ELISA
I-ELISA based on the use of a normal red blood cell antigen (negative antigen) and an *A.marginale* infected red blood cell antigen (positive antigen) has been found to be reliable for the detection of *A.marginale*-positive sera (Ndung'u *et al.,* 1995). Although more cumbersome than tests using only one antigen, this test eliminates those sera that have high levels of nonspecific activity due to iso-antibodies to normal red blood cell components. According to Reyna-Bello *et al.,* (1998) study the test correctly identified all 100 known positive sera taken from cattle up to 3 years after infection, while 3% of negative sera gave false-positive results. But this assay is expensive, complex to perform and not rapid.

Dot enzyme-linked immunosorbent assay/Dot-ELISA
Compared with the I-ELISA, the dot ELISA has the potential advantages of being rapid, inexpensive and simple to perform. While, due to cross-reactivity among *Anaplasma* species, the dot ELISA has a sensitivity of 93% and a specificity of 96% (Torioni *et al.,* 1998).

Indirect fluorescent antibody test/IFAT
Because of the limitations on the number of indirect fluorescent antibody (IFA) tests that can be performed daily, other serological tests are generally preferred. A serious problem encountered with the test is nonspecific fluorescence. Antigen made from blood collected as soon as adequate parasitaemia (5–10%) occurs is most likely to be suitable. Nonspecific fluorescence due to antibodies adhering to infected erythrocytes is the common problem but it can be reduced by washing the erythrocytes in an acidic glycine buffer before antigen smears are prepared (Richey and Palmer, 1990 and Johnston *et al*., 2000).

5. The Common Diagnostic Techniques for Theileriosis and its Challenges

5.1 Identification of the Agent

Giemsa Staining
The macroschizont is a characteristic diagnostic feature of acute infections with *T.parva* and *T.annulata* in Giemsa-stained biopsy or tissue impression smears of lymph nodes, liver and spleen. Schizonts are transitory in *T.mutans* and the *T.sergenti/T.buffeli/T.orientalis* group, in which the piroplasm stage may be pathogenic (Rowlands *et al*., 2000). *Theileria taurotragi* schizonts are not readily detected in Giemsa-stained blood smears. And also for practical purposes schizonts and piroplasms of different *Theileria* species are difficult to discriminate in Giemsa-stained smears (Rowlands *et al*., 2000 and D'Oliveira *et al*., 2006).

Piroplasms of most species of *Theileria* may persist for months or years in recovered animals. Hence, detection of piroplasms in carrier animals is an important epidemiological parameter. However, *Theileria* piroplasms may be difficult to find in stained blood smears. More important, it is generally not possible to discriminate *T.annulata* from nonpathogenic *Theileria* species that may occur simultaneously within the same bovine host (D'Oliveira *et al*., 2006). Negative results of microscopic examination of blood films do not exclude latent infection. Piroplasms are also seen in prepared smears at post-mortem, but the parasites appear shrunken and their cytoplasm is barely visible (Rowlands *et al*., 2000 and Dolan, 2009).

Nucleic-acid-based tests
Recently, PCR was used for detection of the carrier state of *Theileria* infection. However, it appears that PCR performed with blood samples is dependent upon the presence of erythrocytic merozoites and not necessarily of schizonts. So, the detection of latent schizonts in recovered or immunized cattle remains a problem (Rowlands *et al*., 2000).

5.2 Serological Tests

The indirect fluorescent antibody test

The IFAT based on schizonts derived from culture or infected cattle detected considerable levels of antibodies in cattle immunized with attenuated schizonts (Rowlands *et al*., 2000). Although the antibodies detected were not necessarily an indication of immunity to infection, the very fact of the presence of this antibody indicates that a multiplication of schizonts occurred in the vaccinated animals (Pipano and Shkap, 2006).

The IFA test is useful for identifying herds that contain carriers of *T.annulata,* but is not always sufficiently sensitive to detect all infected individuals. This is because of antibodies last for a variable period of time after recovery. Both schizont and merozoites Indirect Fluorescent antigens have failed to detect antibody in some animals carrying patent infection with piroplasms (Rowlands *et al*., 2000). Because of the problems of cross-reactivity among different *Theileria* species (*T.annulata*, *T.parva*, *T.mutans* and *T.taurotragi*), the test has limitations for large-scale serological surveys, particularly in areas where several species overlap. Therefore there is a need for tests, that are more specific, are easy to interpret, and robust enough to be used in field conditions (Rowlands *et al*., 2000; D'Oliveira *et al*., 2006 and Pipano and Shkap, 2006).

6. Common Control Methods of Babesiosis and its Constraints

Disease control and prevention strategies are centered on reliable diagnostic tests for accurately and precisely identifying infected cattle. In most developing countries where babesiosis are endemic, disease control rather than eradication is the only realistic option. Eradication is unlikely to be feasible except in ecologically isolated areas and in advanced countries with the necessary resources. An active control of babesiosis is achieved by three main methods: immunization, chemoprophylaxis and vector control (Radostits *et al*., 2006).

Immunization

The carrier donor system relies on chronically infected animals to supply infected blood for vaccination of susceptible cattle (Bose *et al*., 2005 and Radostits *et al*., 2006). Problems that were encountered with this form of vaccination were; the cattle are not always being protected, unpredictability of reactions and the transfer of other field strains and blood parasites to cattle being vaccinated (Bose *et al*., 2005). The likelihood of vaccine-induced reactions has been reduced with the development of attenuated strains but there is always the risk of reactions when highly susceptible, and adult cattle are vaccinated. Concurrent infections may increase the likelihood of reactions. The fever associated with reactions in pregnant cows may cause abortion and in large bulls a temporary loss of fertility is observed (OIE, 2000 and Holman *et al*., 2003).

In most cases, a single vaccination provided lasting, probably life-long immunity against field infections with antigenically different strains. However, some failures have occurred and are thought to be associated with the choice of vaccine strains, the presence of heterologous field strains, and host factors. There is little evidence of time-related waning of immunity (Bock *et al*., 2000 and Holman *et al*., 2003). Despite the potential severity of vaccination, individuals who survive generally develop immunity against disease, but not against infection, and could remain persistently infected (Holman *et al*., 2003; Jorgensen *et al*., 2004 and Bose *et al*., 2005).

Chemoprophylaxis

Drugs exist for control by therapy and prophylaxis of babesiosis, but they are few and some previously useful products are no longer available (Bose *et al*., 2005 and Radostits *et al*., 2006). For babesiosis infection, diamidine derivatives are frequently used. Imidocarb dipropionate is also recommended for chemoprophylaxis. The mode of action of Imidocarb, diamidine derivatives, with babesiacidal activity is not well known; however, the ultrastructural changes of the erythrocyte-stage parasite following exposure to Imidocarb are described. Although it has been reported that some babesiosis infection have been successfully cleared with Imidocarb, controversy exists regarding its efficacy. Yet, no new therapeutics for this disease is believed to be in development (Bock *et al*., 2000 and De Echaide *et al*., 2007).

Furthermore, if the illness is treated urgently and efficiently following vaccination, and the protozoa are killed before antibodies are produced, no immunity occurs. Drugs can also be used during the period of initial exposure in a method known as chemo-immunization. However the method can be expensive and relies on challenge occurring during the period of drug cover which may create problems under a pastoral system (Jorgensen *et al*., 2004.

Tick Control

Ambitious aims towards eradication of ticks induced several countries to engage in expensive and unsuccessful national campaigns for tick control (De Echaide *et al*., 2007). The two commonly used methods are hand de-ticking and the application of acaricides (Bock *et al*., 2000). Problems with cost, infrastructure and maintenance were important deterrents. Possibly the lack of adequate technology and the availability of material have

accounted for the limited effectiveness of those methods. Acaricides used to control can be toxic to livestock and humans, can create illegal residues in tissues of animals, and can be destructive to the environment if they are not used and handled in a safe and correct manner (Radostits *et al*., 2006 and El-Gaysh *et al*., 2008). Difficulties of getting a complete muster of all cattle on every dipping day also create a problem. In such case, although millions of animals are treated and millions of kilograms of acaricides are used yearly, the safe use should not be taken for granted (El-Gaysh *et al*., 2008 and Chauvin *et al*., 2009). To avoid accidents and misuse, it is necessary to continually review and employ safe use precautions and procedures (FAO, 2006).

It is also difficult to control and eradicate multi-host ticks, which can be infective but temporarily not resident on a beast on dipping day. Control of ticks capable of surviving on both domestic and wild animals presents a major problem (Radostits *et al*., 2006). A major problem is encountered when the protozoan persists through succeeding generations of the vector tick, spread of ticks or infested cattle due to environmental activity, e.g. floods, windstorms as well as illegal movement of cattle without a permit (Chauvin *et al*., 2009).

It is now generally understood that tick control should not only be based on acaricide use. Complementary approaches have been developed and these include resistant cattle, vaccines against the ticks, grazing strategies and fungal biopesticides. In using resistant cattle approach, there is a risk of losing other herd characteristics that are profitable for the farmer and strategies based on breeding programmes are relatively slow to implement and to modify (FAO, 2006).

6.2 Common Control Methods of Anaplasmosis and its Constraints

The eradication of anaplasmosis is unlikely to be feasible with its non-bovine reservoirs and variety of vector species (Radostits *et al*., 2006). Control methods for anaplasmosis have not changed markedly during the past 50 years and active control of anaplasmosis achieved by; arthropod control, chemoprophylaxis, vaccination, and maintenance of an Anaplasma-free herd. Ideally, these methods should be integrated to make the most cost-effective use of each and also to exploit breed resistance and the development and maintenance of enzootic stability (FAO, 2006 and Radostits *et al*., 2006). Control measures implemented vary with geographic location, and depend on availability, cost, and the feasibility of application (Kocan *et al*., 2000).

Immunization

Vaccination has been an effective means of preventing outbreaks of anaplasmosis, but these vaccines, live and inactivated, are dependent on bovine blood as the source of infection or antigen (Richey, 2003). Blood-derived vaccines are difficult to standardize and bear the risk of transmitting other bovine pathogens in apparent at the time of blood collection. Extensive purification is required to remove bovine cell membranes, which may cause side effects. Most importantly, geographic isolates of *A.marginale* are often not cross-protective. Some researchers think that the protection achieved by vaccination is very isolate specific (Kocan *et al*., 2000).

The vaccine does not prevent infection, but aids in the prevention of clinical symptoms or in the reduction of the severity of clinical cases (Breiner *et al*., 2005). Most infected cattle then carry the organism for their entire life. They are "immune carriers." That is to say, they are "immune" to becoming sick from the agent; but, are carriers of the agent and in this way; the organism perpetuates itself. Therefore, use of this vaccine cannot be undertaken lightly (Richey, 2003).

Studies of Breiner *et al*., (2005) shows that calf losses from cows previously vaccinated against anaplasmosis have been noted. The dam can be sensitized by blood elements in the vaccine, if those elements are different than those the cows' possesses. The antibodies formed against the foreign blood elements are concentrated in the colostrum of the cow and passed to the newborn calf during post-partum nursing. The condition, in the calf, known as "Neonatal Isoerythrolysis", NI, or the "yellow calf" syndrome has been developed. It can only occur by vaccinating the dam. Therefore, when using anaplasmosis vaccine as a control method, it would be advisable to vaccinate the cows while they are open or as far from calving as possible. Vaccinating the herd sires does not cause the syndrome in calves (Richey, 2003 and Breiner *et al*., 2005).

Chemoprophylaxis

Until an anaplasmosis problem develops, producers usually are not concerned with control. This program necessitates blood testing and identifying each animal in the herd as a carrier of the disease or as susceptible to the disease (Richey, 2003). It also requires that two separate herds be maintained during the vector season, test the herd and clear up the carriers with tetracycline antibiotics, Continuous chlortetracycline (CTC) medication during the vector season and Continuous chlortetracycline medication the year around (Breiner *et al*., 2005). Because of the need for higher dosages and longer treatment regimens for anaplasmosis, the use of drugs is generally limited to control outbreaks and not to sterilize carriers (Kocan *et al*., 2003).

Arthropod control

The transfer of the agent from a carrier animal to a susceptible animal can occur by a number of routes. One

common way is via ticks (Radostits *et al*., 2006). Additionally, biting insects such as, Diptera (horse flies, stomoxys, etc) are also capable of transmission (OIE, 2000). The wide range of insect vectors and possible environment pollution due to chemical application makes the control complex (El-Gaysh *et al*., 2008).

Not all developing countries and those in transition may have such information available, due to a lack of human, economic and infrastructural resources. In addition to cost, acaricide resistance and the risk of residues in cattle products; poor management of tick control and illegal cattle movement in many countries makes the control of ticks difficult. Repopulation of a region with ticks after only two or three years of freedom can cause serious losses when babesiosis and anaplasmosis return (Radostits *et al*., 2006 and El-Gaysh *et al*., 2008). Additionally, transfer of blood between animals should also be avoided (OIE, 2000 and FAO, 2006).

In addition to chemical control method, a non chemical technology like vaccines has been used. The vaccines that are presently available do not have the knockdown effect as of traditional chemical acaricides. Farmers may initially be disappointed if they treat the cattle and do not see immediate tick deaths, an important marketing constraint. Because there is no natural exposure to the antigens, the present vaccines are not reinforced by tick feeding and have a limited duration of protection. Vaccination is recommended every 10 weeks to 3 months in the tick season (FAO, 2006; Ghosh *et al*., 2007 and El-Gaysh *et al*., 2008).

6.3 Common Control Methods of Theileriosis and its Constraints

Control of Theileriosis is based on a multifaceted approach including pasture management, herd-selection of resistant animals, tick control, and immunization (OIE, 2005).

Antitheilerial drugs

Once an animal is manifesting clinical signs of ECF, treatment is generally considered to be either unsatisfactory or too expensive (Dolan, 2009). Even if a much higher success rate is obtained with the two recently introduced drugs, halofuginone lactate and parvaquone recovered animals may become carriers unless the correct dose is used. These antitheilerial drugs are not generally available and often used without proper diagnosis. Although parvaquone and bu- parvaquone are not able to eliminate infections, they have some efficacy against parasitemia of initial infection. This is due to the fact that, the activity of these compounds is greatest against the schizont stage (OIE, 2005 and Dolan, 2009).

Immunization

Vaccination using attenuated schizont-infected cell lines has been widely used for *T.annulata*, while for *T.parva* control, infection and treatment using tick-derived sporozoites and tetracycline is being implemented in a number of countries in eastern, central and southern Africa (FAO, 2006 and Hashemi-Fesharki, 2008). The immunized cattle remained productive, but different treatment regimens were necessary for different cattle types, depending on the proportion of cattle in their breeding (Radostits *et al*., 2006). For a rational approach to ECF immunization, it is necessary to isolate and characterize *T.parva* stocks from the field before selecting them for immunization. Furthermore, the immunity engendered following ECF immunization is strain/stock specific. So it is necessary to have precise methods for identifying not only different species of *Theileria,* but also different strains of *T.parva* (Kocan *et al*., 2003 and FAO, 2006).

Strategic control plus immunization can markedly reduce the risk of clinical Theileriosis but immunized animals are carriers and all stages of the vector can transmit infection from them to naïve animals. Hence, the risk inherent in the widespread use of such vaccines across national boundaries warrants further consideration (Kocan *et al*., 2003 and Radostits *et al*., 2006).

Tick Control

The successful implementation of rational and sustainable tick control programmes in grazing animals is dependent upon a sound knowledge of the ecology or epidemiology of the parasite as it interacts with the host in specific climatic, management and production environments (Kocan *et al*., 2003). Urgency of need in terms of disease transmission can be illustrated by the need for control of *Rhipicephalus appendiculatus*, which transmits East Coast fever to cattle. Since the disease is transmitted from stage to stage in the life of the tick, an infective adult can infect a susceptible animal within two or three days after it attaches. Therefore, with an acaricide that has a short residual activity; cattle must be treated every three to five days to prevent transmission of the disease (Rowlands *et al*., 2000; FAO, 2006 and Dolan, 2009). Additionally, it is tedious to get considerable information concerning the life history, seasonal appearance and biology of ticks before “strategic” treatment utilized (Rowlands *et al*., 2000 and Kocan *et al*., 2003).

Control of theileriosis is achieved mainly by dipping, but this method is becoming less reliable because of the escalating cost of acaricides (Dolan, 2009). Also, because of the expansion of crop agriculture, some livestock owners can no longer walk their cattle to dips (FAO, 2006 and Rowlands *et al*., 2000). Current problems of the chemical control of ticks, like the risk of residues in meat, milk and their products, the

insecticide resistance and the possible environment pollution are critically outlined (D'Oliveira *et al*., 2006). Furthermore, it has been observed that indigenous cattle, constituting the majority of the herds in some of the affected countries, may lose their endemic stability with intensive dipping (Rowlands *et al*., 2000 and OIE, 2005).

Recent studies have shown that as this all tick control approach may not be cost-effective in terms of increasing the productivity of cattle. Thus, the use of concealed tick antigens for vaccination of cattle has served as the basis of a commercial vaccine, but there are still potential drawbacks that should be addressed. Thus, current concealed antigen vaccines may not prevent damage to bovine hides or the transmission of tick-borne infections (Sathaporn *et al*., 2004).

In many areas of the world, rotation between crops and livestock is a measure that reduces tick populations. This approach needs a prerequisite that knowledge of tick ecology in a region, suitable pasture, animals and fencing. Application can prove difficult for some farmers who do not have sufficient free paddocks. A small proportion of free living eggs and larval stages can often extend for up to 7 or 8 months. These are likely to have very poor or no powers of reinfestation of cattle, and are usually of little practical significance in achieving adequate control of ticks but they do interfere with eradication attempts (Johnston *et al*., 2000).

Tick Control Strategies under Development

Biological control

The biological agents, which potentially could be used for the control of ticks, include some fungi, bacteria, nematodes and ants that attack soil living stages of the ticks. These natural pathogens and predators have not yet been subjected to sufficient field testing or validation and require extensive product development. Nevertheless, preliminary laboratory bioassay trials against *B.microplus* have shown *Metarhizium anisopliae* to have a high level of virulence against ticks (Kirby, 2010).

Myco insecticides are a potentially cost effective, sustainable, environmentally friendly alternative to chemical acaricides that can be applied using conventional technology, thus making them simple for farmers to use. If field trials are successful, more large-scale testing will be needed, including the determination of non-target effects (Kirby, 2010 and Alan, 2011).

It is known that a number of bird species (oxpeckers, cattle egrets, chickens) may contribute to an overall reduction in tick numbers on livestock and in the environment. Their precise effects on tick burdens need further evaluation before they can be considered as significant tick control measures. Until this is known, it is not possible to recommend such alternatives to producers for adoption and practical use in the field (Alan, 2011).

Herbal Remedies

The use of some types of grass or leguminous plants with acaricidal or repellent effects needs greater assessment for possible inclusion in schemes for improving tick control. *Brachiaria brizantha*, *Melinis minutiflora*, *Stylosanthes* species, neem oil, etc., have been shown to have some larvicidal or repellent effect against the larvae of ticks. Extracts of some plants are also active against certain tick species. The feasibility of these species for feeding animals for tick control under field conditions has not yet been adequately studied (Kirby, 2010).

7. CONCLUSION AND RECOMMENDATIONS

The diagnosis of acute anaplasmosis, theileriosis and babesiosis infections in cattle is made by microscopic examination of Wright-Giemsa stained smears. However, the efficiency of the method in the diagnosis of sub clinical infections remains poor. Even though, in recent years, suitable serological tests and specific antigen detecting tests have been described, none of them appears to be adopted for routine diagnostic use in field and to some extent in laboratories. It is hoped that a combination of ELISA, PCR and DNA probes may greatly enhance the present capacity to identify infected animals. The control of the tick vectors is a permanent solution to the TBDs problem but the ever rising costs of acaricides, their effect on the environment, the development of acaricide resistance, and frequent political problems in the affected regions makes the control of TBDs complex. Moreover, live vaccines for tick-borne diseases control have well-known limitations. Some drugs also exist for control by therapy and prophylaxis of TBDs, but they are few and previously useful products no longer available. In line with the above conclusion the following recommendations are forwarded:

- The effort invested in the research and development of high quality standardized diagnostic test should be critically needed.
- Need to improve understanding of factors which cause variant characteristics to arise in parasite populations, and develop ways for predicting and measuring these changes.
- Enzootic stability should be considered in indigenous and exotic cattle.
- Undoubtedly, to implement effective control measures, renewed efforts are needed, particularly to use integrated approaches.
- Where vaccines are used, strict attention is paid to quality control and safety testing.

- In the long term it is inevitable and new therapeutic products will believed to be required.
- The availability of each of the control options, their advantages and disadvantages, and the cost benefit of each strategy should be assessed before deciding on the programme.
- There should be federal regulations pertaining to the interstate movement of TBDs carriers.
- Much greater emphasis is needed to determine the economic importance of TBDs.

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