**Summary:** A survey conducted by the Office International des Epizooties (OIE) on the occurrence of blood parasitic diseases amongst its Member Countries identifies protozoan diseases (trypanosomosis, theileriosis, babesiosis) and rickettsial (anaplasmosis, cowdriosis and ehrlichiosis) as being of economic importance. Each of the diseases listed above had a variable economic impact in the responding countries. The molecular complexity and the exhibition of antigenic variation by some of the causal agents ensures the survival of these parasites.

The specific immune response to the infecting parasites is complex and involves both the humoral and cellular branches of immune systems. In trypanosomosis, parasite growth is primarily controlled through T cell-dependent antibody responses to the variable surface glycoproteins and possibly to other molecules embedded on the surface of the parasites. Cellular immune responses also occur but at the level of immunosuppression directed against B cells. Additionally, a variety of immunomodulatory cytokines are produced during the course of infection. Studies on theileriosis have demonstrated the importance of the humoral response at the level of infecting sporozoites, but once the lymphocytic schizont stage appears, cytotoxic T cells are responsible for clearing parasitised cells. In Babesia infection, T cells are the primary effector cells in protective immunity. To date, there is a lack of information on the control of rickettsaemia in acute anaplasmosis. It has, however, been demonstrated that a T cell subset is critical in protective immunity. Following infection with Ehrlichia and Cowdria parasites, a hypergammaglobulinaemia develops that has no direct correlation to protective immunity, implying an involvement of T cell-mediated immune mechanisms in protective immunity.

This review focuses on the state of knowledge of specific immune responses in blood parasitic diseases, and points to the wealth of information available for three protozoan diseases, in contrast to the numerous gaps in our understanding of specific immune responses to rickettsial infections.

1. **INTRODUCTION**

Blood parasitic diseases have severely hindered development of livestock production in many developing countries, particularly those in sub-Saharan Africa. The bulk of these diseases are caused by vector-borne Protozoa and Rickettsia. The protozoan diseases of veterinary importance are trypanosomosis, theileriosis and babesiosis, of which trypanosomosis is the most widely distributed (Table 1). These protozoan diseases have been encountered in 49 of the 59 OIE Member Countries, located in Latin America, Africa, the Middle East, southern Europe and Asia, that returned the questionnaire sent to them on Blood Parasitic Diseases (Table 1).

It is important to note that trypanosomes, Babesia and Theileria have complicated life cycles. Each developmental stage may have a set of distinctive antigens requiring a specific type of immune response. Control of protozoan diseases by the host is hindered primarily by poor immunogenicity of protozoan antigens and antigenic variation. Furthermore, immune response to one developmental stage may not confer protection to subsequent stages.

Rickettsial infections are a serious problem in sub-Saharan Africa and to a lesser extent in Asia and Europe. The rickettsial diseases of economic importance are anaplasmosis, cowdriosis and ehrlichiosis. These diseases have been encountered in 36 out of 59 OIE Member Countries. The immune response to these organisms involves both the humoral and cellular immune systems. Studies carried out so far in cowdriosis suggest a major role for cellular immune response.

The current state of knowledge of specific immune responses to trypanosomes, Theileria, Babesia, Anaplasma, Cowdria and Ehrlichia, primarily in ruminants, will be discussed.
Table 1: OIE Member Countries reporting blood parasitic diseases

<table>
<thead>
<tr>
<th>Country</th>
<th>Trypanosomosis</th>
<th>Theileriosis</th>
<th>Babesiosis</th>
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<th>Cowdriosis</th>
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Table 1: OIE Member Countries reporting blood parasitic diseases (continued)
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<th>Country</th>
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2. TRYPANOSOMOSIS

Trypanosomosis is a major constraint to livestock production in sub-Saharan Africa (40) and to a lesser extent in Latin America and Asia. The distribution of the disease is influenced by the existence of tsetse and biting flies. Tsetse-transmitted trypanosomosis is encountered in 39 sub-Saharan African countries (Table 1). The non-tsetse-transmitted trypanosomoses occur in Latin America, the Middle East, Asia, eastern Europe and, to a lesser extent, Africa (Table 1). The mechanically transmitted parasites are also found in countries harbouring mainly tsetse-transmitted trypanosomes. The tsetse-transmitted trypanosomes of veterinary importance are Trypanosoma congolense, T. vivax and T. brucei in cattle, sheep and goats, and T. simiae in pigs. The most important pathogenic trypanosome species outside the tsetse belt are T. vivax and T. evansi (mechanically transmitted by biting flies), and the sexually transmitted T. equiperdum. Trypanosoma evansi causes disease in horses, camels, pigs, buffaloes and cattle, while T. equiperdum affects horses and donkeys.

2.1. Humoral immunity

Trypanosomes induce diseases with variable symptoms depending on the infected host, trypanosome species and serodeme. Generally, trypanosomosis is characterised by fever, anaemia, cachexia, reduced productivity, infertility and, if left untreated, animals often die from heart failure or opportunistic infections. The infected animals tend to exhibit persistent fluctuating parasitaemia comprising a series of trypanosome waves expressing different variable surface glycoproteins (VSG) (7, 76). This pattern of parasitaemia exposes the host to a series of antigenically distinct surface antigens of the parasite. The VSG genes encode a family of proteins that exhibit extensive heterogeneity at the N termini, but are fairly similar at the C termini (56, 65). The C terminus is covalently bound to dimyristyl-phosphatidylinositol, which is responsible for anchoring it to the membrane (17). Cleavage of the VSG phosphatidylinositol by endogenous phospholipase C leads to subsequent exposure of a cross-reactive determinant, a cryptic epitope, formed in part by the terminal inositol phosphate (65). It has been shown that the host mounts a humoral immune response to both the N and C termini of VSG. The antibodies directed to the N terminus are specific to the particular VSG, and therefore responsible for the elimination of the parasites displaying on their surface the particular VSG. These parasites are eliminated through opsonisation by macrophages (75). Despite the effectiveness of the anti-VSG-specific antibodies, complete elimination of trypanosomes is hampered by the rapid appearance of those with different variable surface antigens to which the host has not mounted an immune response. The persistence of parasites in circulation leads to continuous stimulation of the host immune system, as evidenced by a marked increase in size and activity of germinal centres, with a concomitant increase in proliferating lymphocytes in the medullary cords and paracortex of lymph nodes, periarteriolar regions and peripheral follicular areas of the spleen (33). Despite the apparent over-stimulation of the immune response organs, the elevated levels of IgM and IgG immunoglobulins occurring in African trypanosomosis are specific to the infecting serodeme/strain in view of the fact that infecting trypanosomes can absorb 85-100% of the generated immunoglobulins (45).

Antibody response to non-VSG invariant antigens also occurs during trypanosome infection (4). Although these responses have no direct correlation to the control of parasitaemia, it has been shown that a predominant IgG response to a cysteine protease, heat-shock protein (hsp 70/BIP) and cryptic VSG epitope could be associated with tolerance to trypanosome infection (3). This would suggest a role for invariant antigens in modulating the infection.

2.2. Cell-mediated immunity

African trypanosomosis and, to a lesser extent, T. evansi infection in water buffaloes, are associated with profound suppression of host immune responses to heterologous antigens introduced following the establishment of infection (5, 61, 62). B cells appear to be one of the targets of immunosuppression as evidenced by greatly reduced IgG1 and IgG2 responses in cattle following vaccination against Brucella abortus (61). Nevertheless, the immunosuppression observed in trypanosomosis varies according to the strain of parasite and animal breed. In mice, the immunosuppression has been attributed to polyclonal activation (75). This polyclonal activation has been linked to the appearance of antibodies to foreign antigens, increased serum IgG concentration, massive plasma cell response in the lymph node and spleen, and a relative reduction in specific antibody responses to vaccines (24). Studies on the causes underlying immunosuppression have clearly demonstrated the role of macrophages, as removal of cells expressing Mac-1 (macrophages, CD5+ B-cell lineage and granulocytes) from in vitro-cultured lymph node cells led to 100% restoration of the proliferative reaction, while the depletion of the Thy-1+ fraction (T cells) failed to restore proliferation (68). The contribution of macrophage lineage to immunosuppression had been demonstrated earlier by Borowy et al. (8) when the authors abrogated immunosuppression in mice through treatment with L-leucine methyl ester. Moreover, supplementation of cultures of spleen cells obtained from infected mice with accessory cells from uninfected ones restored proliferative activity (22, 23).
Interaction of trypanosome-activated macrophages and T cells leads to up-regulation of interferon-gamma (IFN-\(\gamma\)) secretion by CD8\(^+\) T cells with subsequent suppression of interleukin-2R (IL-2R) expression on both CD8\(^+\) and CD4\(^+\) T cells. This effect can be reversed by a 40-45 kDa protein derived from \(T.\) brucei \(brucei\) (49, 50). Apart from modulatory immune responses in the bovine host, activated macrophages also play a key role in the removal of erythrocytes coated with immunoglobulins and even those exhibiting distortion of surface membranes. Erythrophagocytosis by the activated macrophages may be a major factor in the induction of extravascular anaemia (43). Additionally, macrophages produce tumour necrosis factor-alpha (TNF-\(\alpha\)), the secretion of which has been observed in \(T.\) vivax infection in which severe erythrophagocytosis and anaemia were evident (69).

While cellular immune responses have been shown to play a role in immunity to murine trypanosomosis, there is no clear evidence that they perform similar functions in bovine trypanosomosis. It has been demonstrated that trypanosome-infected cattle exhibit an increase in the percentage of CD8\(^+\) and γ\(\delta\) T cells, while there is a decrease in CD2\(^+\) and CD4\(^+\) T cells (29). Even though CD8\(^+\) T cells increase during trypanosome infection, recent data generated following depletion of this subset of cells indicated no modulating effect on the level of parasitaemia or anaemia.

The T cells produce a variety of cytokines which, when bound to specific cell surface receptors, modulate the growth, differentiation or function of the receptor-bearing cells. IL-2 and IFN-\(\gamma\) are secreted by the proliferative lymph node cells during infection (28, 67, 68). IFN-\(\gamma\) is known to stimulate macrophage activity and to promote surface expression of major histocompatibility complex (MHC) class I and II on various cell types. However, the role of IFN-\(\gamma\) in immunity to bovine trypanosomosis remains unclear.

From these observations it is evident that there is an urgent need to identify molecules responsible for the induction of protective immune responses. Identification of these molecules would greatly facilitate the development of an effective vaccine against trypanosomosis.

### 3. THEILERIOSIS

Theileriosis is an important haemoparasitic disease of animals inducing a variety of clinical manifestations ranging from a subclinical presentation to a fatal disease depending, in part, on the animal species, host, age and the species of the microorganism. The disease is widely distributed in tropical and subtropical zones where it is known as East Coast Fever (\(T.\) parva), Corridor disease (\(T.\) lawrencei) and Tropical (Mediterranean) bovine theileriosis (\(T.\) annulata) or Mediterranean Coast Fever. East Coast Fever is of major economic importance throughout eastern, central and southern Africa where losses have been estimated at US$168 million per annum (42). Tropical theileriosis has a wider distribution extending from north Africa to China. There are no recent figures for losses due to this disease; nevertheless it has been estimated that 200 million cattle are at risk of contracting it. The two parasites (\(T.\) parva and \(T.\) annulata) cause a lymphoproliferative disease of cattle characterised by fever, lymph node enlargement, petechial haemorrhages on mucosae and respiratory distress, and often culminates in death. In infections caused by \(T.\) annulata there is also an erythro-destructive stage.

The \(T.\) theileria parasites are transmitted by ixodid ticks (\(Rhipicephalus,\) \(Hyaloma\) and \(Haemophysalis\)), in which a sexual cycle occurs leading to production of sporozoites infective to mammalian lymphocytes. In cattle, the sporozoites first invade the lymphocytes, develop into schizonts and then merozoites, which subsequently infect erythrocytes. In acute cases, death occurs at the macroschizont stage of infection, partly due to destruction of the lymphocytes.

#### 3.1. \(T.\) parva: Humoral immunity

Development of immunity to \(T.\) parva infection is complicated by the existence of parasites in two types of host cells and the parasite's antigenic heterogeneity. Infection by \(T.\) parva induces marked antibody response to the surface antigens of sporozoites, macroschizonts and piroplasms. The antibodies directed against surface antigens, particularly a 67 kDa molecule, can neutralise the infectivity of the sporozoites to lymphocytes (46). This observation led to the evaluation of the 67 kDa protein (p67) as a candidate for a subunit vaccine against \(T.\) parva (44). Although anti-schizont antibodies are readily detectable in cattle following immunisation or infection, it is unlikely that these antibodies can be effective against this stage of the parasite, given its intracellular location. Indeed, there is no evidence that anti-schizont antibodies recognise parasite-specific antigens on the surface of infected cells (12). Once the parasite is established in the lymphocytes, humoral immune responses do not appear to be induced against the surface of parasitised mononuclear cells (12). This finding indicates that antibodies may not play a major role in the removal of infected cells. Furthermore, attempts to protect cattle by the transfer of immune serum have been unsuccessful (41).
3.2. *Theileria parva*: Cell-mediated immunity

The observations described above would suggest that immunity to the schizont is a cell-mediated mechanism. This is supported by adoptive transfer studies with twin cattle demonstrating that cells transferred from the immune to the naive twin protect the latter against a primary infection with *T. parva* (16, 36). *In vitro* experiments conducted by Pearson et al. (53), indicated that bovine peripheral blood mononuclear (PBM) cells from immune cattle proliferated to irradiated autologous parasitised lymphoblasts, and further that a proportion of these responding cells mediated much higher cytotoxic activity against autologous infected cells than against their allogenic counterparts. This initial finding suggested that infection of host lymphocytes with *T. parva* leads to antigenic changes on the cell surface that elicit cell-mediated responses.

By repeatedly stimulating immune PBM cells with autologous infected lymphoblasts, effector cells are enriched for parasite-specific T cells. These effectors, comprising mainly CD4+ and CD8+ T cells, have been shown in monoclonal antibody blocking studies to be restricted by MHC class II and class I gene products, respectively (6, 19). While CD8+ T cells mediate cytotoxic activity against infected targets of appropriate MHC type, the majority of CD4+ T cells are non-lytic. Analysis of T cell lines derived from cattle immunised with different parasite strains indicate the existence of strain-specific and cross-reactive T cells (6, 19, 39). The cross-reactivity could be due to epitopes conserved among parasite strains or the existence of distinct components, some of which are identical between parasite strains.

*Theileria parva* presents an enormous challenge to the bovine immune system owing to its complex strain heterogeneity. Notable features of this heterogeneity are that cross-protection is usually not reciprocal between strains and that protection extends to only a proportion of cattle. Using cloned parasite populations to immunise cattle of different MHC haplotypes, important parameters determining strain-specificity of the cytotoxic T lymphocyte (CTL) response have been investigated (71). It was clear from these studies that two kinds of epitope, strain-specific and cross-reactive, are generated in *T. parva*-infected cells, but one is immunodominant over the other depending on the restricting element and immunising parasite. This was evident in animals that generated strain-specific CTL responses during primary immunisation but exhibited cross-reactive CTL following heterologous challenge. These observations have important implications for the design of subunit vaccines against *T. parva*. One major concern about CTL-based immunity to *T. parva* is its strain specificity. It is possible that by selecting parasite antigens containing cross-reactive epitopes, this shortcoming may be overcome. Current efforts are focused on identifying schizont antigens that are targets for CTL.

The other important consideration with regard to developing a CTL-based vaccine is the definition of factors important for the induction of CTL to defined parasite antigens. Recent studies have indicated that the induction of parasite-specific CD8+ T cells requires helper signals from antigen-specific CD4+ T cells (Taracha & McKeever, personal communication). Work is in progress to elucidate the precise nature of this help and to develop antigen-delivery strategies to meet the requirements for inducing parasite antigen-specific CTL.

3.3. *Theileria annulata*: Humoral immunity

The immune response to *T. annulata* is similar to the one reported for *T. parva*. Humoral immune responses occur following infection with *T. annulata*. These antibodies recognise surface epitopes of the sporozoite and have been shown to block the invasion of mononuclear cells by sporozoites and subsequently to inhibit transformation of the infected cells (21, 80). Efforts to detect antibodies directed to surface molecules of the schizonts, piroplasms and cell surfaces of the infected mononuclear cells and erythrocytes have failed (66). As is the case with *T. parva*, the role of antibodies in protective immunity may be confined to the neutralisation of sporozoites.

3.4. *Theileria annulata*: Cell-mediated immunity

Unlike *T. Parva*, which infects predominantly T cells, *T. annulata* infects B cells. This might account for some differences that may emerge in the host's cellular immune response to the two parasites. To date, the studies conducted in the area of cell-mediated immunity to *T. annulata* have shown the generation of cytotoxic T cells during primary and secondary infection (55). Furthermore, these CTLs are capable of lysing infected lymphocytic cells under the restriction of MHC class I. A second group of cytotoxic, non-MHC class I restricted cells also appears, but are believed to be due to the natural killer cells (70). Additionally, work by Preston & Brown (54) identified the role of macrophages in controlling the multiplication of the parasites in the blood through the production of cytokines that exert strong cytostatic effects on schizont-infected cell lines. Recent work on cell-mediated immunity confirms the involvement of CTLs and macrophages in protective immunity. Further work is required to identify the mechanism(s) that may be responsible for the destruction of
schizont-infected cells. This will facilitate identification of protective antigens and could be used in the development of subunit vaccines against *T. annulata*.

4. BABESIOSIS

Babesiosis is a tick-borne disease of cattle caused by the *Babesia* species. The *Babesia* parasites are transmitted by ticks such as *Boophilus* and *Dermacentor*. The parasites are taken up by ticks while feeding on the definitive host. In the tick, the gametocytes obtained from erythrocytes form zygotes, which differentiate into ookinetes. The ookinetes penetrate the gut epithelium to initiate sporogony leading to the production of sporokinetes and later sporozoites in the salivary glands. The sporozoites become infective to mammalian erythrocytes once inoculated into the blood circulation at 37°C. The sporozoites penetrate erythrocytes to form trophozoites, which subsequently form merozoites and thereafter gametes that are infective to ticks.

The parasites often occur in pairs of pyriform bodies joined at their narrow posterior ends within erythrocytes. The *Babesia* parasites vary in their virulence to the definitive host. This virulence is, in certain cases, related to the geographical origin. For example, *Babesia bigemina* from Australia induces mild clinical signs, while the South African strains are virulent, eliciting peracute and acute cases. Peracute cases exhibit sudden and severe anaemia culminating in jaundice and death, while the acute cases tend to develop high fever, haemoglobinuria with the subsequent appearance of anaemia and jaundice (27). *Babesia bovis* also causes a virulent form of bovine babesiosis characterised by fever, anaemia, anorexia, cachexia, low parasitaemia, generalised circulatory disturbance and often results in high mortality rates among naive cattle. Unlike *B. bigemina*, parasitised erythrocytes sequester in the microvasculature of the brain and lung resulting in cerebral babesiosis and respiratory distress (81). This is believed to be a consequence of the over-production of nitric oxide and inflammatory cytokines, such as TNF-α and IFN-γ, in response to infection (82). *Babesia divergens* causes European bovine babesiosis with clinical manifestations similar to *B. bovis*. *Babesia equi* causes a severe disease in horses characterised by fever, anaemia, icterus and haemoglobinuria. *Babesia equi*, unlike the rest of the *Babesia* species, replicates in lymphocytes to produce schizonts (63). Thereafter, the later stages invade erythrocytes. In this respect it resembles *Theileria*.

4.1. Humoral immunity

Infection by *Babesia* spp. induces production of antibodies. Both IgM and IgG₁,₂ isotypes specific to *Babesia* are produced at the same time, but the IgG persists for a longer period. The *Babesia*-specific antibodies are directed against the surface proteins of the erythrocytic stage and are involved in opsonisation of parasitised erythrocytes (34). Further studies using normal and severe combined immunodeficient (SCID) horses have demonstrated the involvement of antibodies in the control of parasitaemia (25). This confirms findings by Mahoney et al. (32) in which passively transferred *B. bovis* hyperimmune serum or a mixture of IgG₁ and IgG₂ to splenectomised calves infected four days earlier, significantly reduced parasitaemia. The response was restricted to calves exposed to homologous strains of *Babesia*. Reduction of parasitaemia is mediated through opsonisation and subsequent destruction of free parasites and infected erythrocytes by antibody-dependent cell cytotoxic mechanisms (20).

4.2. Cell-mediated immunity

Currently, there is little direct evidence for a protective role of *Babesia*-specific T cells in cattle, but studies in mice have clearly shown that CD4⁺ T cells and activated macrophages are essential in protection against intraerythrocytic parasites of *B. microti* (59, 60). Elimination of *B. microti* infection is normally effected through the production of TNF-α, TNF-β and IFN-α, which activate neutrophils and macrophages leading to enhanced phagocytosis. In cattle, Brown et al. (9) demonstrated the reactivity of those T cells exhibiting immunity to *B. bovis*, using *Babesia* antigens in the presence of adherent cells. In some respects, this suggests a similarity in the pattern of reactivity of T cells in infected cattle and mice. In conclusion, the information available indicates the involvement of T cells in protective immunity through the activity of T helper cells in the initiation of anamnestic antibody responses and as effector cells stimulating macrophages to destroy *Babesia*. Further studies are required to clearly delineate the role of T cells and antibodies in bovine and equine babesiosis.

5. ANAPLASMOSIS

Anaplasmosis is an arthropod-borne rickettsial disease of cattle, sheep and goats, and has a wide distribution. The disease in cattle is caused by *Anaplasma marginale* and *A. centrale*. Infection caused by *A. marginale* is characterised by severe anaemia, cachexia, abortion and death (1), while infection with *A. centrale* induces subclinical to mild disease. *Anaplasma marginale* can be distinguished from *A. centrale* by the location and the characteristics of the inclusion bodies in the erythrocytes (58). *Anaplasma marginale* is transmitted to cattle either cyclically or mechanically.

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by ticks of the genus *Boophilus* and *Dermacentor* and biting flies, respectively. In the tick midgut epithelial and muscular tissue, the parasite undergoes a complex developmental process involving multiplication of large reticulated forms, by binary fission, giving rise to dense bodies (26). Thereafter the parasites gain access to salivary glands where they mature into forms infective to cattle. The infection established in the gut muscle and salivary glands of the male *Dermacentor* ticks has been shown to persist, rendering them reservoirs of anaplasmosis. Once the infective *Anaplasma* organisms are introduced into mammalian blood circulation, the initial bodies enter the erythrocytes by invagination of the cytoplasmic membrane with subsequent formation of a vacuole. The infective forms multiply by binary fission to produce approximately 16 initial bodies that are frequently observed during the acute phase of the disease. The number of parasitised erythrocytes diminishes as the disease becomes chronic.

5.1. Humoral immunity

The intraerythrocytic initial bodies cause structural and biochemical modifications of the plasma membrane of the red cells. The modified erythrocytes elicit production of autoantibodies, which in turn stimulate erythropagocytosis (18, 38). In addition to autoantibodies, Palmer & McGuire (52) have demonstrated the production of antibodies against antigens of *A. marginale*, and six outer membrane polypeptides have been identified (47). These antigens include MSP-1a, MSP-1b, MSP-2, MSP-3, MSP-4, and MSP-5. The studies conducted so far clearly implicate a role for antibody-mediated immunity in anaplasmosis. For example, antibodies to MSP-1 have been shown in vitro to enhance phagocytosis of *A. marginale* by bovine macrophages (11), and specifically to block the binding of the parasite to the bovine erythrocytes (35, 47). The mechanisms of action of the antibodies may involve direct or complement-mediated killing, interference with attachment and penetration of erythrocytes by initial bodies, antibody-mediated opsonisation, and antibody-dependent cellular cytotoxicity. Although the MSP polypeptides in their native form can induce good antibody response and confer protective immunity in cattle (51), recombinant antigens based on these polypeptides have provided inconsistent data on protection. Thesee data suggest that post-translational modifications are necessary for the generation of epitopes responsible for induction of protective immunity.

6. COWDRIOSIS

Cowdriosis is a rickettsial disease of cattle, sheep and goats caused by *Cowdria ruminantium* transmitted by the *Amblyomma* genus of ticks. Cowdriosis is endemic in sub-Saharan Africa and the Caribbean. The disease, which in some cases is peracute, is characterised by high fever, ascites, hydrothorax, hydropericardium and central nervous system signs. The development of these varies depending on the severity of the disease. Infection of wild animals such as bushbuck (*Tragelaphus scriptus*), black wildebeest (*Connochaetes gnou*) and springbok (*Antidorcas marsupialis*) by *C. ruminantium* often induces subclinical disease.


6.1. Humoral immunity

It has been demonstrated that both humoral and cellular immune responses are induced during infection with *C. ruminantium*. Antibodies appear 2 weeks post-infection and, in the absence of rechallenge, persist for variable periods of time ranging from 8 to 30 weeks (64). The period of persistence may be related to continuous low grade stimulation by intermittent parasitaemia in recovered animals (2). Although the antibodies are capable of neutralising infectivity of *C. ruminantium in vitro*, transfusion of immune serum into naive animals does not confer protection. This may be related to the fact that *C. ruminantium*, being a strictly obligate, intracellular pathogen residing in vascular endothelial cells, neutrophils and macrophages, requires cellular immune responses for elimination. Although the specific antibodies generated during immunisation do not confer protective immunity, they may, however, modulate the severity of infection by blocking adhesion to or invasion of endothelial cells in vivo (10).

6.2. Cell-mediated immunity

Early studies by Uilenberg (74) suggested that protective immunity to *C. ruminantium* is mainly cell mediated. This contention was supported by data obtained following transfer of Lyt-2+ T lymphocyte (equivalent to CD8+ T cells) from immunised to susceptible mice, thereby rendering them resistant to *C. ruminantium* infection (15). Furthermore, Totté *et al.* (72) demonstrated that IFN-α, a product of activated macrophages, monocytes and T cells, significantly retards the growth of *C. ruminantium* in vascular endothelial cells. Additionally, cattle
naturally resistant to *C. ruminantium* infection have been shown to produce IFN-α (72). Mahan *et al.* (31) showed that it is possible to inhibit the growth of *C. ruminantium* with supernatant fluid obtained from Concanavalin A-stimulated cultures. In subsequent studies, Mahan *et al.* (30) demonstrated that blocking antibodies raised against IFN-γ abrogated the inhibitory effects of Concanavalin A supernatants on *C. ruminantium* replication. These findings were further supported by Totté *et al.* (73) who used IFN-γ, a product of T cells and natural killer cells, to block proliferation of *C. ruminantium* as well as render host cells refractory to *C. ruminantium* replication. Unlike the situation in mice, the inhibitory effect of the cytokine in cattle is not mediated through nitric oxide production (30).

From the available information, it is apparent that more studies are required in the area of specific immune responses to *C. ruminantium* to delineate the role of CD4+ and CD8+ T cells. More importantly, there is a need to identify antigens/epitopes inducing protective immune response, to facilitate development of a vaccine against cowdriosis.

7. **EHRlichiosis**

Ehrlichiosis, caused by rickettsial organisms, occurs in a variety of animals including humans, dogs, horses, cattle and sheep. *Ehrlichia chaffeensis* infection in humans is characterised by headache, myalgia, anorexia, nausea, vomiting, chills, and in some cases, a rash. Infection is often accompanied by leukopenia, thrombocytopenia, high activated coagulation time (ACT) and aspartate aminotransferase (AST) values (14). *Ehrlichia chaffeensis* is closely related to *E. canis*, the cause of canine ehrlichiosis, a major health problem for dogs especially pure breeds such as alsatians (48). The disease is encountered in tropical and subtropical regions of the world where it is transmitted by the common brown dog tick, *Rhipicephalus sanguineus*. The symptoms vary from mild to severe tropical pancytopenia. Frequently observed clinical manifestations are high fever, leukopenia and thrombocytopenia, lymph node enlargement, cachexia, pneumonia, epistaxis, oedema of the legs and scrotum, vomiting, conjunctivitis and corneal opacity. Extensive haemorrhages are observed prior to death. Equine monocytic ehrlichiosis (EME) is a disorder caused by *Ehrlichia risticii*. EME is characterised by leukopenia, fever, anorexia, diarrhoea, colic, laminitis and death in approximately 30% of the infected horses (77). Bovine and ovine ehrlichiosis are benign infections caused by *Ehrlichia bovis* (cattle), *E. ovina* and *E. phagocytophila* (sheep). There is, however, a bovine petechial fever (Ondiri Disease) caused by *Ehrlichia (Cytoecetes) ondiri* that induces marked capillary damage (13). This infection has only been reported in Kenya and is characterised by widespread petechial and ecchymotic haemorrhages on the mucosal surfaces and throughout the serosal surfaces, high fever, lymph node enlargement and splenomegaly. These changes are often accompanied by hydropericardium. Death occurs in over 50% of the infected cattle.

7.1. **Humoral immunity**

There is very little information available on the nature of immune responses elicited by *Ehrlichia* organisms in the various hosts. To date, induction of neutralising antibodies of the IgG class has been demonstrated, and these antibodies function through antibody-dependent cytotoxicity directed towards infected macrophages (37, 57). In *E. risticii* infection in horses, the appearance of neutralising antibodies in circulation coincides with the clearance of the organisms, suggesting that humoral mechanisms may be important in protective immunity. The neutralising antibodies do not inhibit uptake of *E. risticii* but interfere with survival of the parasite in the macrophage (37, 79).

7.2. **Cell-mediated immunity**

It has been observed that lymphocytes obtained from dogs infected with *E. canis* exhibit cytotoxicity for autologous monocytes, although no antigen(s) involved in this phenomenon has been defined. Involvement of cell-mediated immunity is further confirmed through induction of marked blastogenesis in splenocytes obtained from *E. Risticii*-infected mice on re-exposure to *Ehrlichia* antigens (78). However, there is a need to determine the nature of the T cell involvement in ehrlichiosis in detail to facilitate identification of protective antigens.

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