Potential role of lectins in ticks: *Rhipicephalus appendiculatus* and *Rhipicephalus pulchellus* (Acari: Ixodidae)

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Abstract

The role of lectins in tick vectors was investigated by comparing the presence of lectins in the hemolymph, guts and salivary glands of two tick species: (1) *Rhipicephalus appendiculatus*, the vector of *Theileria parva*, and (2) *Rhipicephalus pulchellus*, which is refractory to this protozoan parasite. There was variation in the agglutination titres with erythrocytes from bovine, mouse and rabbit erythrocytes, suggesting different binding affinities and quantities of lectins. The hemolymph from *R. pulchellus* gave the highest agglutination titer with mouse erythrocytes (1024) compared to that obtained with rabbit cells (8). No agglutination was observed with bovine erythrocytes when assayed with the gut, salivary gland or hemolymph lectins from either tick species. The gut lectin from *R. pulchellus* also demonstrated high titers (1024) with rabbit cells but moderate titers with mouse erythrocytes (256). Hemolymph from *T. parva*-infected *R. appendiculatus* contained double (512) the quantities of lectins compared to uninfected ticks (256) with mouse erythrocytes suggesting an increase in the amount of lectin in the presence of infection and implying a role of lectins in the immunity of these arthropods.

Lectin from the gut of *R. appendiculatus* agglutinated purified piroplasms, a stage in the *T. parva* life cycle ingested by the tick when it feeds on infected cattle. The anti-lectin antibodies conjugated with Fluorescein isothiocyanate (FITC) bound to the piroplasms, suggesting a possible point at which intervention may be developed for the control of theileriosis. The significance of lectins in tick parasite transmission lies in the fact that these molecules play an important part in cell-to-cell adhesion. Defining their binding specificities and quantities in a given tick species may lead to development of a novel type of disease control whose mode of action would be based on competing for the ligands for binding to pathogen receptors or preventing adhesion to host tissues (transmission-blocking), thereby preventing infection.

This study revealed that tick-derived antigens in the form of lectins produced antibodies that recognized the piroplasm as well as schizont stages of *T. parva*. With increasing attention on vaccination of cattle against ticks, there is a need to identify and show target antigens within the tick. The study has elucidated three targets, the salivary gland, hemolymph and the gut. It forms one of the first reports on the effect of lectin antibody on the development and transmission of *T. parva*. This also means that lectins may be used as disease immunogens that can possibly intervene at the piroplasm stage and the schizont stage.

Key words: Lectins, ticks, Transmission blocking vaccine, *Rhipicephalus appendiculatus*, *Rhipicephalus pulchellus*

Introduction

Ticks are well adapted to a large variety of ecological niches and have become disease agent vectors for humans, domestic animals and wildlife. Among ticks, *Rhipicephalus appendiculatus* (Neumann 1901) (Acari: Ixodidae) is a vector of a protozoan parasite *Theileria parva* (Theiler 1911) (Family: Theileriidae) that causes a fatal disease in cattle known as theileriosis. The disease is found in 11 countries in eastern, central and southern Africa (Norval et al. 1992). The potential distribution of this arthropod vector in Africa based on eco-climatic factors covers almost the whole continent and...
is influenced by high temperatures and humidity (Figure 1). This Map illustrates the great potential
danger for Africa if *R. appendiculatus* is not eliminated or controlled. The economic loss due to
theileriosis in Africa is enormous with a mortality of 1.1 million cattle annually (Mukhebi 1992).
*Rhipicephalus appendiculatus* transmits *T. parva* transstadially, either from larva to nymph or
nymph to adult. This tick feeds on a variety of hosts ranging from domestic cattle to buffalo which
form reservoirs for parasites (Walker et al. 2005).

![Map of Africa showing potential climatic suitability for *Rhipicephalus appendiculatus*]

**FIG 1.** Potential climatic suitability for the distribution of *Rhipicephalus appendiculatus* in Africa.
Numbers indicate the ecoclimatic index on a scale of 0–75 where 0 is not suitable, and 75 is very
suitable, for *Rhipicephalus appendiculatus*.

The susceptibility of vectors to vertebrate pathogens has received much attention in the search
for alternative methods of control for vector-borne diseases. The transmission of *T. parva* is
influenced by a number of factors including environment, host, parasite and vector, all of which are
interrelated (Norval et al. 1992). The interaction between the vector and parasite is complex and
evidence suggests that lectin-ligand interactions occur between the vector and parasite (Welburn &
Maudlin 1990; Pathak, 1993; Kamwendo et al. 1995).

According to Hirabayashi (2002), lectins are simply proteins that specifically bind (or cross
link) carbohydrates. Sugars with similar properties are found on the surfaces of many pathogens.
The binding specificity of lectins therefore provides them with the ability to recognize a wide variety
of pathogens. The production of lectins could therefore provide a very effective mechanism of
recognition against a broad range of pathogens. Lectins are important to immunity and immune
responses when parasites invade vectors (Dimopoulos et al. 2001). Agglutination of parasites by
lectins has been reported in many arthropod disease agent vectors and the ability of lectins to react
with microbial glycoconjugates has been used to develop diagnostic probes (Munoz-Crego et al.
Lectins are believed to play a key role in the initiation of infection by bacteria as they mediate adhesion to the epithelial cells of the host (Rudenko et al. 1999).

Lectins also play a role in lectin-phagocytosis relevant in vector and host-tissue interactions (Ofek & Sharon 1988). Studies in arthropods have revealed lectins in the hemolymph that play a role in host-parasite relationships (Welburn & Maudlin 1990). Insects are known to have both humoral (noncellular) immunity, through lectin or lectin-like substances, and cell-mediated (hemocyte) immunity against invading parasites (Pathak 1993). This fact has generated much interest in arthropod vectors of pathogens of both of veterinary and medical importance (Sebitosi et al. 1998). Among Diptera, agglutination of parasites has been reported (Maudlin & Welburn 1994; Mohammed & Ingram 1994; Wallbanks et al. 1986).

Despite reports of lectins in insects and soft ticks, little is known about the role of lectins in hard ticks (Ixodidae) and, in particular, about how these molecules can be used in interventions against the disease agents transmitted by these vectors. In hard ticks, lectins have been isolated in the hemolymph, salivary glands and guts (Sebitosi et al. 1998). These authors suggested further studies into the role of lectins. This article addresses the role of lectins by comparing their quantities in a refractory tick species Rhipicephalus pulchellus (Gerstaker 1873), to those of a vector tick species, R. appendiculatus, by using hemagglutination assays. The term refractory in this sense means resistant (unsusceptible) and refers to the inability of the tick to transmit the parasite. Physiological properties of ticks are important for understanding how T. parva and other microorganisms circulate within the tick and how they are transmitted to vertebrates. In other words, understanding physiological conditions such as lectin composition would help speculate on how the vectors remain unaffected by the parasite inside the vector. This phenomenon could then be applied in explaining the vector-parasite interactions from which possible novel interventions, such as manipulating the susceptibility of ticks to disease agent transmission, could be developed.

This article discusses the interaction of molecules called lectins found in tick hemolymph, salivary glands and gut with different stages of the parasite T. parva, namely piroplasms and schizonts, in order to determine the possible role of lectins. Consequently, targets that could be used as novel antigens for developing a transmission blocking vaccine would be identified for Theileria parva.

The objectives of this article are therefore firstly to compare the quantity of lectins present in refractory (R. pulchellus) and susceptible (R. appendiculatus) ticks. Secondly, the roles played by these lectins in the parasite life cycle are elucidated with a view to identifying possible antigens for novel methods of disease control such as transmission-blocking in theileriosis.

Materials and Methods

Experimental animals, parasites and ticks

Rhipicephalus appendiculatus (Figure 2) of the Muguga stock (RAM, Bailey 1960) originally obtained from Kenya Agricultural Research Institute (KARI) in Muguga in 1978 was the main species investigated. It is found in eastern, central and southern Africa. Figure 1 illustrates the eco-climatic index scale ranges from 0–75 and predicts the distribution of R. appendiculatus in South Africa, West Africa, East Africa and Madagascar. The value 0 denotes unsuitability for survival and 75 great suitability.

For comparative purposes, R. pulchellus (Figure 3), a species that transmits Theileria taurortraagi and not T. parva, was used. Tick species (Figures 2 and 3) were obtained from the International Centre of Insect Physiology and Ecology (ICIPE) and the International Livestock Research Institute (ILRI) colonies in Nairobi, Kenya.
Nonfed adults of *R. appendiculatus* were maintained in 80 x 25 cm flat-bottomed glass tubes plugged with cotton wool and kept in dark incubators at 20°C and 85% RH maintained by including a tray of water and sand in the incubator. Engorged females were maintained at 28°C and at 85% RH to lay eggs, as were eggs and all engorged instars. Nymphs were kept at the above conditions until they molted into adults. The newly molted adults were maintained at 28°C for about three weeks, then put on cattle to feed until engorged. *Rhipicephalus pulchellus* were kept in similar conditions as *R. appendiculatus* (nonfed stages at 20°C and fed ones at 28°C) and all at 85% RH.

Feeding of immature ticks was carried out on New Zealand white rabbits (*Oryctolagus cuniculus*, Linnaeus) using ear bags (Bailey, 1960). The same rabbit species was also used in developing polyclonal antibodies.
Male and female (6–18 months old) Boran cattle (Bos indicus) were used in the experiment. The animals were kept off spray of acaricides and contained no antibodies against *T. parva*. The latter was confirmed using the indirect fluorescent antibody test (Goddeeris et al. 1982). Infected cattle were maintained in the Tick Unit at the International Livestock Research Institute (ILRI), in tick secure, self-contained pens, surrounded by a water moat and fly-proof netting. The animal pens were kept tick-free with dioxathion (Delnav, Coopers LTD, Nairobi, Kenya) and treated with high-pressure steam at 130°C from a generator (Karcher, Germany) to prevent access by extraneous ticks. Balb C mice of either sex (6–8 weeks old) were used in the development of monoclonal antibodies.

**Rearing *T. parva* infected ticks**

*Theileria parva* Muguga stabilate 3087 was used to infect the cattle. Cultures were maintained in liquid nitrogen as described previously (Dolan et al. 1984; Morzaria 1989), thawed in a water bath at 37°C, and allowed to equilibrate for 30 minutes. They were then diluted in a diluent comprising Eagle’s minimum essential medium (MEM) and bovine plasma albumin (BSA) at a ratio of MEM/3.5: BSA/7.5% glycerol at 4°C, to produce a final dilution of 1:20 (Musoke et al. 1992). Cattle were injected with 1 ml on the left ear lymph node and monitored for temperature and parasitosis.

Infected cattle were monitored for temperature rise daily at the same time (08:30 hr), which is an indication of the onset of parasitemia. This was followed by needle biopsies taken from the parotid lymph node, on day five and thereafter. The biopsies were smeared, Giemsa stained and examined for schizonts. Starting from the 10th day, biopsy smears from the contra lateral prescapular lymph nodes and blood smears from the peripheral ear vein were examined for both schizonts and piroplasms. Parasitemia was estimated by counting the number of infected red blood cells per 1000. For this purpose, blood was collected from the jugular vein in EDTA coated vacutainer tubes. The packed cell volume was determined using a microhematocrit. From day 11, when the piroplasm parasitemia was above 4%, ticks were applied. Any animals that became highly infected with East Coast Fever (ECF) were euthanized.

To obtain infected ticks, nymphs were fed on *T. parva* Muguga infected cattle, until the ticks dropped. They were applied in ear bags or in back bags attached by glue. Prior to the application of ticks, the backs of the cattle were shaved using an electric shaver (Osler manufacturing Co. Milwaukee, Wisconsin. USA). Uninfected *R. appendiculatus* (800 nymphs) were applied on days 11, 13, 15 and 17 in two front cloth back patches, adhered to cattle using glue (Pattex, Henkel Chemical, Nairobi, Kenya). On engorgement (5 days later), ticks were weighed and kept in glass tubes (200 per tube) at 24°C; 85 % RH.

To determine infection prevalences in ticks, nymphs that dropped were maintained at 24°C RH 85 % and allowed to molt into adults (3 weeks). They were then kept for a further three weeks at 20°C. About 200 adult *R. appendiculatus* (100 females and 100 males) were selected and applied to rabbit ears for four days. They were then removed, and 30 males and 30 females randomly selected and dissected for determining salivary gland infection of *T. parva*. This protocol of dissection was replicated at least six times.

**Collection of hemolymph and dissection of guts and salivary glands**

Hemolymph was collected from partially fed (day 4) *R. appendiculatus* and *R. pulchellus* adult females maintained at 28°C, 95% RH and 12hr photoperiod. The hemolymph was collected in 10µl micropipettes from excised legs of ticks and pooled in Eppendorf tubes containing phenylthiourea (PTU; Sigma, UK) to prevent melanization. Throughout the process, the tubes were maintained on ice (Sebitosi et al. 1998).

Guts from partially-fed (day 5) adult females were dissected in a Petri dish flooded with distilled water and immediately stored in liquid nitrogen. Eight grams of gut was homogenized in 10 ml buffer...
(0.2M Sodium phosphate; 5M NaCl) containing inhibitors 0.1 M EDTA, 0.1 M Phenyl methyl sulphuric fluoride (PMSF) and 0.01% Sodium azide. The mixture was centrifuged at 900 x g for 30 minutes followed by hand homogenization on ice. It was then sonicated (Branson Sonifier, Model B-30, USA) for 2 minutes, and after 30 seconds it was solubilized in 0.01 % Triton X 100 followed by Ultra centrifugation at 3000 x g for 1 hr (Beckman) at 4°C (Sebitosi et al. 1998).

Salivary glands were dissected in Dulbecco’s Phosphate buffered saline (PBS) and spread on slides. The salivary glands were dissected from ticks that had fed on rabbit ears for three days. Ticks were washed in 70% alcohol and dissected and salivary glands were collected in PBS at pH 7.2 on ice. Salivary glands for hemagglutination assay were stored at -70°C until required and then processed as described above for guts.

In order to determine infection prevalences in salivary glands, the salivary glands obtained through dissection were fixed in absolute methanol for 10 minutes, hydrolyzed in 5M HCL for 1 hr. and then stained in Schiff’s stain for 1 hr. They were rinsed in distilled water and air dried before examination in phase contrast microscope. Positive parasitized salivary gland acini could be identified by the periodic acid Schiff reaction which shows the presence of cells as bright pink areas of sporozoites for T. parva, according to Young & Leitch (1983).

Detection of lectins by Hemagglutination

In order to detect lectins in hemolymph, hemagglutination was carried out using different host red blood cells (rbcs) from rabbits, mice and cattle (bovine). Blood was collected in heparin. The red blood cells were cleaned by washing twice in Tris-HCl buffer (pH 7.2) and centrifuging at 100–200 x g for 15 minutes until clear. Doubling dilutions of hemolymph, each 50 µl in volume, were prepared in Tris-HCl buffer in V-shaped microtiter plates to give final dilutions ranging from 2⁻¹ to 2⁻¹¹ (Sebitosi et al. 1998). Fifty µl of 3% rbcs suspension were added to all the wells and incubated overnight at 4°C or for 2 hr at 22–25°C. The plates were kept moist by placing wet cotton wool inside the plate. The plates were then examined under an inverted microscope and agglutination scored at an arbitrary scale of 3+ (100 % agglutination), 2+ (50 %) and 1+ (10 %) and 0 (no agglutination). End point titters were reciprocals of the dilution that had just caused visible agglutination (Sebitosi et al. 1998; Mohammed et al. 1992; Ingram & Molyneux, 1991). These experiments were carried out in three replicates at least four times. The number of ticks used per experiment ranged between 800–1000 to collect the required quantities of hemolymph or salivary glands and at least 8 gm of gut needed for each experiment. On each microtiter plate, wells were replicated three times.

Blood from the various hosts was washed in PBS (pH 7.2) by centrifuging at 100 x g for 15 min until the supernatant was clear. A 3% suspension of the rbcs was made in PBS containing Ca²⁺/Mg²⁺ pH 7.2. The crude samples were slowly thawed from -70°C (over ice). Fifty µl of PBS was aliquoted into U-shaped 96 well microtiter plates and 50 µl of samples then added and serially diluted. Fifty µl of a 3% rbcs suspension was added to all wells and incubated at 4°C overnight or for 2h at room temperature. The agglutination titters were scored as described for hemolymph (Sebitosi et al. 1998). Two approaches of investigating the role of lectins are described here: one directly agglutinating purified piroplasms, and the second, using antibodies to these lectins conjugated with dyes.

Piroplasm-lectin Interactions: Agglutination of the piroplasms

Blood from calves (6–9 months old) with at least 20% parasitemia of T. parva was collected in Alsever’s solution containing heparin (final conc. 50µl/ml), centrifuged three times (350 x g ; 30 min; Sorval) and theuffy coat removed each time using a Pastuer pipette and a water vacuum pump. Following a fourth centrifugation, the cells were resuspended in Tris-EDTA-NaCl (TEN) buffer, pH 8.0 (20mM Tris; 10 mM EDTA; 100mM NaCl). The packed cell volume was estimated.
by taking blood in 75 µl Hematocrit capillary tubes (Cristaseal, Hawsley & Sons, Ltd, Sussex, UK) and spinning it for 5 min in a micro-hematocrit centrifuge (350 x g). The packed cell volume was recorded from the micro-hematocrit reader in percentages. The cells were then lysed using the detergent saponin (Sigma). The detergent and the cells were both warmed separately in a water bath at 37°C for 30 min. Equal volumes of warm saponin (1mg/ml) and cells were lysed for 30 sec. The mixture was diluted four times the volume with TEN buffer and centrifuged (350 x g; 20 min; Sorval) and the supernatant saved. This was followed by a further spin (1000 x g; 30 min; Beckman ultra centrifuge). The supernatant was discarded. The pellet was then resuspended in TEN buffer and re-centrifuged (1,000 x g; 30 min; Beckman) until the pellet was white (only the soft pellet was taken) which constituted the purified piroplasms.

Agglutination of piroplasms inside red blood cells

Blood from infected cattle of at least 20% parasitemia was collected in heparin/Alsevers and processed in TEN buffer as previously described, except that the cells were not lysed in saponin. The cells were diluted in phosphate buffered saline (PBS) and spotted on a microscope slide (Multispot, superior, Germany). The excess was discarded leaving a thin film. The antigen was then added and incubated for 1 hr at room temperature (22–25°C). The agglutination was checked under a microscope.

Purified piroplasms were diluted in PBS (1:400) spotted on slides (Multispot Superior, Germany) and incubated with similar volumes of the lectin, in serial dilution, for 1 hr at 22–25°C. The agglutination was scored under a microscope as reported in Sebitosi et al. (1998). Controls included blood from a naive cow as well as normal rabbit sera that gave an indication of the non-specific binding.

Visualizing lectin/piroplasm interactions with dyes

Agglutination of live piroplasms was visualized using the 5-carboxyfluorescein diacetate (CFDA). Briefly, the CFDA stock (5mg/ml) was diluted 1:50 and 100 µl added to 250 µl piroplasm mixture, incubated (15 min) in the dark at 22–25°C, then washed once for 5 min at 1000 x g at 4°C to remove the CFDA. The pellet was resuspended in 500 µl PBS using a 26-gauge needle and washed (1000 x G; 5 min; 4°C). The pellet was then resuspended in 90 µl PBS, then ten µl were transferred to a slide. Lectin (10 µl) was added and the mixture was incubated at 22–25°C (30 min). It was then coverslipped and the slide was examined under a fluorescent microscope.

Test sera were precipitated with 50% ammonium sulphate and dialyzed against PBS (Dulbeccos) at pH 7.4 in a cold room at 4°C. A protein A column was equilibrated with PBS at pH 7.4 and the sample loaded. Fractions were eluted with glycine-HCL (2M) at pH 2.6 and neutralized with Disodium phosphate salt. One ml fractions were collected and tested for agglutination inhibition. The “run through” contained the hemagglutination inhibition activity, which means that the anti-lectin was in the IgM fraction since Protein A binds IgG. Further purification was, therefore by size and was carried out by gel filtration on Sepharose 6B. The sample present in the second peak was conjugated with fluorescein isothiocyanate (FITC).

The antiserum was dialyzed against 0.25M Carbonate/bicarbonate at pH 9.6. The protein content of this fraction was determined and then mixed with FITC (0.5 mg/1mg protein) in 10 times the volume of the dye for 15–24 hr or overnight at 4°C. This was carried out using carbonate/bicarbonate buffer pH 9.6. Separation of the conjugated protein from the free fluorochrome was carried out on a Sephadex G 50 column equilibrated with PBS. Since the fluorochrome:protein ratio is only an average determination, some protein molecules would have more fluorochrome and others less. As each fluorochrome molecule is added to the protein molecule, there is a net decrease in charge. Consequently, conjugated antiserum was fractionated by ion exchange chromatography
using an elution gradient of increasing ionic strength. This was carried out on a Diethyl amino ethyl (DEAE) 52 ion exchanger using elution buffer of increasing ionic strength (carbonate/bicarbonate buffer pH 9.6: 0.1, 0.3, 0.4 and 0.8 M NaCl).

The piroplasms incubated with the lectin (1 hr; 22–25°C) were cytospun on a slide, air-dried and fixed in 3.7% formaldehyde (10 min). They were then rinsed in PBS to remove the fixative and the conjugate was applied. The conjugate was diluted 1:50–1:5000 in order to select the best working dilution, which was found to be 1:1000. The conjugate was diluted in Evans blue and applied to piroplasms previously incubated with lectins. They were incubated for 30 min in a moist chamber in the dark and then washed three times in PBS to remove excess conjugate. A drop of glycerol (50% glycerol: PBS) was used as the mounting medium and piroplasms were observed on a Leitz Orthoplan fluorescent microscope fitted with a ploemopak 2 vertical illuminator and a HBO 100 mercury lamp (Leitz, Wetlar, Germany).

Results

Comparison of lectin activity in infected and uninfected ticks

The agglutination activity of the lectins in the hemolymph of R. appendiculatus that were infected with T. parva and those of uninfected R. appendiculatus ticks was variable depending on the erythrocytes used in the hemagglutination assay (Table 1). Bovine erythrocytes for instance, did not agglutinate any of the lectins isolated from either tick species similar to previous reports (Sebitosi et al. 1998). Rhipicephalus pulchellus hemolymph showed higher agglutination titers (1024) compared to those of uninfected R. appendiculatus (256); this was a four-fold lectin activity difference using mouse erythrocytes (Table 1). The gut lectins isolated from R. pulchellus gave different titers with different erythrocytes. For instance, a titer of 1024 was obtained with rabbit erythrocytes compared to 256 with mouse cells. No lectin activity was detected in the salivary glands of R. pulchellus with either bovine or rabbit erythrocytes. Lectins from the hemolymph of R. pulchellus, on the other hand, gave a high titre of 1024 with mouse cells and only 8 with rabbit erythrocytes.

Lectins from salivary glands of R. appendiculatus infected with T. parva showed a titre of 64 with mouse erythrocytes. However, no agglutination was observed with bovine or mouse erythrocytes among uninfected R. appendiculatus. On the contrary, hemolymph from uninfected R. appendiculatus demonstrated lectin activity (512) with rabbit cells. Their infected counterparts showed a titer of only 4 with rabbit erythrocytes. Mouse erythrocytes gave double (512) the agglutination titre with infected R. appendiculatus as compared to the uninfected ones that showed 256 with similar cells. Neither the uninfected nor the infected R. appendiculatus lectins produced any agglutination with bovine erythrocytes.

These results demonstrate the ability of lectins from the hemolymph, gut and salivary glands of R. appendiculatus and R. pulchellus to agglutinate mouse and rabbit erythrocytes. The trends indicate that refractory ticks have more lectins compared to susceptible ones and that infected ticks contain greater quantities of lectins. This is based on the observed high lectin titres in R. pulchellus hemolymph and gut when assayed with mouse and rabbit erythrocytes. Table 1 shows that both infected and uninfected R. appendiculatus hemolymph lectins were agglutinated by mouse rbc s at 512 and 256 respectively. Comparing the two tick species in this regard indicates that refractory species have greater amounts of lectins than susceptible ones. This would further imply that lectin plays a role in modulating or preventing T. parva parasites from developing in this species.

These ticks seem to have different lectins in different tissues (gut, salivary gland and hemolymph) as evident in the different agglutination titers obtained with various rbc s. With mouse
rbc, *R. pulchellus* hemolymph lectins produced titers of 1024 and 256 with the gut lectin. Hemolymph lectins did not agglutinate bovine rbc (0). These results are in agreement with previous reports of variation in titers of agglutination in tick lectins (Sebitosi et al. 1998; Veres & Grubhoffer 1990) and indicate that lectins from the various tissues are different or heterogeneous in nature.

### TABLE 1. Comparison of lectin activity between *Theileria parva* infected and uninfected ticks.

<table>
<thead>
<tr>
<th>Tick species</th>
<th>Infection status</th>
<th>Tick tissue</th>
<th>Source of erythrocytes</th>
<th>Agglutination titres</th>
</tr>
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<tr>
<td>R. pulchellus</td>
<td>_</td>
<td>hemolymph</td>
<td>rabbit</td>
<td>8</td>
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<td>mouse</td>
<td>1024</td>
</tr>
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<td>bovine (cow)</td>
<td>0</td>
</tr>
<tr>
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<td>_</td>
<td>Gut</td>
<td>rabbit</td>
<td>1024</td>
</tr>
<tr>
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<td>_</td>
<td>Gut</td>
<td>mouse</td>
<td>256</td>
</tr>
<tr>
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<td>512</td>
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<td>0</td>
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<tr>
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<td>+</td>
<td>hemolymph</td>
<td>bovine</td>
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</tbody>
</table>

End point titres expressed as the reciprocals of the dilutions (1/n): 0 = negative at 1:2

### Agglutination of piroplasms

The lectins agglutinated fresh piroplasms, which were observed using carbocyanine dye, as green clusters or clumps on the slides (Figures 4A and 4B). Controls treated with PBS buffer only appeared as scattered piroplasms. Agglutination of fresh piroplasms is important as it implies a role of lectins in the recognition or binding/cell-to-cell adhesion in the vector-parasite interactions. Lectins may serve as recognition sites where interventions can be developed.

### Binding on piroplasms

Purified piroplasms incubated with FITC conjugated lectin antibody showed fluorescence demonstrating that lectin antibody bound onto purified piroplasms (Figure 5). The surfaces of erythrocytes in the control group, however, showed no fluorescence.
FIG 4A. Agglutination of purified *Theileria parva* piroplasms following treatment with tick gut lectin and labelling with carboxyfluorescent diacetate dye. Lectins are proteins or glyco proteins that specifically bind (or cross link) carbohydrates and in this case are seen to agglutinating parasites (piroplasms) (phase contrast x 120).

FIG 4B. Control: Phosphate buffered-saline (PBS) Treated piroplasms showing no agglutination. Piroplasms are a developmental stage in the life cycle of a protozoan parasite *Theileria parva* that causes East Coast Fever in Cattle.

FIG 5. Fluorescence of *T. parva* piroplasms with fluorescein isothiocyanate (FITC) conjugated anti-lectin antibody (x 1200)
Discussion

Detection of lectins in ticks

Lectins were detected in the gut, salivary glands and hemolymph of *R. appendiculatus* and *R. pulchellus*, using hemagglutination of the erythrocytes. Similar detection of lectins in the guts of invertebrate disease agent vectors has been shown in mosquitoes (Mohammed et al. 1992), tsetse flies (Ingram & Molyneux 1991; Maudlin & Welburn 1994; Welburn et al. 1989) in argasid and ixodid ticks transmitting spirochetes (Grubhoffer et al. 1993) and in *Rhodnius prolixus*, an important vector of *Trypanosoma cruzi* (Pereira et al. 1981), making hemagglutination a viable technique for detecting lectins in ticks.

Infected and uninfected ticks

The fact that the refractory tick *R. pulchellus* showed higher agglutination titres in its hemolymph compared to the susceptible *R. appendiculatus* that is a vector of *T. parva*, perhaps suggests that refractory species contain more of these molecules than their susceptible counterparts. Among *R. appendiculatus*, *T. parva*-positive individuals demonstrated higher titers (512) compared to 256 in uninfected individuals, showing that different levels of lectins are present in uninfected and infected ticks. This fact indicates that lectins are secreted when ticks are infected, implying that lectins play a role in tick immunity. Hemolymph from the refractory *R. pulchellus* gave an agglutination titre of 1024, which was triple that of infected *R. appendiculatus* ticks (256). Based on these observations, the quantity of lectins seems to be larger in infected ticks compared to uninfected ticks, postulating a possible role of lectins in parasite-vector interactions.

Furthermore, refractory ticks had three times the amount of lectins in their hemolymph, compared to susceptible ticks. This may mean that lectins could play a role in the susceptibility of ticks to pathogens or indeed prevent infections altogether. In other words, the increase of lectins in infected ticks may indicate that lectins play a role in the defense (prevention) of infections. These lectins seem to be produced or induced in infected ticks.

On the other hand, moderate lectin activity titres (64) were detected in the salivary glands of infected *R. appendiculatus* ticks compared to uninfected *R. appendiculatus* implying that lectins are probably induced in large quantities in the presence of infections. Similar reports have been made in other arthropods such as insects (Ingram & Welburn 1994). It is possible that more lectins are synthesized in the presence of foreign bodies like parasites. Variation in titers of agglutination may imply a variation in the amounts of lectins with different affinities, or perhaps different binding affinities for the various rbcs which is common among invertebrates (Fries 1984). Lectins from the hemolymph and gut of *R. appendiculatus* showed different sugar specificities which led to the agglutination of erythrocytes in some species by both lectins but to different titres and with different absorptive capabilities against erythrocytes of other species (Sebitosi et al. 1998). Such a phenomenon is common among invertebrates (Ishiyama et al. 1974).

The role of lectins

It is a challenge to speculate on the possible role of the gut lectin in *Rhipicephalus* ticks since it is most improbable that this lectin would agglutinate cattle erythrocytes in vivo. Earlier studies (Sebitosi et al. 1998) showed that the gut lectin was an integral membrane protein, not present in the lumen. The possible role, therefore, would be to fix the parasite onto the gut wall following lysis of the rbcs where further differentiation would occur. In the present research, lectins were shown to agglutinate purified piroplasms but not those present in the red blood cells, and their antibodies bound to the piroplasms, which is the stage of the parasite ingested by the tick. Lectin-parasite interactions have been reviewed by Jacobson & Doyle (1996). A bipartite model where midgut
Lectins promote cell death, but are essential for trypanosome maturation, was proposed (Jacobson & Doyle 1996). Furthermore, refractory ticks have been shown to have more lectins. In insects such as *Glossina*, lectins have been shown to kill trypanosomes as well as to signal their maturation and establishment (Maudlin & Welburn 1987; Welburn & Maudlin 1990). The events triggering maturation are reported to involve stimuli from lectins of both the midgut and hemolymph, and various strains of trypanosomes seem to require different levels of lectins to induce maturation. Volf et al. (1994) surveyed the hemagglutination activities in the gut and head extracts of various species and geographical populations of phlebotomine sand flies by comparing their lectin quantities and sugar binding specificity. They found that there was a species-specific interaction between lectins and *Leishmania* parasites. Very similar results have been reported by other authors (Wallbanks et al. 1986; Ingram & Molyneux 1991) working on mosquitoes.

The inability of the tick gut and hemolymph lectins to agglutinate bovine erythrocytes was also observed by Sebitosi et al. (1998) and suggests a possible host-vector-parasite evolutionary adaptation. The parasite seems to be well adapted in that it is passed on to the invertebrate host where it is deposited in the gut and from where it migrates to other regions where further development occurs. In the gut, the rbcs are lysed and the piroplasms released (Norval et al. 1992). They further differentiate into micro and macro gametes (Melhorn & Schein 1984; Young & Leitch, 1981) and develop into male and female gametes. Fusion takes place and a zygote is formed in the gut lumen. It then undergoes development in the gut epithelium (Walker 1990). Had the lectin agglutinated the bovine rbcs, perhaps hemolysis and subsequent release of piroplasms would have been prevented thereby eliminating the parasite. In earlier studies by Sebitosi et al. (1998) blood from buffalo, an animal in which the parasite produces no clinical symptoms, was agglutinated by the gut lectins implying a role of lectins in modulating parasite infection. The ability of the parasite to survive in some hosts and not in others is probably due to several factors. In African buffalo plasma and serum for instance, there exists a factor that kills *Trypanosoma brucei brucei*, *T. b. rhodesiense*, *T. b. gambiense*, *T. evansi*, *T. congolense* and *T. vivax* in vitro (Reduth et al. 1994). Similar trypanocidal serum was observed in the eland whereas sera from oryx, waterbuck, yellow back duiker, cattle, sheep, goat, horse, mouse, rat and rabbit was not trypanocidal (Reduth et al. 1994; Nguu et al 1996; Black et al. 1999).

Looking at the carbohydrate specificities of lectins helps to define the structures of the lectins. The inhibition of gut lectin activity in *R. appendiculatus* was demonstrated by Fetuin, a glycoprotein, and the sugars D(-) mannosamine, D (+) glucosamine and D(+) galactosamine (Sebitosi et al. 1998). On the other hand, the hemolymph lectin was found to be inhibited by Xylose, N-acetyl galactosamine and β-d-Fructose by the same authors. This difference in carbohydrate-binding specificity of lectins with respect to simple sugars is common (Sharon and Lis, 1989). This may help explain why, in this study, some of the rbcs were agglutinated and others were not. Lectins recognize carbohydrate structures on the surface of the cells that are present in some cells and absent from others, revealing tick lectins to be heterogeneous with regard to their reactivities towards erythrocyte surface carbohydrates. These properties have also been observed in insect lectins (Pathak 1993).

Lectin amounts seem to vary in different tick species as observed from the differences in agglutination titres with erythrocytes. This variation in lectin quantity may be reflected in the differences in infection prevalences among ticks of the same species, same locality or different ecological zones. Further research is required in this area as it may have implications for the epidemiology of vector-borne diseases.

This study has provided data that are relevant to the efficacy of vaccines that target antigens within the tick and is in agreement with Willadsen et al. (1989). Antibodies to the lectins were demonstrated to bind not only to piroplasms but also to the schizont stages found in cattle. This
means that lectins may be used as disease interventions at the piroplasm stage and possibly the schizont stage. This study has also demonstrated three other important aspects, namely that different lectins exist in the ticks, *R. appendiculatus* and *R. pulchellus*; that these lectins may play an important role in the development of the parasite *T. parva*; and finally, that lectins should be targeted as sub-unit vaccine molecules for the control of theileriosis. The lectins of the gut, salivary gland and hemolymph have been detected in these two species and partially characterized.

Future experiments should concentrate on the localization of the lectins, developing antibodies and elucidating what happens when they are ingested by ticks or when vaccinated into cattle. In animal hosts, studies should look for vaccines against protozoan diseases based on lectins as antigens and clone the genes for these lectins.

**Conclusion**

This study has confirmed that lectins play a role in vector-parasite interactions both in invertebrate vectors, the ticks, and vertebrate hosts, the cattle. Ticks of both species investigated were found to have different lectins in the gut, hemolymph and salivary glands as evident from the different agglutination titers with various red blood cells. This variation in agglutination means that the lectins have different binding specificities and are heterogeneous in nature. Future research should focus on isolating salivary gland lectins in *R. pulchellus* and characterizing them. These results have shown that lectins as antigens could be derived from the gut, salivary glands and hemolymph. More research needs to be done to explain the binding characteristics and any implications for animal husbandry.

This study has also highlighted the role of lectins in ticks. The fact that none of the lectins in refractory or susceptible ticks agglutinated bovine erythrocytes has evolutionally significance. There seems to be an evolutionary adaptation that exists between the vector-parasite and the host. Among refractory hosts, greater quantities of lectins seem to ward off parasite establishment as may be the case for *R. pulchellus* compared to *R. appendiculatus*. In animal hosts, cattle which were susceptible to the disease showed no agglutination with any of the lectins assayed again implying a role for lectins in modulating parasite infections. This fact points to lectins as possible antigens for disease control.

The lectin antibody immune reaction with the piroplasms and schizonts implies that lectins serve as recognition molecules in the immune system and are therefore possible sub-unit vaccine antigens which should be explored. At the piroplasm stage which is ingested by the tick, a transmission blocking vaccine could be developed with hemolymph and gut lectins as antigens. Such a vaccine could then prevent transmission of *T. parva* from cattle to cattle by ticks. At the schizont stage in cattle, studies have shown that lectin antibodies bind to schizonts and therefore are possible antigens for sub-unit vaccines.

Further research is necessary to elucidate antibody development in cattle following inoculation by lectin-derived antigens and the efficacy of such vaccines in blocking transmission. This research has shown the potential for lectins in the control of theileriosis and the urgent need for these molecules to be used as antigens for the control of vector-borne diseases. What remains to be done is to move from theory into practice in order to prevent the loss of animal and human lives due to diseases caused by pathogens and parasites transmitted by vectors by deriving the antidotes (prevention strategies) derived from the very vectors transmitting the disease agents. As the Swahili proverb goes, “Dawa ya moto ni moto” “the antidote for fire is fire or put out fire with fire”.

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