NOVEL TICK RESISTANCE ANTIGENIC INDICATORS (TRAI) FOR HOST ANIMALS

Abstract: A new protein is described which has a molecular weight of about 15,000 daltons and the N-terminus SDY-EFPKPKXRG. The protein is suitable for the control of disease and the protection of grazing cattle from ticks.
TICK VACCINE

This invention relates to antigenic compositions and in particular to vaccines for the control of tick infestations on animals.

In many countries the control of tick infestations on animals, particularly cattle, is a problem of considerable economic significance. Even with mild infestation there is a loss in meat and milk production and hide damage, and with heavier infestations the ticks must be removed to avoid death of the cattle. Ticks are also disease carriers of, for example, *Haemopla* parasites which lead to outbreaks of "tick fever".

The traditional method of control has involved the use of sprays or dipping vats with various chemicals, such as DDT, chlorinated hydrocarbons, carbamates, organophosphates, pyrethroids, and amides. Most of these chemicals have had a limited period of commercial use because various strains of ticks emerged which were resistant to them. In some cases it was possible to extend the usefulness of a particular chemical by increasing the concentration of the chemical in the spraying or dipping fluid, but this increased the cost and frequently toxic symptoms were observed in the cattle. In general the dipping or spraying treatment needs to be repeated every three to four weeks and there are additional high labour costs involved in moving the animals and moving them to and from the dipping or spraying sites.

There are further difficulties associated with the use of these chemicals. Where the chemicals are used in a concrete dipping vat set in the ground there is considerable contamination of the dip contents by urine and faeces from the cattle leading to decomposition and hence additional loss of the chemicals, and necessitating regular chemical analyses to monitor the level of the active ingredient. The dips need to be cleaned periodically and this leads to exposure of workers to potentially hazardous chemicals. Furthermore, the
disposal of dipping fluids has to be done with care to avoid environmental damage.

A further difficulty in the use of chemicals lies in the increasingly difficult task of finding new effective chemicals to combat each new resistant tick strain as it emerges. Part of this difficulty is compounded because it is desirable that the new chemical leaves minimal residues in the meat and milk of the host animal, and that such residues that are left are not harmful to consumers.

Thus for many decades there has been an urgent need for alternative safe and cost-effective means of controlling tick infestations in cattle. Management approaches such as pasture spacing have limited effectiveness and in most cases the required areas of land are simply not available. Attempts to breed tick-resistant cattle have continued for many years with little impact on the problem. Eradication campaigns have been tried and have given limited success in some areas, albeit at high cost, but are impracticable in view of the very large areas which may be involved.

We now have discovered an effective method of tick control which does not require the use of hazardous chemicals, and once a single treatment is effective for extended periods the need for regular medicating is eliminated. This method depends on the capacity of the animal's immune system to prevent tick survival or development on the animal.

One of resistant cattle for the control of cattle tick has been suggested as a supplement to tick control by chemicals. The stimulus of tick infestation is normally required before resistance is manifested; this resistance is "acquired" and is presumed to be immunologically mediated. However, the degree of resistance which an individual bovine can develop is a variable characteristic which varies widely according to breed. Some breeds, for example Fries and part Fries crosses breed, develop high levels of resistance while others, such as
most British breeds, remain largely unexplained despite extensive selective breeding programs, however, this
natural resistance has not provided a satisfactory
alternative or supplement to the chemical control of ticks.

The possibility of developing a tick vaccine has been
suggested previously. Whole tick homogenates, salivary
antigens, gut-associated antigens and certain purified
proteins or enzymes have been used by various workers (See
have produced levels of immunity which are significant but
still inadequate by comparison with chemical methods of
control. Various explanations for this limited success
have been proposed, for example, that vaccines cannot be
expected to produce a greater resistance than the
naturally acquired resistance that is characteristic of
the breed concerned, that whole tick homogenates include
immuno-suppressive components, and that purified antigens
are inadequate and require synergy with other antigens.

Australian patent specification 49906/65 discloses an
attempt to prepare tick antigens by purification of crude
whole tick extracts. However, the reported level of
isolation of useful antigens is low.

The present invention for vaccinating cattle against
cattle tick confers a high long-lasting level of
resistance on naive cattle of breeds which
characteristically develop only a small degree of natural
resistance. The efficacy of the vaccine is such as to
offer a very satisfactory alternative to chemical control,
as well as the advantage of very infrequent treatments.

Accordingly, we provide an antigenic composition which
comprises antigenic material derived from the hypodermis
of a tick, viz.

It is found that antigenic material derived from the
hypodermis (i.e. the central nervous system of the tick)
extends and improves the protection given by other
antigens e.g. those prepared from the tick gut alone,
while we do not wish to be limited by discussion.
of the possible underlying basis for our invention. It is possible that there are materials in the whole body of the

of the tick that interfere with the generation of an effective immune response otherwise stimulated by the antigens of

the tick. Thus, in a further embodiment of our invention we provide a mixture wherein the antigens are
derived from both the body and the gut of the tick.

In another aspect of our invention, we provide a

mixture for the protection of warm-blooded animals against

ticks prepared from antigens which are obtained by
dissolution of sympathetic or other nerve tissues from adult

ticks. The dissected tissues may be homogenized,

sonicated and centrifuged. The resulting mixture of

antigens consists both water-soluble and particulate

materials. We have found the particulate fraction to be

more antigenic.

In a preferred aspect of our invention, the antigenic

materials are obtained from larval ticks. The advantage

of this method lies in the higher proportion of

sympathetic in the body-weight of the larval tick compared
to that of the nymph or adult stages of the ticks. The

larval ticks may be extracted by grinding or homogenizing

or otherwise disrupting the ticks, followed by extraction

with suitable aqueous media, fractionation by suitable

methods of affinity chromatography, gel permeation

chromatography, ion-exchange chromatography, hydrophobic

gel chromatography, electrophoresis, electrosamplating,

collective precipitation, and concentration to provide the

final antigenic materials enriched in the antigenic

components.

In a further embodiment of our invention, antigenic

materials are obtained by disruption of developing tick

eggs at the stage when the materials are first synthesized

in the growing embryos.

The antigenic materials may also be obtained by

disruption and expression of tick genetic material.
In suitable host organisms, including bacteria, yeasts and other microorganisms and in cultured mammalian cells,

in another embodiment of our invention, antigenic materials characteristic of the synganglion are obtained

by in vitro culture of tick cells or tick cells hybridized with other animal cells

In a further aspect of our invention, antigenic materials, including but not limited to those
characteristics of the synganglion, are obtained by
affinity chromatography or immune precipitation or
otherwise using serum antibodies of warm-blooded animals
successfully immunized with tick antigenic material, or
using monoclonal antibodies which bind specifically to
tick antigenic material.

In a further embodiment of our invention antigenic
materials are obtained by binding to immobilized ligands
which are related to known functional molecules of the
nervous system, for example neurotransmitter molecules,
or growth factors characteristic of nervous systems, or
their derivatives respectively.

In a still further embodiment, immune suppressive
components from other tick tissues are inactivated and
then added to the antigen mixture, so as to evoke an
anti-suppressive response.

The antigenic materials are combined with one or more
suitable adjuvants known to those skilled in the art, for
example saponins (or derivative or related material), "Neosporin",
mercury disulfide, thiomersal disulfide, Freund's
complete adjuvant, Freund's incomplete adjuvant, other
water-in-oil emulsions, double emulsions, decaene,
diethylaminoethyl-dextran, potassium alum, aluminum
phosphate, aluminum hydroxide, bentonite, alumina,
sodium lauryl sulfate, retinol, calcium phosphate, protamine,
sarcosine, glycerol, cortisol, propylene glycol,

carboxymethyl polymers available under the registered trade
mark "Carbosol", fixed oils and synthetic esters of higher

- 5 -
forty acids. However, have been found to be particularly effective adjuvants.

The antigenic material may be treated with a suitable preservative including for example phenol "Phenol".

formaldehyde, propylene glycol, glycerol, or after of 5-hydroxypropionic acid, benzoic acid and its sodium salt, hexachlorophene, quaternary germicides, sodium azide and thimerosal used as such or in the form in which it is available under the registered tradename "Mercurochrome".

The antigenic material may be sterilized prior to or after formulation as appropriate by filtration, irradiation or chemical treatment.

The antigenic material may also be coupled chemically or physically to a suitable carrier, such as kinase
particles, or immunopotent macro-molecules, or aqueous heads or sulfate, or it may be formulated as antigen combined with buffer or other adjuvants.

The antigenic material may also be treated with a suitable inhibitor, modifier, crosslinker or denaturant,
or by heat or by defatting, or in a way to conserve or enhance its immunogenic efficacy.

-The antigenic material may also be used in combination with other therapeutic agents such as antigens, for example levamisole or sitømus.

therapeutically acceptable salts, flucytosine and other vaccines, for example diphtheria vaccines.

The antigenic material may also be used in combination with immunostimulatory agents such as levamisole, 
5-ASA, thiopeta, lectins, bacterial lipopolysaccharides, polymyxin, tilosquine, lactones, 
tarantolone, L-lysine, and thyroxine, or with immunoregulatory hormones such as interleukin 1,
insulin and the like, or with combinations of the nature of transfer factor.

The antigenic material may be freeze-dried or
other- - or dried or reconstituted, and reconstituted just prior the use by addition of a suitable liquid medium, for
avable a sterile saline solution, optionally with the addition of one or more adjuvants or other additive materials as above.

The antigenic material formulated by any of the means described in Example formed the vaccine.

The amount of the vaccine administered to the animal will depend on the bodyweight of the animal and the relative activity of the particular preparation of antigenic material. Preferably the vaccine is formulated in such a manner that sufficient antigen to protect the animal is contained in a volume of from 1 to 10 ml of vaccine, and more preferably in 1 to 5 ml of vaccine. The amount of antigenic material required is very small and typically samples of antigenic material containing less than 2 mg of protein (preferably 10-100 micrograms) for cattle, particularly 50-500 micrograms, are sufficient for effective immunization of the animals against tick infestation. For other animals, e.g., dogs or poultry, correspondingly smaller doses would be used.

The preferred mode of administration of the vaccine is parenteral. The term “parenteral” is used herein to mean intravenous, intramuscular, intradermal, and subcutaneous injection. The administration is most conveniently carried out by intramuscular injection. The conventional injection gun used for other therapeutic applications with cattle may conveniently be used.

The vaccine may be used for the control or eradication of, or protection from infestation by, Argasid and Ixodid ticks, including species such as *Parasitengus bennetti*, *Rhipicephalus sanguineus*, *Ixodes scapularis*, *Amblyomma americanum*, *Ornithodorus moubata*, and *Haemaphysalis leporispalustris*.

The vaccine may also be administered in the form of a depot or slow-release device or controlled-release device (implanted in or attached to any part of the animal).

The novel method for the control of ticks provided by the invention has a number of advantages. As mentioned herein, the method of the invention avoids the use of hazardous chemicals with the attendant difficulties of
maintaining and cleaning dipping units and spraying equipment, but there are many additional advantages. These additional advantages include the ease of combining treatment for control of ticks with other therapeutic treatments, the simple labour-saving method required for administration, elimination of possibly toxic insecticides being added to the environment, prevention of the development of resistant strains of ticks, and long periods of protection. This period of protection is advantageous both in reducing the need for regular treatments and because animals are protected at the start of each new tick season when the number of ticks in the pastures is low and likely to go unnoticed. In most situations an annual vaccination will be sufficient to confer year-round protection of animals from tick attack.

The effect of the vaccine is not only to reduce the yield of ticks from infested cattle, but also to reduce the viability and fertility of the surviving ticks so that few eggs, generally of low viability, are produced. This phenomenon accentuates the effect of widespread vaccination in reducing contamination of pastures with ticks.

In the case of the cattle tick, *Boophilus microplus*, which has a single host in its complete life cycle, the extended period of protection will also have the effect of steadily reducing the tick levels in the pastures and this effect will accumulate from year to year. A similar effect will occur with multihost ticks.

The invention is now illustrated by, but by no means limited to, the following Examples.

**Example 3**

Two Hereford calves, without prior exposure to ticks, were vaccinated with antigen preparations prepared by homogenizing guts and ganglia obtained by dissection of adult cattle tick (*Boophilus microplus*). Particulate and water-soluble fractions were obtained by centrifugation. The particulate fraction was administered as a suspension
with sapoïn (Doll 8), the soluble fraction was emulsified with Freund's incomplete adjuvant, and the two were injected subcutaneously at different sites. The vaccines were administered three times, on days 0, 14 and 45 of the trial. The doses included 200 micrograms of soluble antigen protein on each occasion, and 500, 100 and 100 micrograms of particulate antigen protein on the three days respectively.

A similar pair of calves was tested at the same time with the adjuvants only, and a third pair was untreated. The calves were challenged on days 62, 69, 94, 181 and 250 with 20,000 larval ticks on each occasion. All the adult ticks which dropped from the calves were counted and incubated until dispiration was complete. Ticks which were abnormal in size or appearance were also noted and incubated separately.

The table gives the cumulative totals for normal and abnormal ticks and for the eggs produced. The vaccinated calves yielded the following reductions relative to the control calves:

<table>
<thead>
<tr>
<th>Control</th>
<th>Vaccinated</th>
<th>90%</th>
<th>20%</th>
<th>90%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total ticks</td>
<td>- reduced by 85%</td>
<td>- reduced by 95%</td>
<td>- reduced by 98%</td>
<td></td>
</tr>
<tr>
<td>Normal ticks</td>
<td>- reduced by 90%</td>
<td>- reduced by 95%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total eggs</td>
<td>- reduced by 90%</td>
<td>- reduced by 95%</td>
<td>- reduced by 98%</td>
<td></td>
</tr>
<tr>
<td>Mean weight of normal ticks</td>
<td>- reduced by 50%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean weight of eggs produced by normal ticks</td>
<td>- reduced by 66%</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 1**
Workshop II
Preparation of tick vaccines using affinity chromatography to extract protective antigens from larval tick homogenates

Summary
A) Production of Antiserum
- Guts and synganglia are dissected from adult ticks
- Vaccines are prepared from these organs and administered to cattle.
- The cattle are infected with ticks and shown to be highly immune
B) Preparation of Affinity Column
- Serum is prepared from the immune cattle from (A), antibodies are extracted from the serum, and bonded to chromatographic support
C) Chromatographic Extraction of Antigens
- Larval ticks are homogenized and separated into soluble and particulate fractions
- Both fractions are applied to the immobilised serum antibodies from (B)
- Antigens which bind to the immobilised antibodies are eluted and formulated as a vaccine
D) Vaccination of Cattle
- Cattle vaccinated with the antigens obtained from (C) are substantially immune to tick infestation, whereas whole larval tick homogenates are ineffective in vaccination.

A) Production of Antiserum
Collection of tick organs. Female ticks (Rhipicephalus appendiculatus) were collected manually from 2 Hereford cattle following the molt to young adult (day 14-17 of the life cycle). The ticks were embedded in wax and the following organs were dissected out:
1. Synganglion
2. Gut
Organ were snap frozen on dry ice and stored at -10 C.
-20°C until used.

Presentation of antigens
(1) Slices were suspended in 50 mM tris buffer containing 135 mM NaCl and 1 mM MgCl₂, pH 7.2.
(2) Antigen buffer and were homogenized in a manual tissue homogenizer. The homogenates were sonicated 30-60 seconds bursts for a total of 10 minutes. Homogenization was followed by centrifugation at 600 g for 10 minutes.
- Pellets were resuspended in an aliquot of supernatant and sonicated as above for a further 5 minutes. The sonicated preparations were pooled and centrifuged at 15,000 g for 20 minutes and the cell pellet was discarded. The supernatant was then subjected to centrifugation at 100,000 g for 1 h. The supernatant thus obtained was retained (soluble protein fraction) and the membrane pellet resuspended by homogenization in antigen buffer.
- Soluble and membrane fractions were stored at -20°C until used. All steps described above were carried out in ice baths.
- (2) Synganglia were forced through a fine mesh using a pestle. The cell suspension was collected in antigen buffer and frozen at -20°C until used.

Vaccination preparation
(9) Soluble and membrane fractions and cell suspensions were divided into equal vials of fractions into incomplete adjuvant. Membrane protein fractions were suspended in antigen buffer containing 1 mg saponin (Dul 8) per ml.
- All vaccine preparations were made up on the day of immunization.

Administration of vaccines
Vaccine preparations were administered intramuscularly using a 18 g needle as set out in Table 2. Membrane preparations were inoculated in the middle third of the right side of the neck, soluble protein preparations were administered in the middle third of the left neck and cell
Injections were inoculated into the middle third of the
subcutaneous and subcutaneous muscles of the left hind
leg. Control animals were either not inoculated or received
adjacent plus saline. Injections were administered on day
11 and 12 of the experiment.

Blood sample procedures:
Animals were bled from the jugular vein at regular
intervals, serum was collected by centrifugation and
stored at -20°C.

Challenger larva
The cattle were exposed to tick larvae, (ten days old).
30,000 per infestation) which were placed on the cattle on
five occasions at seven-day intervals. All normal and
damaged ticks were collected and counted and their
production of eggs and larvae determined

RESULTS
Table 3

<table>
<thead>
<tr>
<th>Tick Numbers</th>
<th>Egg Mt.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>normal</td>
</tr>
<tr>
<td>Intact controls</td>
<td>17380</td>
</tr>
<tr>
<td>Adjacent controls</td>
<td>17764</td>
</tr>
</tbody>
</table>

Percent protection by section:-
Normal Ticks 99%
Total Ticks 98%
Eggs 97%

9) PREPARATION OF AFFINITY COLUMN

Sera from the 5 normal cattle were precipitated with
50% ammonium sulphate for 4 hours at room temperature.
Following extensive dialysis against affinity coupling
buffer: 0.1 M NaHCO₃, 0.5 M NaCl, pH 8.5; 100 µg of
immunoglobulin (IgG) were coupled to 10 ml of cyanogen
bromide activated sepharose 4B gel. Remaining reactive
sites were blocked with 0.2 M glycine buffer pH 8.8.

Following coupling and washing, gels were packed in 15 ml
columns. Normal Igs from control animals, subsequently
proven to be highly susceptible to ticks, were also
coupled to gel and columns were prepared as previously. In addition, activated Sepharose 4B gel was blocked by 0.2M glycine buffer, pH 8.9, and used to control non-specific absorption of proteins to gel.

C) CHROMATOGRAPHIC EXTRACTION OF ANTIGENS

Large quantities of eggs (10 gms - 100 gms.) collected over a 7-day laying period from batches of female ticks, were hatched in containers. The 20-day old larvae were frozen for 1 hour, separated from the egg debris, and extracted as described for gut extracts (see 1). Antigens were dialyzed in affinity buffer (PBS pH 7.4) and 50 mg protein was applied to the loaded affinity column. Antigen was incubated on the column for 30 minutes, then the column was extensively washed with PBS pH 7.4 at 30 ml/hr for 30 min until baseline O.D. readings at 280 nm were re-established. Glycine buffer 0.1 M, pH 2.1 was applied and fractions were collected until baseline O.D. readings were again re-established. The column was stored in Tris buffer containing sodium azide and regenerated the following day using high and low pH buffer cycles.

Particulate and soluble fractions from larval tick homogenates were both separately chromatographed in this way. The particulate fraction was first treated with Triton X-100 but was not centrifuged. Both soluble and particulate materials were subsequently specifically eluted together.

D) VACCINATION OF CATTLE

Affinity chromatographed larval antigens were formulated as for gut antigens (see C above) and administered to cattle on days 0, 28 and 56 to a total of 500 micrograms of protein per animal.
**Table 1**

<table>
<thead>
<tr>
<th>Group</th>
<th>Percent Protection</th>
<th>Total Ticks</th>
<th>(60%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adult homogenate</td>
<td>10%</td>
<td>9%</td>
<td></td>
</tr>
<tr>
<td>Ovalbumin plus particulates</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Affinity purified fraction</td>
<td>65%</td>
<td>84%</td>
<td></td>
</tr>
<tr>
<td>Ovalbumin plus particulates</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Example (2)**

**Efficacy of Vaccines Based on Dissected Tick Organs**

Cuts and synganglia are dissected from adult ticks as described in Example (1) (A).

- Varies are prepared and administered to cattle, either gut preparation (soluble and particulates) or synganglion (cell suspension) or both together, as described in Example (1) (B) and Table 1.

- Cattle are infected with ticks and immunity assessed as described in Example 2(A).

**Results**

(A) Percent Protection against three successive weekly challenges commenced on day 56 of trial:

<table>
<thead>
<tr>
<th>Total Ticks</th>
<th>Ticks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Synganglion plus gut</td>
<td>95%</td>
</tr>
<tr>
<td>Gut alone</td>
<td>98%</td>
</tr>
</tbody>
</table>

(B) Percent Protection against natural infestations at approximately 6 months after first vaccination:

<table>
<thead>
<tr>
<th>Ticks (ticks)</th>
<th>(ticks)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Synganglion plus gut</td>
<td>6%</td>
</tr>
<tr>
<td>Gut alone</td>
<td>(0%)</td>
</tr>
</tbody>
</table>
Thus whilst the protection given by vaccination plus gut is comparable to gut alone at 56 days, the long term protection at 6 months is markedly superior.

These results are shown in more detail in Figure 1.

Figure 1 shows the cumulative drop of ticks from 2 groups of vaccinated cattle compared to the mean cumulative ticks dropped by controls. Group 1 was vaccinated with antigen derived from both synephrin and gut. Group 2 was vaccinated with antigen from gut alone.

The level of immunity (percent protection) was determined by challenging the two groups and the controls, with pulse infestations of 30,000 R. microplus larvae, and also by natural infestation in tick-infested pasture.

The results show enhanced long-term protection in Group 1 (synephrin and gut) relative to Group 2 (gut alone) relative both to pulse and natural tick challenges. There is also a degree of natural immunity in controls.
Claims

1. An antigenic composition which comprises antigenic material derived from the synganglion of a tick.

2. A composition according to claim 1 wherein the synganglion-containing antigenic material is derived from tick larvae.

3. A composition according to claim 2 which further comprises antigenic material derived from the gut of the tick.

4. A composition according to any preceding claim wherein the antigenic material has been purified by passing over an affinity chromatography substrate having antibodies to tick antigenic material bound thereto.

5. A composition according to any preceding claim wherein the tick is Boophilus microplus.

6. A composition according to any preceding claim which further comprises a saponin as an adjuvant.

7. An antigenic composition which comprises antigenic material derived from tick larvae.

8. A composition according to claim 7 wherein the antigenic material is derived from the synganglion and gut of the tick.

9. A composition according to claim 7 or 8 wherein the antigenic material has been purified by passing over an affinity chromatography substrate having antibodies to tick antigenic material bound thereto.
10. A composition according to claim 7, 8 or 9 wherein
the tick is *Rhipicephalus microplus*.

11. A composition according to any one of claims 7 to 10
which further comprises a saponin as an adjuvant.

12. A vaccine which comprises antigenic material derived
from a tick together with a saponin as a vaccine
adjuvant.

13. A vaccine according to claim 12 wherein the antigenic
material is derived from the synganglion and gut of
the tick.

14. A vaccine according to claim 13 wherein the antigenic
material has been purified by passing over
an affinity chromatography substrate having antibodies
to tick antigenic material bound thereto.

15. A vaccine according to claim 12, 13 or 14 wherein the
tick is *Rhipicephalus microplus*.

16. A vaccine according to any one of claims 12 to 15
wherein the antigenic material is derived from tick
larvae.

17. A process for the production of purified antigenic
material derived from a tick, which comprises:

(i) preparation of an affinity chromatography
substrate having bound thereto antibodies to
tick antigenic material;

(ii) passing crude tick extract over the substrate,
such as to adsorb tick antigen on to the
substrate, and

(iii) eluting purified antigenic material from the
substrate.
18. A process according to claim 17 wherein the crude tick extract is derived from tick larvae.

19. A process according to claim 17 or 18 wherein the antibodies bound to the affinity chromatography substrate are derived from blood serum from an animal successfully immunized against tick.

20. A process according to claim 17, 18 or 19 wherein the antibodies bound to the affinity chromatography substrate are monoclonal antibodies produced from tick antigenic material.

21. A method for the control of tick infestation of a warm-blooded animal which comprises immunizing the animal with an antigenic composition or a vaccine according to any one of claims 1 to 18.