

A Northern NSW Farmer's insight:

The stated purpose of the NSW cattle tick program was:

1. Prevent entry into NSW
2. Eradicate cattle tick in NSW

The current operation of the program does not prevent cattle ticks from entering NSW and does not eradicate them in NSW, instead it puts a big cost and work load on affected farmers without solving the issue. There is no consideration of the abilities of farmers to comply with all the requirements and there is no help or assistance available.

From a business point of view it is impossible to plan as there is no clear time frame when the problem will be solved and restrictions lifted.

The current border control appears to be very weak.

- * How can cameras detect any cattle ticks in trucks or horse trailers passing the border at 100km/h?
- * How could cattle ticks recently re-infest Northern NSW?

The new Owner Treatment Scheme can and will not work. It is wishful thinking and will cause further loss of control. Cattle ticks will get out of hand and spread through NSW. The scheme is out of touch with reality! May be it can be used to eradicate virtual cyber cattle ticks in a farm game on the mobile phone?

Proposal for a solution:

1. **Strict and effective control at the NSW border to prevent cattle ticks coming into NSW.**
2. **Communication** with Queensland to push back the cattle tick area from Southeast QLD.
3. **Support for NSW farmers** who are affected by cattle ticks. It is a shared responsibility! Farmers are not able to handle it without support and the main burden is currently carried by farmers and their families.
4. Introduce **transparency** and communication into the cattle tick program, so that everyone involved knows the status, distribution and when the eradication will be completed.

Australian Trail Horse Riders Association (ATHRA)

Formal Submission: Self-Certification for Movement of Horses into NSW from QLD

Date: 27 November 2025

Submitted To: NSW Independent Biosecurity Commissioner



On behalf of the Australian Trail Horse Riders Association, we wish to raise significant concerns regarding the current framework for self-certification of horses moving from Queensland into New South Wales under the cattle tick program.

1. Background and Current Process

The existing NSW DPI process allows horse owners to self-certify movements by submitting a letter from the property owner stating that no cattle have been present on the property for the preceding or forthcoming 12 months. Once uploaded, this letter enables horses to cross into NSW without chemical treatment, provided they return to Queensland within seven days.

There is no follow-up or verification conducted by NSW DPI to confirm whether the property is genuinely “low risk.” This lack of oversight has created confusion among horse owners and undermined the credibility of accredited certifiers who have invested in training, insurance, chemicals, and equipment to ensure compliance.

2. Concerns Raised by Accredited Certifiers

Accredited certifiers have reported:

- Hostility and frustration from horse owners who believe spraying is unnecessary under the current rules.
- A perception that professional inspection and treatment are redundant, given the ease of self-certification.
- Financial strain on certifiers whose businesses are becoming unviable due to reduced demand and inconsistent enforcement.
- Growing risk of cattle tick outbreaks in NSW, exacerbated by recent flooding events and the absence of robust monitoring.

This situation creates an uneven playing field: some horse owners pay for professional inspection and spraying, while others simply submit a letter and pass through unchecked.

3. Impact on Horse Owners, Clubs, and Cross-Border Events

For horse owners, particularly those travelling from Queensland into NSW for recreational or competitive events, the current system is:

- Costly: Accredited inspections and treatments add significant expense to cross-border participation.

- Cumbersome: The online application process is unwieldy and difficult to navigate.
- Unfair: Horses are widely recognised as low-risk carriers of cattle tick, yet horse owners bear disproportionate compliance costs compared to other livestock industries.

For clubs and associations, these requirements make organising cross-border events extremely difficult. Costs are passed on to participants, discouraging attendance and reducing inclusivity. The administrative burden of ensuring compliance adds further strain to volunteer-run organisations, threatening the viability of interstate gatherings and undermining the spirit of collaboration between Queensland and NSW horse communities.

4. Need for Reform

The cattle tick program was introduced in the 1920s, when livestock owners had limited tools to manage infestations. Today, modern solutions such as Cydectin LA, vaccinations, and advanced spraying options are widely available. The program has not kept pace with these developments, nor with the biological reality that horses are low-risk carriers.

We strongly recommend:

- A comprehensive review and restructure of the cattle tick program.
- Introduction of a streamlined, cross-border self-certification system for horses, recognising their low-risk status.
- Simplification of the online application process to reduce administrative burden.
- Equitable treatment of horse owners, ensuring they are not disproportionately penalised compared to other livestock industries.
- Consideration of the impact on clubs and associations, ensuring that cross-border events remain viable and accessible.

5. Conclusion

The current framework is inefficient, inequitable, and unsustainable. It undermines professional certifiers, imposes unnecessary costs on horse owners, and restricts the ability of clubs to host interstate events. A modernised, risk-based approach is urgently needed to support the horse industry, protect biosecurity, and enable fair participation across state borders.

We welcome the opportunity to contribute further to any review process and to work collaboratively towards a system that reflects contemporary industry practices and realities.

Contact: Claire Tolcher
Chief Executive Officer
Australian Trail Horse Riders Association

To the Biosecurity Commissioner,
Dr Katherine Clift,

Thank you for the opportunity to present a submission on the Cattle Tick Program from the Fletcher family at Cressvale Herefords in Piggabeen in New South Wales.

1. History - Our family has been involved in the cattle tick issue since cattle tick first arrived from Queensland in 1906.
2. Our property has been involved in the program since the Board of Tick Control was instituted in 1920.
3. Where do we go from here?

Priorities:

- Eradication- This is a MUST for the benefit of the entire state of New South Wales.
 - Resistance to chemicals - An ever-present threat that must be avoided.
 - Staff on the Ground- We need experienced, dedicated, field staff allocated to each area and responsible for communicating with each individual stock owner with emphasis on education and cooperation rather than regulation. Only use regulation as a last resort, but always keep it in the back pocket.
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4. The University of Qld vaccine - It sounds promising, we would love to see it given the support it requires and then be introduced. We need to keep our current eradication policy in place and embrace the UQ vaccine if and when available. Potentially it promises to reduce both the amount and cost of chemical usage and the workload and stress on both stockowners and their cattle.
 5. Owner Treatment Program- So many short comings including problems with estimating weight of animals, under-dosing to cut costs, development of resistance to chemicals and many, many more.
 6. Queensland people- Some have bought properties in New South Wales and would like New South Wales to embrace the Queensland system so that they can move cattle freely between states. They apparently have no knowledge of or consideration for the implications of their requests on the entire state of New South Wales.

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7. Hide Damage- Hides of cattle tick infested cattle are perforated, limiting their use and value.
8. Tick Line in New South Wales - Currently the Queensland border fence is the tick line. If it is shifted anywhere else, those inside the line will be highly impacted and those on the tick line will have the same problems as we have now, but without the experience of how to cope.
9. Queensland Certifiers - They were only required to scratch one side of animals presented. Is that still the case? Certifiers can not be expected to find small ticks.
10. Blooding- Blooding is not a vaccination as most people think of vaccinations It actually puts the organism into the blood of the animal. The reality is that blooded cattle are not a problem if there are no cattle ticks, the vectors of tick fever present. Also stockowners can have cattle ticks on their cattle without having deaths. But, put the two together and we have DISASTER. There is a great lack of knowledge regarding the blooding process.
11. Imidocarb- Should be used immediately on animals suffering from tick fever. This is an animal welfare issue and to withhold its usage is negligence.
12. OTP- Appeared to be brought in as an experiment, developed on the run. Implementation was unexplained and haphazard. Who introduces a policy with nothing written down or communicated?
13. Unfortunately, we on the Far North Coast feel that we are out of sight and out of mind. We are a long way from Sydney and Orange. Decisions have been made by people with no real knowledge or experience. This is a box ticking exercise.
14. The Cattle Tick program needs to be in the Department of Animal Health and Welfare. Border surveillance needs to be with Biosecurity. There is a Transport Department pullover check point at Chinderah. It is not open all the time. It would be ideal if the DPI could cooperate with the Transport Department and conduct random checks on vehicles of interest going South. If people knew there was a possibility of being checked they, hopefully, will be more careful about their Biosecurity responsibilities. Word would quickly spread.
15. Buffer Fence - The staff member who looked after the buffer fences has been removed and relegated to other duties. The buffer fences are in dire need of repairs and maintenance, particularly on the southern side. The buffer itself is weed infested. People including bike riders and walkers are freely using this regulated zone. As they are entering from the Queensland side, they can carry seed ticks or any other invasive species on their clothing.

16. Ticks hitch a ride- kangaroos, wallabies, echidnas, wild dogs and snakes add to our risks. Red Deer, a favoured host, have been reported at Mudgeeraba which by crow is only a few valleys away. Fences do not control them. We can treat our cattle for cattle ticks, but we cannot treat the Deer.
17. Desperation- Makes people do things they wouldn't otherwise do. For example, stockowners in the Kyogle area who have not reported the cattle ticks on their properties and have blooded them instead, apparently on the advice of their practicing Vet. Did desperation drive their decisions? If not, what was their motivating force? Their losses have been unacceptable.
18. Misinformation and Disinformation - Unfortunately, we are encountering this far too frequently.
19. Regrets - We nearly had the ticks eradicated in 2004. We hadn't had any tick fever cases for many years. Then the DPI got rid of the tick board, made some very ill-advised decisions and look where we are now. We cannot sacrifice, indeed waste 120 years of hard work, blood, sweat and tears.

Yours faithfully,

The Fletcher Family (Noel, Jan and Alison)

Submission to Dr Katherine Clift, NSW Biosecurity Commissioner

Urgent: Restore Government-led Management of the NSW Cattle Tick Program

Dear Dr Clift,

We welcome your independent review of the NSW Cattle Tick Program and thank you for undertaking this important work. The Biosecurity Act 2015 objectives are to prevent, early detect and eradicate cattle tick from NSW.

This submission strongly urges the Commissioner to prioritise immediate, government-led action to restore the effectiveness of the NSW Cattle Tick Program. The historic, century long buffer zone that protected NSW from cattle tick and tick fever has been eroded. Recent spread, repeated outbreaks of tick fever and increasing cattle deaths demonstrate that leaving control to voluntary, owner led measures is failing and that DPIRD must resume active, enforceable management with adequate resources as soon as possible.

Summary

- For more than 100 years the regulated Cattle Tick Program, including the Tweed buffer zone kept NSW essentially tick free. The Tweed was verified tick free in 2004.
- In recent years infestations and outbreaks of tick fever have increased and moved south and west, driven primarily by inadequate enforcement, lack of DPIRD tick officers on the ground and inconsistent inspections. Lack of working plunge dips available to producers for treating cattle, and lack of compliance to the rules.
- The move towards Owner Management approach cannot be interpreted as neutral policy reform. In effect it appears to be a decision to shift responsibility for a public good onto individual producers rather than addressing the clear gaps in DPIRD resourcing and enforcement.
- Owner Management places impossible expectations on producers. Even when a small number of properties fail to comply with the rules, reinfestation is inevitable that is the reason why government management and enforcement is essential.
- This is an animal welfare crisis. DPIRD must immediately re-establish active management, enforcement and surveillance with clear consequences for non-compliance.

Problem Detail

- The buffer zone QLD/NSW border has functioned as a collective protection mechanism. Due to lack of staff the buffer zone is now compromised by ongoing untreated infestations and by cattle movements from infested areas.
- Enforcement gap: There are currently insufficient DPIRD tick officers to complete the necessary inspections and commence Cattle Tick Program on

properties with infestations. Known infested neighbouring properties have not been inspected, and required to treat, or compelled to implement the Cattle Tick Program, resulting in repeated reinfestation of compliant properties.

- Regular purchases by NSW producers of blooded (hot) bulls and cattle from infested regions in QLD and NSW studs who are wanting to sell to their QLD clients. Movement of “hot” cattle into NSW is increasing with producers unaware of the high risk they are exposing their naïve herd.
- Owner Management failures: Over recent years the responsibility has shifted to the property owners, but many properties do not, cannot or will not meet the required standards. Where the Owner Management Program has been relied upon, reinfestations have continued, demonstrating the model **cannot** replace regulated, resourced government intervention.
- Vaccinating naïve cattle with live tick fever can itself produce fever, anaemia, loss of condition especially in older and pregnant cows causing reduce fertility, abortion and death. These reactions reduce production and welfare increased labour and treatment costs and risk spreading the vaccine organisms via ticks onto naïve tick free cattle.
- Animal Welfare and Economic impacts. Tick fever causes deaths, abortions and long-term production losses. The stress on vaccination is expensive, extremely stressful on naïve stock and not viable large-scale substitute for prevention. Producers cannot absorb the financial and welfare costs if the buffer fails.

Why DPIRD must manage the NSW Cattle Tick Program

- Cattle tick control is a shared public good. Individual compliance only protects neighbours if it is universal. If a few properties remain non-compliant, reinfestation follows. This makes government enforcement essential.
- Proven historical success. Eradication & containment historically succeeded because government led, coordinated and **enforced** the program. **That structure must be reinstated.**
- DPIRD has the statutory authority, veterinary expertise & operational capacity to deliver coordinated surveillance, diagnostics, treatment oversight & rapid response.
- Legal & regulatory responsibility. Biosecurity regulation exists to protect the broader community. If DPIRD continue to scale back enforcement and the buffer zone collapses, the state risks large-scale animal welfare failures and economic losses for which it is responsible.
- There needs to be clear penalties for non-compliance. This will also deter risky behaviour and ensure fairness for producers who have complied to the rules.

This is not a theoretical risk; it is happening now. The erosion of the historic tick-free buffer and the increase in tick fever cases are direct consequences of under-resourcing, reduced inspections and an overreliance on owner management. DPIRD must retake operational control, boots on the ground, enforced inspections, clear

penalties and a transparent program backed by adequate staffing. Without decisive action now, the scale of animal suffering and economic loss will be catastrophic and entirely preventable.

Yours sincerely

Tim & Anna Gilliland

[REDACTED]
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Submission to the Department of Agriculture re Cattle Tick Review – 13th November 2025

It is obvious that the ongoing program of Tick eradication in the Northern Rivers area needs review

- The task of managing Cattle Tick needs to be a shared one
- Producers need to step to the plate – accept that changes are needed and that they need to take more responsibility both on a management front and a financial cost front – there is an attitude amongst many producers the Government should carry the cost
- The reality is that proper management of cattle tick is no different to managing other aspects of Animal Husbandry – it comes at a cost but delivers financial returns in milk production and weight gains – all of which go in the individual producers pocket
- Producers need to stop crying poor – the facts are they are enjoying the highest prices they have ever had and have been doing so since January 2020

Following points

- The concept of Tick Eradication needs to be revisited
- The efforts to eradicate Ticks in the Northern Rivers has been ongoing for 100 years and is unsuccessful
- The Department of Agriculture does not have the resources to cover the work load required to run the program as it exists – frankly it has been stepping away from the program resourcing for many years , I would say since the mid 1980s when the maintenance of dip yards was pushed on to Landowners
- We need to move towards a model of control not eradication
- We have two categorys of Livestock Producers – those who earn their living from breeding , growing and fattening beef cattle – versus Hobby Farmers who are largely small acreage owners and who work off property

- The above is problematic as many hobby farmers have no concept about what is required to manage cattle tick ,are only home of a weekend and are hard for department officers to interact with (as they don't work weekends)
- As adjoining holdings by and large reinfestations are occurring because the small holding factor makes a united targeted approach impossible to achieve
- Straying Livestock has been touted as a major factor in tick infestation s spreading – cattle stray – even through new fences – flood events bring down creek blocks , fences also get old and are very costly to replace
- The department needs to step away from the on farm management of cattle tick and administer tick control from where they can be effective – the point s of exit from the tick zone – such as saleyards and accredited clearing dips- such as prevails in the Queensland Tick Zone
- The measures should apply to livestock leaving the area destined for other holdings ie farms and feedlots
- Cattle going to Slaughter should be exempt

Chemical use

- I think most people would agree dipping is the best way to control cattle Tick , the dip network is non existent
- Using one chemical consistently at 3 week intervals carries two risks – tick resistance and possible residue build ups in the animal hence the meat produced from it
- We are in a changing world where chemical use in food is under a massive focus – consumers are concerned about how their food is produced
- The Department has been sticking with Cydectin pour on as a treatment as it has a nil withholding period , so not exposing the Department to litigation because of stock owner,s potential lack of management of withholding periods – stock owners need to take responsibility for the management of the chemicals used as treatments ,which need to be rotated and have varying withhold periods

Qld Tick Zone

- Landholders manage ticks on their properties – no interference from govt
- To leave the tick zone cattle have to be tick free
- They are inspected , dipped and cleared through nominated clearing dips and dips at saleyards
- The onus is on the Producer to have their livestock clean at the point of sale
- At Saleyards the purchaser is responsible to present the cattle clean of cattle ticks and has too hold and feed them if they are infested untill they are presented clean to leave the tick zone

Animal Welfare

- Treating livestock through winter is difficult
- August September and October are generally the worst months to be treating cattle
- Animal welfare is an issue that is at the forefront of consumers minds in relation to the food they eat
- Bashing livestock through yards during adverse weather periods is unacceptable

Vaccinations

- I am not an expert on the science around this but have the following view
- I am in favour of vaccinating our cattle to give them a level of protection against the Red Water Virus
- As I understand it the herd will develop resistance to the virus if vaccinated
- The cattle born and bred inside the Queensland Tick Zone don't need vaccinations as they have resistance to the virus – this would eventually apply to the Northern Rivers Herd
- Vaccinating cattle on small holdings which are lacking in management will also provide a
- level of protection to adjoining herds

For Context- Who is Greenmountain Group ?

- Operates a vertically Integrated Beef Breeding Fattening and Processing Business
- The Scarrabelotti Family has been farming in the Richmond Valley Since 1880
- Owns the Coominya Food Processing Business in the Brisbane Valley supplying Beef and Veal to Domestic and Export Markets – Processing around 3000 head a week
- Has significant land holdings in the Richmond Valley with properties from Wiangarie North of Kyogle to Just South of Coraki along the Richmond River
- Runs from 4000 to 5000 cattle on these properties depending on weather conditions

[REDACTED]

[REDACTED]

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Submission to the NSW Independent Biosecurity Commissioner Review of the NSW Cattle Tick Program

Submitted on behalf of Norco Cooperative Limited

About Norco

Norco is Australia's last remaining 100% farmer-owned dairy co-operative, established in 1895 and operating continuously for more than 130 years. Our members are predominantly located across Northern New South Wales and South-East Queensland, and the strength of the co-operative rests fundamentally on the viability and resilience of these local dairy farms.

Norco's operations - including processing, manufacturing, domestic supply, and export activity - depend on a reliable, high-quality, and locally sourced milk supply. This supply base has been in long-term decline due to a range of structural pressures, including rising production costs, climatic variability, biosecurity challenges, and market uncertainty. Maintaining the health and productivity of our regional herds is therefore essential not only for Norco's sustainability, but for the broader dairy industry and the regional communities that rely on it.

In this context, a strong, effective, and well-resourced NSW Cattle Tick Program is crucial to protecting the future of dairy farming in northern NSW. The program safeguards herd health, prevents production losses, and protects areas of the state that remain free from cattle tick infestation. For a co-operative that depends on local farmers, the program is a critical pillar of regional biosecurity and industry stability.

Importance of the Current Cattle Tick Program to the Dairy Industry

Cattle ticks pose a severe biosecurity threat to dairy operations. The presence of tick fever organisms can lead to significant animal welfare issues, reduced milk production, increased veterinary costs, and in severe cases, herd mortality. Because dairy cows are often higher-value animals with more intensive management requirements, the impact of cattle tick incursions is particularly pronounced in the dairy sector.

The current NSW Cattle Tick Program plays a vital role in safeguarding tick-free regions and rapidly responding to incursions. The program's surveillance, movement controls, and treatment protocols have been instrumental in containing risk along the Queensland border, an area of particular relevance for Norco and our suppliers.

Norco strongly supports the continuation and strengthening of the program. Specifically, we advocate for:

- Enhanced surveillance capacity, including modern diagnostic tools, improved reporting systems, and enough field staff to ensure rapid responses.
- Maintaining and tightening movement controls, particularly in high-risk border regions, to reduce the likelihood of the pest being transported into dairy production zones.
- Sustained and increased government resourcing, recognising that the cost of prevention is significantly lower than the cost of managing widespread infestation.
- Stronger collaboration between government, industry, and producers, ensuring farmers receive clear guidance and support around compliance, emerging risks, and treatment options.
- Strong enforcement and compliance measures for individuals and businesses that violate bio-security regulations regarding livestock movement and management.
- Ensuring that tick eradication in NSW remains the primary objective of the cattle tick management program as opposed to a program of controlled management. It is our view that any move to controlled management program will ensure deeper and more widespread tick incursions across NSW.

Risks Associated with Reduced Investment in the Cattle Tick Program

Any reduction in funding or capability within the NSW Cattle Tick Program would significantly increase biosecurity and economic risks for dairy farmers and the wider agricultural sector. Key risks include:

Increased Incursions and Spread - Lower levels of surveillance and compliance monitoring would heighten the chance of undetected tick incursions into currently tick-free dairy regions. Once established, eradication becomes extremely costly, protracted, and in some cases unachievable.

Financial Impacts on Producers and the Co-operative - A single cattle tick outbreak can cause substantial losses through reduced milk yield, treatment costs, increased labour demands, and potential loss of animals. For Norco's supply base, already constrained by reduced herd numbers, such burdens may threaten farm viability.

Impacts on Milk Supply Security - A widespread cattle tick incursion would further reduce the local milk supply pool at a time when the NSW dairy industry is already under pressure. Any reduction in local production impacts Norco's processing operations, employment across regional communities, and domestic dairy supply chains.

Market and Trade Consequences - Biosecurity failures can damage market confidence, trigger regulatory restrictions, and impose compliance burdens on producers and processors. For a farmer-owned co-operative operating in competitive domestic and export markets, these impacts are material.

Long-Term Loss of Tick-Free Status - If the program is weakened and incursions become more common, NSW risks losing its long-standing tick-free status. Reversing such a

situation would require significantly greater investment than maintaining the current program, and would impose ongoing costs on producers through increased treatment requirements and long-term productivity losses.

Constrained Growth Options for Farmers and Industry – A key constraint for growth of existing dairy farms, and subsequently the dairy industry, is access to productive livestock. With the majority of Australia's dairy cows located in Victoria the importation of Victorian dairy cows into NSW is commonly adopted to allow farmers to grow their dairy herd. This option becomes a greater risk in regions where risk of tick fever is highest. If tick incursions become more widespread in NSW this will create an additional constraint on business growth for NSW dairy farmers.

Conclusion

Norco strongly urges the NSW Independent Biosecurity Commissioner and the NSW Government to maintain and strengthen the NSW Cattle Tick Program. For dairy producers in northern New South Wales, the program is not optional, it is a foundational safeguard that protects animal health, ensures regional productivity, and underpins the broader viability of the state's dairy sector.

As Australia's last remaining dairy co-operative, Norco carries a unique responsibility to protect the livelihoods of its farmer members and the communities in which they operate. Our business is dependent on a resilient, reliable local milk supply. With herd numbers already declining, the introduction or spread of cattle tick into currently tick-free regions would add yet another destabilising burden.

A strong cattle tick management system is, therefore, not simply a biosecurity program, it is an economic stabiliser, a risk-mitigation system, and a public-good investment that supports the prosperity of regional NSW. Reduced investment would increase the likelihood of incursions, escalate response costs, jeopardise animal welfare outcomes, and ultimately erode the productive capacity of the dairy supply chain. Prevention remains vastly more cost-effective than response, and certainty in program funding and capability is essential for both farmers and processors planning for long-term sustainability.

Norco acknowledges the work already undertaken by NSW DPI and values the commitment to continuous improvement reflected through this review. We encourage the Commissioner to adopt recommendations that:

- Ensure long-term, stable funding for surveillance, compliance, and rapid response;
- Strengthen cross-border coordination with Queensland, where the risk profile is highest;
- Enhance engagement and communication with producers to support best-practice on-farm biosecurity; and
- Any changes made to the program should result in further strengthening of the current program, including enforcement and compliance, in order to maintain NSW's tick-free status and achieve true eradication.

We appreciate the opportunity to contribute to this process and affirm our willingness to support the review with further information, industry insights, or participation in stakeholder consultations. Norco and its farmer members stand ready to work collaboratively with government to ensure that NSW remains a leader in livestock biosecurity and that the dairy industry can continue to thrive.



NSW Farmers submission to the NSW Cattle Tick Program Consultation

November 2025

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Lizzie Sarks



Table of contents

Executive summary..... 1

Recommendations 2

The Current Status of Cattle Tick 2

Alignment with the Biosecurity Act..... 3

Similar Programs in NSW and other jurisdictions..... 4

Previous reviews and evaluations 6

The Beneficiaries of the Program 7

Additional information 8

About NSW Farmers

NSW Farmers is Australia's largest state farming organisation and advocates for issues such as the environment, biosecurity, water, animal welfare, economics, trade, workforce, and rural affairs. Agriculture is crucial, employing more than 75,000 people in NSW and the ACT, and contributing more than \$24 billion to the economy.

Our goal is to reach \$30 billion in output by 2030 by supporting stronger farming businesses. We ensure genuine farmer voices shape policies, and we provide specialist advice, along with valuable member benefits.

Executive summary

Cattle tick remains one of the most significant biosecurity threats to Australia's cattle industry. NSW has successfully maintained a tick-free status for over 100 years through the NSW Cattle Tick Program, safeguarding beef and dairy herds, markets and agricultural businesses.

The case for continuing and strengthening the Program is clear, with eradication being achievable and more cost-effective than alternative approaches.

Any changes to the Program that reduce government's role in delivering the Program would undo decades of progress, expose producers to escalating costs, and jeopardise market access. Conservative estimates show that tick incursions could cost producers millions annually through treatment, prevention, productivity losses, and movement delays - figures that are extremely higher than the spend on the Program in recent years.

Conservative estimates show movement delays as a result of tick infestations alone could exceed \$1 million annually, rising to \$3 million in drought conditions. Treatment and prevention costs could reach \$8 million in the Northern Rivers alone, highlighting the importance of continued investment in the Program.

NSW Farmers strongly supports the NSW Cattle Tick Program, arguing that it must remain government-led and receive increased investment in predictable, long-term funding and skilled personnel to protect industry, animal health and welfare, biosecurity, and to conserve regional jobs and communities that are supported by cattle production throughout the state of NSW.

Additionally, the *NSW Biosecurity Act 2015* sets clear legislative requirements for notifiable pests, of which cattle tick is one, and so a maintained coordinated approach is essential to ensure industry and government meet their General Biosecurity Duty obligations to prevent, eliminate, or minimise biosecurity risks so far as is reasonably practicable.

It must be noted that, although the Program's main area of delivery is concentrated in a small region of north-east NSW, its protective reach extends across the entire state to safeguard the integrity of NSW's livestock industry with the NSW-Queensland border a clear boundary for the cattle tick free area that is the state of NSW; it would be a mistake to consider compromises that have been made in other states and resulted in increasing reports of cattle tick impacts.

Eradication in NSW is achievable, and producers and industry must be engaged and supported in a meaningful way by Government, to ensure the robust delivery and success of the Program in maintaining the state's cattle tick-free status and eradication as the primary objective when cattle ticks are confirmed within the state.

Recommendations

Recommendation: That the NSW Government maintain and strengthen the Cattle Tick Program to ensure NSW retains its tick-free status and eradication remains the primary objective, while enabling both producers and government to meet their General Biosecurity Duty under the *NSW Biosecurity Act 2015*.

Recommendation: That the NSW Government does not consider implementation of cattle tick management models used in other jurisdictions as they are not comparable or appropriate to NSW.

Recommendation: That the Cattle Tick Program remain a key biosecurity defence focus of the NSW Government, with prioritisation of adequate funding, staffing, communication and education for the benefit of livestock industries and regional communities.

Recommendation: That the NSW Government prioritise predictable, long-term funding allocation to the Program and the employment of skilled personnel in the Program to guarantee continuity and high-standard delivery of prevention, surveillance, treatment, and compliance measures.

Recommendation: That the NSW Government leverage existing opportunities, including reinstating usable dip sites.

The Current Status of Cattle Tick

The NSW Cattle Tick Program (the Program) is the frontline defence protecting the state's \$3 billion cattle industry and NSW's cattle tick-free status that has been maintained for decades through coordinated government action.

The Program has a proven track record of success spanning more than a century, having kept NSW largely cattle tick-free despite the pest being endemic just across the border in Queensland. This achievement is the result of sustained government leadership, robust surveillance, and coordinated traceability and eradication efforts including through industry partnerships with stock owners, saleyards and agents.

Producers and industry continue to be overwhelmingly supportive of the Program because of this success. However, there are concerns with the delivery of the Program in recent years. NSW Farmers' members and other industry bodies have raised issue with decreasing Department staff resources to facilitate treatments and monitor compliance and surveillance, as well as communication and engagement issues with current and new cattle owners particularly with the continuing land use changes resulting from peri-urbanisation of parts of the Northern Rivers.

Producers are also concerned about the perception of the Department reducing its responsibility of treatment delivery, leaving producers with increased responsibility but without adequate support or training. Compliance and enforcement of rules, chemical resistance issues, and the risk of tick fever—particularly from vaccinated (blooded) cattle spreading the disease—add further complexity and anxiety.

NSW Farmers is calling on the Government to maintain and improve the current NSW Cattle Tick Program to enhance its delivery and to rebuild industry confidence – any move other than this would



undo decades of progress and expose the state to unacceptable biosecurity, economic, and animal health and welfare risks.

Climate suitability modelling referenced in the consultation paper reinforces the vital importance of the Program. While stray cattle ticks are currently confined to a very small area in the north-east of the state, the modelling shows that large parts of eastern NSW could provide ideal conditions for cattle tick survival and spread. The fact that ticks have not expanded into these areas is proof that the Program works. Without strong, well-resourced eradication measures, ticks could rapidly establish across these high-risk zones, triggering widespread biosecurity and animal health and welfare issues, market access restrictions, and significant economic losses.

In August 2025, when a review of the NSW Cattle Tick Program was announced, the NSW Agriculture Minister said that “*the current program receives more than \$4 million per year in funding,*”¹ which is a fraction of the cost of the Government’s announcement that they have committed \$1 billion to biosecurity² this year in NSW.

However, many producers across the Northern Rivers region of NSW have expressed dissatisfaction with the Program in recent years, citing reduced DPIRD staff to support treatment and surveillance methods, reduced engagement and communication issues. This would appear to correlate with funding reductions to the Program across 2019-2023.³

NSW Farmers acknowledges the re-establishment of DPIRD’s Cattle Tick Consultative Committee (CTCC) in March 2025, and Government’s efforts made with stakeholders to address the issues raised by industry. Since the re-establishment of the CTCC, NSW Farmers understands that more staff have been appointed to the Program, and a communication plan, resources and updated website have been developed and recently launched. These efforts are to be commended. They are in line with calls from industry and they must be given adequate time to be effective and make an impact. The new measures have not yet had this opportunity, and it would therefore be inappropriate to consider any alternate approaches that do not prioritise eradication

Recommendation: That the NSW Government maintain and strengthen the Cattle Tick Program to ensure NSW retains its tick-free status and eradication remains the primary objective, while enabling both producers and government to meet their General Biosecurity Duty under the *NSW Biosecurity Act 2015*.

Alignment with the Biosecurity Act

The NSW Cattle Tick Program is fundamental to ensuring compliance with the *NSW Biosecurity Act 2015*, which establishes biosecurity as a shared responsibility between government, industry, and the community.

Under the Act, each of these bodies have a General Biosecurity Duty to prevent, eliminate, or minimise biosecurity risks so far as is reasonably practicable. The Program provides the structure, resources, and oversight necessary for producers to meet these obligations effectively, while enabling government to uphold its own responsibilities for surveillance, eradication, and enforcement.

¹ Minister for Agriculture. 2025. Media release. New Independent Biosecurity Commissioner tasked with examining state’s cattle tick program. Available at: www.nsw.gov.au/ministerial-releases/new-independent-biosecurity-commissioner-tasked-examining-states-cattle-tick-program

² Ibid

³ NSW Parliament. February 2025. PC 4. Budget Estimates – Additional Questions on Notice. Question 36.

Without a coordinated government-led program, compliance becomes fragmented and risks escalate. To discharge their General Biosecurity Duty effectively, producers need clear guidance and coordinated measures to manage cattle tick risks effectively. This includes timely inspections, and access to accurate, effective treatment, all of which the Program delivers.

Similarly, government must increase its leadership role in the Program to ensure responsibilities under the Act are upheld and that eradication is achieved most efficiently and effectively.

By maintaining a strong, well-resourced Cattle Tick Program, both producers and government fulfill their statutory obligations, protect animal health and welfare, and safeguard the state's cattle industry from significant economic and biosecurity threats.

Recommendation: That the NSW Government maintain and strengthen the Cattle Tick Program to ensure NSW retains its tick-free status and eradication remains the primary objective, while enabling both producers and government to meet their General Biosecurity Duty under the *NSW Biosecurity Act 2015*.

Similar Programs in NSW and other jurisdictions

Cattle tick programs in other jurisdictions

NSW Farmers recognises that some other states and territories in Australia have cattle tick infestations that are considered endemic and managed in zones. Adopting cattle tick management models in NSW that are used in other Australian jurisdictions would be a costly mistake.

Jurisdictions such as Queensland, the Northern Territory and Western Australia have programs that classify ticks as endemic across significant proportions of their territory. This is not the case in NSW and the contextual differences between these jurisdictions – including geography, climate, property and herd size – means the programs are not equitably comparable. Considering the programs as similar would be a disservice to the efforts producers, industry and the Department have contributed to maintaining NSW's tick-free status over nearly 100 years.

This incidence of cattle tick in NSW is confined to a small area of the north-east of the state. There is also a higher density of both beef and dairy producers operating in this area of NSW, with mixed land use, small farms, lifestyle blocks, and urban development interspersed with commercial dairy and cattle operations. This complexity makes self-managed or zonal approaches, like those in Queensland, impractical and risky in NSW, and instead demands strong government coordination to implement effective surveillance, treatment and compliance measures.

Beef production in NSW consists of approximately 20 per cent of the nation's cows and calves, and NSW is the second largest dairy producing state in Australia. This is a significant proportion of national beef and dairy production however there are only 450 infected properties⁴ in the north east corner of NSW – an incredibly small proportion of all producers in the state. This small number means eradication is achievable and far more cost-effective than managing widespread infestations (see financial modelling below). To succeed, the Program requires predictable, long-term funding and skilled personnel to maintain continuity and uphold high standards to ensure biosecurity remains uncompromised.

Recommendation: That the NSW Government does not consider implementation of cattle tick management models used in other jurisdictions as they are not comparable or appropriate to NSW.

⁴ DPIRD. 2025. Consultation Paper – Background on the NSW Cattle Tick Program

Similar programs in NSW

There are a number of programs in NSW, though not identical to the Cattle Tick Program, that have shared biosecurity objectives. These programs range from those that maintain freedom from pests, diseases and weeds found in other jurisdictions, through to federal and state government-operated programs that minimise biosecurity risks such as spread from endemic diseases and pests. Some examples are provided below. These programs provide different outcomes and impacts for agricultural production, but all demonstrate the importance of coordinated, well-resourced biosecurity measures.

Eradication of bovine tuberculosis and brucellosis

Australia's successful eradication of bovine tuberculosis and brucellosis provides valuable lessons for the NSW Cattle Tick Program. Both eradication campaigns demonstrated that long-term, government-led strategies with predictable funding, strong industry partnerships, and rigorous compliance frameworks are essential for success. These programs relied on coordinated surveillance, clear communication, and consistent enforcement to achieve national freedom from diseases that once posed major economic and animal welfare risks. Importantly, they showed that eradication – rather than ongoing management – is both achievable and cost-effective when supported by sustained investment and stakeholder engagement.

Grapevine phylloxera and Mediterranean fruit fly

Both grapevine phylloxera and Mediterranean fruit fly (medfly) are considered amongst the most destructive pests in the world to their hosts, and their management in NSW provides important learnings and precedence for the state's Cattle Tick Program.

Grapevine phylloxera is present in a small area of NSW (Sydney), however, as a result of strong biosecurity efforts and legislation preventing the movement vines, this pest has not spread to other grapevine growing regions of the state.

Medfly was discovered in Perth in 1895, and Western Australia is still the only state in the country to have the pest. This is thanks to strict quarantine measures and biosecurity protocols in every Australian jurisdiction,

The strict biosecurity protocols of NSW, and the classification of these as notifiable pests has protected the state from serious risks, economic and environmental losses, and has emphasised the importance of strong, government-led biosecurity measures.

Footrot program

The NSW Government Footrot Program operated since 1988 when the footrot strategic plan was developed⁵. The Program's objective is minimising the negative impact of virulent footrot on animal welfare, sheep and goat enterprise productivity and social wellbeing of NSW sheep and goat producers. The Program demonstrates government-industry partnership to reduce flocks impacted by footrot, reducing prevalence from 15 per cent of flocks affected in 1991 to less than 1 per cent of flocks by 2009. This successful partnership demonstrates the achievement possible through an appropriately government resourced and funded NSW Cattle Tick Program with strong industry partnerships to continue to maintain NSW's cattle tick free status and eradication of cattle ticks that enter NSW.

National Arbovirus Monitoring Program

⁵ NSW Government, 2023, *NSW Footrot Program 2023 Report*, [accessed online](#) 21 November 2025.

The National Arbovirus Monitoring Program (NAMP) monitors arboviruses of cattle, sheep, goats and camelids, including bluetongue, Akabane and bovine ephemeral fever. NAMP gathers data throughout Australia by serological monitoring of cattle in sentinel herds, surveys of cattle herds and insect vector (for example, mosquitoes, ticks, sandflies and midges) trapping⁶. Data is used to develop and amend the Bluetongue Virus Interactive Zone Map, which is based on World Organisation for Animal Health guidelines and defines areas where bluetongue virus occurs and where occurrence has not been detected for a minimum of two years.

Farmers and other stakeholders can request to be notified of changes to the map as they occur. NAMP monitors the occurrence of these arboviruses to help market access maintenance to arbovirus-sensitive markets.

However, NSW Farmers is aware of concerns with the map changing without notice and subsequent impacts to a producer's ability to export live dairy cattle to arbovirus sensitive markets. This affects business operations as markets can be switched off without warning. Trade and market access implications are the similarity drawn with the NSW Cattle Tick Program, as increased market access can be achieved through improved disease management derived from maintaining NSW's cattle tick-free status and eradication of cattle ticks that enter the state⁷.

Previous reviews and evaluations

The amount of publicly available information on previous reviews of the NSW Cattle Tick Program is quite limited, and so the following comments are restricted to a 2017 report undertaken by the then Department of Primary Industries, Graham Centre for Agricultural Innovation and Charles Sturt University - *Understanding rural landholders' perspectives on the NSW Cattle Tick Program*.⁸

The findings of the 2017 research report remain highly relevant and align closely with the feedback producers are providing today. The report highlighted strong support among landholders for the NSW Cattle Tick Program and its eradication objective, while also identifying the need for improved communication, clearer processes, and responsive service delivery. Producers then - and now - are not calling for less government involvement; they are asking for a stronger, better-resourced program that works in partnership with them to achieve eradication. This consistency over time demonstrates that the Program is valued and trusted, but confidence depends on visible government leadership and operational capacity.

The report also underscored the complexity of the Northern Rivers region, with its mix of commercial cattle enterprises, lifestyle blocks, and urban encroachment, creating unique compliance and surveillance challenges. These findings mirror current concerns about changing land use and the need for tailored engagement strategies. Producers today share the same sentiment expressed in 2017: eradication is achievable, and they are willing to do their part, but government must maintain its commitment and resources. Diluting the Program or shifting responsibility entirely to producers would undermine decades of success and expose NSW to unacceptable biosecurity risks.

⁶ Animal Health Australia 2025, National Arbovirus Monitoring Program, [accessed online](#) 21 November 2025.

⁷ Meat and Livestock Australia, 2025, *P.PSH.1076 – Product development of a new cattle tick vaccine*, [accessed online](#) 21 November 2025.

⁸ 2017. DPI, Graham Centre for Ag Innovation and CSU - Understanding rural landholders' perspectives on the NSW Cattle Tick Program. Available at: https://researchoutput.csu.edu.au/ws/portalfiles/portal/21717192/21122283_Research_report_OA.pdf

The Beneficiaries of the Program

The entire state of NSW is a beneficiary of the Cattle Tick Program. The Program is not a localised issue to the Northern Rivers - it is a state-wide safeguard for the cattle industry. Every beef and dairy producer, processor, and exporter in NSW relies on this program to keep markets open and herds healthy. This extends both nationally and internationally, with live beef export markets being disrupted for 6-12 months in the event of a tick fever outbreak, and dairy cattle potentially being unable to produce milk for their whole lactation cycle,⁹ impacting the availability of chilled products for export.

While it is primarily delivered in the Northern Rivers region of NSW, its benefits extend to beef and dairy producers and processors across the state. Maintaining NSW's cattle tick-free status is critical to protecting the integrity of the entire livestock industry. If the pest is not eradicated, and spreads further into NSW the consequences would be devastating resulting in increased production costs, disruption to domestic trade, loss of market access, and widespread animal welfare concerns. Tick infestations lead to animal and owner stress, increased costs and reduced productivity.

The Whole NSW Cattle Industry

As aforementioned, the NSW Cattle Tick Program is not just a regional safeguard¹⁰ - Maintaining NSW's tick-free status:

- ensures market access,
- protects herd health, and
- prevents the significant economic losses that would occur if ticks spread beyond the Northern Rivers.

Every beef and dairy producer across NSW benefits from the Program's success.

Northern Rivers Beef and Dairy Producers

Producers in the Northern Rivers are on the frontline of eradication efforts given their proximity to Queensland. The presence of cattle ticks in Queensland will likely always mean Northern Rivers beef and dairy producers face the greatest risk of an incursion of tick, and deadly tick fever, and the Program provides critical surveillance, treatment, and compliance measures to protect their animals and livelihoods.

The Entire Supply Chain

The benefits extend far beyond the farm gate. Processors, transport operators, feedlots, and exporters all rely on a tick-free NSW to maintain smooth operations and market access. Infestations would trigger movement restrictions, disrupt logistics, and increase compliance costs throughout the supply chain, ultimately impacting consumers and export competitiveness. Both Australian and international consumers trust the quality of Australian beef and dairy products, and the Cattle Tick Program helps protect this reputation.

Regional Communities and Jobs

⁹ QLD Government. 2025. Managing tick fever in cattle. Available at: <https://www.business.qld.gov.au/industries/farms-fishing-forestry/agriculture/biosecurity/animals/diseases/cattle-tick-fever/manage>

¹⁰ DPIRD. 2024. Livestock – Beef Cattle. Available at <https://www.dpi.nsw.gov.au/about-us/publications/pdi/2024/beef-cattle>



The cattle industry is a cornerstone of regional economies, supporting thousands of jobs in production, processing, transport, and associated services. If ticks were to spread, the resulting economic shock would ripple through rural communities, threatening employment and local businesses that depend on a strong livestock sector.

The Social Fabric of Regional Communities

Cattle and horses are central to the cultural life of regional NSW, particularly through agricultural shows and community events. If tick infestations prevent livestock from attending shows, it would undermine these traditions and the social fabric of rural towns. Regional shows are more than entertainment – they are vital for community cohesion and local economies. Losing them would erode the lifeblood of many communities.

Additional information

Transitional arrangements

NSW Farmers does not support any transitional arrangements. The Cattle Tick Program needs to remain a deliverable biosecurity and animal health and welfare activity of the Government, with the prioritisation of adequate funding, staffing, communication, education¹¹ for the benefit of livestock industries and Australia's agricultural production and trade.

Recommendation: That the Cattle Tick Program remain a key biosecurity defence focus of the NSW Government, with prioritisation of adequate funding, staffing, communication and education for the benefit of livestock industries and regional communities.

Recommendation: That the NSW Government prioritise predictable, long-term funding allocation to the Program and the employment of skilled personnel in the Program to guarantee continuity and high-standard delivery of prevention, surveillance, treatment, and compliance measures.

Financial modelling

The financial impact of cattle tick on NSW producers, even under conservative estimates, is significant and demonstrates why the NSW Cattle Tick Program must continue. Based on ABARES data,¹² non-sale cattle movements of up to 3,000 head could be affected annually if ticks disrupt movements, meaning cattle have to be held longer on-farm. At a conservative cost of \$140 per head¹³ for holding cattle longer, this equates to \$420,000, rising to over \$1 million in a heavy tick year. In drought conditions, when supplementary feeding costs can reach \$975 per head,¹⁴ the impact could escalate to \$3 million. These are significant cost concerns for producers which will only worsen if ticks in NSW are not eradicated.

¹¹ NSW Farmers Policy 4740. 23AC - NSWFA seek the prioritisation of adequate funding, staffing communication, education and the regulation of the ticks and tick fever for the benefit of the livestock industries and support the NSW Cattle Tick Program and re-establish the NSW Border biosecurity checkpoints

¹² ABARES. 2025. Farm Data Portal. Available at: <https://www.agriculture.gov.au/abares/data/farm-data-portal>

¹³ DPIRD. 2025. Beef Cattle Gross Margin Budget. Available at: https://www.dpi.nsw.gov.au/_data/assets/pdf_file/0005/1622975/Beef-enterprise-feeder-steers-gross-margin-July-2025.pdf

¹⁴ QLD DPI. 2020. Feeding Cattle. Available at: <https://www.dpi.qld.gov.au/business-priorities/biosecurity/animal-biosecurity-welfare/welfare-ethics/drought-feeding/feeding-cattle>

Beyond movement delays, productivity losses compound the problem:

- MLA estimates treatment and prevention costs at \$20 per head.¹⁵ For the Northern Rivers region alone, with an average beef herd size of up to 400,000, this represents \$8 million in costs. Factoring in a statewide beef herd size of over 5 million animals, the potential costs of treatment and prevention across the state, for beef alone, would be astronomical if ticks were to become endemic in NSW.
- Research shows cattle impacted by tick can suffer an average weight deficit of 15 kilograms. At current liveweight prices – \$5.37 per kilogram for steers and \$3.80 per kilogram for cows¹⁶ – this equates to a loss of approximately \$80.55 per steer and \$57 per cow. When scaled across herds in tick-affected areas, these losses quickly accumulate into hundreds of thousands of dollars in foregone income. Combined with additional costs for treatment, prevention, and delayed movements, the financial burden on producers is significant.

It should be noted that these figures do not factor in the cost of department resources, staff and materials; nor do they factor in the impacts that the delays in stock reaching market would have on supermarket shelves and increased prices to compensate for producer costs.

Consequently, these figures underscore that the current expenditure on the Program by Government is a fraction of the economic losses NSW would face without continuing eradication efforts.

Producer mental health

The mental health toll of cattle tick on producers cannot be overlooked. Watching livestock suffer from cattle tick infestations is deeply distressing for farmers who take pride in animal welfare. This emotional strain is compounded by the financial pressure of treatment, prevention, and lost productivity. The constant worry about potential outbreaks, market disruptions, and the viability of their business adds layers of stress, often leading to anxiety and burnout. For many producers, these challenges are not just economic - they affect family wellbeing and community resilience. A coordinated, government-led eradication Program is therefore critical, not only to protect livestock and livelihoods but also to safeguard the mental health of those who feed the nation.

NSW Farmers commitment to success of the program

NSW Farmers is committed to working collaboratively with government, producers, and the broader livestock industry to ensure the NSW Cattle Tick Program remains effective and sustainable. Our priority is to maintain NSW's tick-free status through a coordinated, government-led approach that protects biosecurity and supports producers. Such a program needs predictable, long-term funding and skilled personnel needed to deliver high-standard surveillance, treatment, and compliance measures to uphold the integrity of the state's dairy and cattle industries.

Recommendation: That the Government prioritise predictable, long-term funding and skilled personnel to guarantee continuity and high-standard delivery of surveillance, treatment, and compliance measures.

Further opportunities

¹⁵ Meat and Livestock Australia. 2022. Priority list of endemic diseases for the red meat industry - 2022 update. Available at https://www.mla.com.au/globalassets/mla-corporate/research-and-development/final-reports/bahe0327_final-report_revised.pdf

¹⁶ Agora Livestock. November 2025. Casino Cattle Report. Available at: <https://agoralivestock.com.au/saleyard-casino-cattle/>

Dips

NSW Farmers is aware of a significant number of dip sites throughout the North Coast of NSW (1,648).¹⁷ While it is acknowledged that approximately 254 of these have been demolished there remains an opportunity for any dips that are usable, to be reinstated for use as part of the Program.¹⁸ When used correctly, dips are an effective method of treatment to control and kill cattle ticks.

Cattle tick vaccine

Early trials of a cattle tick vaccine developed by the University of Queensland have shown promising results in disrupting the life cycle of a tick.¹⁹ Such a vaccine could be considered as a tool to support biosecurity strategies to maintain NSW's tick-free status. While not a complete solution, the vaccine's potential to integrate into the future of the Program as a supportive tool reinforces the need to continue to pursue eradication. It is important to note that this vaccine is entirely different from tick fever vaccines, which carry risks of spreading fatal tick fever to unvaccinated cattle.

Recommendation: That the NSW Government leverage existing opportunities, including reinstating usable dip sites.

¹⁷ DPIRD. 2017. Arsenic and DDT residues at cattle dip yards. Available at:

<https://www.dpi.nsw.gov.au/agriculture/chemicals/animal-chemicals/cattle-dip-yards>

¹⁸ NSW Farmers Policy 4971. 25AC: NSW Farmers requires the DPIRD (or relevant government agency) to facilitate the ease of dipping cattle through fully funding the maintenance of existing dips and exploring the establishment of strategically placed, new facilities and/or re-opening de-commissioned facilities.

¹⁹ University of Queensland. 2024. Media Release: Ticking the boxes to defeat a deadly parasite. Available at: <https://news.uq.edu.au/2024-06-19-ticking-boxes-defeat-deadly-parasite>

Submission to Dr Katherine Clift, NSW Biosecurity Commissioner

Urgent: Restore Government-led Management of the NSW Cattle Tick Program

Dear Dr Clift,

We welcome your independent review of the NSW Cattle Tick Program and thank you for undertaking this important work. The Biosecurity Act 2015 objectives are to prevent, early detect and eradicate cattle tick from NSW.

This submission strongly urges the Commissioner to prioritise immediate, government-led action to restore the effectiveness of the NSW Cattle Tick Program. The historic, century long buffer zone that protected NSW from cattle tick and tick fever has been eroded. Recent spread, repeated outbreaks of tick fever and increasing cattle deaths demonstrate that leaving control to voluntary, owner led measures is failing and that DPIRD must resume active, enforceable management with adequate resources as soon as possible.

Summary

- For more than 100 years the regulated Cattle Tick Program, including the Tweed buffer zone kept NSW essentially tick free. The Tweed was verified tick free in 2004.
- In recent years infestations and outbreaks of tick fever have increased and moved south and west, driven primarily by inadequate enforcement, lack of DPIRD tick officers on the ground and inconsistent inspections. Lack of working plunge dips available to producers for treating cattle, and lack of compliance to the rules.
- The move towards Owner Management approach cannot be interpreted as neutral policy reform. In effect it appears to be a decision to shift responsibility for a public good onto individual producers rather than addressing the clear gaps in DPIRD resourcing and enforcement.
- Owner Management places impossible expectations on producers. Even when a small number of properties fail to comply with the rules, reinfestation is inevitable that is the reason why government management and enforcement is essential.
- This is an animal welfare crisis. DPIRD must immediately re-establish active management, enforcement and surveillance with clear consequences for non-compliance.

Problem Detail

- The buffer zone QLD/NSW border has functioned as a collective protection mechanism. Due to lack of staff the buffer zone is now compromised by ongoing untreated infestations and by cattle movements from infested areas.

- Enforcement gap: There are currently insufficient DPIRD tick officers to complete the necessary inspections and commence Cattle Tick Program on properties with infestations. Known infested neighbouring properties have not been inspected, and required to treat, or compelled to implement the Cattle Tick Program, resulting in repeated reinfestation of compliant properties.
- Regular purchases by NSW producers of blooded (hot) bulls and cattle from infested regions in QLD and NSW studs who are wanting to sell to their QLD clients. Movement of “hot” cattle into NSW is increasing with producers unaware of the high risk they are exposing their naïve herd.
- Owner Management failures: Over recent years the responsibility has shifted to the property owners, but many properties do not, cannot or will not meet the required standards. Where the Owner Management Program has been relied upon, reinfestations have continued, demonstrating the model **cannot** replace regulated, resourced government intervention.
- Vaccinating naïve cattle with live tick fever can itself produce fever, anaemia, loss of condition especially in older and pregnant cows causing reduce fertility, abortion and death. These reactions reduce production and welfare increased labour and treatment costs and risk spreading the vaccine organisms via ticks onto naïve tick free cattle.
- Animal Welfare and Economic impacts. Tick fever causes deaths, abortions and long-term production losses. The stress on vaccination is expensive, extremely stressful on naïve stock and not viable large-scale substitute for prevention. Producers cannot absorb the financial and welfare costs if the buffer fails.

Why DPIRD must manage the NSW Cattle Tick Program

- Cattle tick control is a shared public good. Individual compliance only protects neighbours if it is universal. If a few properties remain non-compliant, reinfestation follows. This makes government enforcement essential.
- Proven historical success. Eradication & containment historically succeeded because government led, coordinated and **enforced** the program. **That structure must be reinstated.**
- DPIRD has the statutory authority, veterinary expertise & operational capacity to deliver coordinated surveillance, diagnostics, treatment oversight & rapid response.
- Legal & regulatory responsibility. Biosecurity regulation exists to protect the broader community. If DPIRD continue to scale back enforcement and the buffer zone collapses, the state risks large-scale animal welfare failures and economic losses for which it is responsible.
- There needs to be clear penalties for non-compliance. This will also deter risky behaviour and ensure fairness for producers who have complied to the rules.
- My property was officially placed under quarantine on 23/5/2023 , as were most of the neighbouring properties once inspected and ticks found, the majority

worked hard and got our properties and livestock clean, 2 properties which are direct neighbours to infested properties in the immediate area , still are yet to have their livestock inspected for the first time 2 and a half years later , 1 at Kunghur Creek , the other at the end of Midginbil road, yet all properties that were inspected as clean are back in quarantine for the second time around, wallabies and wild dogs carry ticks from property to property. Being in tick quarantine has placed a significant financial burden on my business, as all my cattle movements and work pre-quarantine were conducted on a weekend , so as not to interfere with my mechanical contracting business. During the treatment period , treatments were conducted on a week day which meant losing a day of contracting work. To move livestock off the property requires the cattle to be dipped which is only possible on a week day, another day of lost income. The DPI needs extra funding to put more officers on the ground and stop behaving like a toothless tiger in enforcing livestock owners to yard their cattle for inspection so we can conduct a blanket program and have a crack at eradicating cattle tick in this area.

This is not a theoretical risk; it is happening now. The erosion of the historic tick-free buffer and the increase in tick fever cases are direct consequences of under-resourcing, reduced inspections and an overreliance on owner management. DPIRD must retake operational control, boots on the ground, enforced inspections, clear penalties and a transparent program backed by adequate staffing. Without decisive action now, the scale of animal suffering and economic loss will be catastrophic and entirely preventable.

Yours sincerely

Darrell Plater

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Livestock Immunity to Ticks

Johann Schröder



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5 Adaptive Immunity Elicited by Vaccination

Abstract

Vaccination can induce an adaptive immunity to ticks. Depending on the chosen antigen or antigen combination, this immunity can interfere with tick feeding, survival, reproduction and/or pathogen transmission. Various techniques have been used to select from a rich source of candidate subunit antigens in tick secretions, and in structural and functional proteins. Coevolution of ticks and their hosts means that ticks can avoid or neutralize many of the natural immune mechanisms they encounter. Having to empirically test the protective immunogenic efficacy of candidate vaccines, and comparing antigens with each other, has uncovered a new field of scientific endeavour. Formulating the antigen(s) in a vaccine that will optimize the degree and duration of protection presents additional challenges.

Vaccination is a cornerstone of health management, due to its low cost, proven efficacy, duration of effect, safety profile and ease of delivery (Meeusen *et al.*, 2013). Research into immunological control of ticks follows two main approaches – investigating the antigens eliciting the complex physiological and immunological reactions to tick infestation, and the effects of immune reactions that do not occur as a result of natural infestation (Willadsen and Jongejan, 1999). Tick bites elicit both innate (cutaneous basophil hypersensitivity, leucocyte infiltration) and adaptive (T- and B-lymphocytes, plasma cell, macrophage and DC involvement) immune responses, with an important role ascribed to cell-mediated immunity. It is desirable to develop vaccines with the broadest possible application (Trimnell *et al.*, 2005).

Translational biotechnology over the past three decades has uncovered many tick–host–pathogen interactions at the molecular level and offers the promise of several avenues towards better tick control, with engagement of the immune system a major player (de la Fuente, 2021).

The objective of vaccinating against ticks is worth considering. The reviewed literature investigates many antigens that will elicit a

protective immune response which interferes with tick physiology, ultimately leading to the demise of the tick. It requires ticks to ingest host antibodies raised against internal antigenic epitopes (hidden or concealed antigens: shifting the ‘battlefield’ into the tick). There is the alternative paradigm of immunizing the host against the harmful secreted components of tick saliva, including the pathogens they may transmit (waging the ‘battle’ inside the host). In the latter case, interfering with the tick’s vector competence will most probably again require an ingested immunoglobulin (Merino *et al.*, 2013a). A vaccine containing a mixture of antigens seems to offer the promise of achieving both these goals and against multiple tick species, to broaden its global appeal (Parthasarathi *et al.*, 2021).

It is also vitally important not to forget that resistance to ticks has many effector mechanisms, of which the innate and adaptive immune response is one (Jonsson *et al.*, 2014). In addition to using a vaccine to reduce reliance on chemical acaricides, integrated tick management will include efforts to select for and breed tick-resistant cattle, using additional resources such as genomic and proteomic profiling (see sections 2.3 and 4.4.2, this volume).

5.1 Early Successes

5.1.1 Protozoa and helminths

The concept of eliciting adaptive immunity to parasites is not new. Immunizing livestock against parasites was first achieved in the case of *Eimeria* spp. in chickens (Chapman, 2014). Irradiated third-stage larvae of lungworm (*Dictyocaulus* spp.) have been used for a long time to immunize cattle and sheep against husk (Cornwell, 1961; Benitez-Usher, 1975; Benitez-Usher *et al.*, 1976). Protozoa of the genus *Babesia* are still being attenuated by serial passage before being used to immunize cattle, with or without chemical intervention against any possible adverse vaccine reaction (de Vos and Jorgensen, 1992). The infect-and-treat method (ITM) achieves immunity in cattle against East Coast fever and tropical theileriosis, without attempting to attenuate the parasites (Irvin, 1985). A live attenuated isolate of *Toxoplasma gondii* is available as a commercial vaccine in New Zealand (Kelly, 2018). Although a protective immunogenic replica could not be expressed by recombinant technology, a gut antigen of the gastrointestinal helminth *Haemonchus contortus* was isolated and a vaccine successfully commercialized (Smith, 1993; Smith and Smith, 1993; Smith *et al.*, 1994, 2001).

Technological advances enabling the recombinant expression of gene products and precision fermentation have led to rapid advances in the development of vaccines with subunit antigens against infectious disease, but less so in the case of helminth, protozoal and arthropod parasites.

5.1.2 Ticks

Immunizing guinea pigs and cattle in Canada with antigens extracted from the gut and other internal organs of partially fed ticks, collected before they had completed their bloodmeal (Allen and Humphreys, 1979), followed soon after by reports of immunizing rats against *D. variabilis* with tick midgut extracts (Ackerman *et al.*, 1980), opened the door to a revolution in tick control of livestock.

In preparation for further work, a sequential histological examination traced the development and function of gut cells in pharate (unmoulted) and feeding nymphs and adult female [*B. microplus*] *R. australis*. Stem cells develop into secretory and digest cells, the former containing glycoprotein granules and the latter packed with endosomes filled with ingested host cells and fluid (Agbede and Kemp, 1985). This was followed by an ultrastructural study to confirm the identity of the secretory cell types identified earlier, their origin and the formation of their granules (Agbede and Kemp, 1987).

Engorged female ticks were collected 18–20 days after larval infestation, suspended in buffered saline and disrupted in a blender before the suspension was filtered to remove the tick cuticles and centrifuged. In a series of three experiments, centrifuge supernatant and pellet material were used singly or combined, with Freund's complete adjuvant, to vaccinate Hereford (European) and Droughtmaster (composite Zebu) cattle by various regimes. Vaccinated cattle were exposed to induced infestations with varying numbers of [*B. microplus*] *R. australis* larvae. The effect of vaccination was evaluated by indirect radioimmunoassay and gel diffusion of antibody levels, histopathology of tick gut mucosae, numbers of ticks engorging and laying eggs, and visible signs of damage (Fig. 5.1). The histological lesions had not previously been seen in ticks from cattle with naturally acquired resistance. Antibody titres in individual cattle were not correlated with their immunity. Tick survival, engorgement and ovipositioning were compromised to varying degrees. Larvae feeding on vaccinated cattle were largely unaffected (Agbede and Kemp, 1986; Johnston *et al.*, 1986; Kemp *et al.*, 1986).

A different approach was taken with the dissection of synganglia and midguts from semi-engorged [*B. microplus*] *R. australis* removed from cattle and processing these to yield three fractions. Vaccines were prepared by emulsifying soluble gut antigens in Freund's incomplete adjuvant, suspending particulate gut antigens in saponin or suspending disrupted synganglion cells in Freund's incomplete adjuvant. Hereford cattle were vaccinated three times in 6 weeks and challenged with larval infestations 8, 9 and 10 weeks later. Vaccinated cattle dropped fewer engorged ticks that weighed less and laid fewer eggs than those from unvaccinated animals. The



Fig. 5.1. Evidence of damage to gut epithelium in *R. microplus* adults feeding on vaccinated cattle, with host erythrocytes leaking into haemolymph causing red leg discolouration. From Kemp *et al.* (1986) with permission of Elsevier.

'gut vaccine' gave 87% protection, compared with 80% when a gut-synganglion combination was given. A synganglion-alone vaccination did not provide protection. Antibody titres were demonstrated by ELISA (Opdebeeck *et al.*, 1988).

Differing from naturally acquired immunity directed largely at tick larvae, vaccination with antigens extracted from adult [*B. microplus*] *R. australis* results in direct damage to the adult tick, decreasing replete weight and affecting egg laying. Numerous purification steps were required for final identification of the antigen(s) responsible for inducing the immunity (Willadsen *et al.*, 1988).

Salivary gland extracts of *H. a. anatolicum* were used to vaccinate European/Zebu cross-bred cattle twice, 2 weeks apart, prior to challenging them with unfed adult ticks. Whole extract (as opposed to centrifugal sediment or supernatant) administered with Freund's complete adjuvant was most effective in reducing weight and egg production (Banerjee *et al.*, 1990), but no distinction was made between antigen(s) that may have been secreted or constitutional.

5.1.3 Bm86

The successful cloning in *E. coli* of a cDNA encoding for a tick gut epithelium surface glycoprotein was a landmark achievement. The responsible tick gut antigen was designated Bm86. It is present in very low abundance in extracts of partially engorged adult female ticks and has an affinity for wheat germ lectin. Microgram amounts of this antigen were shown to induce effective protection in cattle against [*B. microplus*] *R. australis*, as shown by the decreased survival of ticks on vaccinated cattle and a reduction in engorgement weight and severely impaired reproductive capability of the survivors. Antisera to the antigen react with the surface of digest cells in the tick gut, strongly and rapidly inhibiting their endocytotic activity, which is a critical step in bloodmeal digestion. A number of peptides from this antigen were sequenced and one was found to have homology with the epidermal growth factor (EGF) precursor and a second to be homologous with

a putative protective antigen from *Plasmodium falciparum* (Willadsen and Kemp, 1988; Rand *et al.*, 1989; Willadsen *et al.*, 1989, 1992). Improvements and refinements followed, such as high yield expression by *Pichia pastoris* with improved immunogenicity (Rodríguez *et al.*, 1994).

Glycosylation is common in cell surface proteins such as on the surface of tick gut cells and protects against proteolysis. It would also be expected for some salivary proteins secreted at the tick attachment site. This explains why antibodies from animals infested with ticks will react with tick-derived Bm86 (through the carbohydrate epitopes) but not with recombinant Bm86 which only has the polypeptide epitope. The damage to the gut of [*B. microplus*] *R. australis* and the decrease in engorgement weight and egg production are much less in cattle with naturally acquired immunity than in vaccinated cattle (Willadsen and McKenna, 1991; Willadsen *et al.*, 1993a).

The recombinant Bm86 vaccine Gavac™ ('garapatas de vacca' – tick of the cow) was tested in dairy and beef cattle exposed to natural tick (this is assumed to be *R. microplus*, although it is not explicitly stated in the publication) infestations in Brazil. In a field study in Cuba, tick-naïve calves were vaccinated with Gavac, kept on natural pasture and challenged with an induced *R. microplus* larval infestation. In both cases, the antibody response was monitored by ELISA and efficacy by the numbers of engorged ticks, which were substantially reduced (Rodríguez *et al.*, 1995a, b).

Gavac was initially commercialized in Cuba, Colombia, Dominican Republic, Brazil and Mexico. In field trials, it was 55–100% effective in controlling infestations of [*B.*] *R. microplus* for 12–36 weeks (de La Fuente *et al.*, 1998, 1999). It led to an 82% reduction in the use of chemical acaricides in Cuba within 8 years, and a dramatic drop in the numbers of clinical cases of babesiosis (Valle *et al.*, 2004; de la Fuente *et al.*, 2007a). A similar reduction (83.7% within 2 years) in acaricide use was seen in Venezuela (Suarez *et al.*, 2016) but Gavac has been less successful commercially in Mexico, where cattle are concurrently infested with *Amblyomma cajennense* which is not affected by the vaccine (de la Fuente *et al.*, 2007a).

Marketed as TickGARD® (with the antigen expressed by *E. coli*) and later TickGARD®PLUS

(expression by *Pichia pastoris*) (Tabor, 2021), Bm86 was not a commercial success in Australia for several reasons. Having to inject cattle more than once a year to maintain an effective antibody titre is impractical in northern Australia, where cattle face the greatest challenge from [*R. microplus*] *R. australis* but are routinely mustered only once a year.

Australian dairy cattle vaccinated with TickGARD®PLUS in southern Queensland carried fewer ticks, with lower tick reproductive efficiency. The cows gained more weight than unvaccinated controls and had lower milk somatic cell counts (Jonsson *et al.*, 2000). On Friesian cattle immunized with two TickGARD injections and challenged with [*B.*] *Rhipicephalus annulatus* larvae in Israel, the ticks did not develop to nymphs and adults. Larvae derived from *Babesia bovis*-infected female ticks transmitted the infection to vaccinated cattle but nymphs from females infected with *Babesia bigemina* did not, supporting the observation that Bm86 does affect the larval stage of *R. annulatus* (Pipano *et al.*, 2003).

More than 30 years after its registration (de la Fuente, 2021), the commercial success of the Bm86 recombinant antigen against infestations of *R. microplus* has not yet been equalled, but its success is hampered by a narrow efficacy spectrum, genomic variation and limited duration of immunity (de la Fuente *et al.*, 2017).

Cattle vaccinated with Bm86 were exposed to induced infestations of [*B.*] *R. microplus* and *R. decoloratus* simultaneously and displayed 74 and 70% reduction in reproductive capacity, respectively. Induced infestations with *H. a. anatolicum*, *H. dromedarii*, *R. appendiculatus* and *A. variegatum* were affected in varying to non-significant extent (de Vos *et al.*, 2001). Bm86 confers partial protection against *Hyalomma* and *Rhipicephalus* spp. that are phylogenetically closely related but fails to protect against the phylogenetically more distant *Amblyomma* spp. (Almazán *et al.*, 2003). Camels and cattle were vaccinated with Bm86 and challenged with induced infestations of *H. dromedarii* and *A. cajennense*. The effect on the various assessment criteria for *H. dromedarii* nymphs and adults was variable, but no effect against *A. cajennense* was seen (Rodríguez-Valle *et al.*, 2012a).

This limited success has evidently not hampered the search for an improved solution,

and a host of alternative antigens have been investigated (de la Fuente *et al.*, 2017). More is said below (see section 5.2.5) about Bm86 orthologues in other tick species. Table 5.1 lists some examples of subunit antigens investigated in vaccines against ticks.

5.2 Finding the Right Antigen

Ectoparasitic arthropods are large and complex, with genome sizes not unlike those of their hosts, and spend most of their lives outside or on the surface of their hosts. This poses a considerable challenge to vaccine development (Meeusen *et al.*, 2007). The tick genome is large (Jongejan *et al.*, 2007), with the genome of *R. microplus* estimated to be 7.1 gigabase pairs, more than twice the size of the human genome (Fogaça *et al.*, 2021), but it also contains significant amounts of repetitive DNA, potentially problematic for genome assembly. Whole-genome sequencing (WGS) relies on inbred colonies with minimal polymorphism, which is not available for many Acari. Evolutionary distance between ticks and insects may limit homology-based gene predictions (Van Zee *et al.*, 2007). Having access to an annotated tick genome has been valuable in the discovery of potential antigens (Bellgard *et al.*, 2012) but hampered by the fact that 60% of the (in this case, *R. microplus*) genome lacks similarity with sequences from other organisms, making transcriptomic analysis difficult. Cross-species microarray technology was used to identify genes shared by *R. microplus* and *R. decoloratus* (van Zyl *et al.*, 2015), both of which infest cattle in southern Africa and should ideally be controlled by the same vaccine.

Whether by chemical or immunogenic means, when tick management interventions are devised, the chosen gene function(s) target must be sufficiently conserved to ensure an acceptable species spectrum of susceptibility, but not so well conserved that anti-tick measures will have an unintended adverse effect on the host. Subolesin (see section 5.2.5) is an example of a gene conserved in arthropod parasites and vertebrate hosts, raising the potential for inducing autoimmune reactions. The expectation that the antibody response will be directed at non-self epitopes, thus reducing the risk to the

vaccinated host, seems to have been validated (de la Fuente *et al.*, 2011).

The sources of identified antigen candidates for an anti-tick vaccine can be grouped as egg, salivary gland, midgut, Malpighian and cement associated (Abbas *et al.*, 2023).

Messenger RNA (mRNA) purified from salivary glands dissected from feeding female *A. variegatum* was used to make random cDNA clones, whose nucleotide sequences were analysed. Sequences that encode for protein components of tick cement, proteins that contribute to anticoagulant and anti-inflammatory responses, and potential immunosuppressive molecules were found. It was surmised that interference with the function of these proteins might disrupt the life cycle of *A. variegatum*, helping to control it and reduce its disease transmission capability (Nene *et al.*, 2002). Genes transcribed in the salivary glands of female *R. appendiculatus* infected with *Theileria parva* did not differ greatly from those expressed in uninfected ticks. The recognized genes coded for previously characterized tick salivary proteins, including an immunosuppressive protein (Nene *et al.*, 2004).

Because T-cell responses are important in immunity to parasites, T-cell epitope identification is an important first step in developing T-cell-directed vaccines. Mapping T-cell epitopes for BoLA MHC peptide ligands has been done with mathematical algorithms, offering an advantage in the development of T-cell-directed vaccines. T-cell epitopes can be used as vaccine components or can help with the selection of antigens for inclusion in a vaccine (De Groot *et al.*, 2003).

Twenty-two or more antigenic molecules have already been tested in trials for vaccinating cattle against *R. microplus*. Despite considerable interest in antigenic proteins in tick cement and saliva (Zhou *et al.*, 2006), concealed and dual antigens seem to predominate in vaccination experiments (Leal and Ferreira, 2021).

Characterizing the haemolymph proteome of *Hyalomma asiaticum* by liquid chromatography tandem mass spectrometry yielded 311 proteins. Thirteen of these were immunity related and included PRRs, signalling proteins and effectors. Serpins and other proteins that may participate in coagulation were also identified. These discoveries could facilitate the

Table 5.1. Examples of subunit tick antigens.

Protein	Function	Species	Author	Hidden?
Akirins; subolesin; Q38, Q41 chimeras	NF- κ B gene expression, innate tick immunity	Conserved	de la Fuente <i>et al.</i> , 2011, 2013; Merino <i>et al.</i> , 2011; Carreón <i>et al.</i> , 2012; Naranjo <i>et al.</i> , 2013; Trentelman <i>et al.</i> , 2019; Contreras <i>et al.</i> , 2020; Lee <i>et al.</i> , 2020; Mendoza-Martinez <i>et al.</i> , 2021	Y
Aquaporin	Water channelling	<i>I. ricinus</i>	Campbell <i>et al.</i> , 2010	Y
ATAQ proteins?	Bloodmeal digestion	<i>R. microplus</i> <i>R. appendiculatus</i> <i>H. longicornis</i>	Nijhof <i>et al.</i> , 2010 Kiper, 2013 Adjou Moumouni <i>et al.</i> , 2023	Y
Bm86	Bloodmeal digestion	<i>R. microplus</i> <i>R. australis</i> <i>R. decoloratus</i>	Willadsen and McKenna, 1991; Willadsen <i>et al.</i> , 1993a; Rodríguez <i>et al.</i> , 1995a, b; de Vos <i>et al.</i> , 2001	Y
Bm86 orthologues		<i>R. annulatus</i> <i>H. a. anatolicum</i> <i>H. m. marginatum</i> <i>H. excavatum</i> <i>H. dromedarii</i> <i>I. scapularis</i>	Canales <i>et al.</i> , 2008; Azhahianambi <i>et al.</i> , 2009; Canales <i>et al.</i> , 2009a; Ben Said <i>et al.</i> , 2012a; Koči <i>et al.</i> , 2021	Y
Bm91		<i>R. australis</i>	Jarmey <i>et al.</i> , 1995; Nijhof <i>et al.</i> , 2007	Y
Bm95		<i>R. microplus</i>	García-García <i>et al.</i> , 2000	Y
BmVDAC	Mitochondrial porin	<i>R. microplus</i>	Ortega-Sánchez <i>et al.</i> , 2020	Y
Calreticulin	Ca-binding, anticoagulant	<i>H. longicornis</i>	Zheng <i>et al.</i> , 2022; Parizi <i>et al.</i> , 2023	N
Cathepsin	Proteolytic processes	<i>R. microplus</i>	Leal <i>et al.</i> , 2006a	Y
Cement protein RH50	Attachment, feeding	<i>R. haemaphysaloides</i>	Zhou <i>et al.</i> , 2006	N
Cement protein 64P	Attachment, feeding	<i>R. appendiculatus</i>	Havliková <i>et al.</i> , 2009; Saimo <i>et al.</i> , 2011	N
Cystatin	Blood digestion Immune modulation	<i>R. appendiculatus</i> <i>R. microplus</i> <i>I. scapularis</i> <i>O. moubata</i>	Rangel <i>et al.</i> , 2017; Lu <i>et al.</i> , 2020; Parizi <i>et al.</i> , 2020	
Ferritin 2	Fe metabolism	<i>I. ricinus</i> <i>R. microplus</i> <i>R. annulatus</i>	Hajdusek <i>et al.</i> , 2010	Y

Continued

Table 5.1. Continued

Protein	Function	Species	Author	Hidden?
GPCRs	Signal transduction	<i>R. microplus</i>	Brock <i>et al.</i> , 2019	Y
Lipocalin	Histamine binding	<i>I. persulcatus</i> <i>H. longicornis</i>	Mans <i>et al.</i> , 2003; Konnai <i>et al.</i> , 2011; Wang <i>et al.</i> , 2021; Pérez-Sánchez <i>et al.</i> , 2022	N
Metalloproteases	Anticoagulant	<i>R. microplus</i>	Ali <i>et al.</i> , 2015	N
Paramyosin	Immune modulation	<i>R. microplus</i> <i>H. longicornis</i>	Leal <i>et al.</i> , 2013; Wu <i>et al.</i> , 2021	Y
Ribosomal phosphoproteins	Protein synthesis	<i>R. microplus</i> <i>A. mixtum</i> <i>R. haemaphysaloides</i> <i>H. longicornis</i>	Rodríguez-Mallón <i>et al.</i> , 2012, 2015, 2020a, b	Y
Serpins	Anticoagulant	<i>A. americanum</i> <i>R. australis</i> <i>R. haemaphysaloides</i>	Rodríguez-Valle <i>et al.</i> , 2012b; Kim <i>et al.</i> , 2016a; Xu <i>et al.</i> , 2020	Y
Silk	Cement component	<i>R. microplus</i>	Zivkovic <i>et al.</i> , 2010a; Antunes <i>et al.</i> , 2014	N
TIM	Glycolysis	<i>H. longicornis</i>	Liang <i>et al.</i> , 2022	Y
TROSPA	Pathogen transmission	<i>R. microplus</i>	Merino <i>et al.</i> , 2013b	Y
Ubiquitin	Protein turnover	<i>I. scapularis</i> <i>A. americanum</i> <i>D. variabilis</i> <i>H. longicornis</i>	de la Fuente <i>et al.</i> , 2007b; Choy <i>et al.</i> , 2013; Bullard <i>et al.</i> , 2016b; McClure Carroll <i>et al.</i> , 2019; Wang <i>et al.</i> , 2020	Y

identification of new targets for control of tick-borne pathogens (Yuan *et al.*, 2022).

5.2.1 Immuno-screening

A cDNA library constructed from mRNA extracted from partially fed female *H. longicornis* was immuno-screened in rabbits. The cDNAs of two genes encoding salivary gland proteins were cloned and their expression sites determined by RT-PCR on dissected tick salivary glands and midguts. The recombinant version of one of the genes was expressed *in vitro* in *E. coli*. The feeding of immature and adult ticks feeding on rabbits vaccinated with the recombinant protein was impaired (Tsuda *et al.*, 2001).

Another cDNA expression library was constructed from an *I. scapularis* cell line initially derived from tick embryos. Mice were immunized with pools of the cDNA clones and challenged with *I. scapularis* larvae, in a process labelled expression library immunization (ELI). Clones in pools that induced immunity were sequenced and individual antigens identified. Interpreting the results of this work can be complicated. Failure to demonstrate an effect of immunization could be due to a cellular rather than a humoral effect (i.e. no antibodies transferred from bloodmeal to haemolymph). Or immunization could have unintended consequences, such as promotion of tick feeding because the expressed antigens encode for tick immunosuppressants or anticoagulants, for example. A

protective antigen (4D8) from one of the cDNA subpools was later designated subolesin (see section 5.2.5) (Almazán *et al.*, 2003).

5.2.2 Reverse vaccinology (RV)

This process, aimed at producing an immunogenic recombinant subunit antigen, starts with an annotated map of the organism's genome sequence, working backwards to find proteins essential for the organism's function and survival, determining their molecular structure and site(s) of expression *in silico*, cloning these genes and expressing them in a suitable system, testing the immunity they elicit, and its utility as a protective mechanism (Goodswen *et al.*, 2012). The *in silico* process of selection can be refined by using machine learning algorithms to reduce the number of false candidates (Goodswen *et al.*, 2013) and by using predicted scores for peptide-MHC molecule binding to T-cell receptors (Goodswen *et al.*, 2014a) and can be accelerated by automation (Goodswen *et al.*, 2014b). The probability of failure is high because protective immunity will clearly only be elicited by antigenic proteins which are accessible to the vaccinated animal's immune response.

Reverse vaccinology cannot replace experimental work, but computationally helps to narrow down the candidates by attempting to predict, from a sequence of letters, whether the three-dimensional (3D) protein they encode for will induce the memory helper T- and B-cells needed in a protective immune response. RV has become a reality thanks to an exponential increase in the amount of publicly available molecular biological data, and an exponential increase in computer processing, data storage and communication, among others (Goodswen *et al.*, 2023).

Effective vaccines have been produced by the RV approach against a number of infectious diseases, but multicellular parasites such as ticks present the obstacles of genome size and complexity, antigenic variability and probably the need for more than just a humoral immune response (Meeusen *et al.*, 2007; Lew-Tabor and Rodriguez-Valle, 2016; Bhakta *et al.*, 2022).

The tick vaccine project in the adaptation and cattle welfare programme of the Australian

Cooperative Research Centre for Beef Genetic Technologies (Beef CRC3) ran from 2005 to 2012. By means of RV, it identified approximately 20 vaccine candidates (short peptides representing 'non-concealed' B-cell epitopes). Twelve of these were tested in a series of efficacy trials against induced tick infestations in Australia (*R. australis*) and Brazil (*R. microplus*). To compensate for the low immunogenicity of the short peptides, initial vaccine booster doses were formulated with Freund's complete adjuvant, and the second booster with Freund's incomplete adjuvant. Three antigens achieved efficacies of 63–66% when administered singly, but mixing of the peptides resulted in lower efficacy rather than the anticipated additive/cumulative, or possibly synergistic, effect (Tabor *et al.*, 2017).

5.2.3 Gene silencing and editing

In the absence of an annotated tick genome, an alternative approach to RV for establishing the importance of a gene or its expression product for tick survival, and thus its potential as a vaccine antigen, was to either silence the gene by a process called RNA interference (RNAi) (Lew-Tabor *et al.*, 2011; de la Fuente and Kocan, 2022) or to remove the gene from the tick's genome by so-called gene editing, or CRISPR/Cas-9.

Clustered regularly interspersed short palindromic repeats (CRISPR) and their associated protein genes serve as a prokaryotic adaptive immune system providing protection against foreign DNA, such as from bacteriophages. They function by incorporating short DNA spacers, homologous to the invading DNA sequences, into a CRISPR array that is then transcribed to interfere with the phage replication. Genetic manipulation of CRISPR arrays has enabled their use as targeted genome editing tools (Hynes *et al.*, 2014).

First described for use in ticks in 2002, RNAi involves the introduction of double-stranded RNA (dsRNA) into the organism by various means. Tick salivary glands have been incubated with dsRNA to demonstrate disruption of gene transcription *in vitro* or dsRNA injected into whole female *A. americanum* to affect their

feeding pattern *in vivo* (Aljamali *et al.*, 2002). The first report of RNAi in the single host [B.] *R. microplus* described the injection of dsRNA into engorged females and the consequent silencing of Bm86, Bm91 and subolesin genes in eggs and larvae (Nijhof *et al.*, 2007). Unless care is taken with the size of the dsRNA, off-target effects may occur. Smaller dsRNA fragments reduce the risk of off-target effects (Lew-Tabor *et al.*, 2011).

Recombinant dsRNA was injected into ticks to investigate their feeding mechanism and the importance of tick cement (Hollmann *et al.*, 2018). Ubiquitin and Bm86 dsRNA were fed to individual ticks in various combinations of whole blood and serum and the knockdown confirmed by the quantitative reverse transcription polymerase chain reaction (qRT-PCR) (Lew-Tabor *et al.*, 2014).

5.2.4 Secreted and excreted antigens

Immunological control of ticks requires a vaccine antigen not normally involved in host responses to infestation. Salivary gland-derived molecules introduced to the host during tick engorgement are not the ideal antigens for an optimal immunization strategy. The induction of an intense host skin reaction to tick feeding is to be avoided (Wikel, 1988). Coevolution over millennia has led to ticks developing various mechanisms for avoiding and modulating the host immune response (see sections 3.4 and 4.6, this volume).

Despite this intuitively logical argument, the development of a vaccine targeting tick saliva components became the focus of much tick vaccine development research (Zhou *et al.*, 2006). Perceived benefits of exposed over concealed antigens lay in their expected greater spectrum of efficacy (species and stages, due to greater conservation), longer lasting immunity (repeated boosting from natural infestation) and deterrent action against tick attachment (thus also reducing pathogen transmission) (Nuttall *et al.*, 2006). The express objective of the Beef CRC3 was to identify antigens that were not concealed (Tabor *et al.*, 2017). Many attempts at immunizing against ticks using secreted and excreted tick antigens have been described and have met with varying degrees of success.

Tick saliva contains hundreds of proteins, depending on tick species, developmental stage, feeding stage and host. Because these proteins facilitate successful tick attachment and engorgement, potentially disabling them with a host immune reaction makes them an attractive port of call in the search for novel antigens. Some of them have been shown to be immunologically more antigenic, inducing a robust protective tick immunity (Černý *et al.*, 2020). Salivary glands in adult ticks have three (females) or four (males) types of acini to secrete this variety of molecules (Havlíková *et al.*, 2009). A vaccine against tick salivary proteins can interfere with tick attachment or pathogen transmission (Bhowmick and Han, 2020).

The composition of tick saliva has been investigated through analysis of salivary gland transcriptomes which revealed more than 1000 polypeptides for hard ticks. But there is a discrepancy between this number of coding sequences and the number that can be inferred from the genome sequences of *R. zambeziensis* and *I. scapularis* for lipocalins and metalloproteases. These discrepancies can be blamed on different technologies and analytical methods, differences in expression rates between strains of the same species or elevated mutation rates in highly expressed protein families (Guizzo *et al.*, 2023). Soft ticks may secrete up to 200 different proteins, belonging to different protein families, during the feeding process (Cheng, 2010). Using urea and sodium dodecyl sulfate extraction, 654 proteins were found just in the attachment cement of *R. microplus*, a metastriate hard tick of the Brevirostrata (short mouthparts) (Villar *et al.*, 2020).

Choosing the right protein(s) in tick saliva to incorporate into a vaccine is further complicated by the fact that the composition of tick saliva changes in the course of the tick's sojourn on the host (Bullard *et al.*, 2016b; Kim *et al.*, 2016b; Abbas *et al.*, 2023). The immune response to an individual salivary antigen probably has a limited window of opportunity within which to exert its effect and a high degree of redundancy probably means that the effect on the secreting tick might be minimal (Antunes and Domingos, 2023).

Comparing the salivary gland transcriptomes of *R. microplus* fed on tick-susceptible (Holstein) and -resistant (Nelore) hosts revealed

137 differentially expressed genes. Proteins expressed by upregulated genes in ticks from susceptible cattle were involved in the stress response mechanism during feeding. Ticks fed on susceptible cattle expressed a molecule with a sequence similar to that of a small heat shock protein (HSP) cloned from the salivary glands of *R. annulatus* and shown to have a strong immunogenic effect in rabbits. Increased expression of HSPs during feeding indicates that they may be required to stabilize tick proteins during ingestion of warm blood. They have also been found to play a role in fibrinogenolysis during feeding. This study was expected to shed new light on possible vaccine targets (Giachetto *et al.*, 2020).

Proteomic characterization demonstrated that the composition of tick saliva differs from that of tick cement in *Ixodes scapularis*, with vitellogenin-like lipoproteins the most abundant proteins in cement. Partially engorged ticks were removed from rabbits and the cement material adhering to the hypostomes manually removed and cleansed prior to freezing. The material was not washed and assumed to be contaminated with some non-cement saliva. Saliva was collected from replete detached ticks by stimulation with pilocarpine (Lynn *et al.*, 2022). This may necessitate a reassessment of earlier work examining tick salivary gland secretions.

Tick cement

Because they are essential for tick attachment and feeding, the protein components of the cement from metastriate ixodid ticks, assumed to have short hypostomes, seem to be logical candidates for vaccination against tick infestation. The cDNA encoding one such protein in the *e* cell granules of type III salivary gland acini of *R. appendiculatus* was cloned. The recombinant protein, expressed by *E. coli*, reacted with sera from cattle infested with *R. appendiculatus*. This work stopped short of attempting to demonstrate protective immunity in vaccinated host animals (Bishop *et al.*, 2002).

Even though *Ixodes* spp. are prostriate ticks with long hypostomes, considered not to need cement for attachment (Francischetti *et al.*, 2009; Hall-Mendelin *et al.*, 2011), guinea pigs were immunized with cement extract from *I. scapularis*, causing premature tick detachment and up to 41% reduction in engorged tick

weights (Lynn *et al.*, 2022). This might be similar to the decline in tick infestation seen in response to naturally acquired adaptive immunity (see Chapter 4, this volume).

Cement consists of many carbohydrate components and interacting proteins. Purified extracts of cement proteins are immunogenic and immunoprotective. Cement protein 64P was identified on its amino acid composition and shown to have close compositional similarity to mammalian skin keratin and collagen, most likely to avoid rejection by the host's immune system during tick feeding. Sera from guinea pigs with naturally acquired adaptive immunity to *R. appendiculatus* did not react with the protein. It was shown by *in situ* hybridization to be expressed in the salivary gland of *R. appendiculatus* females at the beginning of feeding, but not in unfed females, and waned as feeding progressed to engorgement. Female ticks expressed 64P mRNA in type II and III acini (Havlíková *et al.*, 2009). As many as 160 proteins were identified in tick cement cones recovered from the mouthparts of manually detached *A. americanum*. These were classified as protease inhibitors (serpin, Kunitz serpins), lipocalins, storage (ferritin, vitellogenin, calmodulin), detoxification and housekeeping proteins (Hollmann *et al.*, 2018). Some proteins found in tick cement might actually not be actively secreted but contaminants from sloughed salivary gland cells (Zhou *et al.*, 2006).

Tick recombinant protein 64 (Trp64) from *R. appendiculatus* cement, expressed in *E. coli*, in vaccine form has been shown to elicit an immune response that affects both exposed and hidden tick antigens, and in more than one tick species. Antisera from vaccinated guinea pigs cross-reacted with cement cone extract, salivary glands, haemolymph and midgut of *R. sanguineus* nymphs. Gel electrophoresis results also demonstrated cross-reactivity with extracts from *I. ricinus*, *A. variegatum* and [B.] *R. microplus* (Trimnell *et al.*, 2005). This is in contrast with attempts to immunize rabbits with *R. appendiculatus* Trp64 expressed by baculovirus in insect cells against induced infestation with larvae, nymphs and adults. There was no effect against immature ticks and the effect on adult tick mortality and engorged weight was not statistically significant (Saimo *et al.*, 2011).

The salivary gland cDNA libraries from Breviostoma ticks contained more sequences of glycine-rich proteins similar to silks of true spiders than those from Longirostrata ticks (Maruyama *et al.*, 2010). Very long glycine-rich proteins, similar to spider silk proteins, found in metastrata (Breviostoma) ticks may function as cement proteins (Francischetti *et al.*, 2011). The glycine-rich protein, similar to spider silk, found in the feeding cement cone was assumed to give the cone its strength and insolubility (Bullard *et al.*, 2016a).

The gene encoding for flagelliform silk protein (100Silk) was confirmed by real-time RT-PCR to be downregulated in tick salivary glands infected with *A. marginale*. Silencing of this gene resulted in reduced *A. marginale* infection in both tick salivary glands and cultured BME26 cells, making it a candidate for development of a vaccine (Zivkovic *et al.*, 2010a). Rabbit polyclonal antibodies against silk protein added to bovine blood fed by capillary tube to engorging female *R. microplus* reduced tick weight and egg production but had no effect on infection with *A. marginale* or *B. bigemina* if these were included in the blood (Antunes *et al.*, 2014).

Tick toxins

Tick toxins potentially contain proteins that lend themselves as antigens in vaccines. Monoclonal antibodies against the paralysis-inducing toxin of *R. e. evertsi* were shown by Western blot to also recognize similar toxins from *I. rubicundus* and *A. (P.) walkerae* and were demonstrated to protect chickens from *A. (P.) walkerae* paralysis. This raised the possibility of developing a vaccine against both spring lamb and Karoo paralysis, based on a recombinant antigen (Crause *et al.*, 1994).

Masina (1999) isolated the cDNA sequence of the HT-1 neurotoxin of *I. holocyclus*, successfully expressed the toxin as a maltose-binding fusion protein (MBP) in an *E. coli* host system, and attempted expression in a baculovirus system. The recombinant fusion protein partially protected mice against the native holocyclotoxin but was not protective in dogs challenged with live ticks. At that stage, the native neurotoxins HT-2 and HT-3 had not been characterized, nor their genes isolated, which was seen as a prerequisite if a vaccine against tick paralysis were

to be developed (Masina and Broady, 1999). Subsequent investigations with monoclonal antibodies indicated that the MBP had been incorrectly folded, when compared with the native toxin. It was decided to use a ubiquitin fusion protein, which was smaller than the MBP. Despite having the correct folding conformation and being recognized by commercial dog anti-tick serum, it was found not to be protective in rabbits and mice (Chung, 2001).

Recent investigation of the *I. holocyclus* salivary gland and viscera transcriptome revealed 19 holocyclotoxin moieties (Rodriguez-Valle *et al.*, 2018). Eight of these were included in an experimental cocktail vaccine. Dogs vaccinated three times over a 9-week period were monitored for holocyclotoxin-specific IgG induction and when challenged with an unfed adult female *I. holocyclus* 2 weeks after the third vaccination, were shown to be protected from toxicosis (Rodriguez-Valle *et al.*, 2021).

Calreticulin

Calreticulins are calcium-binding proteins secreted in the saliva of several tick species, potentially interfering with platelet aggregation and the classical complement pathway of the innate immune response (see sections 3.4.1 and 3.4.2, this volume). They would seem to be potential vaccine antigen candidates, but recombinant *H. longicornis* CRT expressed by *E. coli* and administered to mice and rabbits failed to elicit a humoral response (measured by ELISA) or any effect on tick engorgement, moulting (nymphs to adults) or egg production (Zheng *et al.*, 2022). There is controversy regarding the utility of CRT as a biomarker for human tick bites, with different results obtained for *A. americanum* and *H. longicornis* recombinant CRT (Kim *et al.*, 2015a; Zheng *et al.*, 2022).

Lipocalins

Lipocalins are low molecular weight proteins secreted from tick salivary glands. They are best known for their anti-inflammatory effect by binding histamine (see section 3.4.3, this volume) and play important roles in blood feeding, immune response and reproduction (Paesen *et al.*, 2000). There are at least 14 lipocalin genes in *I. ricinus*. One of them

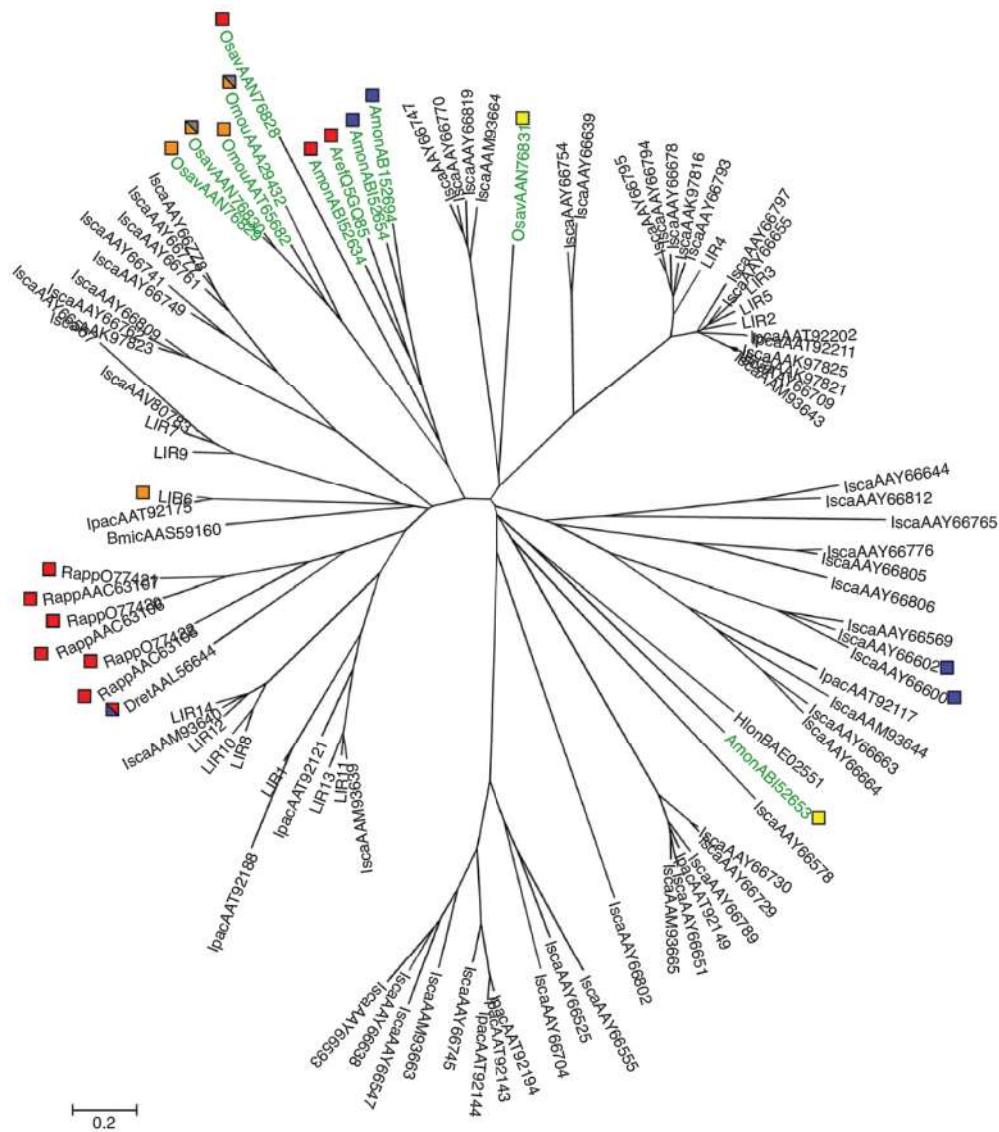


Fig. 5.2. A radial phylogenetic tree of the ixodid tick lipocalin superfamily. From Beaufays *et al.* (2008a) under the terms of the Creative Commons Attribution License.

specifically binds leukotriene B₄, inhibiting neutrophil chemotaxis (Beaufays *et al.*, 2008b). Fig. 5.2 illustrates the phylogenetic relationships of tick lipocalins (Beaufays *et al.*, 2008a). They are sufficiently different from those of the rest of the lipocalin family to be considered as possible vaccine targets (Mans *et al.*, 2003).

Recombinant lipocalin from *I. persulcatus* expressed by *E. coli* was formulated with Freund's

adjuvant (complete for the initial, incomplete for the booster injections) and used to vaccinate mice three times at 14-day intervals. Blood collected 7 days after the last injection contained antibodies to the recombinant lipocalin, demonstrated by Western blotting. Numbers of attaching and engorging nymphal ticks fed on immunized mice were not significantly different, but they took longer to reach engorgement and

their replete weight was significantly reduced (Konnai *et al.*, 2011).

A lipocalin most abundantly expressed in the salivary gland of *H. longicornis* and with close sequence homology to *I. persulcatus* lipocalin was cloned and expressed by *E. coli* and used to immunize rabbits. This prolonged the blood-meal and reduced replete weight, ovipositioning and egg hatchability of an induced subsequent tick infestation, amounting to 60.17% efficacy (Wang *et al.*, 2021).

Savicalin is a non-salivary lipocalin, first described in *O. savignyi* haemocytes, midgut and ovaries. Its functions are unclear but its expression relates to postfeeding events such as development, ecdysis or bacterial challenge (Cheng, 2010; Cheng *et al.*, 2010). Savicalin has since also been found in *Ornithodoros erraticus* saliva and mooted as a potential vaccine antigen (Pérez-Sánchez *et al.*, 2022).

Other tick salivary proteins

Tick saliva contains a pharmacopoeia of bioactive molecules aimed at modulating or suppressing the host's innate and adaptive immune response (see sections 3.4 and 4.6, this volume). The idea was mooted to target tick-derived immunosuppressants with an immune response, acknowledging that redundancy is probably also a feature of tick-mediated suppression of host immunity (Wikel, 1999). Analysis of the sialome of *R. pulchellus* revealed differences between the genders (Tan *et al.*, 2015), which might necessitate closer scrutiny of salivary proteins considered as vaccine antigens.

The gene encoding a glycine-rich 50 kDa protein, differentially expressed in the salivary glands of *Rhipicephalus haemaphysaloides*, was designated RH50, cloned, expressed in *E. coli* and used to immunize rabbits, which were subsequently challenged with induced infestations of nymphs and adult female ticks. The protein was also found in *R. haemaphysaloides* cement and surmised to be a membrane-bound matrix sloughing off from salivary gland cells and contaminating the tick saliva. The number of ticks attaching to immunized rabbits was markedly suppressed but mortality and feeding in those ticks that did attach were unaffected (Zhou *et al.*, 2006).

Tick-naïve calves were vaccinated four times at 15-day intervals with a recombinant *R. microplus* metalloprotease (BrRm-MP4), cloned from tick salivary glands and expressed in *E. coli*, before being challenged with an induced tick infestation. Antibodies elicited against BrRm-MP4 were confirmed by ELISA and overall protection calculated at 60% based on reductions in survival and engorgement (42.9%), fecundity (17.5%) and fertility (14.8%) (Ali *et al.*, 2015).

Proteins with α -Gal modifications (see section 1.3.4, this volume, meat allergy) may hold promise as protective antigens for prevention and treatment of anaphylactic reactions (de la Fuente *et al.*, 2017).

A vaccine cocktail of three histamine-binding proteins, two cement cone protein antigens and a subolesin homologue from *R. appendiculatus* was formulated in Montanide ISA 50 V adjuvant and used to vaccinate Friesian or Friesian/Ayrshire-cross calves. All the selected antigens had a published history of promise as potential anti-tick vaccine candidates. The calves' humoral response was monitored by indirect ELISA and the anti-tick effect evaluated by induced infestations with nymphs and adults. Despite demonstrable antibody titres to each of the antigens in the cocktail, no significant anti-tick effects were observed. A number of theories were advanced to explain the failure. Redundant salivary proteins from the challenge ticks may have circumvented the immune response. Antigenic competition was considered as unlikely, because of the natural complexity in the protein make-up of tick saliva. The wrong choice of adjuvant and protein expression system (*E. coli*) was raised as a possibility (Olds *et al.*, 2016).

Saliva collected from *I. scapularis* by pilocarpine stimulation was fractionated in an ion exchange column and each fraction separated by gel electrophoresis. The 15 fractions were grouped into five pools of three fractions each and used to immunize guinea pigs prior to induced infestation with *I. scapularis* nymphs. The fractions differed in their ability to elicit antibody production, as confirmed by ELISA, and in their effect on tick feeding, judging by nymphal tick engorgement and detachment. Antibody responses to the fractions were also different between guinea pigs vaccinated by injection and rabbits immunized by feeding ticks. Defining

the subset of salivary proteins that might serve as vaccine targets will require refinement and further testing (Černý *et al.*, 2020).

Three salivary proteins from *A. sculptum*, possibly involved in haematophagy, expression of their mRNA detected in larvae, nymphs and adults, with expression levels increasing as feeding progressed, were cloned and expressed in recombinant form. All three recombinant proteins (rAsKunitz, rAs8.9kDa and rAsBasicTail) inhibited haemolysis and thrombin activity, whereas the latter two also inhibited the enzymatic activity of coagulation factor Xa and trypsin. Mice were immunized and shown to be protected against challenge by adult female ticks by 59.4% (rAsBasicTail) to more than 85% (rAsKunitz, rAs8.9 kDa). When challenged by nymphs, the efficacy was 70–100%. Thrombin inhibitors with Kunitz-like domains had previously been identified in *R. microplus*, *H. longicornis* and *I. scapularis* (Costa *et al.*, 2021).

5.2.5 Concealed ('hidden') antigens

Immunization against a hidden antigen requires a paradigm shift, because the engagement between the immune system and the pathogen no longer occurs inside the host. The product of the immune response is raised within the host but the battle is fought externally, inside the tick attached to the host's skin.

An immune response against a subunit internal ('concealed/hidden') antigen is something the tick will not have encountered before and would thus be less likely to withstand or circumvent. The tick can be damaged by the immune response if the host is first vaccinated. It might act synergistically with naturally acquired immunity (Willadsen, 1987; Willadsen *et al.*, 1993a).

Two hidden parasite antigens have thus far been commercialized – recombinant subunit Bm86 against ticks (TickGARD®, TickGARD®^{PLUS}, Gavac; see section 5.1), and a gut antigen extracted and purified from the abomasal sheep nematode *Haemonchus contortus* (Barbervax®) (Smith, 1993; Smith and Smith, 1993; Smith *et al.*, 1994). They both require repeated administration to make up for the lack of 'boosting' by natural reinfestation.

One of the essential characteristics of a hidden antigen in a tick vaccine is its accessibility to host antibodies ingested during the bloodmeal. Immunoglobulins in ingested blood enter the tick's haemolymph and bind to internal organs, such as the ovary and salivary gland, without evidence of their degradation (Jasinskas *et al.*, 2000). The antigen also needs to be important in the tick's physiology, with limited functional redundancy or alternative pathways to achieving the same physiological outcome (Hope *et al.*, 2010). Failure of guinea pig immunization with *I. scapularis* midgut extract, the preparation of which is not described (Lynn *et al.*, 2022), may have been due to either or both these criteria not being met.

The location of the target of the vaccination plays a role in the selection of the ideal antigen for a tick vaccine. An antigen expressed closer to the tick's mouthparts will enable engagement with the host's immune response earlier in the bloodmeal, thus possibly thwarting not just tick infestation but potentially also the transmission of any tick-borne pathogen. Antigens further into the tick's digestive tract or in the internal organs will require ingestion of antibody-laden blood, by which time the tick may already have had the opportunity to secrete not only attachment cement but also saliva containing anticoagulant, anti-inflammatory and immunosuppressive molecules, toxin and possible viral, bacterial and/or protozoal pathogens.

This distinction was demonstrated by comparing the responses to tick infestation in mice immunized with either tick cement protein or midgut Bm86. The former evoked a delayed type of skin hypersensitivity response, interrupting tick feeding and preventing transmission of tick-borne encephalitis virus, whereas the latter affected tick survival but did not prevent virus transmission (Labuda *et al.*, 2006).

Two fractions of midgut protein, isolated from *A. variegatum* by detergent solubilization, were used to vaccinate rabbits and shown to reduce the reproductive capacity of a subsequently induced infestation by 48–77%. Gel electrophoresis and immunoblotting demonstrated the complexity of the antigens but more work was needed for their characterization (Kinyua *et al.*, 2002).

Different host responses to the same antigen were revealed when cattle and sheep

were vaccinated with recombinant 5'-nucleotidase, a membrane-bound enzyme in the tick gut (Willadsen *et al.*, 1993b). When challenged with induced infestations of freshly moulted adult [*R. microplus*] *R. australis*, sheep with high antibody titres were protected. Cattle challenged with larvae showed no significant protection. Differences in antibody titre were found to be an unlikely reason for the discrepancy (Hope *et al.*, 2010). Despite scepticism about tick-host specificity (see section 1.2.4, this volume), sheep are not normally regarded as a preferred host for the single-host *Rhipicephalus* spp. (*R. australis*, *R. decoloratus*, *R. microplus*) (De Rose *et al.*, 1999) and may not have been the appropriate model in this instance. Inducing an infestation with freshly moulted adults of a single-host tick would involve removing them from the host on which they moulted and transferring them to a different host. This is also unconventional. Another reason for the discrepancy might be that adults (used to challenge vaccinated sheep) and larvae (cattle) might not be equally vulnerable to the antibodies that were raised.

Bm86 orthologues

Homologues are genes related by common descent. Orthologues are homologues derived from a single ancestral gene, and typically perform equivalent functions (Koonin, 2005).

Two Bm86 homologues in [*B.*] *R. decoloratus* were found to have 86% and 85% amino acid sequence identity with Bm86 and were designated Bd86-1 and -2, respectively. Native Bd86 protein in *R. decoloratus* midgut was shown by indirect immune-peroxidase staining to react strongly with sera from TickGARD-vaccinated cattle. When challenged with *R. decoloratus* larvae, the weight of engorged female ticks collected from these cattle was reduced by 55.6% and tick egg production by 61%. The vaccination had no effect on challenge infestation with adult female *R. appendiculatus* (Odongo *et al.*, 2007).

Variations in the efficacy of Bm86 vaccination against different isolates of *R. microplus* led to the notion of cloning and expression of Bm86 from local tick strains to eliminate geographic regional differences. Standardizing the expression system from *E. coli*, *Aspergillus nidulans* or *A. niger* to *P. pastoris* was also expected to improve the antigenicity and immunogenicity

of the recombinant antigen. The genes encoding Bm86 (*R. microplus*), Ba86 (*R. annulatus*) and Bd86 (*R. decoloratus*) were cloned and expressed in *P. pastoris*. Rabbits were immunized with the recombinant proteins, using Montanide ISA 50 V as the adjuvant, and their sera evaluated by Western blot to demonstrate cross-reactivity between the three orthologues (Canales *et al.*, 2008).

In order to test the cross-reactivity between Bm86 and Ba86 previously demonstrated serologically, cattle were vaccinated with recombinant Ba86 or Bm86 from two different *R. microplus* strains. Ba86 vaccination reduced infestations of *R. annulatus* and *R. microplus* by 71% and 40%, respectively. The two Bm86 proteins were more effective than Ba86 against *R. annulatus* but varied in their efficacy against *R. microplus* (Canales *et al.*, 2009a).

A Bm86 orthologue was cloned from the eggs of laboratory-reared *H. a. anatolicum*, expressed as a fusion protein in *E. coli* and designated rHaa86. The recombinant protein was formulated with Montanide 888 in mineral oil and used to immunize tick-naïve calves three times, 30 days apart. The calves were challenged after another 37 days with unfed adult *H. a. anatolicum*. The calves' antibody titres were confirmed by ELISA and found to peak 124 days after the first vaccination. Vaccinated calves yielded 73.8% fewer engorged female ticks than controls (Azhahianambi *et al.*, 2009). Subsequent work demonstrated that vaccination with rHaa86 could serve as a component in integrated control of *H. a. anatolicum* and the *Theileria annulata* it transmits (Jeyabal *et al.*, 2010).

In the belief that the homologous form of Bm86 in each tick species will be better than heterologous cross-protection, homologues of Bm86 were isolated from *O. savignyi*, *A. variegatum*, *D. reticulatus*, *D. variabilis*, *Haemaphysalis elliptica*, *H. marginatum*, *I. ricinus* and *R. e. evertsi*. These were amplified, which led to the identification of a structurally related protein (see *ATAQ proteins* below) (Nijhof *et al.*, 2010).

Orthologues of the Bm86 gene were isolated from strains of *Hyalomma scupense* (syn. *H. detritum*) isolated in Tunisia. The recombinant protein rHd86 from one strain was expressed in *P. pastoris* and found to display 35 to 40% nucleotide sequence diversity from Bm86 and

Bm95 proteins. Homologue Hd86 proteins from different *H. scupense* strains showed limited intra-specific diversity of 1.7%, indicating its suitability as a vaccine candidate against this tick species (Ben Said *et al.*, 2012a). Further investigation of Bm86 orthologues in *Hyalomma* spp. in Tunisia revealed 60–66% amino acid sequence agreement between Bm86 and Hm86, He86 and Hdr86 proteins from *H. m. marginatum*, *H. excavatum* and *H. dromedarii*, respectively. There was closer agreement with the sequence in the previously expressed Hd86 protein of 91%, 88% and 87%, respectively. This reaffirmed the indication that rHd86 will be a better vaccine candidate against *Hyalomma* spp. than Bm86 (Ben Said *et al.*, 2012b). Phylogenetically, the amino acid sequence variations in Bm86 orthologues from different *Hyalomma* spp. were seen as a tool to augment morphological species identification (Ben Said *et al.*, 2015).

Homologues of the Bm86 gene were found in *R. appendiculatus* and designated Ra86. Analysis of the sequences of different field populations and a laboratory strain indicated that they clustered into four major phylogenetic clades (Kamau *et al.*, 2016). This work did not investigate immunogenetic differences possibly attributable to the genetic variations.

Multiple orthologues of Bm86 with at least three identifiable epidermal growth factor (EGF)-like domains have been discovered in *Ixodes scapularis*. Although upregulated during *Borrelia burgdorferi* infection, RNAi knockdown of the Is86 genes did not seem to affect tick engorgement or *B. burgdorferi* persistence in mice. Immunization of mice with recombinant EGF antigens marginally reduced tick engorgement and skin spirochaete loads, but insufficiently for them to be considered as likely vaccine candidates (Koči *et al.*, 2021).

Bm95

The discovery of sequence variations in the Bm86 locus in *R. microplus* strains from Mexico, Argentina, Venezuela and Cuba and *R. australis* from Australia (García-García *et al.*, 1999) and the appearance of isolates of [*B.*] *R. microplus* that were less susceptible to Bm86 vaccination led to the isolation and cloning from an Argentinean strain of the tick a gene encoding an antigen designated Bm95 and expressing it

in *P. pastoris* (García-García *et al.*, 2000). There was an inverse relationship between the efficacy of vaccination with Bm86 and sequence heterogeneity in the Bm86 locus. Diversity in the Bm86 amino acid sequence greater than 2.8% was calculated to confer resistance to vaccination (García-García *et al.*, 1999). Vaccination with Bm95 in Montanide 888 adjuvant protected cattle from infestation with *R. microplus* larvae of the Cuban Bm86-sensitive Camcord, and of the Argentinean Bm86-refractory isolate (García-García *et al.*, 2000).

Bm91

A glycosylated membrane protein was discovered in the salivary gland and gut of adult female [*B. microplus*] *R. australis* and designated Bm91. Partial amino acid sequence data for the protein show striking similarities to that of mammalian carboxypeptidases or angiotensin-converting enzymes, suggesting that it may have an enzymatic function (Jarmey *et al.*, 1995). It was of relatively low abundance, with approximately 300–400 µg being recovered from 1 kg of semi-engorged adult female ticks. Absence of recognition by sera from cattle exposed to natural tick infestation indicated that this was another example of a concealed antigen (Riding *et al.*, 1994). Cattle were vaccinated either with Bm86 alone or in combination with Bm91 and challenged with [*B. microplus*] *R. australis*. The addition of the Bm91 antigen led to statistically significantly enhanced efficacy over that with Bm86 alone (Willadsen *et al.*, 1996). Knocking out the Bm91 gene by RNAi through injection of dsRNA into freshly moulted and engorged female *R. microplus* reduced gene transcription by 90% in both the unfed and replete female ticks, but had no effect on engorged tick weight, mortality, ovipositioning or egg hatchability (Nijhof *et al.*, 2007).

Ubiquitin

Ubiquitin contributes to several intracellular processes in addition to protein turnover, such as endocytosis, DNA repair, transcription and immunity. It is known to occur in arthropod disease vectors such as mosquitoes, lice and ticks. Among others, ubiquitination regulates the IMD pathway (see section 3.1.4, this

volume) in *I. scapularis* (Choy *et al.*, 2013). Another function of ubiquitin is to be attached as a label to a protein destined for destruction in a proteasome, a discovery for which the Nobel Prize in Chemistry was awarded in 2004 (Anon., 2004). The 'ubiquitin machinery' is under the control of a collection of activating, conjugating and ligase enzymes. Ubiquitination involves ubiquitin-activating and -conjugating enzymes and a ubiquitin-protein ligase, and is countered by de-ubiquitinases removing ubiquitin from molecules (Choy *et al.*, 2013). Ubiquitin was identified as the most consistently expressed household gene in *A. americanum* salivary glands and hence the most suitable reference gene (Bullard *et al.*, 2016b).

The p47 enzymatic substrate of the E3 ubiquitin ligase X-linked inhibitor of apoptosis (XIAP) is a positive regulator of the *I. scapularis* IMD network, acting as a molecular rheostat (McClure Carroll *et al.*, 2019). It restricts bacterial colonization: disruption of p47 expression through RNAi increased colonization of *I. scapularis* by *B. burgdorferi* and *A. phagocytophilum* (Severo *et al.*, 2013; McClure Carroll *et al.*, 2019). Silencing the genes encoding, for example, ferritin, subolesin and ubiquitin in *D. variabilis* inhibited infection by *A. marginale*, while injection of ubiquitin dsRNA increased tick mortality (de la Fuente *et al.*, 2007b).

Knocking down the tick homologue of the *D. melanogaster* ubiquitin-63E gene in [*R. microplus*] *R. australis* with 594 bp dsRNA caused differential expression (down-/upregulation) of 280 ESTs, the functions of more than half of which were unknown or hypothetical. Ubiquitin knockdown was associated with changes in proteolytic pathways, as evidenced by the down-regulation of several proteases. Gene-silenced ticks did not lay eggs. Refining the gene silencing and limiting the off-target effects with shorter (~100–200 bp) dsRNA meant that the ESTs could be selected more predictably (Lew-Tabor *et al.*, 2011).

Bites by *H. longicornis* were shown by quantitative proteomics to give rise to differentially ubiquitinated proteins (DUPs) in rabbit skin. These DUPs were concluded to be involved in protein degradation, metabolic processes and immune responses. Differential ubiquitination of, for example, MHC-II can have a regulating effect on the ubiquitin–proteasome system and

the MHC-I and MHC-II antigen-presenting pathways (Wang *et al.*, 2020).

G protein-coupled receptors (GPCRs)

G proteins are a family of guanine nucleotide-binding regulatory proteins that serve an essential role in transducing receptor-generated signals (e.g. olfactory and light sensory signals) across the plasma membrane (Hepler and Gilman, 1992). The GPCRs are cell surface membrane receptors considered as poor antigens for anti-tick vaccines, but little is known about their physiological role in ticks (Brock *et al.*, 2019). Neuropeptides, produced in the arthropod synganglion, are the main ligands of GPCRs, making them candidate targets. The *R. microplus* synganglion transcriptome was analysed to gain information on their encoding gene sequences, in support of research into novel tick control technologies (Guerrero *et al.*, 2016; Waldman *et al.*, 2022).

Kinins are multifunctional neuropeptides that regulate diuresis, feeding and ecdysis in insects but their physiological roles in ticks are unclear. The kinin receptor in *R. microplus* is a GPCR and silencing it reduces the tick's reproductive fitness (Xiong *et al.*, 2019).

RNAi in two strains of unfed female *R. microplus* was used to investigate the function of the leucokinin receptor (LKR), one of the GPCRs. Immunohistochemistry was used on fed female ticks to localize expression of the LKR to the midgut. There were strain differences but gene-silenced ticks were found to spend less time taking a bloodmeal and weighed 30% less, their egg production was 28.3% less, and their egg hatchability was 36.7% compared to 56.9% in unsilenced ticks (Brock *et al.*, 2019).

Bioinformatic analysis of the sequenced genome of *I. scapularis* led to the annotation of a number of neuropeptide GPCR genes. One of these encodes for corazonin, which plays a central role in the tick's physiology and behaviour. The genomic organization is similar to that of the human gonadotropin-releasing hormone (GnRH) receptor gene. An unexpected discovery was that the corazonin receptor gene encodes for two splice variants of the receptor that vary in their amino acid sequences and could have different roles (Hauser *et al.*, 2023).

Octopamine is a neurotransmitter that binds to GPCRs and is found in insects. An amino acid sequence similar to that of the octopamine receptor in insects was found in [*B. microplus*] *R. australis*. Amitraz is a formamidine acaricide and a specific agonist of the octopamine receptor. Emerging resistance to amitraz was suspected of being the result of a mutation in the nucleotide sequence of the octopamine receptor, but this was shown not to be the case (Baxter and Barker, 1999). Subsequent investigations have revealed consistent and strong associations between GPCR mutations and resistance to amitraz in the USA, Latin America and South Africa (Robbertse *et al.*, 2016; Jonsson *et al.*, 2018).

Cystatins

Cystatins are a group of cysteine peptidase inhibitors present in lysosomes and extracellular fluids. In ticks, they modulate blood acquisition and digestion, host immune responses and tick immunity (see sections 3.4, 4.1.1, 4.6.3, 4.6.5, 4.6.8, this volume), all functions that would prevent ticks from feeding if they were to be disabled.

Three novel cystatins were described from *I. persulcatus*. Their transcripts were detected in different tissues and instars, indicating their widespread expression during tick development. Hamsters immunized with a recombinant cystatin from *R. microplus* developed a serologically detectable humoral immune response but were not protected against challenge with adult *I. persulcatus* (Rangel *et al.*, 2017).

Antibodies raised against recombinant *R. microplus* cystatin cross-reacted with *R. appendiculatus* cystatin, and vaccine trials with recombinant cystatins impaired engorgement of *O. moubata* and *I. scapularis* nymphs (Parizi *et al.*, 2020). A novel *R. microplus* cystatin, Rmcystatin-1b, was found to be highly expressed in the midgut of partially and fully engorged female ticks. RNAi-mediated knockdown resulted in increased midgut proteolytic activity. Recombinant Rmcystatin-1b was expressed in *E. coli* and inhibited cysteine peptidases, which included a cathepsin L-like protease. A possible role in interaction with the apicomplexan parasite *Babesia bovis* during its proliferation, preventing deleterious effects to the tick, was suggested for this peptidase (Lu *et al.*, 2020).

Cathepsins

Cathepsins are a group of proteases involved in many cellular processes (see section 4.1.1, this volume). *R. microplus* egg yolk contains an aspartic protease designated *Boophilus* yolk pro-cathepsin (BYC). It contributes to tick embryogenesis by its involvement in the degradation of vitellin. Cattle immunized with either native or recombinant (expressed in *E. coli*) BYC mounted a humoral immune response that offered modest protection against subsequent challenge with [*B.*] *R. microplus* larvae. The authors suggested that BYC be considered as a candidate antigen in a multicomponent vaccine (Leal *et al.*, 2006a, b).

Akirin, subolesin and chimeras

A gene called akirin (from the Japanese 'akiraka ni suru' – 'making things clear') was discovered in *Drosophila melanogaster* by RNAi, shown to act in parallel with the NF- κ B transcription factor downstream of the IMD pathway, and to help defend against Gram-negative bacteria. Akirin (AKR) is localized in the nucleus and highly conserved, with two homologues in the human genome. Knockout of both homologues in mice revealed the essential downstream function in TLR, TNF and IL-1 β signalling pathways towards production of IL-6 (Goto *et al.*, 2008a, b).

Subolesin (SUB) was discovered in *I. scapularis* larvae by ELI of mice, identified as an endopeptidase and designated 4D8 (Almazán *et al.*, 2003). It is the orthologue (non-identical sequence but similar function) of akirin in insects. The evolutionary history reveals that not only sequence and structure but also function in relation to biological processes and pathogen infection were conserved in AKR and SUB (Artigas-Jerónimo *et al.*, 2018). SUB has since been cloned from 10 tick species representing six genera and was shown by RNAi to play a role in multiple cellular processes. Gene knockdown ticks showed tissue degeneration in guts, salivary glands and reproductive organs. It was given the generic name 'subolesin' from the Latin *suboles*: offspring, progeny (de la Fuente *et al.*, 2006a, 2008) and shown to be involved in tick innate immunity to pathogen infection (Zivkovic *et al.*, 2010b; Moreno-Cid *et al.*, 2013; Naranjo *et al.*, 2013).

The description of a SUB orthologue with conserved epitopes in *A. albopictus* (Canales *et al.*, 2009b) leads one to surmise that SUB/AKR may in future be referred to by a collective name that acknowledges their conserved status in insects and ticks.

Silencing the SUB gene in engorged female *R. microplus* led to the production of abnormal eggs, suggesting a possible role of this protein in embryonic development (Nijhof *et al.*, 2007). RNAi in replete females of three three-host tick species (*A. americanum*, *D. variabilis* and *I. ricinus*) also affected subolesin expression in eggs and larvae (Kocan *et al.*, 2007).

When used as an antigen, SUB induced an immunity which decreased tick survival and reproduction rates, and caused degeneration of gut, salivary gland and reproductive tissues. Conserved protective antigens such as SUB/AKR were considered to show promise for inclusion in a universal vaccine to control arthropod vectors and reduce pathogen transmission, but further research was needed (de la Fuente *et al.*, 2011, 2013; Naranjo *et al.*, 2013).

Silencing the SUB gene impaired the tick's vector capacity for *Anaplasma marginale* and *A. phagocytophilum* (de la Fuente *et al.*, 2006b, 2010). The SUB-induced immune reduction of tick burdens was shown to decrease *Babesia bigemina* infection in vaccinated cattle (Merino *et al.*, 2011). *Hyalomma lusitanicum* infected with *B. bigemina* in Sicily had upregulated levels of SUB. Vaccines using tick proteins involved in vector–pathogen interactions could achieve reductions in both tick burdens and pathogen infection (Zivkovic *et al.*, 2010b; Merino *et al.*, 2013b) but SUB/AKR vaccines had no effect on TBEV infection and transmission (Merino *et al.*, 2013a).

Recombinant SUB expressed by *E. coli* was used to vaccinate rabbits before challenge with *H. longicornis*. Using the usual parameters of replete weight and egg production, the calculated efficacy was 37.4% (Lee *et al.*, 2020). Whereas SUB on its own might lack a convincing protective immune response, its use in combination with other antigen(s) holds promise. A synergistic effect was shown when larvae of *R. microplus* were fed *in vitro* on serum from cattle vaccinated with both Bm86 and SUB, and SUB is a routine inclusion in many experimental tick vaccines (Trentelman *et al.*, 2019; Estrada-García,

personal communication, Matanzas, Cuba, 28 March 2022), with the possibility of broadening the efficacy spectrum. There is clearly still some controversy, in that SUB alone was more effective than either Bm86 alone or a SUB/Bm86 combination against natural *R. microplus* infestation (Mendoza-Martínez *et al.*, 2021).

Three separate experiments investigated the immune responses of red deer (*Cervus elaphus*) and white-tailed deer (*Odocoileus virginianus*) to vaccination with either Bm86, SUB and/or mosquito AKR. The assessments were based on antibody responses, control of induced *R. microplus* infestation and antibody response and tick counts after natural tick exposure. Bm86 and SUB were similarly effective (76–83%) against induced *R. microplus* infestations on white-tailed deer. AKR reduced counts of natural infestations of *Hyalomma* spp. and *Rhipicephalus* spp. on red deer by 25–33% 14–20 weeks after immunization (Carreón *et al.*, 2012).

Biosynthetic antibody-binding sites can be connected by amino acid linkers to form a single chain (Huston *et al.*, 1988; Aguilar-Gurrieri *et al.*, 2023). The SUB/AKR chimeras Q38 and Q41 were designed by combining recombinant protective epitopes identified with sera from sheep and rabbits immunized with *I. scapularis* SUB and *Aedes albopictus* AKR. Q38 was the result of combining selected SUB and AKR epitopes with GGGS (glycine, serine) amino acid linkers. In Q41 SUB and AKR were linked by cysteins, forming disulfide bridges. SUB and AKR expressed in *P. pastoris* and the chimeras expressed in *E. coli* were used to vaccinate mice infested with *I. ricinus*, *A. albopictus* and *Phlebotomus perniciosus* in an attempt to control multiple vector species. SUB vaccination had the highest efficacy across all vector species, Q41 the highest efficacy in mosquitoes and Q38 the highest effect on reducing mosquito and sand fly egg production (Moreno-Cid *et al.*, 2013). A Q38 vaccine was 99.9% and 46.4% effective against *I. ricinus* and *D. reticulatus* larvae respectively fed on rabbits (Contreras and de la Fuente, 2016). A wild population of roe deer (*Capreolus capreolus*) in Andalucía was vaccinated once a year with Q38 over a 3 year period, demonstrating an antibody response and a reduction in natural infestation with *H. marginatum*, *H. lusitanicum*, *R. bursa* and *I. ricinus* (Contreras *et al.*, 2020).

BmVDAC

An investigation of the molecular interactions between *R. microplus* midgut cells and *Babesia bigemina* led to the discovery of a mitochondrial voltage-dependent anion-selective channel (VDAC), also known as a mitochondrial porin, and a central component of the apoptosis process. Porins interact with intracellular parasites, regulating the permeability of the outer mitochondrial membrane. The VDAC-encoding gene was identified and sequenced (Rodríguez-Hernández *et al.*, 2012). BmVDAC was shown by RT-PCR to be expressed at higher levels in *R. microplus* when infected with *B. bigemina* and considered to be a potential anti-tick vaccine antigen candidate (Rodríguez-Hernández *et al.*, 2015). The protective effect of recombinant BmVDAC was demonstrated by an 82% efficacy against *R. microplus* infestation on immunized cattle. This effect was moderated, with efficacy of only 34% against ticks on cattle infected with *B. bigemina* (Ortega-Sánchez *et al.*, 2020).

Ferritin-2

Blood-feeding ticks have a copious amount of free (non-haem) iron in their bloodmeals to utilize, detoxify, store and transport. Ferritins are involved in iron metabolism and the intracellular ferritin-1 was first described in *I. ricinus* and *O. moubata*. Secreted ferritin-2 (Fer2) is required for iron transport from the tick gut to ovaries and salivary glands. Silencing of the Fer2 gene impairs the ability of ticks to feed, making it a promising candidate for a tick vaccine (Hajdusek *et al.*, 2009). Fer2 sequences were amplified from *I. ricinus* and *R. microplus*, cloned into the expression vector pET100, expressed in *E. coli* and formulated with Montanide ISA 50 V. Vaccinated rabbits infested with unfed adult *I. ricinus* yielded fewer, lighter and less fertile engorged adult female ticks. Vaccinated cattle were infested with *R. microplus* and *R. annulatus* larvae and protected to a similar level as with Bm86 vaccination (Hajdusek *et al.*, 2010).

Fer2 is also secreted by *H. longicornis* where it was shown to be present in ovaries and eggs. Silencing the Fer2 gene induced a reduction in engorged tick weight, increased mortality and decreased fecundity, demonstrating the crucial role of this protein in blood feeding and

reproduction (Galay *et al.*, 2013). Fer2 was extracted from eggs of *H. longicornis* at various stages of embryogenesis and assumed to act as an iron transporter and conserver (Hernandez *et al.*, 2023).

Analysing the Fer2 sequence revealed conserved antigenic regions among various tick species. Fer2 was derived from *I. persulcatus* and shown to have a high iron-binding capacity, lending further support to its possible utility in a vaccine (Githaka *et al.*, 2020).

TROSPA

A midgut receptor for outer surface protein A (TROSPA) has been found in *I. scapularis*, (Pal *et al.*, 2004), *R. annulatus* (Antunes *et al.*, 2012), *I. ricinus* (Figlerowicz *et al.*, 2013) and *R. microplus* (Merino *et al.*, 2013b). TROSPA binds and facilitates midgut colonization and subsequent pathogen transmission to the host (Chmelař *et al.*, 2016). RNA interference with this gene impairs infection of the tick by *B. burgdorferi* (Pal *et al.*, 2004; Figlerowicz *et al.*, 2013) and *B. bigemina* (Antunes *et al.*, 2012; Hajdušek *et al.*, 2013; Merino *et al.*, 2013b), but not *A. marginale* (Merino *et al.*, 2013b). Cattle were vaccinated with recombinant TROSPA, expressed in *E. coli* and formulated with Montanide ISA 50 V, infested with *R. microplus* larvae and coinfecting with *A. marginale* and *B. bigemina*. Vaccination did not interfere with tick infestation or egg laying but reduced the amount of *B. bigemina* DNA recovered from collected replete ticks (Merino *et al.*, 2013b).

Paramyosin

Paramyosin is a muscle protein found in a large number of invertebrates and, beyond its structural function, has been implicated in modulation of the host's immune system by inhibiting the classical complement pathway (see section 3.1.3, this volume) and binding of IgG. Recombinant [B.] *R. microplus* paramyosin (rBmPRM) was expressed in *E. coli*. Expression analysis indicated the presence of BmPRM in all tested tissues (e.g. fat body and salivary glands) and developmental stages, but not in saliva. It was not yet known if BmPRM binds IgG because it acts as an Fc receptor (see section 3.1.3, this volume; Ferreira *et al.*, 2002).

The IgG response of cattle exposed to natural infestation with *R. microplus* was determined by ELISA, using both recombinant paramyosin (BmPRM) expressed in *E. coli* and salivary gland protein extract used as the antigen. The comparative reactions were more highly correlated in Nelore (Zebu) than in Hereford (European) cattle. The expression of paramyosin was determined by qRT-PCR in various tick stages and tissues and found to be highest in the adult female fat body and the gut in partially engorged adult female ticks. The fact that tick-infested cattle displayed a serological response to what is considered to be a concealed antigen presented an enigma. The authors concluded that BmPRM is an antigen to consider for inclusion in a 'cocktail' vaccine against *R. microplus* (Leal *et al.*, 2013).

After cloning the cDNA encoding paramyosin in *H. longicornis*, the recombinant protein was expressed in *E. coli* and vaccinated into rabbits, providing them with 50% immune protection against tick infestation. The protective epitope was analysed and identified as a peptide and conjugated with keyhole limpet hemocyanin (KLH). The cumulative (engorgement, fertility and fecundity) immunoprotective efficacy of the two vaccines in rabbits challenged with infestation by adult *H. longicornis* was 60.4% and 70.9%, respectively (Wu *et al.*, 2021).

Serpins

Serpins in tick saliva modulate the host's innate immune response by interfering with blood coagulation (see section 3.4.1, this volume) and inflammation (see section 3.4.3, this volume), and the adaptive response by inhibiting T-cell activation and cytokine secretion (see section 4.6.5, this volume). They seem to bridge the gap between secreted and concealed antigens, depending on where the immune confrontation occurs. Clearly, antibodies acting in the host against tick salivary protein at the bite wound are addressing a secreted antigen. But if the effect of the immune response occurs in the tick at the site of the serpin expression before it is secreted, the antigen would be regarded as concealed.

Rapid amplification of cDNA ends (RACE) was used to isolate and clone the cDNA coding for a [*R. microplus*] *R. australis* serpin. The

recombinant serpin (rRMS-3) was secreted by *P. pastoris* and demonstrated by qRT-PCR expression analysis to be mainly expressed in the salivary glands of female adult ticks. Only sera from tick-resistant bovines recognized the serpin's B-cell epitope, and semi-engorged ticks fed sheep sera with antibodies to the epitope by capillary tube showed an 81.16% reduction in reproduction capacity (Rodriguez-Valle *et al.*, 2012b).

Serpin 19 is characterized by a reactive centre loop that is 100% conserved in ixodid ticks. The specific reactivity of recombinant *A. americanum* serpin 19 makes it a potential candidate antigen for a universal tick vaccine (Kim *et al.*, 2015b). Serpin 19 was confirmed to be important to *A. americanum* fitness and feeding by mRNA disruption with RNAi. Treated ticks took smaller bloodmeals, were deformed and died during subsequent incubation, bolstering the opinion that it was a potential component of a 'cocktail' tick vaccine (Kim *et al.*, 2016a).

A serpin involved in vitellogenesis (egg yolk formation) was found in *R. haemaphysaloides*, labelled RHS8, and its gene cloned into a pET-30a vector and expressed in *E. coli*. Antiserum against the recombinant rRHS8 was produced in vaccinated mice. Knockdown of the RHS8 gene by RNAi in unfed adult female ticks resulted in reduced engorged weight and egg production and hatchability. RHS8 is worth considering in the development of a control strategy (Xu *et al.*, 2020).

Ribosomal phosphoproteins

Ribosomal phosphoproteins P0, P1 and P2 are located within the 60S ribosomal subunit, are highly conserved between species and considered to play an essential role in protein synthesis. Their phosphorylation could potentially influence their antigenicity. Approximately 15% of patients with systemic lupus erythematosus develop autoantibodies against these ribosomal proteins (Hasler *et al.*, 1991), with ribosomal protein P0 considered the classic lupus antigen (Frampton *et al.*, 2000). Their conserved nature mitigates against their suitability as a vaccine candidate (Hassan *et al.*, 2020), but a relatively poorly conserved 20 amino acid synthetic peptide used as a vaccine in rabbits protects against infestation by *R. sanguineus*, a three-host

tick. The calculated efficacy was 90%, based on a reduction in the viability of moulted nymphs and numbers of engorged adults and their egg production (Rodríguez-Mallon *et al.*, 2012).

The vaccine displayed an efficacy of 96%, based on the same criteria, against *R. microplus* (Rodríguez-Mallon *et al.*, 2015), and *Amblyomma mixtum* on rabbits and *I. ricinus* on cattle. The PO peptide was conjugated with Bm86 and KLH to enhance the spectrum and level of efficacy prior to vaccinating cattle against *R. microplus* (Rodríguez-Mallón *et al.*, 2020a, b). Another combination of PO was with recombinant *Rhipicephalus haemaphysaloides* subolesin. In vaccinated rabbits challenged with adult *H. longicornis*, the efficacy was 86.5% (Hassan *et al.*, 2020).

For unknown reasons, the PO conjugated with KLH or Bm86 (with Montanide ISA50 as adjuvant in the latter case) was disappointing when tested against *R. australis* (Mahony, 2021).

ATAQ proteins

Analysis of Bm86 homologues from argasid and ixodid ticks led to the discovery of a novel protein in metastriate ticks, named ATAQ after a part of its signature peptide. ATAQ proteins are structurally related to Bm86, contain multiple EGF-like domains and were shown by qRT-PCR to be expressed both in midguts and Malpighian tubules (Bm86 orthologues are only expressed in tick midguts) and more continuously over the life stages of *R. microplus* and *R. appendiculatus*. The vaccine potential of ATAQ proteins against tick infestations was yet to be evaluated but because they are more conserved than Bm86 orthologues, were thought to possibly confer protection against heterologous tick infestations (Nijhof *et al.*, 2010).

A yeast two-hybrid screening system was used to analyse the protein–protein interactions of Bm86 and ATAQ from *R. microplus*. They were shown to share two (aldehyde and retinol dehydrogenase) interacting partners, but Bm86 was shown to also have a Kunitz-like protein component. The three moieties are all involved in retinol (the alcohol form of vitamin A) metabolism that helps regulate the immune responses (Kiper, 2013). The ATAQ genes of seven Mexican isolates of *R. microplus* were sequenced and found to have a high degree of conservation

(98–100% for nucleotides and 97.8–100% for the encoded proteins), but to differ for 20 amino acid sequences from a *R. microplus* isolate from Mozambique. It would be necessary to gather more information on diversity in the ATAQ gene in order to form an opinion on its suitability as a vaccine candidate (del Castillo *et al.*, 2020).

A homologue of *R. microplus* ATAQ was found in *H. longicornis* (rHLATAQ), expressed throughout the midgut and in Malpighian tubule cells and containing multiple EGF-like domains. It was genetically distant from previously reported ATAQ proteins and expressed throughout all life cycle stages, increasing steadily during feeding. *H. longicornis* females fed on rabbits immunized with rHLATAQ took longer to complete their engorgement, weighed more, produced more eggs over a longer period and their eggs took longer to hatch. It was concluded that antibodies against ATAQ might disrupt tick engorgement and ovipositioning (Adjou Moumouni *et al.*, 2023).

Aquaporins

Aquaporins are water channels enabling ticks to deal with a bloodmeal 100 times their body weight, while returning 75% of the ingested water and electrolytes to the host via their saliva. An aquaporin in the gut, rectal sac and salivary glands of *I. ricinus* was identified from a cDNA library and cloned (*IraQP1*) for production of dsRNA and subsequent RNAi in unfed adult female ticks. Their feeding was assessed *in vitro*, using a membrane feeding technique (Kröber and Guerin, 2007) and *in vivo* on guinea pigs. Silencing the *IraQP1* gene dramatically affected the volume of blood ingested. It was not lethal, thus ruling out this protein as a potential target (Campbell *et al.*, 2010).

Triosephosphate isomerase (TIM)

Triosephosphate isomerase is a key enzyme in glycolysis and a potential candidate antigen for a vaccine. The TIM homologue gene of *H. longicornis* was found to be expressed in all developmental stages and in all tissues of the unfed adult female. The gene was cloned and inserted into a vector to obtain the recombinant protein. Vaccinated rabbits displayed a humoral immune response confirmed by Western blot,

demonstrated by ELISA, and 50.9% efficacy against unfed adult *H. longicornis*, as evidenced by their reduced replete weights, egg production and egg hatchability (Liang *et al.*, 2022).

5.2.6 Epitope diversity

Variable efficacy of various versions of Bm86 as a vaccine against *R. decoloratus*, *R. microplus*, *R. australis* and *R. annulatus* has been one of the stumbling blocks to greater commercial success. The system used for expressing the antigen (*E. coli*, Baculovirus, *Pichia pastoris* or pKLAC2-*Kluyveromyces lactis*) (Tabor, 2021) is no doubt partly to blame. But another problem bedevilling the search for recombinant subunit tick antigens that will offer a wide spectrum of protection is sequence polymorphism in the genes that encode the proteins, and in their amino acids, leading to variable and unsatisfactory vaccine efficacy (García-García *et al.*, 1999, 2000; Cunha *et al.*, 2011).

The midgut Bm86 and Bm85 genes of 30 [B.] *R. microplus* strains from Brazil, Argentina, Uruguay, Venezuela and Colombia were sequenced. Variations of up to 3.65% were seen in the nucleotide sequences of the Bm86 gene, and 6.08% in the amino acid sequences of the Bm86 protein. Amino acid sequence variations greater than 2.8% are deemed sufficient to confer vaccine inefficiencies (Sossai *et al.*, 2005). Sequencing the Bm86 cDNA gene from 125 field isolates revealed 3.1–7.4% divergence from the Yeerongpilly reference vaccine antigen, which was thought to account for the poor efficacy of the vaccine in many parts of Mexico (Martínez-Arzate *et al.*, 2019). Bm86 homologues cloned from [B.] *R. decoloratus*, sequenced and expressed by *E. coli*, contained two conserved linear peptides that helped to explain the significant reduction in engorged female weight and egg production in *R. decoloratus* ticks fed on Bm86-vaccinated cattle.

Rhipicephalus microplus and *R. annulatus*, both vectors of bovine and equine babesiosis, share midgut Bm86 antigen sequences but with enough amino acid divergence to render vaccine protection ineffective. Bm86 antigens from vaccine strains of *R. australis* and *R. microplus*, Australian Yeerongpilly and Cuban Camcord,

compared with Bm86 from South Texas isolates of *R. annulatus* and *R. microplus* ('cattle fever ticks'), displayed 10–10.2% divergence in the amino acid sequences (Freeman *et al.*, 2010).

Ra86 homologues of Bm86 from a laboratory strain and four field isolates of *R. appendiculatus* were sequenced. The laboratory strain contained two alleles, based on amino acid insertions and deletions (indels), and the field isolates three more. The amino acid sequence identity between the sequence types varied from 74 to 97% (Kamau *et al.*, 2016), which is bound to adversely affect the consistency of effect of a putative vaccine in the field.

Considering the multitude of tick species and the plethora of potentially immunogenic structural and secreted epitopes and their different functions, the prospect of a single vaccine that can offer protection on this wide front is daunting (Hassan *et al.*, 2020). It seems inevitable that the successful vaccine will contain several antigens (Parthasarathi *et al.*, 2021). A vaccine composed of three recombinant antigens (*R. microplus* Bm86 and *H. anatolicum* subolesin and tropomyosin), injected into cattle three times over 60 days gave 86.7% protection against challenge with *R. microplus* and 87.2 and 86.2% efficacy against *H. anatolicum* larvae and adults. A significant antibody response against all three antigens was recorded for up to 140 days (Parthasarathi *et al.*, 2023).

Another combination of relatively well-conserved antigens was used to overcome the vulnerability of a single antigen to genomic variability. SUB, calreticulin and cathepsin-L genes of *H. anatolicum* and *R. microplus* were sequenced and analysed for intra- and inter-species homogeneity. Intraspecies sequence homology was >97%, while between species it was >77% and >85% for SUB and calreticulin respectively, and >57% for cathepsin-L. Cattle were vaccinated with the individual recombinant antigens and challenged with newly hatched larvae of the two tick species. The efficacy percentages are summarized in Table 5.2 (Kumar *et al.*, 2017). Although the individual percentages, by antigen or tick species, are disappointing at first glance, it is intriguing to consider what the cumulative (or possibly synergistic) effect of an antigen combination against a multispecies infestation challenge might be in a field situation.

Table 5.2. Summarized efficacy of three recombinant antigens against *H. anaticum* and *R. microplus* on cattle. Adapted from Kumar *et al.* (2017).

Antigen	% Efficacy	
	<i>H. anaticum</i>	<i>R. microplus</i>
Subolesin	65.4	54.00
Calreticulin	41.3	37.56
Cathepsin-L	30.2	22.21

5.2.7 A double-barrel approach

Immunity to the vector might imply protection against the pathogens it transmits, but a vaccine that offers protection against both the vector and its transmitted pathogen may be worth considering. A multiantigen vaccine offering broad-spectrum immunity against multiple tick genera seems entirely feasible, but a practical difficulty might be the selection of the pathogen whose antigen(s) should be combined with those of the tick. New technology might allow the nimble approach of ordering small quantities of a bespoke vaccine for a specific geographic region based on the tick(s) and tick-borne disease(s) that prevail there.

Immunoinformatic approaches were taken for the *in silico* design of a multivalent vaccine that would target more than one immunodominant epitope of structural proteins of CCHFV. This would be combined with tick salivary gland subolesin to impart dual activity. Linear and conformational B-cell, helper and cytotoxic T-cell epitopes from the viral proteins were incorporated, with appropriate linkers and adjuvant. The proposed vaccine construct was expected to induce immune protection against both the CCHFV pathogen and its tick vector (Shrivastava *et al.*, 2020).

Alternatively, a vaccine targeting and neutralizing the tick molecules essential for transmission of tick-borne infections will protect against infection and avoid the need for vaccination against every tick-borne pathogen (Brossard and Wikel, 2004). An example of this was the vaccination of cattle with tick proteins involved in vector–pathogen interactions, such as SUB, TROSPA, silk and the Q38 chimera. They were subsequently infected with *Anaplasma marginale* and *Babesia bigemina*, before being infested with

R. microplus larvae. Vaccination with TROSPA did not affect the tick parameters, whereas Q38, silk and SUB were 75%, 62 and 60% effective. *B. bigemina* transmission was reduced by Q38, TROSPA and SUB and that of *A. marginale* by silk and SUB vaccination (Merino *et al.*, 2013b).

5.3 The Vector Microbiome

Disturbance of the microbiome equilibrium (dysbiosis; see section 2.1.7, this volume) not only causes anomalies in tick development but also impairs the tick's chances of survival and its vector competence (Estrada-Peña and Cabezas-Cruz, 2019; Wu-Chuang *et al.*, 2022). *I. scapularis* larvae infected with *B. burgdorferi* had a smaller microbiome redundancy, with 15.4% fewer functional pathways than uninfected nymphs (Estrada-Peña *et al.*, 2020).

The realization that the tick's gut microbiome might present a hitherto neglected source of hidden antigen was an exciting discovery (Narasimhan and Fikrig, 2015; Wu-Chuang *et al.*, 2022). Vaccines against tick microbiota alter the structure of the microbial community (Fig. 5.3), vector feeding, the functional profile of their microbiome and the development of pathogens in their vectors (Mateos-Hernández *et al.*, 2020, 2021; Maitre *et al.*, 2022), as in the case of *B. afzelii* infection in *I. ricinus* (Wu-Chuang *et al.*, 2023). The functional redundancy of the tick microbiome (15.7–19.2% of pathways provided by more than 100 genera in uninfected *I. scapularis*; Estrada-Peña *et al.*, 2020) provides a sobering background to the selection of vaccine antigens from this potential source.

5.4 The Promise of New Technology

5.4.1 Synthetic antigens

The Q38 and Q41 SUB/AKR chimeras (see section 5.2.5) were not the first examples of biosynthetic epitopes.

Taking note of the sequence variation in recombinant Bm86 (see section 5.2.5), synthetic peptides were designed *in silico* with

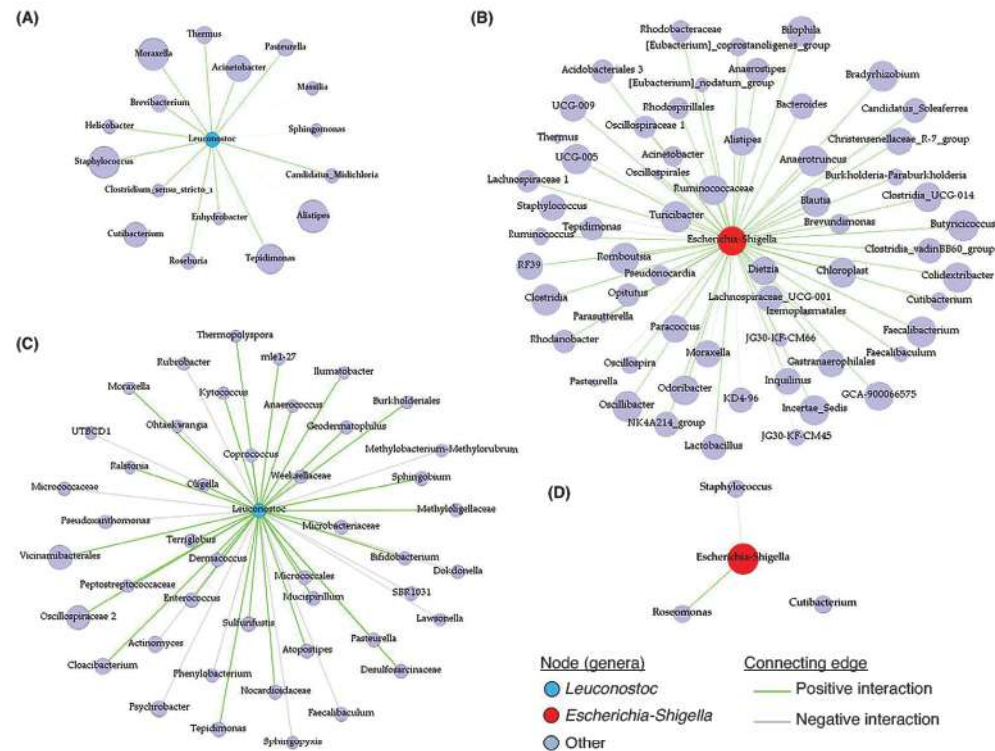


Fig. 5.3. Animals vaccinated against the tick microbiota display an impact on the structure of the tick's microbial community. From Mateos-Hernández *et al.* (2021) under the terms of the Creative Commons Attribution License (CC BY).

selected amino acids, mapping the T and B epitopes of the Bm86 protein, expressed in *Komagataella (Pichia) pastoris* and refined to one candidate. The chosen amino acid sequences of the immunogenic epitopes were found to be conserved in *R. microplus* strains from different parts of the Americas. The synthetic peptide is designated SBm7462® and has been patented in several countries (Patarroyo *et al.*, 2002, 2009, 2020). Tick-naïve calves vaccinated with the recombinant peptide with saponin as adjuvant were challenged with newly hatched *R. microplus* larvae after the vaccination. The efficacy of immunization as assessed by the usually combined parameters of engorged tick numbers, egg production and fertility was 72.5%. The immune response of the vaccinated calves was monitored by ELISA confirmation of IgG production. Peripheral blood mononuclear cells (PBMC) were isolated, cultured and stimulated with concanavalin A for detection

of cytokine gene expression. RNA was extracted and cDNA obtained by reverse transcription for quantitative transcription profiling of IL-4, IFN- γ , IL-10, IL-12 and TNF- α expression by qPCR. IL-4 was found to have been upregulated and TNF- α downregulated. The site of action of antibodies to SBm7462 in the tick gut was displayed by immunohistochemistry (Fig. 5.4), and changes in regional lymph node germinal centres were measured by histomorphometry. These results point conclusively to a type two adaptive immune response elicited by SBm7462 (Patarroyo *et al.*, 2020).

Tick subolesin have two different epitope types in addition to the linear B-cell epitope (Prudencio *et al.*, 2010). B-cell-reactive epitopes were identified in the mapped peptide array of SUB from three different tick species. If these epitopes can be related to the protection and efficacy of SUB-based vaccines, it could lead to the design of new chimeras (Contreras *et al.*, 2022).

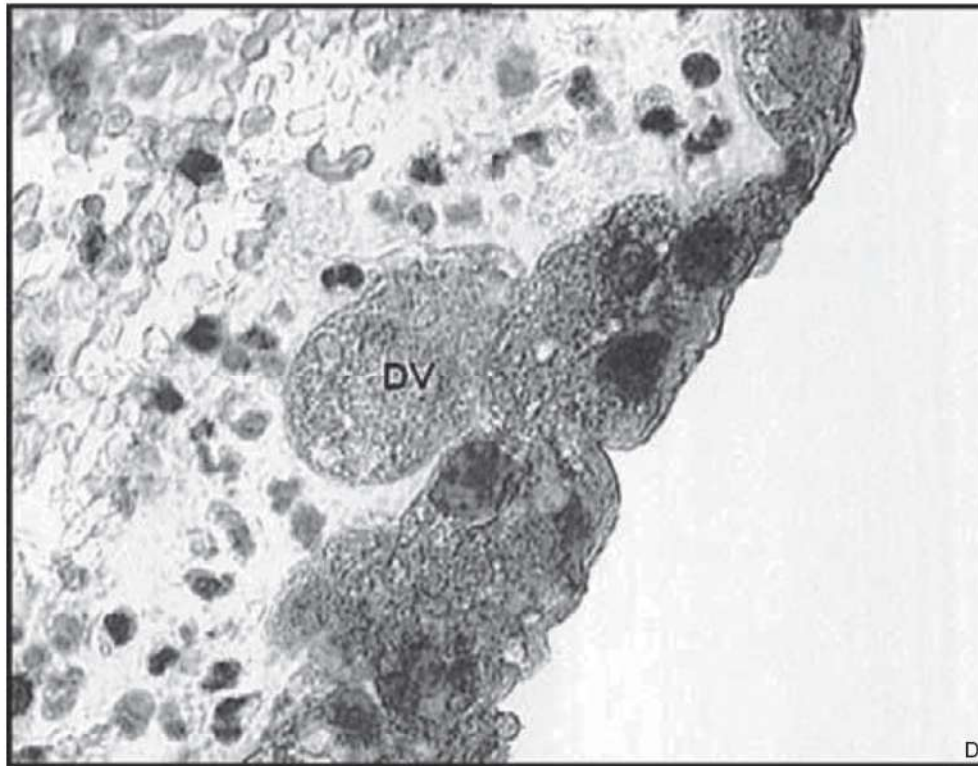


Fig. 5.4. Microphotograph of indirect immunoperoxidase staining of Bm86 protein on gut cells of [*B.*] *R. microplus* feeding on a calf vaccinated with the synthetic peptide SBm7462. From Patarroyo *et al.* (2002) with permission of Elsevier Science B.V.

5.4.2 Antigen delivery and presentation

Exogenous antigens need to be processed and presented to receptors to elicit an immune response. Virus-like particles (VLPs) are structural proteins devoid of native virus genome and are efficiently processed by dendritic cells for presentation by MHC class I molecules to cytotoxic T-lymphocytes, and by MHC class II molecules for activation of T-helper cells. They can also be considered as typical T-cell-independent B-cell antigens. They are thus capable of activation of innate and adaptive immune responses, both humoral and cell mediated. Recombinant VLPs were considered as veterinary immunogens against a variety of pathogens, all of which were viruses at the time (Liu *et al.*, 2012).

DNA tattoo vaccination

The skin is rich in APCs, and dermal delivery of DNA vaccines by tattooing induces higher specific humoral and cellular immune responses than intramuscularly injected DNA. The tattoo procedure causes many minor mechanical injuries followed by haemorrhage, necrosis, inflammation and regeneration, thus stimulating the innate immune system (Pokorna *et al.*, 2008; Quaak *et al.*, 2009). Plasmid DNA vaccines are easier and simpler to produce than recombinant protein and highly flexible with regard to modification of the expression cassette (Van der Berg *et al.*, 2014).

Tattoo vaccination of mice against *B. burgdorferi* induced high IgG titres and provided near total protection against challenge with infected *I. scapularis* nymphs. Tattoo vaccination

with four recombinant *I. scapularis* salivary proteins induced low to moderate IgG titres and did not provide protection against infestation (Klouwens *et al.*, 2021). The same antigens will have to be tested by conventional administration before tattoo vaccination against ticks is rejected as a possibility.

Transgenic plants

Plants expressing recombinant immunizing proteins could offer the possibility of delivering the first and booster doses of an oral vaccine with considerable benefits, such as low cost, minimal animal handling and ease of repeated administration.

Three transgenic plant lines expressing the spike protein of transmissible gastroenteritis virus (TGEV) were used to immunize pigs. Expression of the gene products was confirmed by ELISA and Western blotting, while the pigs' TGEV-specific immune responses were determined by ELISA and virus neutralization (Tuboly *et al.*, 2000). Emulating these results against tick infestation in a ruminant might bear investigation.

Oral administration

Recombinant *R. microplus* SUB produced in *E. coli* was mixed with heat-inactivated *Mycobacterium bovis* in phosphate-buffered saline and administered orally to European cross-bred calves. The animals were challenged with an induced infestation of *R. microplus* larvae 3 weeks after the second vaccination. IgG antibody responses were assessed by ELISA and the expression profiles of their genes involved in immunity (complement 3, TNF- α , IL-2, IL-12) were analysed by qRT-PCR. Vaccination reduced the number of engorged female ticks by 51% and the hatchability of the eggs they produced by 30% (Contreras *et al.*, 2019).

The principle of a multivalent (four different epitopes) Semliki Forest virus replicon-based mRNA vaccine (see *Endogenous expression* below) administered orally by way of a *Salmonella typhimurium* mutant was proven in mice and hamsters protected against SARS-CoV-2 infection (Jawalagatti *et al.*, 2022).

Endogenous expression

The concept of transfecting APCs, or muscle or skin cells, with the DNA encoding for an antigen was tested by constructing expression vectors containing the ovalbumin gene alone, or linked to cytokine genes, including granulocyte and macrophage-colony stimulating factor (GM-CSF), IFN- γ , IL-2, IL-4, IL-12 or a sequence encoding nine amino acids of IL-1 β , and injecting these into mice. The results indicated that humoral, cytokine and cytotoxic responses to DNA vaccination can be altered by certain cytokine constructs (Maecker *et al.*, 1997).

Prior to this, mouse cells had been successfully transfected *in vitro* with liposome-mediated luciferase messenger RNA (mRNA), resulting in greatly increased luciferase protein expression (Malone *et al.*, 1989). Development and refinement of the technology was well under way, and it had been acknowledged as a promising alternative to conventional vaccines (Scorza and Pardi, 2018), by the time the global human Covid-19 pandemic thrust the concept of endogenously expressed antigens, prompted by mRNA administered by various means, into lay public awareness.

Although the initial use for livestock is likely to be dominated by infectious diseases (Mahony *et al.*, 2024), the relative ease with which virtually any genomic sequence can be designed *in silico*, customized, synthesized and delivered as an mRNA vaccine, for expression of the antigen as an intracellular, secreted, or membrane-bound protein, holds promise for the future immunogenic management of ticks on livestock. Antigen-encoding mRNA vaccines have been shown to elicit antigen-specific humoral and cellular immune responses (Chahal *et al.*, 2016; Maruggi *et al.*, 2019; Abbas *et al.*, 2023).

The risk of antigenic drift or genomic diversity (see section 5.2.5) is justification for using an mRNA that encodes for a well-conserved epitope or multiple epitopes. Covid-19 vaccines have shown efficacy varying from 60% to 95%, attributed to the fact that they encode for epitopes in the virus spike protein which is prone to frequent mutation (Piadel *et al.*, 2022).

Exogenous mRNA is recognized by a variety of innate immune receptors, and cells have a propensity to limit the duration of expression,

which limits the stability and activity of conventional mRNA (Chahal *et al.*, 2016; Maruggi *et al.*, 2019). This is potentially advantageous if it provides adjuvant activity, promoting DC maturation and eliciting robust T- and B-cell responses. But this innate immune response might also inhibit antigen expression and negatively affect the immune response (Pardi *et al.*, 2018), as well as possibly inducing toxicity. Ideally, mRNA vaccines need to be delivered intracellularly (Chahal *et al.*, 2016). The mRNA can be encapsulated for protection in a variety of lipid, polymer or dendrimer nanoparticles. The innate immune activation can also be decreased and the protein translation increased by modifying the mRNA nucleosides (Pardi *et al.*, 2018; Scorza and Pardi, 2018). Elimination of exogenous mRNA by the innate immune system can also be circumvented by mRNA replicons (Sethna *et al.*, 1989).

The hypothesis that primary vaccination with the gene for Bm86 with or without the genes for the cytokines GM-CSF and IL-1 β would extend the antibody response to a booster vaccination with Bm86 could not be proven in sheep (De Rose *et al.*, 1999). Safety concerns about the possible stable transfection of DNA into somatic or germ cells were allayed by indications that the majority of plasmid DNA injected into skeletal muscle remains at the injection site. Various groups of researchers have attempted to develop a DNA vaccine against tick infestation (Abbas *et al.*, 2023).

Guinea pigs were vaccinated with a 19ISP vaccine and subsequently challenged with *I. scapularis*. Bite wounds displayed early erythema, the ticks' engorgement was curtailed and their transmission of *B. burgdorferi* was impeded. The mRNA in the vaccine encoded 19 *I. scapularis* proteins, known to be expressed in salivary glands, many of them secreted in the bite wound. The nucleoside sequences had been varied and the mRNAs were encapsulated in lipid nanoparticles (Sajid *et al.*, 2021). In a subsequent refinement, the number of mRNAs was reduced to 12, and the 12ISP vaccine shown to provide similar protection. None of the antigens administered individually elicited a protective immunity. Salp14 was the component responsible for the strongest tick bite erythema response. The authors conclude that salivary antigens not included in the 12ISP cocktail might

contribute to its efficacy, as may the inclusion of mRNA encoding for hidden gut antigens (Matias *et al.*, 2023). The improved protection elicited by a combination of mRNA-encoded antigens had previously been demonstrated, admittedly for a different pathogen (Freyn *et al.*, 2020).

5.5 Formulating the Vaccine

There appear to be conflicting opinions on the importance of antibody titre level and the extension of titre half-life to enhance vaccine efficacy. In both instances, the simulation models were based on the life history of the single-host tick [*B.*] *R. microplus*. Simulations based on European breed cattle in Australia indicated that control was improved with increases in both antibody titre and titre half-life, but with a flattening off and diminishing returns for antibody titre and linearly increased vaccination efficacy from extended half-life (Floyd *et al.*, 1995). Using vaccination data gathered in Cuba and Brazil, returns from increased titre half-life flattened earlier, and vaccination efficacy improved linearly with increased titre levels (Lodos *et al.*, 2000).

The difference is bound to be one of mathematics, with differences in the models used (TICK2 vs TICKSIM) and the assumptions underpinning these. But it highlights the potential pitfalls in designing a vaccination regime that will be effective against different tick species with single- and multi-host life cycles on the same host, which is the norm rather than the exception.

5.5.1 Enhancing the immune response: the role of adjuvants

The refinement of vaccine antigens, moving from whole organisms to extracts, toxoids, or single proteins or peptides expressed by recombinant technology, has been accompanied by a decline in immunogenicity, which can be remedied by an adjuvant (Brewer, 2006) – an ill-defined substance that non-specifically triggers the innate immune system and accelerates, prolongs and/or enhances the immune response. An adjuvant can cause clustering

of antigens, which enhances the activation of B-cells and boosts antibody titres. The migration pattern and cellular uptake of vaccine particles are affected by their size. Large particles remain in tissue at the injection site and have to be transported to the lymph node by monocytes, neutrophils and DC. Smaller particles are taken up more efficiently by DC or can drain directly into lymphatic vessels draining to the lymph nodes (de Veer and Meeusen, 2011).

There are different types of adjuvants, with different modes of action. They can be grouped as aluminium salts, liposomes, emulsions, agonists of the stimulator of interferon genes (STING), TLR ligands, RLR and CLR. Liposomes can encapsulate both the antigen and immunostimulatory molecules, enhancing uptake of antigen and stimulation of immune cells and releasing entrapped antigens slowly over an extended period. Freund's adjuvant is a water-in-oil emulsion either with (complete) or without (incomplete) killed *M. tuberculosis* bacteria. They not only improve antibody titres but also enhance the specific cytotoxic T-lymphocyte response (Wu and Liu, 2021).

Aluminium adjuvants (alum) have been used in human vaccines since the 1920s but until recently, the physicochemical interactions between aluminium compounds and antigens were poorly understood (Brewer, 2006). Dynamic alterations in antigen processing and presentation underlie enhanced DC function induced by alum. *In vitro*, alum induces increased antigen accumulation and presentation and reduces protein degradation by DC (Ghimire *et al.*, 2012). This survey did not find any mention of alum in conjunction with a tick antigen.

Keyhole limpet hemocyanin (KLH) is a copper-containing respiratory protein from the marine gastropod *Megathura crenulata*. Although clinically used for treatment of carcinoma in human medicine, it has also become a generalized vaccine component and carrier of conjugated haptens (Harris and Markl, 1999). KLH was conjugated with the PO peptide and Bm86 to vaccinate cattle against *R. microplus* (Rodríguez-Mallón *et al.*, 2020a, b; Mahony, 2021).

Heat-inactivated *Mycobacterium bovis* activates the innate immune response through complement three and was used to enhance the

protective response to a *R. microplus* SUB vaccine in an oral formulation (Contreras *et al.*, 2019).

The use of nanotechnology offers a new communication medium with the immune system. Nanoparticles mimic the size of microorganisms (bacteria and viruses) and by incorporating key molecules involved in immune processes, nanocarriers can be taken up by the immune cells and modulate their responses (Dacoba *et al.*, 2017). The size, shape and surface properties of mesoporous silica nanoparticles (MSNs) can be manipulated (Frickenstein *et al.*, 2021) and they can be used as self-adjuncting vaccine carriers with remarkable potential for the delivery of a variety of antigens. The adjuvant effect of nanoparticles results in higher IgG levels than from conventional alum-adsorbed vaccines (Prego *et al.*, 2010). Silica vesicles, one class of MSNs, have been tested as self-adjuncting vaccine delivery systems targeting infectious and protozoal bovine diseases and [*R. microplus*] *R. australis*. Bm86 expressed by *E. coli* and amplified in recombinant baculovirus was experimentally formulated in silica nanovesicles as carriers, and its distribution pattern in mice demonstrated by fluorescent imaging (Mody *et al.*, 2021).

5.5.2 Duration of effect: the need for a sustained stimulus

In the absence of a boosting effect from natural tick challenge when a concealed tick antigen is used in a vaccine, repeat vaccination is a prerequisite for maintaining a protective immunity. Under extensive production systems, where mustering cattle is a considerable financial impost, a vaccine that will provide prolonged immune cover is needed, reducing the frequency of booster vaccination. There have been several attempts but few have thus far been commercially successful.

Chitosan is a non-toxic linear polysaccharide derived from chitin by deacetylation, found in the exoskeletons of crustaceans and insects, and structurally similar to cellulose. Because of its biocompatibility and biodegradability, chitosan is commonly used for many applications in pharmaceutical and medical fields, such as vaccine delivery systems in both solution and

particulate forms (Chua *et al.*, 2012; Duttagupta *et al.*, 2015). A biodegradable vaccine depot with the antigen incorporated into the chitosan component induced antibody titres lasting for up to 55 weeks from a single injection in mice (Chua *et al.*, 2015). An injectable biopolymer providing initial rapid release followed by sustained delivery of a biologically active agent, with chitosan as one of the possible components, was patented in 2017 (Al Kobaisi and Mainwaring, 2017) and tested with Bm86 in cattle against *R. australis*. When challenged with tick larvae 56 days after the vaccination, cattle enjoyed 76.9% protection. Immunoglobulin titres increased dramatically to a booster injection 1 year later, but there was an apparent allergic skin reaction at the booster injection sites (Mahony *et al.*, 2019) which would have a profound negative impact on producer acceptance of this product.

Subcutaneously injected clay nanoparticles formed nodules with loose structures from which conjugated antigen was sustainably released over several weeks. The nanoparticles actively and continuously recruited immune cells and stimulated a stronger immune response than alum or QuilA as alternative adjuvants (Chen *et al.*, 2018).

The synthetic peptide SBm7462 was encapsulated in biodegradable microspheres made from polyesters of lactic and glycolic acids and injected subcutaneously in mice. Although the *in vitro* release profile over 51 days was encouraging, this was not reflected in the immune responses of the mice. IgG titres elicited by the microencapsulated antigen were significantly lower than those resulting from immunization with the antigen emulsified in saponin over a period of 21 weeks (Sales-Junior *et al.*, 2005).

The continued expression of therapeutic proteins for as long as 7 weeks *in vivo* can be advantageous where continuous therapeutic levels of the encoded protein are needed, moderating the need for repeated injections (McCafferty *et al.*, 2021), e.g. therapeutic oncology vaccines (Maine *et al.*, 2021). Messenger RNA replicons function as a mechanism for mRNA self-amplification (Sethna *et al.*, 1989), leading to sustained translation. It has been suggested that an mRNA liquid nanoparticle formulation could enable slow continuous antigen delivery (Abbas *et al.*, 2023). This will clearly be desirable for a concealed-antigen vaccine

against tick infestation in cattle. RNA replicons with genetic switches that allow control of timing and magnitude of their translation after *in vivo* delivery would be desirable in the case of vaccines, where a measured dose of antigen is usually preferable. Such a switch was encoded on an RNA replicon derived from Venezuelan equine encephalitis virus, and turned off by the administration of trimethoprim, at a serum level 45-fold below what is used in human therapy (McCafferty *et al.*, 2021).

5.6 Efficacy Testing Methodology

Although a vaccine has to be tested under field conditions to confirm its efficacy, the imperatives of cost, animal ethics and sustainability are strong incentives towards developing and adopting laboratory model and *in vitro* screening and testing methods for the early stages of tick vaccine development. As in the case of chemical acaricides (Holdsworth *et al.*, 2006), the effect of an immune response on parasitic tick survival, feeding, egg production (fecundity) and hatchability (fertility) has to be assessed (Costa *et al.*, 2021) in order to judge a vaccine candidate's suitability. Calculation of the efficacy of a vaccine that incorporates these parameters will need to be standardized in order for thresholds to be set and candidate products compared equitably. The protective antibody titre threshold and the duration of this protective effect also need to be known, as the immune response wanes without stimulation or boosting.

The various methods of evaluation of the effect of chemicals on ticks are well established, but innovation and standardization are required for the variety of ways of exposing the tick to the immune response – *in vitro*, *in vivo*, in the laboratory and in the field, at different life cycle stages.

Agreement on and standardization of test methods will contribute to the ability to collaborate and compare research results on a level playing field. An encouraging attempt towards this goal was a small meeting in Morocco in 2015, giving rise to formation of the cattle tick vaccine consortium (Schetters *et al.*, 2016; Parizi *et al.*, 2023). This was predated by the creation in 1984 of the Ibero-American development programme of science

and development (CYTED). Funding of its INCOGARR project (immunogens compatible with integrated management strategies in tick control) commenced in 2017 (Rodríguez-Mallon *et al.*, 2018).

Guinea pigs, rabbits and deer mice were used in early experiments to demonstrate acquired immunity to ticks (Trager, 1939). Rabbits are popular laboratory animals and can yield valuable *in vivo* data in tick vaccine experiments. Vaccination with *A. variegatum* midgut protein fractions was used to demonstrate reduced egg production and hatchability from ticks applied to rabbits' ears and confined with ear bags (Kinyua *et al.*, 2002).

Ornithodoros spp. argasid ticks were fed successfully on the air sac membrane of embryonated chicken eggs to study the transmission of tick-borne pathogens (Burgdorfer and Pickens, 1954; Burgdorfer, 1956). Larvae of [*B. microplus*] *R. australis* were successfully cultivated to the nymphal stage on the air cell membrane of embryonated hens' eggs (Pierce and Pierce, 1956). In one instance, a colony of *O. moubata* was maintained entirely by feeding on the exposed membranes of embryonated hens' eggs (Hood *et al.*, 1976). It remains to be seen if this could be an inexpensive alternative for maintaining laboratory tick colonies and possibly testing the effect of immune products.

Capillary tube artificial feeding of partially engorged *R. microplus* females weighing 25–60 mg has been used to test the effects of immunoglobulins on the tick. The ticks were immobilized with double-sided adhesive tape and microhaematocrit capillary tubes filled with citrated cattle blood placed over their mouthparts. The tubes were replaced every 3 hours and engorgement took ca. 28 hours. The authors suggest that monoclonal antibodies can be used to identify antigens that can be used as vaccine immunogens (Gonsioroski *et al.*, 2012).

In a variation of this method, [*R. microplus*] *R. australis* were exposed either to antibodies or to RNAi achieved by feeding dsRNA. Self-drawn Pasteur pipettes were used instead of capillary tubes. The test ticks were fed blood containing antibodies raised by cattle vaccinated twice 1 month apart with recombinant Bm86. Ticks were fed on 100 µl of this mixture, followed by 100 µl whole blood before incubation for assessment of efficacy, which was calculated at 83%.

A separate experiment of this investigation was the feeding of ubiquitin and Bm86 dsRNA. Knockdown of the two genes was confirmed by qRT-PCR. The ubiquitin dsRNA was highly effective against the ticks, but the effect of the Bm86 dsRNA was minimal (Lew-Tabor *et al.*, 2014). It is difficult to envisage this rather laborious technique being upscaled for screening of large numbers of antigens and their formulations.

Variations in infection levels between ticks make capillary feeding an imperfect method for characterizing the effect of tick–pathogen interventions (Antunes *et al.*, 2014; Bhowmick and Han, 2020).

Amblyomma americanum ticks were successfully fed on defibrinated blood *in vitro* through silicone-coated lens paper membranes in order to collect their cement cones uncontaminated by host skin or hair (Bullard *et al.*, 2016a). This method might lend itself to *in vitro* screening of antigen and vaccine candidates.

5.7 Loss of Vaccine Efficacy

Because immunity to concealed tick antigens is not boosted by natural tick infestation, the induced protective immunity is short-lived (Trimnell *et al.*, 2005). There is also the question of the correlation of antibody titre with resistance to ticks, and the efficacy of anti-tick immunity in the face of varying levels of tick infestation challenge (Leal and Ferreira, 2021). It is easy to envisage the more rapid depletion of protective antibodies by a higher number of blood-sucking ticks in the absence of a boosting stimulus.

Aside from a decline in immunity in the absence of adequate boosting stimuli, possibly hastened by the removal of circulating antibodies by feeding ticks, it seems there are at least two ways in which vaccine efficacy could wane over time, due to repeated use. One is the selection, by vaccination, of tick strains with diverse midgut antigen amino acid sequences, rendering them non-susceptible to host antibodies. The other could be spontaneous antigenic drift due to mutations, the rate of which might be calculated using a mutation fixation index (MFI) for the site where the antigen is expressed. At the turn of the 20th century, the MFI for the Bm86 locus

was calculated as between 0.02 and 0.1 amino acids per year. This is higher than the rates of human DNA and RNA mutations.

Because amino acid sequence diversity of up to 2.8% did not result in significant tick strain differences in sensitivity to the Bm86 vaccine, it was surmised that amino acid diversity greater

than 2.8% would be needed to affect vaccine efficacy. It was concluded that selection of vaccine-resistant tick strains could take from 200 to 1000 years (García-García *et al.*, 1999). The possibility of repeated exposure to vaccine antibodies accelerating the rate of antigen mutation probably also needs consideration.

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