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ILRI
INTERNATIONAL LIVESTOCK RESEARCH INSTITUTE
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CABI
Nosworthy Way
Wallingford
Oxfordshire OX10 8DE
UK

Tel: +44 (0)1491 832111
Fax: +44 (0)1491 833508
E-mail: info@cabi.org
Website: www.cabi.org

CABI
745 Atlantic Avenue
8th Floor
Boston, MA 02111
USA

Tel: +1 (617)682-9015
E-mail: cabi-nao@cabi.org

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10 Ticks and Their Control

Peter Willadsen
Brisbane, Australia

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Executive Summary

The problem

Ticks are bloodsucking external parasites. They are responsible for decreased productivity due to blood loss from the host animal and 'tick worry', the irritation resulting from their feeding activity. Other negative effects include the injection of toxins, transmission of endemic and emerging diseases (such as heartwater, African swine fever and Congo-Crimean haemorrhagic fever) and tick-associated disease (such as dermatophilosis). Tick-borne pathogens affect 80% of the world's

cattle population and are ubiquitous in the tropics and subtropics. Countering these negative effects requires expensive control measures. Global costs associated with ticks and tick-transmitted pathogens in cattle alone were estimated years ago at above US$13.9 billion and US$18.7 billion, respectively (de Castro, 1997) and costs have doubtless increased in this century. Climate change and transboundary trade in livestock have recently begun to drive ticks into new areas where animals have less resistance to both ticks and tick-borne diseases. In Africa, ticks and tick-borne disease appear at the top of several rankings of important livestock diseases; notably, diseases transmitted by parasites, such as trypanosomiasis and East Coast fever (ECF), ranked high in International Livestock Research Institute (ILRI) prioritizations of livestock disease across regions and production systems of sub-Saharan Africa (Perry *et al.*, 2002).

The most common control methods are the use of genetically resistant animals and the application of acaricides. Acaricides may be applied through dips, sprays or pour-on formulations as well as intra-ruminal boluses, ear tags and footbaths. Resistance to acaricides is the ability in a strain of ticks to tolerate doses of acaricides that would prove lethal to most individuals in a normal population of the same species, and this is a major and growing problem. An anti-tick vaccine is commercially available for only a single tick species. Pasture management also has a role in integrated control.

ILRI's role in the global context

Because of its geographical location, abundant infrastructure and technical expertise, ILRI was and remains in a powerful position to contribute to the global understanding of African ticks and tick-borne diseases. In 1979, the International Laboratory for Research on Animal Diseases (ILRAD) Tick Unit was established as a resource – a provider of skills and materials that could be used in ILRI's research and, potentially, by others as well. The unit was built primarily to support research on ECF. The control of ECF and other such diseases is inextricably linked to ticks and their control, so ILRI, although it lacked a systematic tick research programme – a programme aimed at some specific component of tick biology or control – was soon involved in diverse areas of tick research.

Following flagship projects at ILRAD, ILRI conducted important research on tick biology, tick population dynamics, the impact of ticks and tick control using chemicals. Apart from ECF, a major ILRI research theme, other haemoparasites and, more recently, viral pathogens were studied.

Scientific impacts

Sustainable strategies for the control of ticks and tick-borne disease involve a complex interplay between parasite and host species, available control technologies and a range of environmental factors. In this challenging situation, ILRI's contributions were a continuum from laboratory-based science, through field experimentation, to practical advice and policy recommendations.

ILRI made early and ongoing contributions to our understanding of 'endemic stability'. This occurs when rates of infection are sufficient to maintain a level of acquired immunity that minimizes clinical disease in a population. The concept was developed to describe patterns of tick-borne disease in cattle but has since been applied more broadly in veterinary and human health.

ILRI's field experiments in Kenya, Ethiopia and Uganda led to recommendations for on-farm tick control. For example, in Kenya, acaricide treatment improved weight gains where keeping unvaccinated cattle without acaricides was uneconomical. Rotation of acaricides could potentially mitigate resistance, so ILRI scientists conducted the first field examination of acaricide rotation, finding it was advantageous.

More laboratory-based research into tick biology was greatly facilitated by the existence of the Tick Unit, described above. ILRI developed an artificial feeding system for ticks and methods for maintaining tick colonies (four stocks for over 30 years, and six for more than 20 years). In addition, tick-breeding research established high- and low-infectivity lines for ECF: seven different stocks and lines of ticks from eastern and southern Africa, which include low (refractory) and high (susceptible) genetic tick lines. Vector biology studies included understanding population genetics of ticks in East Africa and molecular taxonomy of Afro-tropical ticks, tick ecology,

and disease dynamics at the wildlife and livestock interface. ILRI developed maps of tick distribution and models that explained the spread and the subsequent disappearance of an especially problematic tick species after its introduction to Zimbabwe.

The Tick Unit has adapted protocols for detecting and quantifying chemical resistance in ticks to backstop similar efforts by regional veterinary services. Pen and field trials of new acaricide formulations are being tested as alternatives to existing compounds to mitigate acaricide resistance in tick vectors of veterinary importance.

ILRI has played a significant role in the international effort to apply a range of molecular technologies to improve the scientific understanding of ticks and future means of their control. As genomic technologies emerged globally, ILRI's scientists played a valuable part in sequencing tick genomes as they did for ECF. Significant research was conducted on anti-tick vaccine evaluation and antigen identification, vector genomics and vector–pathogen interactions. The involvement in the identification of novel tick antigens continues.

According to Altmetric (www.altmetric.com/; accessed 24 February 2020), ILRI contributed to 2% of the research outputs on ticks.

Development impacts

The most visible development achievement is an ECF vaccine, which is covered in Chapter 6 (this volume). The Tick Unit and the tick research have been essential components of this major commitment by ILRI. The organization has played a key role in the development of a recombinant ECF vaccine and an important part in the development of the infection-and-treatment regime, which has been shown to have a significant impact in Tanzania. A significant and practical outcome of the Tick Unit and ILRI's research includes sharing knowledge and advice with the Centre for Ticks and Tick-Borne Disease (CTTBD), an animal vaccine production facility in Malawi.

Studies of heartwater, an important tick-borne disease affecting small ruminants, generated evidence on economic impacts and a potential market for a vaccine.

ILRI developed recommendations on tick and tick-borne disease control and acaricide usage for farmers but since uptake of this information is indirect, via extension services, its final impact is not known.

Policy influence and advice

ILRI generated evidence on the adverse consequences of stopping state-supported tick control in Zimbabwe and advised on better control approaches. A consultancy to the Director of Veterinary Services in Zimbabwe recommended tick control strategies at a critical time for livestock production in that country.

Capacity development and partnerships

ILRI has built capacity in tick research and control for students, extension workers and farmers. Supported by the Wellcome Trust, ILRI and government officials set up meetings between farmers and researchers, to identify problems associated with ticks and tick-borne disease, including tick sampling with farmers. In addition, tick scientists from Sudan, Kenya, Malawi and Ghana have come to ILRI to be trained on tick dissections and tick management. To strengthen future research capacity, the institute has trained more than 20 PhD and MSc students and Fellows. The close link on the ILRI campus between advanced molecular sciences and practical tick culture and control have often been a key competitive advantage.

National and international partners have included the Wellcome Trust, the UK Biotechnology and Biological Sciences Research Council (BBSRC), the Brazilian Federal Agency for Support and Evaluation of Graduate Education (CAPES), Genesis Labs, the University of Bern and the Directorate of Veterinary Services in Kenya.

Introduction

From the early days of ILRI, the impact of African tick species on animal productivity, the complexity of their control in the field and their activity as vectors of diseases such as ECF, babesiosis, anaplasmosis and heartwater have all engaged ILRI's scientists. Supporting these practical issues has been research into fundamental

aspects of tick biology. The aggregate contribution of ILRI's scientists to tick research over the decades has been considerable. However, from the beginning, the major focus of animal health research in ILRI has been on a small number of tightly defined areas such as vaccination against ECF and trypanosomiasis. Perhaps for that reason, ILRI has typically lacked a single, focused tick programme, and its engagement in tick research has often seemed to be ambivalent. Increasing this ambivalence may have been a reluctance to intrude where other African institutions could claim involvement. An additional consideration may have been the fact that tick control depended largely on either the use of indigenous livestock by smallholders or the application of chemical acaricides manufactured by global animal health companies. Hence, the role of an institution like ILRI was not so clear.

The lack of a coherent strategy had two broad consequences. First, as the research activities that were undertaken reflected either the specific needs of other projects or a scientist's personal interest, ILRI's contributions to tick research cover an extremely diverse set of topics. In contrast with other, more focused areas of activity within ILRI, it is therefore harder to point to major, quantifiable impacts. This is the downside of the somewhat piecemeal approach ILRI took to tick research. On the upside, as the contributions were mostly a result of individual initiatives, they were typically dependent on external collaborations. They therefore constitute an excellent example of ILRI's capacity to engage productively in multi-organizational projects. The number of collaborating institutions involved in ILRI's published tick research is large. In what follows, the focus is on areas where, subjectively assessed, ILRI's contributions have been major or at least essential to the final result.

In reviewing this diverse effort, it is convenient to break it generally into two components. The first is work that has been largely field based, focusing on the practicalities of tick control. The second is work that has been more laboratory based. Significant contributions have been made to practical tick control by quantifying the impact of ticks, in understanding the dynamics of tick populations on a local and landscape scale, and in providing input to the effective use of pesticides (acaricides), including the economic cost and benefit of their application. The more laboratory-based research has focused on developing research capacity through the ILRI Tick Unit and the acquisition of techniques such as *in vitro* tick feeding and genomic technologies. In turn, this has led to application in the development of vaccines for ECF, an understanding of the genetic diversity of the major ECF vector species, *Rhipicephalus appendiculatus*, and progress in the development of anti-tick vaccines. Each of these areas will be addressed in turn.

A note on nomenclature: *Boophilus* ticks were long classified as a genus and in all of the literature pre-2001 are cited as such. Approximately 15 years ago, the suggestion was made, on the basis of molecular evidence, that the genus should be considered a subgenus of *Rhipicephalus* (Barker and Murrell, 2004). The coherence of the genus *Rhipicephalus* as it stands has also been questioned. Hence, in the literature a species may be described as *Rhipicephalus decoloratus*, *Rhipicephalus* (*Boophilus*) *decoloratus* or *Boophilus decoloratus*. In what follows, to cover all possibilities, the *Boophilus* ticks will be referred to as *Rhipicephalus* (*Boophilus*) spp. or *R.* (*Boophilus*) spp.

Practical Tick Control

The impact of ticks as vectors of disease has been quantified by groups working on these diseases, principally, in the case of ILRI, ECF (Minjauw and McLeod, 2003). The direct effect of ticks themselves has been less examined, although it is a major concern in countries such as Australia and much of Central and South America. There were, however, several early attempts to quantify production losses due to ticks. De Castro *et al.* (1985a) used Boran cattle immunized against *Theileria* spp. to examine the effects of acaricide treatment. Five tick species were found to infect the cattle, although in smaller numbers on acaricide-treated animals, which also showed higher live weight gains. Overall, however, the conclusion was that it was possible to keep Zebu cattle without chemical tick control, once immunized against *Theileria* spp. Estimates of the direct impact of *R. appendiculatus* on productivity were low. In another study published in 1985, only relatively small and transient effects were found from the infestation of cattle with up to 400 *R. appendiculatus* a week for 24 weeks.

There was some suggestion of acquisition of immunity to the ticks (de Castro *et al.*, 1985b). Morzaria *et al.* (1988) returned to the question, finding that, for cattle vaccinated against ECF, acaricide treatment improved weight gains, while keeping unvaccinated cattle without acaricide treatment was simply uneconomical.

In Africa, as throughout the rest of the world, if tick control is consciously applied, it is most likely to be through the application of synthetic acaricides. The scientific challenge is to identify the optimal acaricide treatment regime, one that balances the negatives of cost and potential environmental and health impacts with the positives of the control of tick-borne disease and the minimization of production losses through the direct effects of ticks themselves. Within this question lies an extremely complex set of issues. Are the animals to be treated – usually cattle – indigenous or exotic breeds? Which are the most important tick species and what is their distribution, both spatially and temporally? Which acaricides are available and what is their efficacy? The choice of acaricide may be affected by issues such as the cost and the occurrence of acaricide resistance. 'Acaricides' will include not only commercial products of certified quality but also market-derived treatments of uncertain origin, quality and efficacy. This, in turn, is affected by national and regional registration procedures, which may be rigorous or disturbingly lax. All these issues may be regarded as essentially scientific or technical. The final set of complexities relate to infrastructure and human behaviour: the availability of dips or other treatment facilities, the distance between the farmer and the treatment facility, and the fact that acaricide application, regardless of the efficacy of the chemical itself, is frequently done so poorly that treatment is ineffective.

An event in recent African history provides a good example of the benefits and risks of tick control, especially in the absence of a scientifically sound and enforceable policy. Andy Norval, who was head of the ILRI Tick Unit from 1987 to 1989, had in earlier work noted that the most common tick in Zimbabwe in overgrazed tribal areas was *R.* (*Boophilus*) *decoloratus*, while in well-managed commercial farms, the diversity of species was greater but the economically most important one was *R. appendiculatus*. Until the early 1970s, ticks and tick-borne diseases were controlled efficiently through a programme of intensive dipping. The low incidence of tick-borne disease led indirectly to overgrazing in communal areas. Then, between 1973 and 1978, a weakening of the dipping programme was followed, typically after 1–3 years, by increases in the population of *R.* (*Boophilus*) *decoloratus* and hence outbreaks of babesiosis and anaplasmosis. Cattle numbers plummeted; then, with reduced animal numbers, grazing pressure reduced, grass cover increased and *R. appendiculatus* and *Amblyomma hebraeum* re-established in these areas. This, in turn, was followed by outbreaks of theileriosis and heartwater (Norval, 1979; Lawrence *et al.*, 1980). These disease outbreaks were attributed to a loss of immunity to the tick-borne diseases, i.e. a loss of endemic stability because of a long-term lack of exposure to ticks and the diseases they transmit. Subsequently, serological surveys in 1981–1982 suggested that endemic stability was being regained in cattle on communal land, and the suggestion was made not to reintroduce intensive dipping (Norval *et al.*, 1992).

Over the years, ILRI has made a number of contributions to such complex situations. Although there has been little direct involvement in the evaluation of acaricides in straightforward field situations, there are exceptions. For example, in 2003, there was a 1-year longitudinal study of 92 smallholder dairies in Kenya to evaluate the efficacy of deltamethrin in the control of the major tick-borne diseases and trypanosomiasis. Four application regimes were compared – biweekly, monthly and bimonthly treatment and untreated controls – and the conclusion was reached that monthly treatment could reduce the incidence of tick-borne diseases to a statistically significant degree (Muraguri *et al.*, 2003). In 2003 and 2004, the efficacy of cypermethrin on four tick species was investigated in Ethiopia and it was found that application every 3 weeks provided good protection (Mekonnen *et al.*, 2004).

The complex issue of optimal frequency of acaricide treatment in the absence of pesticide resistance has been examined by ILRI and their collaborators in a variety of production settings. The population dynamics of four major tick species on indigenous and cross-bred cattle in a dry or semi-arid area of Uganda were examined for cattle given biweekly, monthly or no acaricide treatment. There were effects of lactation and

year-on-year variation but only on the cattle given biweekly treatment were the overall numbers of ticks reduced significantly (Okello-Onen *et al.*, 1999). In a companion paper published in 1998, the variable costs of acaricides, drugs and labour, and the benefits in live weight gains, were together used to calculate the most economically beneficial treatment frequency for indigenous cattle. The conclusion was that a biweekly dipping strategy did not offer benefits commensurate with costs, while the monthly treatment gave clear economic benefit (Okello-Onen *et al.*, 1998), a conclusion that seemed somewhat at variance with the finding on tick numbers. The authors returned to the question in 2003, again examining the effect of biweekly and monthly dipping on indigenous breeds over a period of almost 3 years. The biweekly dipping improved milk offtake and pre-weaning growth rates (Okello-Onen *et al.*, 2003). As they currently stand, such discrepant results would be difficult to translate into a clear message for farmers.

Scientifically more interesting was a paper derived from an ILRI–Kenya Agricultural Research Institute (KARI) collaboration that addressed an unresolved issue in acaricide usage (Kamidi and Kamidi, 2005). *Boophilus* spp. in particular have a pronounced capability to develop resistance to a range of acaricides. The example of resistance in *Rhipicephalus* (*Boophilus*) *microplus* is astonishing and very well documented. It is known that, with the possible exception of amitraz, resistance once acquired can persist for a very long time, even in the absence of chemical selection pressure. Commonly, the market offers an odd mix of different acaricides that are used by farmers in a fairly random way. On occasion, attempts have been made to regulate a chaotic situation by enforcing the serial use of acaricides with the idea of exhausting one before applying another. An alternative approach would be to use acaricides in a structured rotation, although the benefits of this for tick control have not been established.

Rotation of pesticides from different chemical groups has been used in the management of some crop pests to reduce the probability of the emergence of strong resistance. This has never been systematically studied with acaricides for tick control. There has been one published laboratory-based test of the idea that the emergence of resistance could be delayed by the rotational use of two different acaricides in a controlled way. The results were encouraging (Thullner *et al.*, 2007). However, the work reported by Kamidi and Kamidi (2005) is perhaps the only published example where this has been examined in a field situation. The work described the consequences of three different acaricide treatments on a single smallholder farm with exotic dairy cattle over 7.5 years. Initially, ticks were controlled by weekly spraying with amitraz. Then, when resistance developed after 3 years, this was replaced by fortnightly treatments with an organophosphate, although it was known that resistance to this chemical group occurred in the district. A dramatic increase in tick-borne disease followed. The final strategy was a regime of fortnightly spraying, using amitraz and the organophosphate in rotation. This resulted in the lowest incidence of tick-borne disease of the three strategies.

These observations raise interesting questions. First, it is striking that, although both acaricides were expected to achieve at best partial tick control used separately, the rotation seemed to be effective. Second, the cost of acaricide was, inevitably, very high, although perhaps less than might have been incurred with an increased incidence of tick-borne disease. Finally, of course, the long-term financial and biological sustainability of intensive pesticide treatment in the face of known resistance must be doubtful.

Several publications have attempted to take the issue of ticks from the field to the landscape scale. During the 1990s, the evolving situation in Zimbabwe was a focus, and the approaches included: (i) examination of the field distribution of particular species, often supplemented by ecological and climatic modelling; (ii) studies on current acaricide usage and practices; and (iii) economic analysis of preferred strategies. For example, factors contributing to the spread of *R. appendiculatus* in Zimbabwe after its introduction and its subsequent disappearance were examined using a climate model. It was concluded that the chief causal factor was a wet–dry climate cycle (Norval and Perry, 1990).

On a number of occasions, these concerns evolved into policy advice. The focus on Zimbabwe may have been a result of the dramatic effects of a failure in tick control during the civil unrest. In 1990, Perry and Mukabeni of ILRI, and Norval, who had recently shifted to the University of Florida, together with Barrett, in the

Department of Veterinary and Tsetse Control Services, submitted a report containing recommended tick control strategies to Zimbabwe's Director of Veterinary Services (Perry *et al.*, 1990a). Twenty-five years later, the report still encapsulates many of the typical issues: political imperatives, changing disease epidemiology, the cost of acaricides, the paucity of methods to control tick-borne disease, and so on. To briefly recap this history: prior to the independence war of the 1970s, intensive acaricide treatment had led to the eradication of ECF and the control of other tick-borne diseases. The highly regulated system of treatment was, however, increasingly unpopular. It collapsed during the war, as noted previously, with consequent severe outbreaks of disease and the death of approximately 1 million cattle. Subsequently, there was evidence that endemic stability to these diseases was re-established or in the process of being re-established, and there seemed to be the opportunity to pair satisfactory disease control with reduced use of expensive (state-purchased) acaricides. The catastrophic loss of cattle, however, had reawakened a demand for intensive dipping, which was reintroduced for political reasons. By the time of the ILRI consultancy, the costs were becoming unsustainable.

The policy advice that flowed from this consultancy was complex. It divided communal lands into four categories, based largely on the probable but different impacts of babesiosis, anaplasmosis, heartwater and theileriosis. A reduction in acaricide treatment with a move, where possible, to minimal acaricide application was proposed. The target was to achieve endemic stability to the various diseases, supported by vaccination. Significant cost-saving was envisaged, although the costs of vaccinations were uncertain (Norval *et al.*, 1992).

These ideas were revisited again in 1994. The concern was specifically with heartwater and its vectors, the ticks *A. hebraeum* and *Amblyomma variegatum* (Norval *et al.*, 1994). *A. hebraeum* was then widely distributed in the dry southern Lowveld with some foci in the wetter areas of the Highveld, while *A. variegatum* occurred in the Zambezi Valley and surrounding dry Lowveld areas. The distribution of *A. hebraeum* had changed over the preceding 70 years, while that of *A. variegatum* had remained static. The distribution patterns of both species in Zimbabwe displayed anomalous features: the ticks occurred in areas of lowest predicted climatic suitability for survival and development and in areas where the densities of cattle, the most important domestic host, were lowest. The only factor favouring the survival of the species in the Lowveld habitats in which they occurred was the presence of alternative wildlife hosts for the adult stage. Norval *et al.* (1994) concluded:

> Their absence from more climatically favourable Highveld habitats appears to have been the result of intensive acaricide treatment of cattle over a long period and a historic absence of significant numbers of wildlife hosts. Eradication of *A. hebraeum* and *A. variegatum* by intensive acaricide treatment of cattle can be achieved in the absence of significant numbers of alternative hosts, because of the long attachment and feeding periods of the adults of these tick species. However, eradication becomes impossible when alternative hosts for the adult stage are present, because a pheromone emitted by attached males attracts the unfed nymphal and adult stages to infested hosts. The unfed ticks are not attracted to uninfested hosts, such as acaricide-treated cattle.

In the face of a probable reduction in intensive acaricide treatments, due to the cost to the government, the authors suggested two potential alternative strategies: to establish a buffer zone to restrict disease spread, or to allow the ticks to spread and to control heartwater by immunization.

By 1998, there was evidence that the heartwater vectors were, in fact, spreading (Peter *et al.*, 1998). A study reporting the results of a survey in 1995–1996 (Perry *et al.*, 1998) returned to the question of *A. hebraeum*, *A. variegatum* and heartwater. The dynamics of tick control were changing. The government, under financial pressure, had abandoned intensive dipping, and wildlife species were moving back into the Highveld. Suggestions were made on how to deal with the increased disease risk. The overuse of acaricides remained a concern. It was noted that intensive acaricide treatment (i.e. more than 30 times a year) was associated with a higher risk of heartwater than on farms using more strategic dipping.

Given the very strong focus in ILRI on ECF, the distribution of *R. appendiculatus* was a long-standing interest. The example of the spread of *R. appendiculatus* in Zimbabwe is given above. A broader consideration of the distribution of

R. appendiculatus in Africa based on climate and vegetation was published by Perry *et al.* (1990b). The starting point in this research was a predictive model of tick distribution based on climatic factors. The model was originally developed by the Commonwealth Scientific, Industrial and Research Organisation (CSIRO) to better understand the distribution of *R.* (*Boophilus*) *microplus* in Australia (Sutherst, 2003). Here, it was to be applied to *R. appendiculatus*. The authors found that the ecoclimatic indices of suitability correlated well with known tick distributions in eastern Africa but were insufficient as a single-factor explanation in central and southern Africa. Cold stress and vegetation microenvironments were also considered to be important. Factors such as cattle and wildlife distributions and acaricide control (if any) were hypothesized to be relevant, although the data to test that hypothesis were insufficient. Predictably, too, this focus on tick distribution and climatic factors was combined into an attempt to understand the epidemiology of *Theileria parva* using additional information from geographic information systems (Lessard *et al.*, 1990).

Development of Research Capacity

A mix of fundamental methodology, facilities, equipment and hands-on experimental expertise underpins all scientific research. ILRI played a significant role in developing such scientific capacity in at least two areas of tick research. The first was through the establishment of a Tick Unit for the development and maintenance of tick colonies together with the acquisition of techniques needed for tick and tick-borne disease research. The second was via the involvement of ILRI staff in tick genomics and other molecular technologies.

The ILRI Tick Unit

The ILRI Tick Unit was built around experimentally valuable infrastructure: fly- and tick-proof animal isolation rooms (pens) with a capacity for 16 cattle, tick culture and incubation rooms, small-animal facilities and associated laboratory space. More important were and are the biological resources. Currently, the ILRI Tick Unit has in culture representatives of three genera of ticks, including the subgenus of *Boophilus*. These genera are *Rhipicephalus*, *Rhipicephalus* (*Boophilus*), *Hyalomma* and *Amblyomma*. The species in culture are *R. appendiculatus*, *Rhipicephalus zambeziensis*, *Rhipicephalus evertsi*, *A. variegatum*, *R.* (*Boophilus*) *decoloratus*, *R.* (*Boophilus*) *microplus* and *Hyalomma anatolicum*. The diversity of *R. appendiculatus* cultures is a focus of the collection, with seven different lines from eastern and southern Africa, including selected lines derived from the Kiambu stock that differ in susceptibility to *T. parva* infection and lines from South and eastern Africa selected for experiments on the impact of diapause on disease transmission, as well as geographically diverse isolates, including two from Zimbabwe and three from Zambia. The expertise of the ILRI Tick Unit currently is in tick culture, performance of animal trials under controlled conditions and the process of producing sporozoites for ECF projects. These skills continue to underpin basic research into *T. parva* (e.g. Henson *et al.*, 2012).

Ticks share with many other parasites the irritating characteristic that, while they are exceedingly difficult to kill in the wild, they are often very difficult to maintain under controlled conditions. Hence, it is an achievement that of the *R. appendiculatus* stocks, four have been maintained for over 30 years and six for more than 20 years. Two of the other species have been in culture for over 30 years and one for more than 20 years. Admirable though this is, it is also a cause for concern. Experience with *R.* (*Boophilus*) *microplus* in Australia showed that a much-used reference tick colony, maintained for even longer, showed declining viability and then sudden collapse.

Artificial feeding systems

To understand and, if necessary, dissect the process of tick feeding on a mammalian host (cattle) and thus acquiring a disease organism such as *T. parva*, it would be experimentally useful to be able to feed ticks in a less physiologically and biologically complex situation than on the natural host. For *R. appendiculatus*, this problem was tackled in the 1990s. Slightly earlier work at

ILRI with *A. variegatum* showed that it was possible to feed the ticks on rabbit or cattle skin membranes, with high carbon dioxide concentrations and a temperature of 37°C being important for success. Adult female and male ticks were fed to engorgement over a period of up to 16 days. All stages of the tick fed successfully and although engorgement weights were less than on natural hosts, egg laying was successful. Ticks fed *in vitro* successfully transmitted *Theileria mutans* and *Ehrlichia ruminantium* to cattle (Voigt *et al.*, 1993).

For a number of reasons, use of an artificial membrane rather than animal skin is desirable, but success with such membranes is easier to achieve for ticks with long mouth parts than for ticks with short ones, such as *R. appendiculatus* (Young *et al.*, 1996a). Nevertheless, in 1995, successful feeding of nymphal *R. appendiculatus* on an artificial membrane was described. When the system was used to feed nymphal ticks on blood infected with *T. parva* piroplasms, the prevalence of infection was high and comparable with results achieved with ticks feeding on blood donor cattle (Waladde *et al.*, 1995). The status of such feeding systems was reviewed (Waladde *et al.*, 1996; Young *et al.*, 1996a).

Genomics and molecular biology

By the middle of the first decade of the 21st century, the biological revolution precipitated by full-genome sequencing of many organisms was well under way. Just as important as the genome sequences themselves were the new experimental approaches and techniques that evolved, drawing on the enormous quantities of new information. For ticks, the start was slower than for many other organisms, including a variety of other parasites and disease organisms. The lower priority attached to veterinary diseases, particularly of developing economies, and the smaller size of the research community were probably two reasons for this. More significant, however, was the sheer size of the tick genomes. Depending on species, size estimates varied from one-third that of the human genome to more than twice the size. The first full tick genome sequencing project to be launched was for one of the smaller genomes, that of the North American tick, *Ixodes scapularis* (Hill and Wikel, 2005), which was of only indirect relevance to African issues. By 2016, this first tick genome had been thoroughly explored and now undoubtedly represents an important resource for ongoing research into all tick species (Gulia-Nuss *et al.*, 2016). By 2006, high in the priority list for tick genomes was *R.* (*Boophilus*) *microplus* because of its economic importance. In this case, the size of the genome, considerably larger than the human genome, was daunting (Ullmann *et al.*, 2005; Guerrero *et al.*, 2006). Nevertheless, by 2010, considerable advances had been made in assembling sections of the genome. In this international effort, ILRI's scientists played a valuable part (Guerrero *et al.*, 2010).

In the specific area of genome sequencing, African tick species lagged behind. Nevertheless, large quantities of interesting sequence data for African species were being accumulated. The focus usually was on the salivary gland, a logical choice. The pharmacologically and physiologically active factors necessary for maintaining the tick's attachment site pass through the salivary gland. The salivary gland is also the route of transmission of tick-transmitted diseases as well as often being a site for their development. In 2002, a complementary DNA (cDNA) library was generated from the salivary gland of feeding *A. variegatum* females. Sequencing of random clones from the library gave more than 2000 non-redundant sequences, 39% of which could be tentatively identified with known proteins based on sequence similarities. Abundant families of cement proteins, anti-haemostatics and also possible anti-inflammatories were identified, all proteins expected to be important to the success of tick feeding (Nene *et al.*, 2002).

Somewhat more targeted approaches were adopted in two subsequent studies. In 2004, cDNA libraries were prepared from the salivary glands of *R. appendiculatus* infected with *T. parva* and from uninfected salivary glands. Over 9000 sequences were collected from each sample, with the intention of identifying genes specifically up- or downregulated as a result of the *T. parva* infection. No major differences between the abundantly expressed genes in the two samples were found, although the sequences themselves represent a repository of useful information (Nene *et al.*, 2004).

Secreted proteins are often identified by a signal sequence, a relatively short peptide sequence necessary for their export from cells. A novel method using a 'signal sequence trap' was used to identify possible secreted proteins from both *R. appendiculatus* and *A. variegatum*. Given the already extensive databases of known proteins existing in 2005, it was interesting that of 61 *R. appendiculatus* sequences, only 15 could be tentatively identified. For *A. variegatum*, the proportion was just one out of seven (Lambson *et al.*, 2005). This underlines, if such emphasis was needed, the paucity of fundamental information about tick genes and proteins.

The reason for the sheer size of the tick genomes continues to be of interest. A recent ILRI shed some light on this question. Relatively small segments of high-molecular-weight DNA from *R. appendiculatus* were shown to have large numbers of degenerate transposable elements. By extrapolation, the authors suggested that there could be about 65,000 copies of a single family of these repetitive elements; in basic terms, this means that the size of the tick genomes could be, in part, due to the accumulation of such elements of unknown (if any) function over evolutionary time spans (Sunter *et al.*, 2008).

A recent and rather different 'molecular' study offers a new solution to an old problem. This chapter has already described historical changes in tick distribution that affect the occurrence of tick-borne disease. These are not isolated instances. Rather, the speed, scale and potential impact of such changes in tick distribution appear to have increased, while climate change, animal movements, trade and the multiplicity of associated factors will probably ensure that such changes continue.

Coping with changes in tick distributions demands reliable knowledge of the identity and distribution of ticks. This requires the collection of large numbers of ticks across broad areas and then the accurate identification of species. This has previously been done either morphologically or using DNA-based analysis or a combination of both. The former requires a trained scientist to distinguish closely related species. DNA-based methods tend to demand expensive equipment and reagents, time and expertise. Rothen *et al.* (2016) described an alternative, the use of matrix-assisted laser desorption/ionization (MALDI-TOF) mass spectrometry. The essential piece of scientific equipment needed for such analyses is complex and expensive, while the skill needed to operate it is considerable. The intuitive response to the idea of using MALDI-TOF to identify ticks could well be that the idea is scientifically interesting but impractical. However, preparation of samples for MALDI-TOF analysis is simple and cheap, the instruments are high throughput and analyses are commonly carried out in a specialized, central facility (in the case of this study in Japan). These factors together have the potential to change the feasibility of using this technology. As reported, a collection of 398 African ticks of the genera *Rhipicephalus*, *Rhipicephalus* (*Boophilus*), *Hyalomma* and *Amblyomma*, in total ten species, were identified morphologically and by cytochrome C oxidase DNA sequencing. Of these, 48 individual ticks were then used to construct a reference database of mass spectra. Subsequently, the remaining ticks were identified by mass spectrometry using this database. Overall, a sensitivity of 96.1% and a specificity of 99.7% were achieved. The potential usefulness of this methodology for field surveys is considerable.

Application of Research Capacity

Ticks as vectors, with a focus on *R. appendiculatus* as the vector of *T. parva*

Ticks and their biology are inextricably linked with the epidemiology, impact and control of tick-borne diseases. The special complex of *R. appendiculatus* and *T. parva* (i.e. ECF) has been central to ILRI's animal health research from the institute's beginning.

ECF is the subject of a major chapter in this book (Chapter 6). Given that, this section will deal only with a few specific questions where the dominant research issue concerned the biology of the tick vector rather than that of *T. parva*. Some aspects have been described already. Disease epidemiology depends on knowledge of the incidence and distribution of the tick vectors; ECF control in most areas relies heavily on tick control via the use of acaricides. ILRI's contributions to both these areas have been discussed.

A major practical achievement by ILRI has been its contribution to the development of an infection-and-treatment method (ITM) vaccine for ECF. The ability to produce such a vaccine depends on quality-controlled tick colonies and the technical ability to perform all stages of vaccine production (Patel *et al.*, 2016). ILRI remains a resource of knowledge and hands-on expertise for this vaccine production.

As with many other vector-borne diseases, in the case of ECF, the tick is not merely a biological syringe. Rather, it is the site of complex developmental changes in the *T. parva* organism involving a range of specific and poorly understood interactions between the two species. A number of papers in the early- to mid-1980s addressed various aspects of the tick–*T. parva* interaction. Some were practical. Irvin *et al.* (1981) established a rapid method for staining salivary glands of *R. appendiculatus* infected with *T. parva*, and then used it to follow the dynamics of infection and parasite maturation. Later, a method of transplanting *T. parva* kinetes to establish infection in a single tick salivary gland acinus was described, a method that the authors suggested could be applied to the development of cloned parasites (Fujisaki *et al.*, 1988). Other research was more fundamental. The site of *T. parva* developmental changes, the type III acinus in the tick salivary gland, was first examined (Fawcett *et al.*, 1981) before the ultrastructure of the sporogony of the parasite in the salivary gland was investigated (Fawcett *et al.*, 1982). Then, in 1993, it was shown that both a crude extract of tick salivary gland and interleukin-2 as a single, pure cytokine could enhance the susceptibility of bovine lymphocytes to infection by *T. parva* sporozoites (Shaw *et al.*, 1993). In a general sense, this ability of salivary gland material from the tick vector to facilitate infection was subsequently rediscovered for a number of tick-transmitted viral diseases (Nuttall *et al.*, 2008).

The fact that vector competence of *R. appendiculatus* varies with tick isolate and that these differences are heritable has been examined several times. In 1995, estimates of heritability were obtained for *T. parva* infecting two tick stocks, Kiambu and Muguga (Young *et al.*, 1995), and the point was made that the heritabilities were high enough to allow selection of tick strains for high and low susceptibility to infection. This was, in fact, done, as noted in the discussion of the tick stocks of the ILRI Tick Unit. Subsequently, the competence of seven different stocks of *R. appendiculatus* and *R. zambeziensis* as vectors of two different stocks of *T. parva* was examined (Ochanda *et al.*, 1998). Reproducible differences were found.

In a slightly earlier paper with significance for ECF epidemiology, the transmission of *T. parva* by nymphal and adult *R. appendiculatus* was examined using two isolates of *T. parva*. With both, infection levels were much higher in adult than nymphal ticks, a contributory factor being the large difference in the number of requisite structures, type III acini, in the salivary glands of the different tick instars (Ochanda *et al.*, 1996). The authors suggested that transmission by nymphal ticks, representing a lower challenge dose of *T. parva*, might cause milder infections and lead to immunity in the cattle host rather than death, a factor in the establishment of endemic stability. Field evidence from Zimbabwe was consistent with this hypothesis.

Then, in 2009, there was a re-examination of the differences between ticks using the same Muguga and Kiambu isolates, although this time the differences were examined by the modern techniques of quantitative and nested polymerase chain reaction (PCR) (Odongo *et al.*, 2009). These techniques were shown to be more sensitive than traditional microscopy, and it was confirmed that, for the Kiambu isolate, a higher proportion of ticks became infected and parasite numbers within adult salivary glands were also higher. It is interesting that these differences between tick isolates persisted through an additional 20 years of continuous tick culture. These observations would make possible the examination at the level of gene expression of factors that might explain the differences in *T. parva* susceptibility.

Finally, an older paper exemplifies the use that can be made of large databases in understanding disease. Much information had been recorded on the transmission of the Muguga stabilate of *T. parva* to Boran cattle using two isolates of *R. appendiculatus*. Between 1986 and 1991, 1241 records of tick batches harvested from 286 infected cattle had been compiled, from which a total of 812 records were selected as suitable for analysis. This database was interrogated using statistical modelling procedures searching for relationships between a long list of

factors and the prevalence, abundance and intensity of salivary gland infection in both female and male ticks (Young *et al.*, 1996b). Twenty-four factors or interactions were found to be statistically significant. Some factors might have been intuitively expected; others were more unexpected. Consistently among the most important factors, however, were the piroplasm levels on the day of harvesting ticks and the month in which the ticks were harvested. The latter finding is surprising, although a tentative correlation between time of nymphal engorgement and the climatic conditions (temperature and humidity) at the time was noted. The final models were complex and with significant error margins, with the consequence that they had limited predictive value. There does not appear to have been subsequent follow-up using this statistical approach.

The genetic complexity of *R. appendiculatus*

As described above, it had long been known that strains of *R. appendiculatus* had various capacities to act as vectors of ECF and that this capacity was heritable. This raises the question of the genetic complexity of this tick species and the extent to which it relates, for example, to geographical distribution.

Kanduma *et al.* (2012) utilized the database of expressed genes coding for salivary gland proteins developed by Nene *et al.* (2004) to identify 29 polymorphic markers. These they then used to discriminate among populations of *R. appendiculatus* and among *R. appendiculatus* and four other *Rhipicephalus* spp. using ten field populations and ten laboratory stocks (Kanduma *et al.*, 2012). Distinguishing *R. zambeziensis* from *R. appendiculatus* was difficult, although the other species were satisfactorily separated. Within *R. appendiculatus*, clustering into two populations occurred, which did not, however, correlate with a field/laboratory culture division. The authors proposed two preferred sets of markers, one optimal for distinguishing between species and the second for intraspecies comparisons.

Subsequently, the question of genetic diversity in *R. appendiculatus* populations was addressed again, this time using two mitochondrial genes, 12S ribosomal RNA and cytochrome C oxidase subunit 1, as well as a third gene that, ultimately, was not informative. Analysis of sequence variation in these genes in ticks sampled from different geographical locations, from different hosts and from laboratory and field isolates (the three chief variables examined) identified 28 haplotypes clustering into two haplogroups. These groups could not be defined by geographical origin, host species or the laboratory/field divide. Based on these observations, the authors suggested that two major genetic groups of *R. appendiculatus* existed in Kenya, with possible broader distribution in eastern and southern Africa. It was considered possible that the existence of two genetic groups could have had implications for the spread of the tick and for the transmission dynamics of ECF (Kanduma *et al.*, 2016a).

The authors returned to the issue in a second publication in the same year (Kanduma *et al.*, 2016b). Ticks from ten locations in Kenya were collected, associated with three grazing systems: cattle, cattle and wildlife co-grazing, and wildlife without livestock. Numerous individual ticks from ten closed laboratory colonies plus ticks of five other *Rhipicephalus* species were included in the analysis. On this occasion, the conclusions appeared to be somewhat different. There was a low degree of genetic differentiation in the field samples, and no relationship, as before, between the genetic diversity and either geographical location or host species. The ten laboratory strains, as well as the other species, were strongly differentiated. In addition, some of the laboratory strains were differentiated from current field samples taken from the same geographical point of origin as the original laboratory strain. The key conclusion, in terms of disease transmission and control, was that there was in fact little genetic diversity in the *R. appendiculatus* populations, perhaps as a result of livestock and wildlife movements through the country. This conclusion can also be compared with the results of analysis of the diversity of protein sequences in a single protein, Ra86, which will be discussed in the following section on vaccines. Even more recently, an array of gene markers was used to look at the broader phylogeny of *Rhipicephalus* and *R.* (*Boophilus*) species, with the results demonstrating once again that much remains to be resolved (Kanduma *et al.*, 2019).

Tick vaccines

There has been occasional interest in the development of tick vaccines since the early days of ILRAD, although initially this was as a minor contributor to antigen discovery research initiated elsewhere. Certainly, this was the case with an anti-tick vaccine effort driven by the International Centre of Insect Physiology and Ecology (ICIPE) in Nairobi (Mongi *et al.*, 1986).

From the late 1990s onwards, there was a more consistent interest in the field. Three factors account for this. First, as had long been the case for all parasite vaccines, the ability to express recombinant proteins offered at least a potential pathway to practical application of antigen discovery programmes. This was as true of ticks as it was of ECF. Second, the steady accumulation of tick genomic information, referred to previously, was a useful source of novel information. Third, in 1988 and 1989, an Australian group patented an antigen from the tick *R.* (*Boophilus*) *microplus* that, when expressed as a recombinant protein in a commercially viable way, gave useful protection against field infestations of ticks. Soon two commercial vaccine developments were under way, one in Australia and the second in Cuba, leading to the release in the early 1990s of two vaccines, TickGARD and GAVAC (Willadsen, 2004).

There were several aspects to ILRI's response. The Australian work gave a stimulus to antigen discovery in a number of laboratories and countries. ILRI collaborated, although as a minor partner, in the evaluation of several antigens, for example, work on the recombinant p29 antigen from *Haemaphysalis longicornis* (Mulenga *et al.*, 1999) and other work that remains unpublished. ILRI has also engaged in *de novo* antigen discovery. An early effort led to the identification of a cement protein, RIM36, from *R. appendiculatus* (Bishop *et al.*, 2002). This was shown to be the target of a strong antibody response by cattle naturally exposed to the tick but, apparently, was of little use as a protective antigen. ILRI has contributed to ongoing, basic research on tick proteins (Costa *et al.*, 2017; Seixas *et al.*, 2018), some of which had no obvious connection to vaccine research. Recently and more encouragingly, it was shown that recombinant glutathione *S*-transferase from *H. longicornis* induced 67% protection against *R. appendiculatus*, although it was ineffective against *Rhipicephalus sanguineus* (Sabadin *et al.*, 2017). This is not only positive news for control of the tick of greatest interest to ILRI but also another example of the unpredictability of cross-immunity among tick species, something explored in greater detail by ILRI and other laboratories with respect to the Bm86 antigen.

Rather more effort went into exploring the potential of Bm86 homologues in the control of endemic African tick species. This was (and remains) a tantalizing scientific and practical issue. It was shown early on that the sequence of the Bm86 molecule was quite strongly conserved across tick genera and species, although with variation (Willadsen, 2004). Even within isolates of *R.* (*Boophilus*) *microplus* itself, sequence differences of up to about 5% have been reported. Sequence differences did not, however, translate into differences in protection against tick infestation in any predictable way. The most striking example was *Rhipicephalus* (*Boophilus*) *annulatus*, a species closely related to *R.* (*Boophilus*) *microplus*. Vaccination with recombinant Bm86 typically gave 80–90% protection against *R.* (*Boophilus*) *microplus* on cattle, but effectively total protection against *R.* (*Boophilus*) *annulatus*, protection that was also sustained for a longer period. The reason is still unclear. Practically, however, the obvious question was: what is the protection of Bm86 against *R.* (*Boophilus*) *decoloratus* or *R. appendiculatus*?

First, it was shown by Odongo *et al.* (2007) that antisera to Bm86 reacted with the *R.* (*Boophilus*) *decoloratus* tick gut, the location of the antigen in *R.* (*Boophilus*) *microplus*, and that *R.* (*Boophilus*) *decoloratus* feeding on vaccinated cattle showed vaccination effects similar to those recorded for *R.* (*Boophilus*) *microplus* on TickGARD-vaccinated cattle: reduced tick numbers and reduced overall fertility, measured as total egg production, reductions of 46 and 61%, respectively. Although less than the effects seen with *R.* (*Boophilus*) *microplus*, these results were certainly encouraging. The presence of two variants of Bm86 was shown, Bd86-1 and Bd86-2, with amino acid sequences that were 86% and 85% identical to the original Bm86. Recombinant Bd86-1 reacted strongly with antisera from TickGARD-vaccinated cattle. Two linear peptide epitopes shared between the Bd86 molecules and Bm86 were identified that, it was suggested, could be the basis of a peptide-based vaccine.

Unfortunately, the Bm86 vaccine had no effect on feeding *R. appendiculatus*. The obvious question was whether a sequence difference between the Bm86 antigen used for the vaccination trials and the homologues in *R. appendiculatus*, named Ra86, was sufficient to explain the lack of protection against that tick species. One problem was the presence of at least two variants of Ra86, which themselves showed only 80% amino acid sequence identity, exceptionally divergent for the 'same' protein (Kamau *et al.*, 2011). Evidence was obtained that both variants could be present in a single genome but that when both were present, one of the two would be transcriptionally dominant.

Further research showed the situation to be even more complex. Nineteen Ra86 sequences were obtained from the laboratory Muguga strain of *R. appendiculatus*, defining two alleles differentiated by insertions or deletions (indels) and different in length by 39 amino acids. Then a further 20 sequences of Ra86 were obtained from each of four field sites in central and western Kenya, revealing a further three different size types, differentiated by 39–49 amino acid indels and hence a total of five indel-defined genotypes. The longest sequence was found only in the laboratory strain. Analysis clustered all Ra86 sequences and Bm86 into four major clades based on amino acid substitutions. Although there was evidence that selection contributed to the sequence variation, there was no evidence that the groupings correlated with geographical separation of tick populations (Kamau *et al.*, 2016).

With such diversity – which has not been reported in *R.* (*Boophilus*) *microplus* in Australia or Central and South America – is the idea of vaccination using a Bm86 homologue impractical? Olds *et al.* (2012) vaccinated cattle with a mixture of two recombinant forms of Ra86 and challenged them with Muguga strain ticks. There was no significant effect on adult mortality, engorgement weight or weight of eggs laid, although there was an interesting decrease in egg hatching, which grew more pronounced in late-laid eggs. There was a slight but statistically significant decrease in moulting of nymphs to adults. These figures, incorporated into a tick population model, showed the potential for a useful, although extremely gradual, decline in tick numbers. There was also a slight decline in the infection levels of ticks fed on *T. parva*-infected cattle vaccinated with Ra86 compared with non-Ra86-vaccinated controls. Clearly, a more efficacious vaccine would be necessary, as such a vaccine, even if theoretically useful, would be unattractive to farmers.

A recent study examined the potential of an antigen cocktail to give protection against tick infestation and/or *T. parva* transmission (Olds *et al.*, 2016). The cocktail used antigens selected from the literature: the tick antigens chosen were subolesin, TRP64 (the cement protein from *R. appendiculatus*) and three tick histamine-binding proteins, while from *T. parva*, the sporozoite antigen p67C was selected. All of these antigens had separately shown promise in vaccination trials, although the tick species, host and challenge model all varied. In this trial, cattle were vaccinated, produced good antibody responses and were challenged with the normal Muguga isolate of *R. appendiculatus* and the Muguga 'low-line' ticks that had been infected with *T. parva*. No significant effects on either tick engorgement and fertility or disease transmission were found. There were, however, some differences in the susceptibility of the two isolates of tick used, emphasizing the potential importance of isolate variation in the field. The authors stressed the desirability, in future work, of early assessment of any tick antigen in the natural tick–host relationship.

Another aspect of this deserves to be noted. The idea of antigen cocktails as a means to improve the efficacy of vaccines is frequently touted, not only for tick vaccines but for many other anti-parasite vaccines as well, to the extent that it has become an important but poorly acknowledged rationale for much antigen research. The concept is experimentally testable, but reports of tests are scarce and those of success even scarcer (Willadsen, 2008). The fact that ILRI carried out such a test, and reported failure, is a worthwhile contribution.

The tantalizing dream of tick vaccine research is a vaccine that protects against multiple species. The partial cross-protection induced by Bm86, despite the variability of protection across species, seemed to offer some hope. This was explored further in a study that used a peptide from Bd86 to raise monoclonal antibodies that were found to cross-react with *R.* (*Boophilus*) *microplus*, *R.* (*Boophilus*) *decoloratus*, *R. appendiculatus* and

Hyalomma anatolicum anatolicum (Kopp *et al.*, 2009). The degree to which such cross-reactivity translates into protection is unknown.

Recent Developments

As the 21st century progressed, ILRI's interest in ticks, with the exception of molecular approaches to tick biology and vaccine development, appeared to decline. The result was that pointers to the future, when they came, were from other institutions and scientists. Three issues emerged: the impact of climate change, the spread of *R.* (*Boophilus*) *microplus* through East and southern Africa and its accidental introduction into West Africa, and finally the evidence of significant acaricide resistance in African tick species.

Today, any discussion of the future of disease control is likely to begin with the anticipated impacts of climate change. The idea, supported by a growing body of evidence, that shifts in vector populations will greatly change the epidemiology of many diseases has been well explored. Changes to tick distributions and hence, for example, to ECF are expected (Grace *et al.*, 2015).

ILRI had earlier played a role in understanding the dynamic nature of ticks and tick-borne disease. The strongest indication that this would be an ongoing if not escalating problem came from *R.* (*Boophilus*) *microplus*. In contrast to other tropical and subtropical parts of the world including Australia, Central and South America and large parts of south and eastern Asia, Africa was until recently fortunate in being spared any major impact from this tick species. The situation has changed over the last two decades. From small populations in eastern South Africa (Natal), the tick has spread through much of South Africa, displacing endemic *R.* (*Boophilus*) *decoloratus* (Tønnesen *et al.*, 2004). In Tanzania, tick surveys conducted between 1998 and 2001 were compared with historical (40-year-old) data, with the results showing that, while *R.* (*Boophilus*) *decoloratus* had largely retreated to high-altitude areas in northern and central Tanzania, *R.* (*Boophilus*) *microplus* had invaded all but the driest and coldest parts of the country (Lynen *et al.*, 2008). This seemed not to have happened by 2007 in Rwanda (Bazarusanga *et al.*, 2007).

In a review published in 2006, Estrada-Pena *et al.* (2006) identified only limited confirmed records of *R.* (*Boophilus*) *microplus* in Africa, all in the south-east. West Africa was still considered free of the tick. Then, in 2007, its discovery was reported in Ivory Coast as a result of an accidental introduction (Madder *et al.*, 2007). A second independent introduction was found to have occurred in Benin in about 2005. Since then, it has continued to spread (Madder *et al.*, 2011, 2012; de Clercq *et al.*, 2012), apparently displacing other *Boophilus* spp. as it invades. The tick has now spread throughout Ivory Coast and far into the north of Benin. In November 2012, *R.* (*Boophilus*) *microplus* was discovered as intense field infestations in south-western Burkina Faso and Mali (Adakal *et al.*, 2013). It continues to spread within Burkina Faso. Its spread has been accompanied by decreased milk production, uncontrolled tick populations, inappropriate acaricide use and cattle deaths (Madder *et al.*, 2011; Adakal *et al.*, 2013).

Surveys in Cameroon in 2013 at a number of sites, involving the identification of 20,000 ticks, showed that *R.* (*Boophilus*) *microplus* seemed not to have reached that country. However, there appeared then to be no ecological reason why it would not eventually (and probably rapidly) spread through much of West Africa, south of the Sahel (de Clercq *et al.*, 2013, 2015)[1]. This expectation has regrettably been confirmed. Recent evidence, which has involved several ILRI scientists, has shown that the species is now present in large numbers throughout much of Cameroon, apparently displacing *R.* (*Boophilus*) *decoloratus* as it spreads (Silatsa *et al.*, 2019a,b).

In more detail, *R.* (*Boophilus*) *microplus* has become the dominant tick species on cattle in south-western Burkina Faso and southern Benin. A full year survey at three sites in Benin and three in Burkina Faso showed that the abundance of *R.* (*Boophilus*) *microplus* was over 50% of all ticks collected of all species at five of the six sites. When tick counts were averaged over the full year, the daily numbers of *R.* (*Boophilus*) *microplus* collected were Gogounou (38), Ouangolodougou (113), Farnifaso (164), Okpara (249), Kpinnou (465) and Kimini (710). At Gogounou, in the northern part of Benin, *A. variegatum* was the most abundant species at 26% of the total count, although the relative abundance of *R.* (*Boophilus*) *microplus* was almost identical at 25%, suggesting that *R.* (*Boophilus*) *microplus*

is continuing to expand its range towards drier areas. Ongoing spread along the tropical, wetter near-coastal regions is, of course, almost certain to occur.

No scientific studies have as yet been carried out to measure the impact of such tick numbers on local cattle. However, based on Australian experience, the blood loss from a daily tick count of 100 or above would have significant effects on productivity, and the higher numbers could easily result in mortality, particularly at times of nutritional stress. Anecdotal evidence from farmers confirms this expectation.

The next evolving problem is that of the spread of acaricide resistance. In work led from Obihiro University of Agriculture and Veterinary Medicine in Hokkaido, Japan, and involving a number of collaborating institutions in Uganda, ticks were collected from 30 farms across Uganda, all having a history of acaricide failure (Vudriko *et al.*, 2016). Such reported failures can be due to acaricide resistance, as is usually assumed, but other factors are commonly part of the explanation. The major tick species were *R. appendiculatus* and *R.* (*Boophilus*) *decoloratus*. Acaricide resistance assays showed that 90% of the samples were resistant to synthetic pyrethroids and 60% of the total were highly resistant, i.e. the acaricides had effectively no lethal activity. Resistance was high against a combined organophosphate/synthetic pyrethroid product and was significant against organophosphates and amitraz. Resistance to multiple acaricides was detected in about half of the samples. Over a 2-year period, three-quarters of the farms had used two or more acaricides, and of these, over half had used chemical rotations that made no sense scientifically.

The work is significant for a number of reasons. Most obvious is the worryingly high frequency of resistance and the occurrence of multiple resistance to diverse chemical groups. Second, the study underlines the importance of incorrect acaricide usage and the frequency with which it happens. Third, there is the fact that resistance to acaricides was found in over 40% of the *R. appendiculatus* samples. It was once a common speculation that resistance would evolve slowly, if at all, on multi-host ticks compared with the single-host *Boophilus* spp., with the alternative hosts perhaps providing a chemical-free refuge. Clearly, under the conditions of cattle farming in Uganda, this has not been the case.

The Future

Given the diversity of tick-related problems, where might ILRI best make its future research contributions? Its greatest competitive advantage over most other research providers is the close juxtaposition of facilities for large-animal experimentation and parasite culture with laboratories capable of advanced molecular research. This is more than a huge experimental advantage. In a more indefinable but still important way, it brings together the molecular scientist and the livestock target of the scientist's research. This juxtaposition could be utilized in many ways, but three examples suffice. First, as is clear throughout this chapter, current tick and tick-borne disease control depends heavily on synthetic acaricides. Resistance to these is an increasing problem. No effective resistance management strategy can be developed until resistance can be rapidly and accurately diagnosed, which itself presupposes an understanding of resistance mechanisms. Current methods of diagnosis have not changed significantly in decades, are often slow and are too frequently inaccurate. To do better is a challenging research problem.

The second area where the close association of scientist/laboratory/experimental animal is beneficial is in vaccine research. This is as true of anti-tick vaccines as it is of ECF vaccines. Interest in this approach to tick control for African ticks is developing. Despite ILRI's worthwhile contributions to this area, the institute has always lacked a tick vaccine programme. As described above, the wealth of genomic information and associated experimental techniques together offer abundant new opportunities and approaches (de la Fuente *et al.*, 2016). If the promise of these is yet to be convincingly realized, it is undoubtedly true that the combination of new science, traditional biology and large-animal evaluation is a powerful combination that ILRI is well placed to exploit.

A third possibility is the investigation of the genetics of within-breed and between-breed variation in the ability of livestock, especially

cattle, to become resistant to ticks. This has been a major component of the management of *R.* (*Boophilus*) *microplus* both in Australia and South America. In the African context, questions of fundamental scientific interest and practical importance are unanswered. For example, to what degree are indigenous cattle resistant to ticks? Are there between-animal differences in heritability high enough to be useful? To what degree is the acquisition of resistance effective against multiple tick species? ILRI possesses the scientific skills, the biological resources and, importantly, the infrastructure and field stations to address such questions.

Each of these options would be about developing technologies, at best partial solutions to practical problems. As has been described above, a robust solution to the control of ticks and tick-borne disease requires an understanding of tick distributions and economic impacts; the current and future effects of climate change; and the regulatory system and the production environment in which tick control is to be applied. ILRI has skills in all of these areas and a strong focus on at least one target group, the smallholder livestock farmer. Thus, ILRI has, in principle, not only the potential to develop new technologies but also the infrastructure and experience to facilitate their effective adoption.

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Note

[1] The TickRisk project, led by Maxime Madder and Eva De Clerq and based largely in Benin, and the WecatiC project, led by Hassane Adakal, have provided the above data, mostly collected during 2012–2013.

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