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Ticks and tick-borne diseases in Asia with special emphasis on China

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Abstract An international conference on the control of ticks and tick-borne diseases sponsored by the European Commission was held in Shanghai between 11 and 15 September 2000. Participants from China, Europe, Australia, India, Iran, Israel, Japan and Thailand attended the meeting. The main objectives were: (1) determining the current situation concerning the epidemiology, economics, diagnosis and integrated control measures of ticks and tick-borne diseases caused by protozoan and rickettsial pathogens; (2) the creation of regional and international research networks and priority settings; (3) the creation of training possibilities for young scientists; (4) the identification of new research directions and priority settings in relation to vaccine development and diagnostics. The papers presented during this meeting focused on parasite characterisation and the description of yet unidentified *Theileria* and *Babesia* species pathogenic for large and small ruminants in China. In addition, progress made in immunity and vaccine development against the pathogens mentioned above and their vector ticks was also discussed.

Introduction

The European Commission has approved, within the framework of the International Cooperation with Developing Countries (INCO-DEV) research programmes, funding for two closely related projects. First, the International Consortium on Ticks and Tick-borne Diseases (ICTTD-2), and second, the molecular and immunological characterisation of *Theileria* species in small ruminants in China: Application for the Development of Diagnostics and Vaccine (ADDAV).

One of the first goals of these newly established projects was to organise a China and European Commission International Conference on the Control of Ticks and Ticks-borne Diseases which was held in Shanghai between 11 and 15 September 2000. Participants from China, Europe, Australia, India, Iran, Israel, Japan and Thailand attended the meeting. The objectives were:

1. The establishment of the current situation concerning the epidemiology, economics, diagnosis and integrated control measures of ticks and tick-borne diseases caused by protozoan and rickettsial pathogens.
2. The creation of regional and international research networks and priority settings.
3. The creation of training possibilities for young scientists.
4. The identification of new research directions and priority settings in relation to vaccine development and diagnostics.

In this article we will concentrate on the current situation regarding tick-borne diseases (TBD).

Epidemiology

TBD of small ruminants are widely distributed in China. Yin Hong and his colleagues from the Lanzhou Veterinary Research Institute (Gansu, China) reported on the economic loss of wool and meat production in sheep and goats caused by TBD. To date, 109 tick species have been identified in China. Most of these infest small ruminants and some of them are respon-

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sible for the transmission of several pathogens. *Babesia motasi*, *Babesia ovis*, *Anaplasma ovis* and a *Theileria* species which is highly pathogenic for small ruminants, once thought to be *Theileria hirci* (= *Theileria lestoquardi*), have been recorded in China. Schnittger et al. (Research Center Borstel, Borstel, Germany), in cooperation with Yin Hong, analysed the sequence of the ribosomal small-subunit RNA gene sequence of the Chinese *Theileria* species and showed that this parasite is most closely related to *Theileria buffeli* and is clearly divergent from *T. lestoquardi*. This suggests that it is an as yet undefined *Theileria* species (Schnittger et al. 2002).

The group of Yuan Zhengpu (Animal Husbandry and Veterinary Medicine Institute, Ganan, Tibet) described the epidemiology of ovine theileriosis in the Ganan region of Gansu province, China. Local Tibetan sheep make up 75% of the small ruminants in this region. The main season for sheep theileriosis is spring (March–May) with some disease incidence in autumn. Animals infected with the disease in spring did not become ill again in autumn. The average incidence of infection in sheep was 28% and in goats 13%. The mortality rate was high in young animals, being about 80% for both sheep and goats, while in adults it was 65–70%. Crossbred and local herds have approximately the same disease incidence. The occurrence of this disease is closely related to the seasonal activity of *Haemaphysalis quinhaiensis*.

Parviz Hooshmand-Rad (Razi Vaccine and Serum Research Institute, Tehran, Iran) spoke about sheep theileriosis caused by *T. lestoquardi* in Iran. This is a leukoproliferative disease with high morbidity and mortality. The infection is acute in yearling lambs, with high fever, rapid weight loss, lymph node enlargement, anaemia and jaundice. The subacute form is seen mostly in adult sheep that have had the disease before. They show mild fever of short duration with some lymph node and spleen enlargement. In Iran, it is a seasonal disease of late spring and early summer, corresponding to the highest seasonal activity of the main tick vector. This vector, *Hyalomma anatolicum anatolicum*, is widely distributed, but the disease is limited to certain regions for reasons that are not at present understood.

Anil Nichani (All India Co-ordinated Research Project, Haryana, Hissar, India) reported on the epidemiological situation of tropical theileriosis in India. Young calves of all breeds are highly susceptible to this disease, followed by crossbred and exotic adults. Nichani studied the effect of vaccination with a *Theileria annulata* schizont cell culture vaccine on the epidemiology of tropical theileriosis. During the observation period of two disease seasons, 36.7% of vaccinated and 63.3% of non-vaccinated animals became carriers. Four cases of clinical theileriosis were recorded in the non-vaccinated group, but no clinical disease was observed in the vaccinated animals.

Diagnosis, identification and discrimination

In addition to morphological examination, serological assays such as IFAT and ELISA have been traditionally used for broader, cross-sectional field studies. Using the latex agglutination test, Yao Baoan (Department of Veterinary Medicine, Huazhong Agricultural University, China) and his colleagues demonstrated that an average of 75% of buffaloes from Hubei province in China were infected with *Babesia orientalis* (Bao et al. 2002). The group of Yin Hong has established an effective ELISA based on native antigens for the demonstration of antibodies in serum samples of small ruminants infected with the yet undefined Chinese *Theileria* species (Guo et al. 2002). However, the use of polymerase chain reaction (PCR) in a reversed line blotting (RLB) framework could provide additional epidemiological information, particularly where multi-pathogen infections are likely to occur. Kosum Chansiri (Department of Biochemistry, Faculty of Medicine, Srinakharinwirot University, Bangkok, Thailand ,) spoke on a *Theileria* species which is an indigenous hemoparasite of dairy cattle in the south of Thailand. His group used the thymidylate synthetase gene (TS) for the phylogentic analysis of *Theileria* sp. (type Thung Song), *T. buffeli* and *Theileria sergenti* and found that the latter two parasites are closely related and that *Theileria* type Thung Song is distantly related to them (Chansiri and Nopporn 2002). Pylogentic analysis of all *Theileria* 18S ribosomal RNA genes allowed for a subdivsion into eight clusters. Some of these clusters contain multiple, species, whereas others appear to contain parasites with similar biological properties whose specific status is at present unresolved (Gubbels et al. 2002).

Protective immunity to and pathogenesis of TBD

Evidence was presented for the role of both innate and adaptive immune mechanisms against TBD. While MHC-class I restricted CTL responses have been observed in *Theileria parva*- and *T. annulata*-infected cattle, direct evidence for the existence of CTL activity against other tick-borne pathogens is still missing. There is growing evidence that $CD4^+$ T cells play a crucial role in the development of protective immunity against a number of pathogens such as *Anaplasma marginale*, *Cowdria ruminantium*, *Babesia bovis, Babesia bigemina* as well as *T. annulata* and *T. parva*. IFN-γ produced by $CD4^+$ T cells and natural killer cells activates macrophages for an enhanced phagocytosis and cytokine and nitric oxide production. These molecules either kill or inhibit the growth of the parasites. In addition, memory T-helper cells provide help for the synthesis of opsonising anti-parasite IgG2 antibodies. On the other hand, the overproduction of cytokines, particularly tumour necrosis factor alpha leads to an exaggeration of the

clinical symptoms and pathological reactions associated with TBD (Ahmed 2002).

P. Preston (Institute of Cell, Animal and Population Biology, University of Edinbrugh, Edinburgh, Scotland) and her colleagues reported on results which indicate that lethal infections with *T. annulata* in Friesian calves were accompanied by the generation of suppressor macrophages which inhibited the proliferation of antigen-specifically and non-specifically activated lymphocytes (Preston et al. 2002). Differences in breed resistance to *T. annulata* were associated with differences in the interaction between lymphocytes and macrophages in Friesian and Sahiwal calves. These differences may account for the ability of Sahiwal calves to contain a *T. annulata* infection better than Friesian calves.

During the meeting, it became evident that there is a great gap in our knowledge concerning the immune mechanisms underlying the protection and pathogenesis of TBD in small ruminants. To overcome this problem, collaboration should be established between the groups cooperating within the recently established INCO-DEV research projects and with other groups involved in ovine immunology.

Vaccination

The meeting addressed questions regarding vaccine delivery and novel approaches to vaccination. The most significant point emphasised was that vaccines against intracellular parasites generally need to be formulated so that the antigens are processed through the so called endogenous pathway. This results in antigen presentation in association with MHC class I and stimulates particularly $CD8^+$ T-cell responses. Naked DNA in association with pox viruses in the so-called prime-boost approach was advocated by Roger Hall (Department of Biology, University of York, UK). Particular attention was drawn to the relevance of this strategy for vaccination against *Theileria* species (Hall et al. 2002).

The question of whether the approach of eluting peptides from MHC class I recognised by CTL as a source of detecting target antigens was raised. This approach was thought to be potentially important, but has been tried over the last few years in several laboratories with limited success. This is probably due to the technical complexity of this approach. Another issue raised was the levels of protection which have been induced by recombinant antigens of *T. annulata*. It was acknowledged that while vaccination trials with recombinant antigens have given encouraging results, thus far nothing comparable with the gold standard set by the cell line vaccine has been achieved.

Varda Shkap (Division of Parasitology, Kimron Veterinary Institute, Beit-Dagan, Israel) described the use of in vitro culture technology for *B. bovis* and *B. bigemina* to produce a vaccine (Shkap et al. 2002). Pure stocks of field isolates were attenuated by either passage in cattle, long-term cultivation, or by in vitro cloning. The long-term cultivation of *B. bovis* and *B. bigemina* produced parasite populations with decreased virulence, while in vitro cloning resulted in the selection of an avirulent, but still immunogenic, parasite population which engendered solid immunity against virulent blood challenge. The results presented emphasised the ability to attenuate and produce effective vaccines without passage through cattle as is used for the production of current conventional vaccines.

Wu Jainsan (Animal Quarantine Institute, Ministry of Agriculture, Qingdao, China) obtained encouraging results using exoantigens of cultured *B. bigemina* and *B. bovis* in combination with Quil A, showing the potential of these antigens for the development of a vaccine. J.L. Zhao (Huazhong Agricultural University, Wuhan, China) reported on successful preliminary studies using culture-derived exoantigens to immunise buffaloes against *B. orientalis*.

Parvis Hoshmand-Rad stated that the Iranian *T. lestoquardi* became attenuated and is suitable for vaccine production after 100 passages. The vaccine led to a mild reaction 13–14 days after inoculation and resulted in a solid immunity. About 200,000–300,000 doses of this vaccine are used annually.

Leonhard Schnittger described a gene of about 1,246 bp which was cloned by screening a cDNA library from *T. annulata* using an antiserum raised against a schizont preparation. The gene contains an open reading frame of 948 bp coding for a polypeptide of 315 amino acids and has an unusually long 5′-non-coding sequence of 259 bp. A search for sequence pattern and motifs within the amino acid sequence revealed the existence of three membrane spanning regions implying that the protein is located at the parasite surface membrane. This and other published genes (*T. annulata*: SPAG1, TAM1 and TAM2) were discussed and considered as possible vaccine candidates.

Peter Willadsen (CSIRO Livestock Industries, Queensland, Australia) summarised the current status of the development of vaccines against ticks. The number of demonstrated and potential antigens from several species continues to increase, though few antigens have as yet been shown to be effective as recombinant proteins. For the commercial TickGARD and GAVAC vaccines which use the antigen Bm86, the question of the link between sequence variation of the antigen and vaccine susceptibility remains unresolved. Frans Jongejan (Utrecht University, Utrecht, The Netherlands) briefly presented some results on the cross-protection afforded by the *Boophilus microplus* recombinant vaccine against other tick species. Equivalents of the *B. microplus* antigen Bm86 have been found in several tick species by immunological and molecular biological methods. The *H. a. anatolicum* gene showed 77% homology with Bm86 at the nucleotide level and 60% at the amino acid sequence level. In vaccination trials, there were significant effects on *Boophilus decoloratus*, but not on *Rhipicephalus appendiculatus* or *Amblyomma*

variegatum nymphs or adults. In contrast, there were significant effects on *H. anatolicum* nymphs and a 40% reduction in the mean weight of recovered ticks when immature and adult ticks were both fed on vaccinated animals. The effects with *Hyalomma dromedarii* were even more striking, with a 95% reduction in the numbers of engorging nymphs. Sugimoto and his colleagues (Hokkaido University, Sapporo, Japan) described the results of experiments aiming at the immunisation of rabbits with an immunodominant 29 kDa *Haemaphysalis longicornis* tick salivary gland protein. Significant protection could be induced against this tick. There was a significant reduction in the engorgement weight of adult ticks as well as an increase in the respective mortalities (40% and 56%) for larvae and nymphs which fed on immunised rabbits.

Perspectives

It is clear that there are a number of emerging species of haemoprotozoan parasites in China. The current difficulty appears to be the identification of the individual organisms and their differentiation from known parasites. Before large-scale field studies of the epidemiology of the individual parasites in question can be undertaken, it is essential to be able to accurately diagnose, identify and discriminate these organisms.

In the context of an epidemiological investigation, it is worth considering the use of currently existing technologies (e.g. RLB) to determine whether or not hosts which are infected with these "new" organisms are not simultaneously hosting other species. Although the positive identification of infections with known parasites does not preclude the possibility of mixed infections with newly identified organisms, the demonstration of the absence of such concurrent infections would strongly support the conclusion that these are newly emerging infections and may even be considered essential in further studies including:

1. The determination of the natural history of infection in the host and the corresponding life-cycle of the parasite including the identification of the natural and/or susceptible host species as well as the corresponding vectors.
2. Carrying out well designed transmission studies on appropriate numbers of hosts to determine pathology, predilection sites, etc. As part of these studies it is also essential to consider the biology of the infection in the vector. For example, when transmission studies are being conducted it is worth focusing some attention and resources on the simultaneous examination of the vectors. This could be through relatively simple and inexpensive techniques such as dissection and direct examination in the first instance, progressing to more advanced methods when the methodologies (e.g. PCR) become available.
3. The determination of the geographical distribution of these parasites and their vectors. There may be significant advantages in using RLB, which allows the direct identification of the carrier status for a variety of TBD organisms and may be comparable to ELISA (which is restricted to the identification of specific immune responses to single organisms) in terms of cost, efficiency and the requirement for technical expertise.
4. The simultaneous collection of information on vector numbers and prevalence and on the intensity of infection. Regardless of the diagnostic assay chosen, it is envisioned that such surveys could be conducted through the infrastructure supplied by government veterinary services. However, it is vital that appropriate study design be considered a priori, such that valuable ancillary information can be collected at minimal additional cost (e.g. host species, age, production system, geographical location, and management system).

Currently, the control of TBD in China is based on tick eradication, chemotherapy and in some cases on vaccination with attenuated parasites. Immunoprophylaxis is lacking for the recently identified *Theileria* and *Babesia* species and the development of vaccines must therefore be given a high priority. Until subunit vaccines are available, attenuated parasites should be used for the immunisation of small and large ruminants against TBD.

An essential prerequisite for a molecular vaccine is adequate knowledge of the nature of the protective immune response and the use of appropriate protective parasite proteins with the capacity to activate T cells and to induce opsonising IgG2 antibodies. The selection of such antigens can effectively be achieved if test systems are established. In this context, T-cell lines and clones, as well as specific antibodies, are suitable probes for the identification of putative protective antigens by screening a large number of parasite antigens and their fractions. The encoding genes of the candidate antigens should be cloned and used in DNA vaccination.

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