Tropical theileriosis
Summary

Introduction

1. This note provides a brief summary of the Disease and Product analysis prepared by a DISCONTOOLS group of experts covering Tropical Theileriosis. This summary is limited to the impact of *Theileria annulata* in cattle although other species exist in cattle and small ruminants. They reviewed the current knowledge on the disease, considered the existing disease control tools, identified current gaps in the availability and quality of the control tools and finally determined the research necessary to develop new or improved tools. Full details are available on the web site at http://www.discontools.eu/ and can be downloaded by selecting Disease Database, then the specific disease and highlighting the variables of interest. This is completed by selecting "create a report" which can then be downloaded as either a PDF or Excel spread sheet.

Disease profile

2. *Theileria* spp. are obligate intracellular tick-borne protozoan parasites infecting mammalian hosts. Several *Theileria* spp. species infect cattle; the two most important species are *T. parva* and *T. annulata*. *Theileria parva* causing East Coast fever (ECF) is restricted to sub-Saharan Africa. Tropical theileriosis, by *T. annulata* is present from North Africa (from Mauritania to Sudan and in Ethiopia) and Southern Europe through the Middle East and into Southern Asia. Sporadic cases of *T. annulata* are reported in northern Spain (Atlantic climate zone) where the vector suspected to be *Hyalomma lusitanicum*. *Theileria* sporozoites are transmitted to animals through the saliva of the feeding tick. Ordinarily *T. annulata* sporoblasts only mature into sporozoites and enter the saliva after the tick attaches to a host and a tick must usually be attached for a few days before it becomes infective. Once infected, assuming recovery, the animal becomes a carrier and can be infective to ticks for months or years (11 years and probably more).

3. *T. annulata* can occur in cattle, yaks, water buffalo and camels and is transmitted by ticks of the genus *Hyalomma*. Tropical Theileriosis is more severe in European breeds, with a mortality rate, in non-treated animals of 40 – 90% while the mortality rate in indigenous cattle breeds from endemic areas can be as low as 3%. The clinical signs associated with infection by *T. annulata* are dependent on the acuteness of infection. However, carrier infection, even in *B. taurus* x *B. indicus* crossbreeds may cause substantial economic loss.

Risk

4. There is no current evidence that *T. annulata* is a hazard to humans although *Hyalomma* can attach to humans but *T. annulata* has never been recorded in humans. Human theileriosis has been diagnosed mainly in USA and is caused by *T. microti* being transmitted by ticks of the *Ixodes* genus with mice as reservoir.

5. One of the potential risks is the spread of the disease to other previously uninfected areas due to expansion of tick habitat as a result of climate change. Global warming may influence the geographical distribution of the tick, the tick abundance and the vectorial capacity of the tick which in turn will affect the distribution and incidence of the disease. This could happen in Corsica where *Hyalomma scupense* was reported in 2015. This vector could cause a risk of *T. annulata* introduction and requires further close monitoring. *Hyalomma* ticks are known to occur in drier biotopes compared to most other tick genera, so climate change or global warming might favour *Hyalomma* survival and spread to new areas.

6. The second important impact relates to the severe constraint on the ability to significantly develop or increase production capacity of indigenous stock in the currently affected areas in order to avoid food shortages resulting from human population growth.

Diagnostics

7. Serological tests as IFA, ELISA, PCR assays and lateral flow devices (LFD), together with identification of schizonts in Giemsa-stained smears from blood or lymph node biopsies are used for diagnosis. ELISA's have been successfully developed for the detection of antibodies to *T. annulata* and have shown that they can detect antibodies for a longer period in affected animals.

Unfortunately there are no ELISA commercial kits. The detection of carrier animals remains a challenge as they harbour low parasitaemias that are difficult to detect using parasitological and even molecular methods. These animals do not always carry detectable specific antibodies. With the development of subunit vaccines for the surface/$CD8^+$ T cell antigens it may be possible to develop marker vaccines and thus there would be a requirement to develop diagnostics that can differentiate vaccinated and naturally infected animals (DIVA approach). This should be feasible using recombinant DNA technology.

Vaccines

8. Live vaccines produced by attenuating parasite-infected leukocytes have been used to immunise cattle against *T. annulata*. They are commercialised in Turkey and India (Tayledoll ®, Turkey and Rakshavac–T ®, India). In more recent times subunit vaccine research has been focused on surface antigens (recombinant SPAG-1 and Tams1 surface antigens of *T. annulata* sporozoites and merozoites, respectively). It has been shown that SPAG-1 recombinant protein can act synergistically with live attenuated vaccine to confer increased protection against challenge. Recent work has identified genes expressed within tick stages, providing candidates for transmission blocking approach, similar to that being developed for *Plasmodium.* Such an approach potentially combine with anti-tick control/vaccines is required to prevent carrier infection and associated losses.

9. The main challenge to prevent clinical infection is to stimulate cell-mediated immunity, which is presumed to be achieved using live vaccines. Live vaccines present difficulties in storage and delivery which are the main constraints although quality control and production are also issues. These vaccines are effective against homologous strains, and partially against heterologous strains. New vaccine developments should either focus on storage improvement or developing a completely new vaccination type such as DNA vaccines (recombinant or sub-unit vaccines). Recent studies showed that a sporozoite antigen can provide a synergistic response with an attenuated cell line implying that the cell mediated mechanisms may not be directed against specific antigens. Antigen delivery and adjuvant development are probably required. There is currently a limited market in Europe for vaccines against theileriosis caused by *T. annulata*. Despite identification of immunodominant $CD8^+$ T cell antigen targets, generation of protective immunity *via* recombinant antigen has yet to be achieved.

Pharmaceuticals

10. Chemotherapeutic compounds such as buparvaquone (and in some countries parvaquone) with theilericidal properties have been used but tend not to completely eradicate the infection thus leading to the development of carrier states persisting during several months and even years. Buparvaquone is registered in Turkey but not in EU in spite of its need in Spain and Portugal for instance. Resistance against this drug has been reported from Tunisia and cases have been identified in Turkey. The emergence of documented resistant populations in Tunisia (2010) and Iran (2012) might justify the development of new drugs with the same level of efficiency. It may be possible to develop compounds that could be continuously available in the blood of the cattle by administering *via* the feed for example such that when ticks feed, the sporozoites are neutralised before having time to infect. This would inhibit the expansion of infected cells thereby enhancing control by the immune response. Loss of buparvaquone efficacy combined with breakdown of tick control is a concern.

Knowledge

11. As with many other infections there remain significant areas of uncertainty in the understanding and knowledge about Tropical Theileriosis. These relate to genetics, epidemiology, pathogenesis, immunology, vaccinology and control. There remain many unanswered questions including **i)** Which climate change parameters would promote spread and increase of the *Hyalomma* vector ticks? **ii)** How is vectorial capacity affected by climate change? **iii)** What is the current epidemiological situation of *T. annulata* in the EU? with cases recently reported in Portugal and Spain. Is *T. orientalis* a threat given recent reports of outbreaks in Australia and New Zealand **iv)** What is the prevalence of disease and distribution of competent

vectors within endemic and bordering regions/countries? **v)** What is the risk of introduction of the parasite in new European regions (mainly South Europe).

12. The impact of host cell type on disease pathology is not fully understood but is likely to play an important role, as are host genetic differences in cellular activation and response to infection. The full complexity of how the parasite manipulates its host (mainly the macrophages) to cause disease is not well understood. Research is needed to validate the parasite antigens that have been identified can confer protective immunity and develop optimal delivery routes, to identify the relative importance of different arms of the immune response and to assess the ability of the parasite to manipulate the immune response. The molecular mechanisms that control sporozoite production and parasite stage differentiation are not fully understood but blocking this event or production of piroplasm-infected erythrocytes would effectively block the potential to infect animals. The potential to block tick-transmission and prevent infection should be investigated.

Conclusions

13. Some 240 million cattle over a region extending from the Mediterranean basin through the Middle East and across South Asia are at risk of infection. Livestock are a key resource for the production of milk, meat, labour, leather and manure as well as generating daily cash for resource-limited farmers in the tropical and sub-tropical regions of the world. It should not be overlooked that indigenous cattle from these regions have a much lower risk of mortality due to *T. annulata* and that even cross breeds are more susceptible to loss of condition from infection. Studies in Tunisia have indicated that subclinical infection has most impact on both milk yield and meat production. Management level will be instrumental in choosing the appropriate approach to disease control; either low input farming with indigenous cattle, or high input farming using high productive animals combined with effective control. The cost of control may be substantial.