FINAL TECHNICAL REPORT

Research on the incidence, economic importance and causal agent(s) of bovine cerebral theileriosis in semi-arid pastoral livestock systems in Ngorongoro, Monduli and Simanjiro District in Northern Tanzania.

R8022

Executive Summary

Bovine cerebral theileriosis (BCT) has been ranked as the highest disease priority by pastoralist communities in Northern Tanzania and is considered a severe constraint to increased livestock production. The study incorporated a participatory rural appraisal survey carried out from September - November 2001, followed by longitudinal studies in selected study sites, field monitoring and treatment of affected cases and collection of post-mortem samples at slaughter slabs in pastoral areas. Gross pathology and histo-pathology work was carried out in collaboration with Turin University.
The planned outputs of this research programme were to confirm the incidence of the disease; to assess its true social and economic impact; and to record the presence of haemoparasites and other possible disease agents and risk factors. Appropriate control methods were to be developed based on a thorough understanding of the epidemiology of BCT.

During the project lifespan, the extent of the problem (both in socio-economic aspects and geographical occurrence) has been clearly outlined. The disease has been confirmed in the relevant sites in northern Maasai land and spreading of the disease towards the south (Morogoro region) has been confirmed. Some of the possible risk factors, predisposing to the expression of Cerebral Theileriosis, have been identified. However, the researchers have been unable to clearly illustrate the dynamics and epidemiology of the disease due to limitations in the diagnostic tools currently available. On the other hand, the set-up of PCR/ RLB activities in the molecular diagnostics labs at Sokoine University of Agriculture (SUA), Morogoro and at the Veterinary Investigation Centre (VIC), Arusha, will allow continuous monitoring of both the presence (Arusha region) and the spread (Morogoro region) of cerebral theileriosis, and allow rapid response and diagnosis if requested from the field.

Treatments of clinical cases have been successful with overall recovery rate of 69%. However with (at this time) limited confirmation of *T.taurotragi* as causal agent in the respective clinical cases, evaluation of individual drugs or combination therapy remained difficult. However, due to the severity of the problem and high recovery rates observed with the use of 30% oxytetracycline long-acting, the pastoral communities in the project area have already adopted these interim results with regards to treatment strategies.

Dissemination of information concerning BCT to relevant stakeholders was carried out through reports, posters, presentations and meetings with stakeholders. Research results have primarily reached end-users in the project area and beyond. Target institutions for promotion and extension are updated on the current status of the results, but with several critical questions still unanswered, an active role in control-strategy formulation has been postponed pending further molecular work and final analysis of results.

Since the research activities implemented under the AH R8022 Programme were a direct response to a disease prioritisation by the (poor) pastoralist communities, interim results of all aspects under investigation have been closely followed by the direct beneficiaries, i.e.

pastoralists. Discussions on those results as well as on the provisional control options have at least prepared the ground for rapid adoption of control strategies once final analysis of all data has been completed. The long-term impacts of the control strategies can be measured through the monthly disease reports from the high-risk areas, which have been clearly identified.
The project outputs will contribute directly towards LPP and AHP purposes. The research is demand-driven and the project addresses researchable disease constraints on production and associated effects on the livelihoods and security of pastoralists. Key-roles of in-country collaborative institutions and NGOs in pastoral areas will guarantee uptake of the research results.

Background

Bovine cerebral theileriosis (BCT) has been ranked as their highest disease priority by pastoralist communities in Northern Tanzania and is considered a severe constraint to increased livestock production. The demand for BCT research was initiated by Maasai respondents in Kiteto, Ngorongoro and Simanjiro Districts with a supporting letter of the Honourable Minister for Water and Livestock Development (letter of 11th December 2000), signed by all Members of Parliament (MP) of the respective Districts. Additional supportive letters were provided by other pastoralist NGOs (letter of Ereto project, 12 December 2000) which were quoting reports indicating 23% overall mortality in Ngorongoro Conservation Area due to Ormilo.

The planned outputs of this research programme were to confirm the incidence of the disease; to assess its true social and economic impact; and to record the presence of haemoparasites and other possible disease agents and risk factors. Appropriate control methods were to be developed based on a thorough understanding of the epidemiology of BCT. Evaluation of the benefits of controlling BCT would emerge from the integrated analysis of the data, allowing the research priority of this problem to be properly assessed, and provide a framework for further research into the epidemiology and control of BCT. Meanwhile, improved knowledge of BCT and its contributing factors would assist pastoralists in decision making on disease control strategies, reducing their vulnerability to BCT and improve livestock production. Confirmation and or identifications of (all) the causal agent(s) of Ormilo would eventually lead to an appropriate treatment regimen.

The direct beneficiaries were expected to be Pastoralist livestock keepers, owning/managing small numbers of livestock and/or restocking livestock numbers in Ngorongoro, Monduli and Simanjiro District in Northern Tanzania for whom livestock are the most important component of livelihood and food security. Benefits are expected to be long-term. Intermediary institutions working on behalf of the pastoralists will apply the research results and the Ministry for Water and Livestock Development will ensure promotion and application of research results.

Project Purpose

Benefits for poor people generated by application of improved knowledge and management of livestock diseases in semi-arid rangelands

The project was to improve livestock production of pastoralist households in Ngorongoro, Monduli and Simanjiro District through improved knowledge on risk factors, incidence, economic impact, transmission and control of BCT. It was anticipated that short-term benefits would already be seen from increased awareness of BCT and correct therapeutic interventions, resulting in reduced mortalities in young adults, increased availability of trade stock and offtake for slaughter. This would allow poor households greater choice in

marketing animals and milk, leading to increased income generation, and improved human health and welfare.
Increased livestock production should lead to greater security and increased income for pastoralist households. Increased access to best available information will empower pastoralist livestock keepers to make decisions about potential livestock health. At the same time, responding to a demand-driven request with active participation of Maasai livestock keepers in most of the research activities, would also foster improved relations and trust between pastoralist livestock keepers and veterinary support staff.

Research Activities

The Bovine cerebral theileriosis (Ormilo) study started in July 2001 and incorporated a participatory rural appraisal survey carried out from September - November 2001, followed by longitudinal studies in selected study sites, field monitoring and treatment of affected cases and collection of post-mortem samples at slaughter slabs in pastoral areas. The Pathology of the disease was investigated in collaboration with Turin University (Italy), while most of the molecular diagnostic work was carried out at the University of Pretoria, with back stopping of Utrecht University. The planned outputs of this research programme were to confirm the incidence of the disease; to assess its true social and economic impact; and to record the presence of haemoparasites and other possible disease agents and risk factors. Appropriate control methods were to be developed based on a thorough understanding of the epidemiology of BCT.

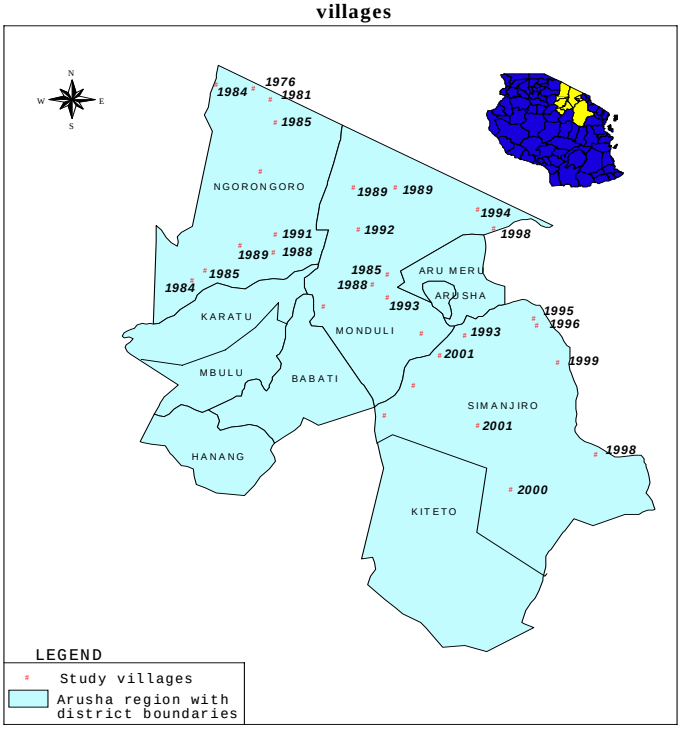
Outputs

1. Economic impact assessment of BCT in pastoral livestock systems in Northern Tanzania (PRA survey)

A 3-months Participatory Rural Appraisal survey was carried out in the pastoral Districts of Arusha region between September and November 2001. The Integrated Tick and tick-borne disease project (ITTBDCP-Tanzania) -Ministry of Water and Livestock Development, in collaboration with the Extension Department of Sokoine University of Agriculture (SUA), conducted the survey. The objective of the survey was to record the history of the problem and establish the extent of the problem, identify critical areas for the planned longitudinal study and record the understanding of the pastoral communities with regards to Ormilo. A full report (65 pp) has been produced and was circulated.

Figure 1. Historical occurrence of Ormilo in Northern Tanzania

Years of first reported Ormilo cases in the surveyed villages



The survey confirmed and highlighted the extent and gravity of the Ormilo disease problem in Northern Maasai land. Maasai respondents clearly identified high-risk and low-risk areas for Ormilo, with a more detailed understanding of the problem in the high-risk villages. Results from PRA and questionnaire data showed that Ormilo is 10 X more likely to occur in *Osupuko* areas (cool, wet highland areas) than *Ilpurkel* areas (dry, arid areas). Ormilo was reported most often in adult cattle over 3 years of age and in both sexes, while temporal patterns suggest an overlap in seasonal distribution of Ormilo cases, rains and ticks. The pastoralists observed significant associations between the presence of ECF and occurrence of Ormilo as well as the presence of certain wildlife species (buffalo, eland, wildebeest) and the occurrence of Ormilo.

Figure 2. Diseases reported by herd owner

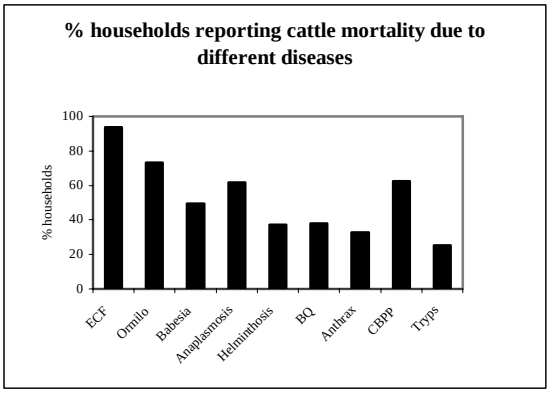
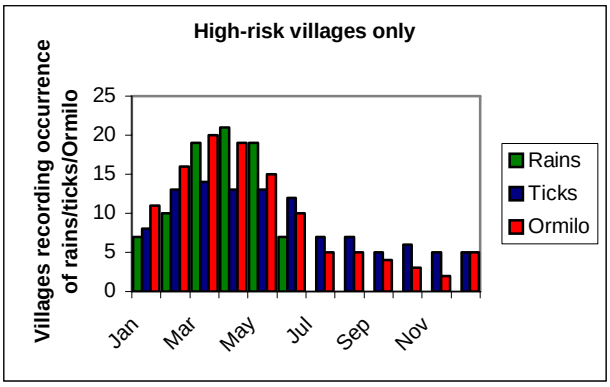


Figure 3. Ormilo seasonality



The survey study clearly outlined the severe negative impact of this disease on livelihood of pastoral households and the failure of control strategies attempted thus far. It underscored the basic understanding of pastoralists on the seasonality and age groups affected, while clearly highlighting their inability to reverse the outcome of clinical cases or cope with the resulting mortalities as the mean case-fatality rate was 80% (SE 3%).

Figure 4. No Ormilo cases/deaths reported

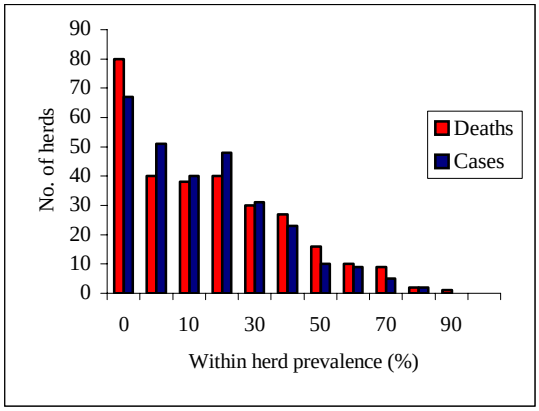
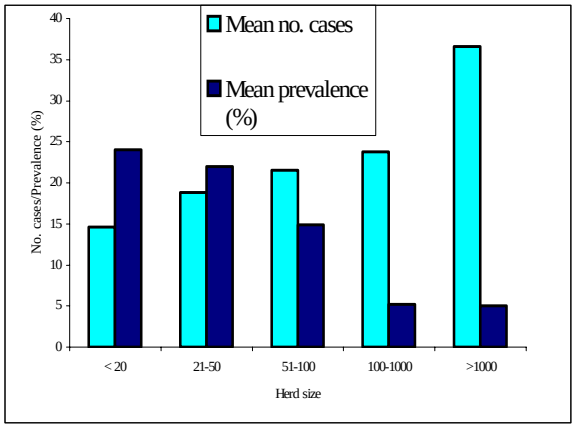


Figure 5. No Ormilo cases by herd size



2. Longitudinal study

A Longitudinal study was carried in 4 sentinel herds in high risk areas (as identified by the PRA survey) and 1 sentinel herd was followed as a control in a non-Ormilo pastoral area between August 2001 and April 2003. The non-Ormilo monitoring herd in Longido was sampled for 11 months after which work was cancelled since the herd owner was reluctant to continue. The sentinel herd in Monduli-juu was abandoned in December 2002 due to poor compliance of the livestock owner, and replaced with a new herd in September-02, which was followed until April 2004. The work in Loliondo was stopped in November 2002 as the herd was moved out of the area because of poor grazing conditions.

The Research Field Officer (FO), Dr Lynen, carried out fortnightly visits to all monitoring sites and data and specimens collected included:

a) Fortnightly tick collections from 5 sentinel animals
b) Monthly sera collection of all 30 sentinel animals for TBD serology
c) Monthly whole blood (citrated) collection for molecular diagnostic work
d) Monthly live weight records
e) Quarterly, faecal sampling and calculation of worm burden
f) Sampling of clinical cases (blood smear, lymph node (LN) smear, whole blood)
g) Collection of post-mortem samples from slaughter slabs (brain, spleen, LN, liver)
h) Treatment and Rx records of clinical cases

Additional community animal health workers (pastoralists) were trained by the FO and provided with the necessary equipment to continue sampling and treatment of clinical cases as well as sample collection from slaughter slabs between visits.

Microscopic examination of all field samples was carried out by the FO, while TBD ELISA was carried out at the Veterinary Investigation Centre (VIC), Arusha. Samples for molecular diagnostic work to identify the causal agent were forwarded both to University of Pretoria (UP), South Africa and Utrecht University, The Netherlands. Database development and data analysis was carried out with assistance of Reading University, UK. Gross pathology and histo- pathology work was carried out in collaboration with Turin university and Italian students joined the field team in Endulen to assist in sample collection.

2.1. Tick and TBD investigations (ITTBDCP-Tanzania)

TBD Serology and molecular diagnostics

TBD serology (ELISA) has shown that all the sites are confirmed as ECF endemic areas with high *T.parva* seroprevalence throughout the year. A slightly lower seroprevalence for *T.parva* is recorded for the Longido site during the dry season (August- November). Seroprevalence however increases immediately with the onset of the rains in Late November, indicating a stronger seasonal occurrence/challenge of East Coast fever compared to the other sites with a year-round challenge.

Identical results were obtained for *B.bigemina* and *A.marginale* while the sites record very low prevalence (either epidemic or free of the disease) of *B.bovis* with the exception of the site in Simanjiro District, which shows an endemic stable situation for *B.bovis*. *E.ruminantium* seroprevalence is low and clearly seasonal.

Figure 6. TBD seroprevalence Endulen (Loliondo) sites

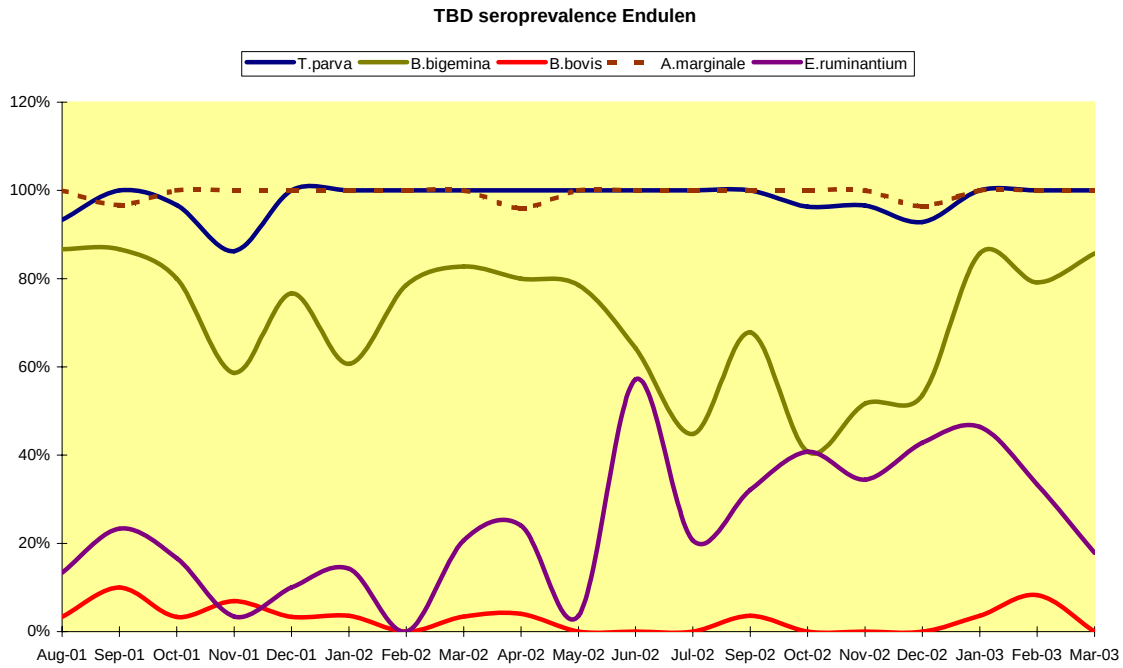


Figure 7. TBD seroprevalence Monduli site

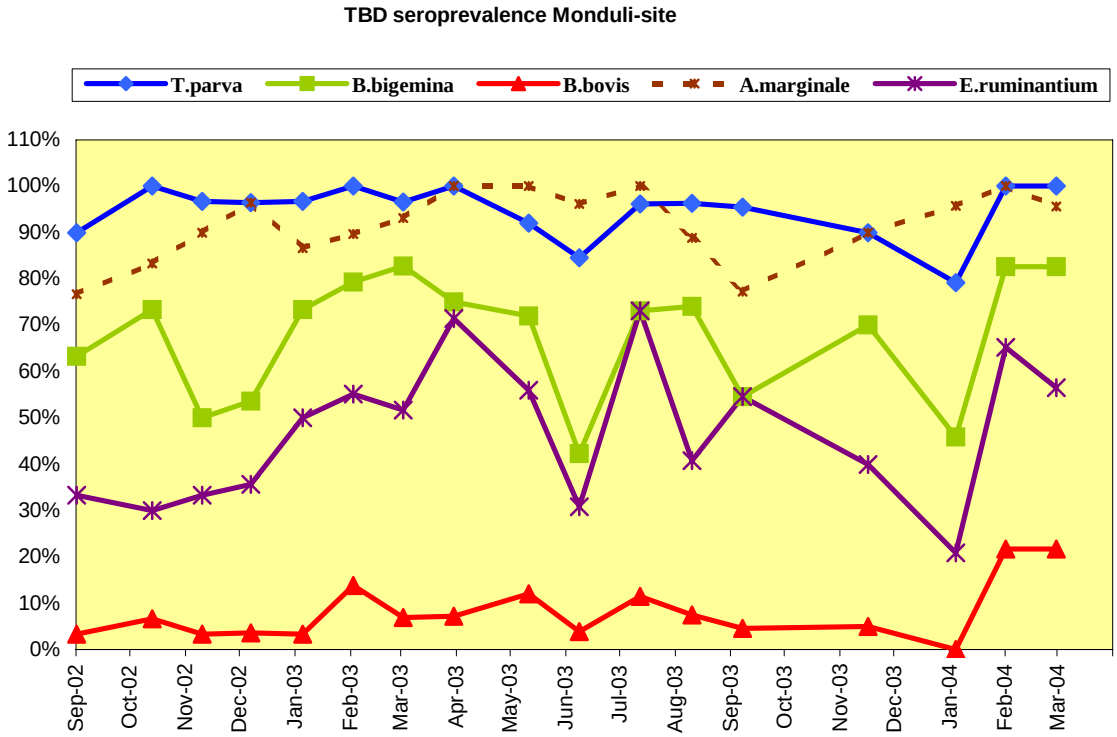


Figure 8. TBD seroprevalence RuvuRemit site

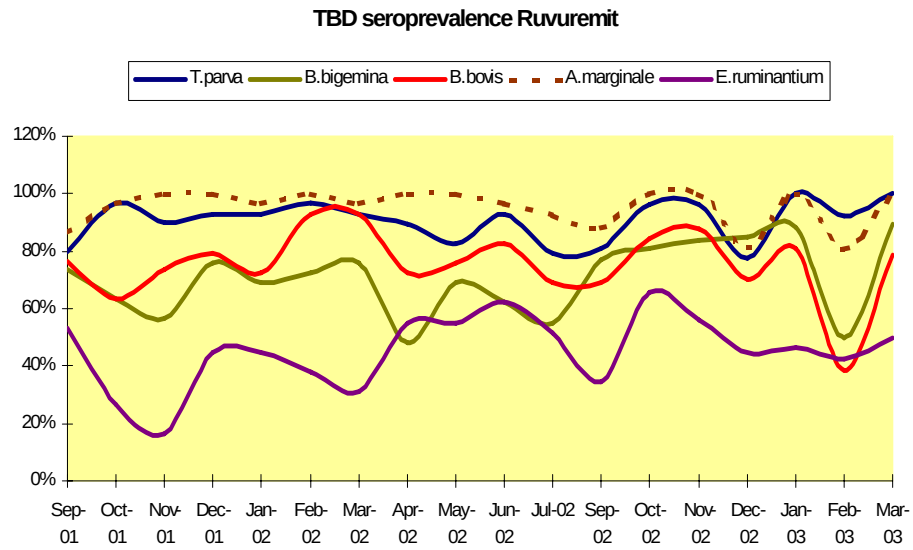
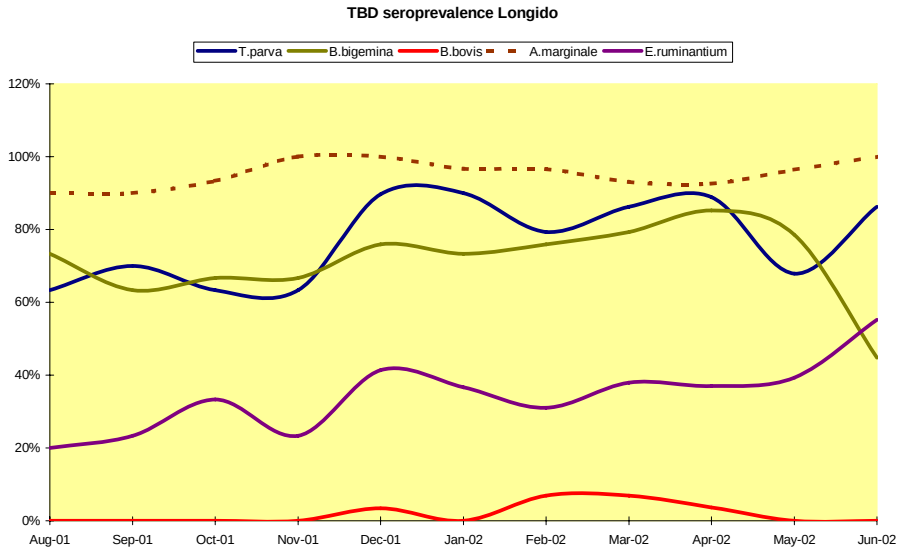


Figure 9. TBD seroprevalence Longido - Ormilo-negative site: Control Herd



All monitoring herds recorded very low *T.taurotragi* parasite prevalence (PCR/RLB) in the monthly blood samples. It is at this stage not clear whether this is due to (i) low carrier capacity and/or intermittent presence of the parasite, (ii) low sensitivity of the *T.taurotragi* probe used or (iii) inherent low detection capacity of *T.taurotragi* using PCR (as has been reported for *B.bovis*).

Table 1. PCR detection of tick-borne parasites in the sentinel herds

Site	Month	tauro	parva	mut	buff	velif	E.all	A.mar	B.big	B.bov	B.alls	lesto	sable	T.MSD	Tspbuffalo
ML	Aug-01	5	0	29	0	27	10	22	0	0	13	nd	nd	nd	nd
	Nov-01	6	0	28	1	27	0	0	0	0	0	0	27	2	1
	Jan-02	4	1	27	0	26	10	0	0	0	0	0	26	0	nd
	Mar-02	5	1	28	0	27	10	0	0	0	0	0	27	0	nd
	May-02	4	0	18	0	18	10	0	0	0	0	0	18	0	nd
NL	Aug-01	2	1	29	22	20	1	26	0	0	11	nd	nd	nd	nd
	Nov-01	1	1	19	20	14	0	0	0	0	0	0	12	12	nd
	Jan-02	1	1	28	23	24	0	0	0	0	0	0	21	22	nd
	Mar-02	2	4	25	23	18	0	0	0	0	0	0	17	25	nd
	May-02	2	1	24	22	19	0	0	0	0	0	0	19	26	nd
SI	Sep-01	0	0	29	2	26	0	0	0	1	4	0	0	nd	nd
	Nov-01	1	0	24	1	23	0	0	0	0	0	0	1	nd	nd
	Jan-02	0	0	26	1	23	0	0	0	0	0	0	3	nd	nd
	Mar-02	1	0	25	0	24	0	0	0	0	0	0	2	nd	nd
	May-02	0	0	26	0	28	5	3	0	4	0	0	7	nd	nd

Some irregularities have been observed with PCR/RLB results for the monitoring herds, which needs further testing, i.e.

1. Non-reproducibility of *A.marginale* carrier status between Aug/Nov-01 samples
2. Low detection level of *T.parva* (in well established ECF endemic areas)
3. Low detection level of both *Babesia bigemina* and *B.bovis* from endemic sites
4. Highest levels of *T.taurotragi* presence recorded in the negative control-herd

Fortnightly tick collections

Results of the fortnightly tick collections are in line with TBD seroprevalence but with statistically lower numbers of *R.appendiculatus* recorded in Longido (Non-Ormilo control herd in North Monduli). While for all other herds, the brown ear tick *R.appendiculatus* (both adults and nymphs) constituted the majority of ticks collected at any given date, Longido recorded significantly lower numbers of R.appendiculatus but high numbers of *R.pulchellus* and *R.praetextatus*. Ruvuremit recorded significantly higher numbers of *Boophilus* ticks.

Figure 10. Tick population dynamics Endulen (Loliondo, Monduli) with regards to *R.appendiculatus*, *Boophilus* and *Amblyomma* ticks

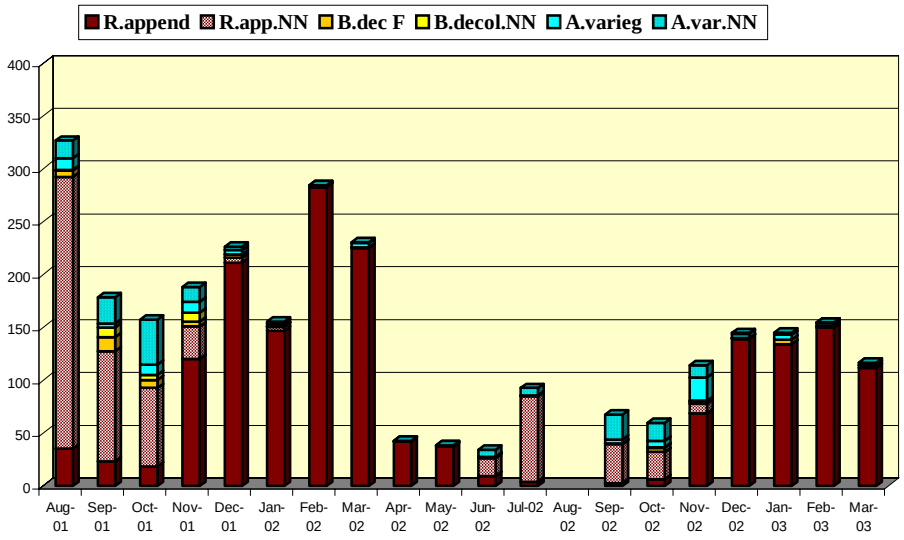


Figure 11. Tick population dynamics Ruvuremit site

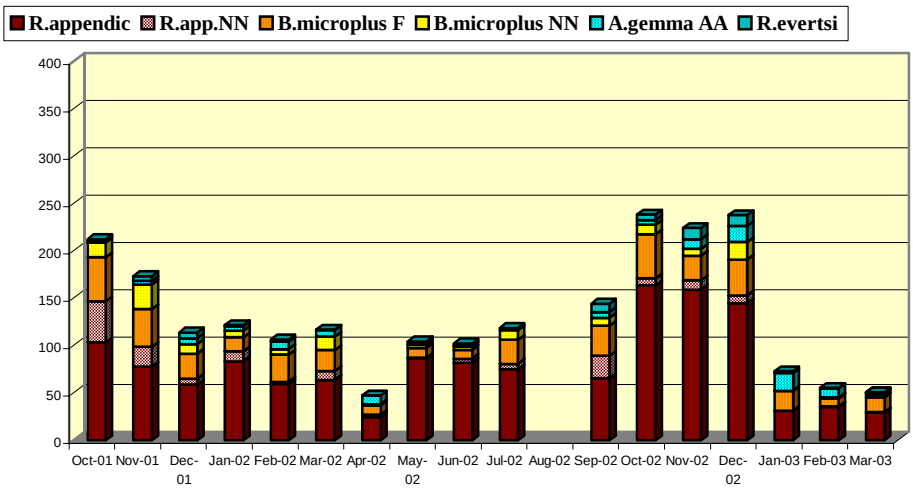
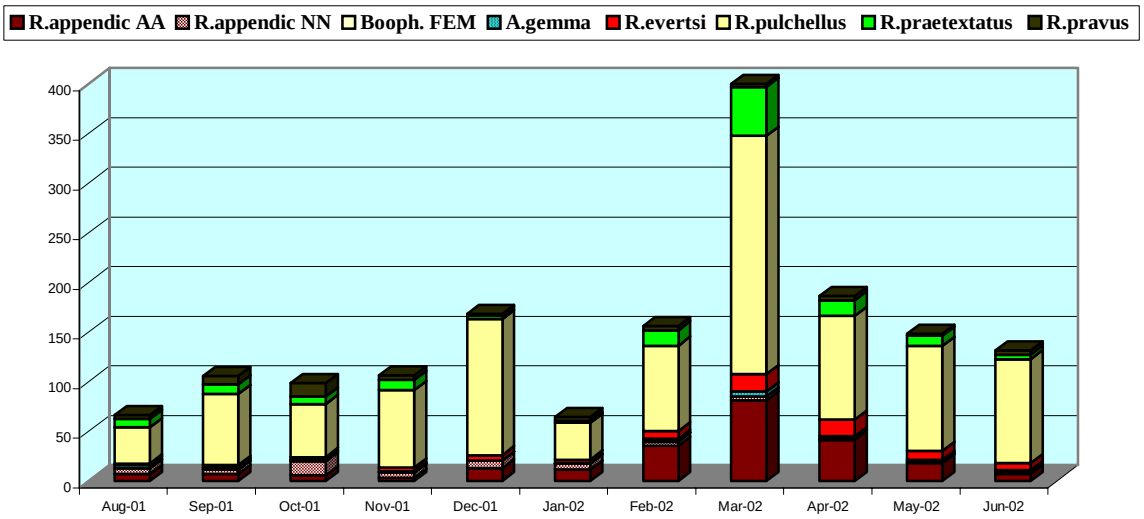


Figure 12. Tick population dynamics Longido site



For the Ngorongoro and Monduli sites, the R.appendiculatus collections (both adult and nymphs) constitutes > 80% of all ticks collected on any given date. The opposite was true for Longido site, where *R.pulchellus* was the most prominent tick collected.
Likewise, while only minimal numbers of other tick species were collected in the Ngorongoro and Monduli sites, the Longido and Ruvuremit collections showed a more diverse tick presence.

Figure 13. Longido site : other ticks collected

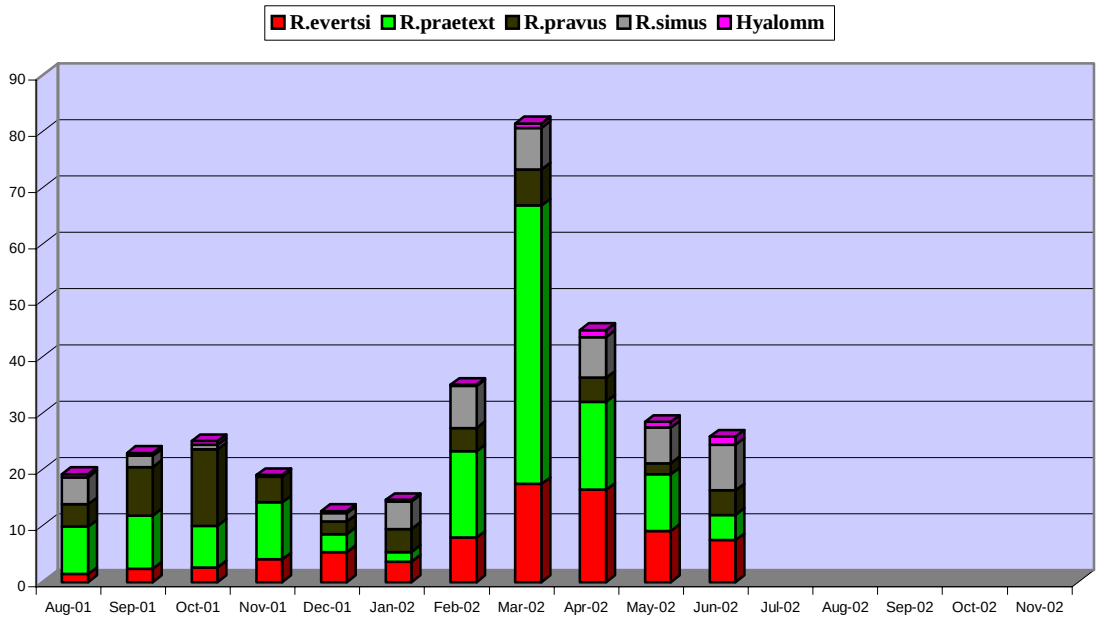
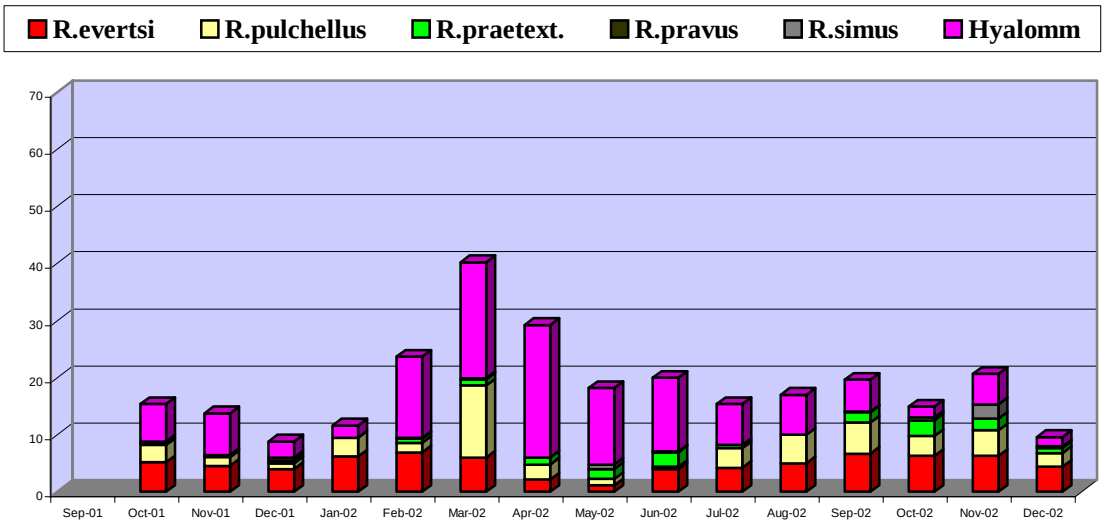


Figure 14. Ruvuremit: other ticks collected



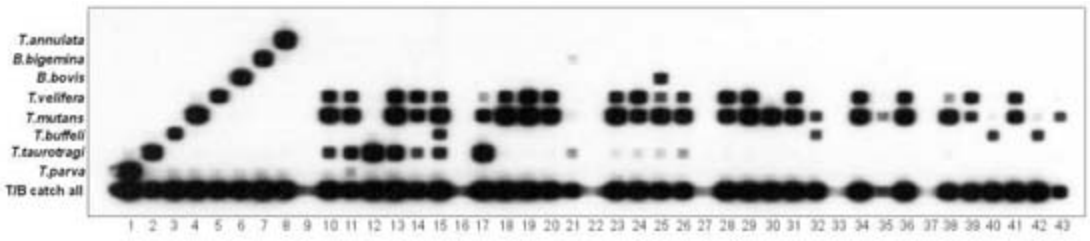
2.2 Identification of possible causal agent(s) of BCT (UP, Utrecht, VIC Arusha) and factors predisposing towards the disease (ITTBDCP-Tanzania).

Identification of causal agent

Provisional identification (2000) of *T.taurotragi* as causal agent of BCT on PCR-RLB was further strengthened through confirmation of the presence of the parasite in a number of clinical cases and several post-mortem samples collected up till March-04. The disease presence was confirmed in all clinical sites. Carrier status could not be established using the current available tools.

a) **Clinical cases:** confirmation (PCR/RLB) of the parasite has been possible in a limited number of clinical cases. Whole blood collection and the need for cold chain has limited the numbers of samples (32) collected from pastoral areas and confirmed (9) in this way. A filter paper approach was tested and started in the field from September 2002 onwards. Approximately 130 additional samples have been collected and have been send to UP. Results (*T.taurotragi* confirmation of suspected clinical cases) are pending.

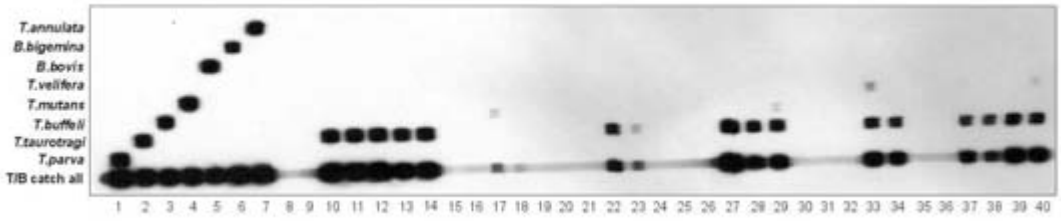
Figure 15. PCR/RLB results clinical samples/ sentinel herd samples



Samples 10-26: clinical cases, Samples 28-43: sentinel herd samples

b) **Post-mortem samples** collected up till March-02 were stored in 10% buffered formaline and proved unfit for PCR work. Post-mortem samples for the period April 2002-March 2003 were stored in 70% methanol as well as buffered glycerine and have been sent to OVI in March 2003. Results show high level *T.taurotragi* confirmation from brain-tissue samples. Few spleen tissue samples tested positive, while none of the lymph node tissue samples of the same animals recorded a positive signal for T.taurotragi, confirming the very specific tissue tropism of the parasite. The disease presence has been confirmed in all clinical sites.

Figure 16. PCR/RLB results post-mortem samples



Samples 8-9,15-16,19-20,24-25,30-31 and 35-36: negative controls
Samples:10-14 Endulen site (Ngorongoro)- brain tissue samples
Samples: 16-18: Oloirobi (Ngorongoro)- brain tissue samples
Samples: 21-23: Loliondo (Ngorongoro)- brain tissue samples
Samples: 26-29, 33-34: Monduli - brain tissue samples
Samples: 30-32: Monduli -lymph node tissue samples of cases 26-29
Samples 37-40: Ruvuremit (Simanjiro)- brain tissue samples

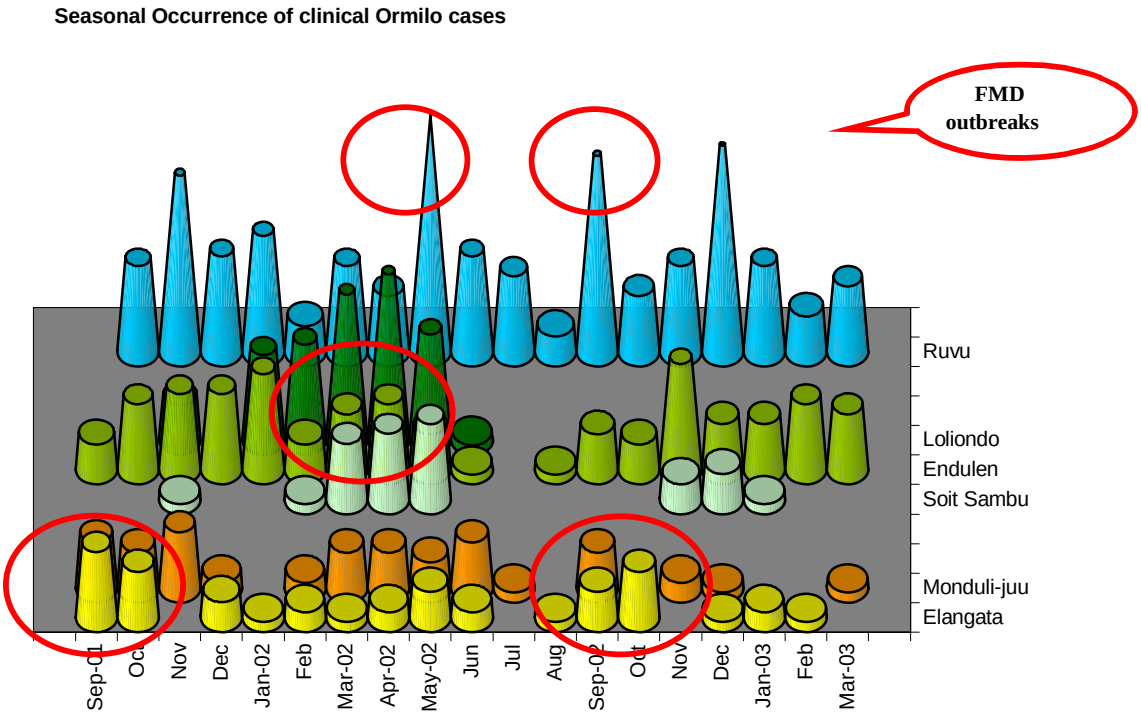
c) Epidemiological work with regards to seasonality, age-group affected etc., was highlighted during the PRA survey and subsequently confirmed in the longitudinal study. Epidemiological work with regards to disease transmission and dynamics (identification of carrier status/ tick vector) has been largely unsuccessful due to low sensitivity of the parasite on PCR/RLB and unavailability of serological test as an alternative. A molecular workshop, organized by EMBO/ICCTD-2 in October 2003 looked at the problem of low sensitivity of the parasite on PCR/RLB s and recommended a change in approach (i.e. nested PCR, etc.) to increases sensitivity of the molecular diagnostics. So far limited improvements have been achieved but fine-tuning of methodology is continuing. Off all tick samples tested (> 1000 ticks), only 2 tests (*R.appendic* female ticks) came up positive for *T.taurotragi*, while all other samples, and all other ticks came up negative. The identification of carrier status of animals and tick-vectors through improvement of molecular diagnostics tools currently in use needs to be continued.

Most of the molecular diagnostic work was carried out at University of Pretoria (RSA) with back stopping from Utrecht University (Netherlands). Planned collaboration with Sokoine University of Agriculture only materialised at the last minute with participation of SUA at the Embo/ICTTD-2 Workshop held in October 2003 at UP. From January 2004 onwards some of the PCR/RLB work was carried out at SUA as well as at the Veterinary Investigation Centre, Arusha, Tanzania.

Factors predisposing towards the expression of Cerebral Theileriosis.

Concurrent infections most prevalent during outbreaks of Ormilo are still the other tick-borne diseases (ECF, babesiosis), however without pointing to a causal relationship between the two. On the other hand, several outbreaks of Ormilo concurred with or immediately after foot & mouth disease (FMD) outbreaks in the respective herds in 2002, 2003. Continuous surveillance in the field and case history of clinical cases strengthened the hypothesis that FMD infections (either clinical or FMD vaccinations) increase the clinical incidence of BCT.

Figure 17. Seasonal occurrence of Ormilo and FMD outbreaks



The most obvious link between FMD and Ormilo was observed in the herd in Monduli-juu, where a FMD outbreak in September 2001 resulted in 5-6 Ormilo cases within the week, and where in September 2002, when the livestock owner vaccinated against FMD, 7 cases of Ormilo occurred, all starting within 1-2 days from each other. This is an abnormal picture as Ormilo cases due occur within the same herds but individual cases are reported with several weeks' intervals.

Furthermore, the presence of neutrophilia in blood smears of clinical cases supports possibility of concurrent viral infections. However, if supposedly viral infections play a role in the expression of cerebral theileriosis, no indicators have been identified thus far to explain the specific age-group susceptibility in relation to Ormilo.

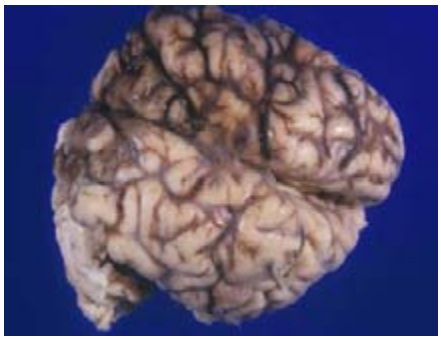
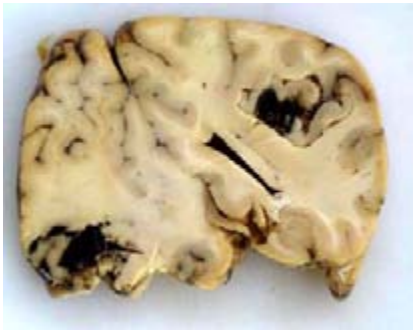
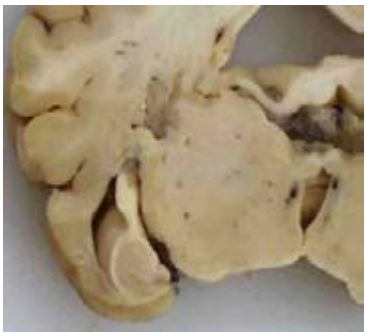
Follow-up on ECF immunised calves more than 2-3 years after immunization against ECF indicated that ECF immunisation at early age decreases the risk of contracting BCT at a later stage.

2.3. Pathology work (ITTBDCP, Turin University)

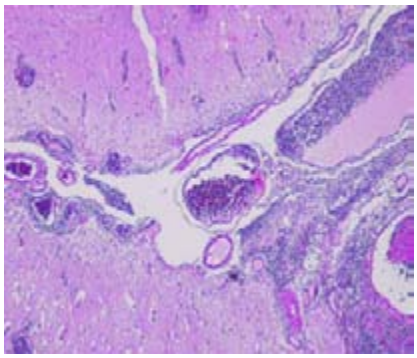
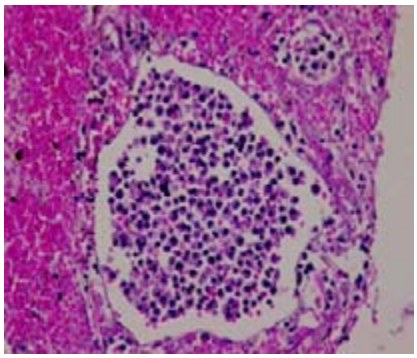
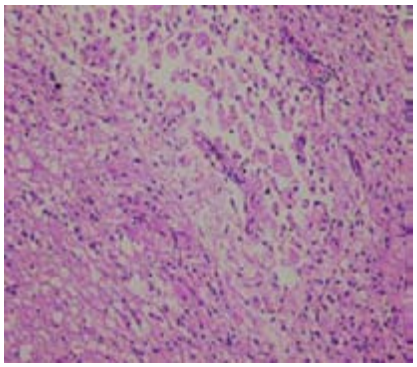
Pathology work was carried out in collaboration with Faculty of Veterinary Medicine, Turin university, Italy, Department of Animal Production, Epidemiology and Ecology and Department of Animal Pathology. Collaboration, initially sought with University of Pretoria for the pathology work did not materialize as the MSc student (UP) cancelled her proposal and subsequent visit to Tanzania.

Interim results on the Gross pathology and histo-pathology work were presented by the Italian colleagues at the 21st Annual meeting of the European Society of Veterinary Pathology, Dublin, September 2003. Histological investigations of acute and chronic cases indicated that the pathology is characterized by massive congestions of meningeal vessels, severe haemorrhages and plasmorrhages especially in choroids plexuses, and severe accumulation of lymphoblasts in the cerebral and meningeal blood vessels in acute cases, while chronic cases revealed disseminated malacic areas. PCR analysis confirmed *Theileria taurotragi* infection in 21 of the brain samples examined. Ultra structural investigations revealed the parasitic involvement of the lymphoid cells.

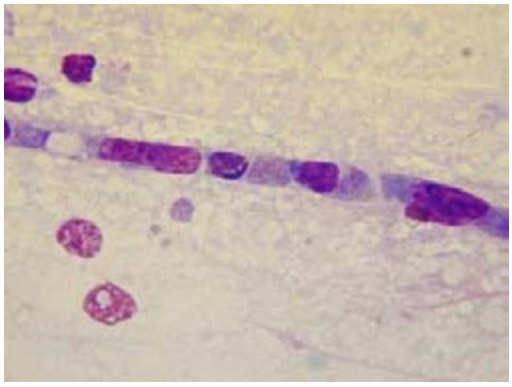
Gross pathological lesions

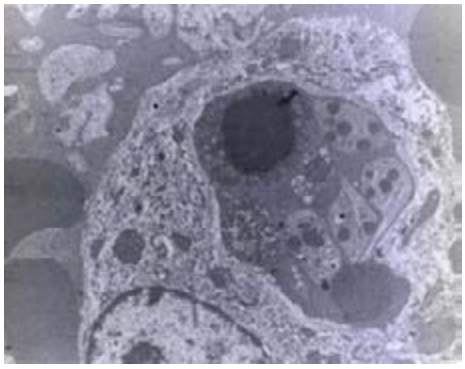
Histopathological lesions

Microscopic confirmation of parasite presence



Electron-microscopic confirmation of parasite presence



Cerebral observed lesions resemble those reported by other authors in BCT caused by *T. taurotragi*. The pathogenesis of this disease remains unknown. Further studies are recommended to establish why the pathological lesions are confined to the brain and the meaning of the intravascular agglutination of lymphoid cells. These data will be useful to distinguish this theileriosis from other diseases causing CNS symptoms and especially to activate an epidemiological surveillance and an effective control of this disease in Tanzania.

Immuno-histo-chemistry work has been started at Turin University to identify the T-cell sub-populations most heavily involved in the pathogenesis of the cerebral symptoms. In the initial phase (Sept-2003 onwards), similar problems have been observed, i.e. the fact that formaline fixation of brain samples, while the preferred method for histological examinations, did not allow for IHC work. New samples are now fixed in formaline for a maximum of 4 days only, after which they are sliced, washed with buffer and 90% alcohol, and ultimately preserved in 90% alcohol.

2.4. Treatments of clinical cases

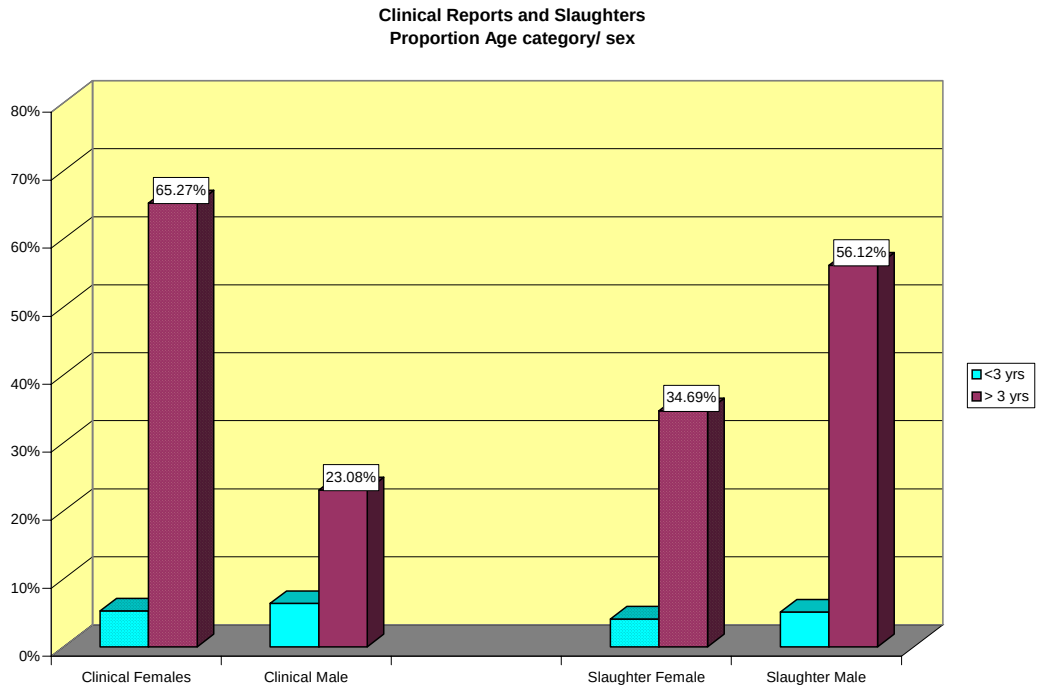
Following treatments were provided in the field by the research team (including extension officers and CAHW) in chronological order as cases were presented:

Order of presentation	Treatment given
1st case	Oxytetracycline 30% (OTC)
2nd case	Butalex (but)
3rd case	OTC + BUT
4th case	Fruvexon
5th case	placebo
6th case	OTC x 2
7th case	OTC + BUT (x 2)
8th case	Start again as 1st case….

A total of 917 clinical cases have been attended to, while an additional 275 cases were recorded as post-mortems but with information of "farmer's treatment", most often OTC 10%, single dose.

In overall, the majority of all animals reported sick for BCT or brought to slaughter slabs for emergency slaughter because of BCT were adult animals (> 3 years). Farmers were more "desperate" to continue trying out Rx for female animals, or even keeping female animals on despite showing nervous symptoms. Male animals ended up significantly more at slaughter slabs, bearing in mind that the normal pastoral herds have a 1/5 ratio males/females.

Figure 18. Clinical cases and slaughters by age/sex

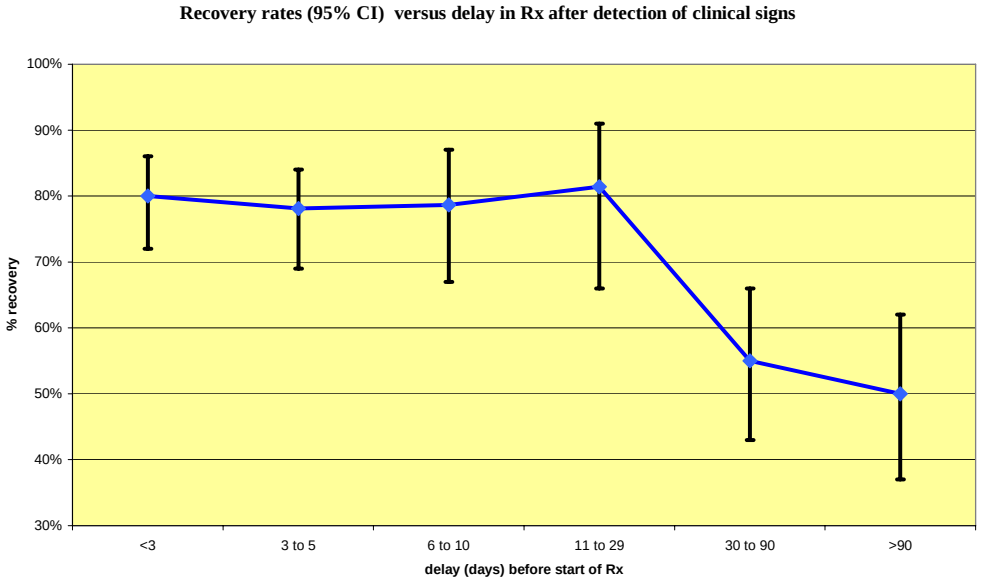


Treatment regimes by the research teams have been successful with overall recovery rate of 69%. However an impressive recovery rate (35 %) had been reported in animals treated with a placebo compared to the 20% recovery rates reported from Farmers Treatment. With (at this time) limited confirmation of *T.taurotragi* as causal agent in the respective clinical cases, evaluation of individual drugs or combination therapy remained difficult.

Interim results show that recovery rates are;

a) Adversely correlated with delay of treatments after onset of cerebral symptoms

Figure 19. Recovery rates against delay in Treatment

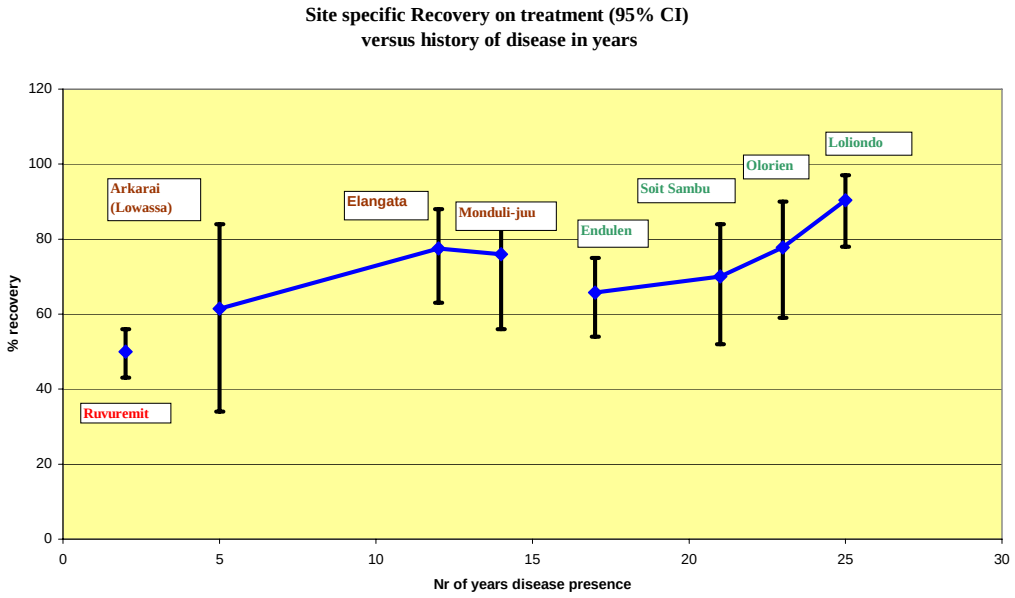


b) and Long-term presence of the disease in the area, decreased number of clinical cases reported and increased recovery rates of clinical cases

Table 2. Cases reported by different field sites

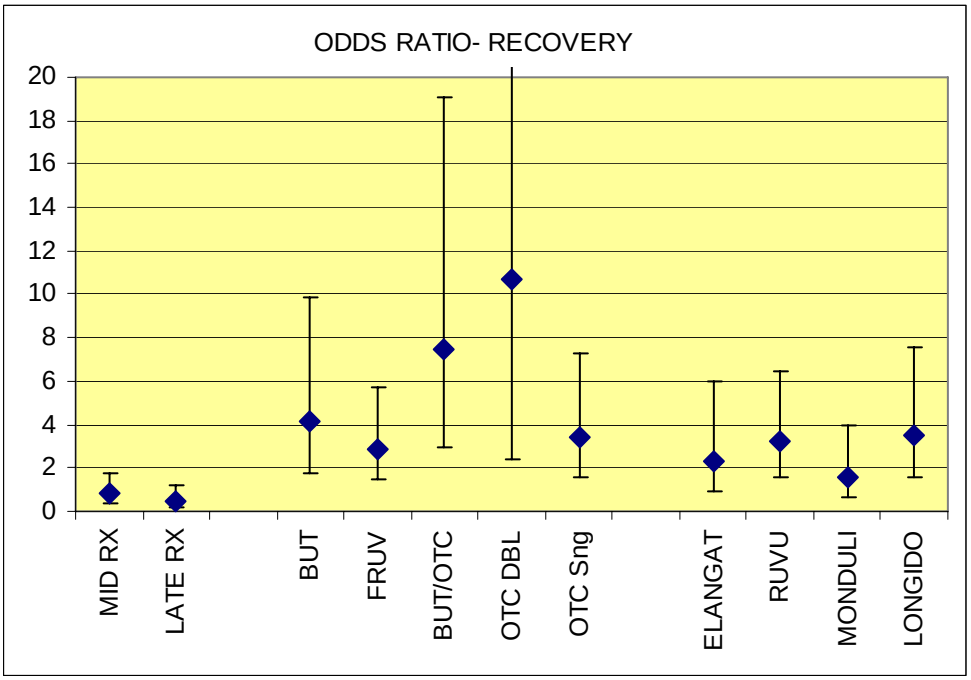
Site (District)	Year of first Disease Report	Nr Clinical cases reported at field site during 2002-2003
Loliondo (Ngorongoro-North)	1977	51
Olorien (Ngorongoro-North)	1980	34
Soit Sambu (Ngorongoro- North)	1981	42
Endulen (Ngorongoro-South)	1985	148
Elangata (Monduli-North)	1990	100
Emariete (Monduli-central)	1995	25 (Sept-2 onwards)
Ruvuremit (Simanjiro-East)	2000	254
Kilosa (Morogoro region)	2001	> 500

Figure 20. Recovery rates versus history of disease in different sites



c) While multivariant analysis indicated that OTC 30% at double Rx (Day 0- Day 6) was the most efficient with the highest odds-ratio for recovery of 10.72.

Figure 21. Odds ratio-recovery



The clinical and post-mortem database was analysed in collaboration with the Veterinary Economics and Epidemiology Unit (VEERU), Reading University. However, at this time, the major limitations in both analysing the treatment regimes as well as the clinical occurrence of Ormilo is the low percentage of both post-mortem and clinical cases actual confirmed to be *T.taurotragi* .

From January 2001 till March 2002 all post-mortem samples (162) were collected in 10% formaline. While the initial identification (2000) of *T.taurotragi* was done on formaline preserved tissue samples, the long-term storage of tissue samples in formaline rendered them unfit for molecular diagnostic work and only limited nr of post-mortem cases have been confirmed (9) from the period Jan-2001 till March-2002 using Giemsa stained Brain crush smears of the respective cases. Meanwhile, collection of whole blood from suspected clinical cases was only possible if the Veterinary field officer was present, i.e. every 14 days (resulting in 32 blood samples collected from 516 suspected clinical cases, i.e. 6% of cases only) since storage of blood in the Maasai areas was not feasible. A filter paper approach to collect blood from clinical cases for PCR purposes was tested in 2002 and adopted in September 2002. Approximately 130 additional samples (out of 180 clinical cases) have been taken, but PCR/RLB results are still pending.

Some of the suspected Ormilo clinical and post-mortem cases were removed from the Ormilo Analysis as microscopic examination revealed other causes of disease/death. In the second phase (year) of the field studies, a higher accuracy in collecting suspected Ormilo cases/post-mortems was observed with fewer animals rejected from the final analysis. In overall the majority (93%) of post-mortem cases were included in the final analysis while fewer (75%) clinical cases were accepted. The latter was inevitable as drug treatments were provided free of charge and livestock owners brought animals for treatment under the disguising it as being an Ormilo case.

Table 3. Proportion of samples submitted for PCR/RLB and subsequent identification

Period	**Number Post-mortems**	Included in Ormilo analysis	PCR done	No positive ***T.taurotragi***	%
Jan 2001- March-02	162 (59%)	148 (91%)	22 (15%)	9	41%
April-02 till Mar-03	114 (41%)	109 (96%)	78 (72%)	46	59%
Total	276	257 (93%)	100 (39%)	55	55%

Period	**No clinical cases**	Included in Ormilo analysis	PCR done	No positive ***T.taurotragi***	%
Jan 2001- Aug-02	516 (74%)	385 (75%)	32 (8%)	16	50%
Sept-02 till Mar-03	180 (26%)	148 (82%)	130 (88%)	**results pending**	
Total	696	533 (77%)	100	**results pending**	

So far, 55% of all post-mortem cases submitted for PCR/RLB have been confirmed for *T.taurotragi* with a 59% conformation for the last year's samples. Confirmation of clinical cases for the first period was 50% but PCR results of the 130 filter papers submitted from Sept-02 onwards are still pending.

Contribution of Outputs

The project was to improve livestock production of pastoralist households in Ngorongoro, Monduli and Simanjiro District through improved knowledge on risk factors, incidence, economic impact, transmission and control of BCT. Short-term benefits through increased awareness of BCT and correct therapeutic interventions, resulting in reduced mortalities in young adults, increased availability of trade stock and offtake for slaughter have been realised. This has allowed poor households greater choice in marketing animals and milk, leading to increased income generation, and improved human health and welfare.
Increased access to best available information has empowered pastoralist livestock keepers to make decisions about potential livestock health. At the same time, responding to a demand-driven request with active participation of Maasai livestock keepers in most of the research activities, relations and trust between pastoralist livestock keepers and veterinary support staff have significantly approved. Furthermore, the significant correlations between the observations recorded during the PRA survey and the subsequent longitudinal studies has acknowledged both the indigenous knowledge of pastoralists with regards to livestock diseases and the usefulness of these communities in participatory research activities.

Research results have primarily reached end-users in the project areas and beyond. Dissemination of information resulted in additional requests from non-project areas to assist in field investigations but due to financial constraints only some of these requests were followed upon.

Target institutions for promotion and extension are updated on the current status of the results, but with several critical questions still unanswered, no active role has been taken in control-strategy formulation and in the dissemination of these results.

It is imperative that all pending results will be processed on PCR/RLB and results disseminated to allow a final database analysis. The direct beneficiaries, i.e. pastoral communities in the project area have adopted the interim results with regards to treatment strategies, i.e. use of 30% oxytetracycline long-acting, but a once the information on the dynamics of the disease are revealed, a long-term strategy needs to be developed to prevent spread of the disease to new areas.

The set-up of Reverse Line Blot activities in the molecular diagnostics labs at Sokoine University of Agriculture (SUA), Morogoro and at the Veterinary Investigation Centre (VIC), Arusha, will allow continuous monitoring of both the presence (Arusha region) and the spread (Morogoro region) of cerebral theileriosis in Tanzania, and allow rapid response and diagnosis if requested from the field. However, since most of the research is based on molecular diagnostic work, sufficient funds need to be committed for this activity to take place. With financial constraints present at national level, further donor support needs to be sought to continue this investigative work. Both Sokoine University as well as the veterinary investigation Centre, Arusha should assist in this effort to allow full realisation of the potential of the molecular diagnostic labs on their premises.

While notions regarding the severity of livestock diseases often differ greatly between the pastoralists and the veterinary departments, and therefore impact and adoption of animal health technologies is often poor, the current research activities implemented under the AH R8022 Programme were a direct response to a disease prioritisation by the pastoralist communities. The direct beneficiaries have closely followed interim results of all aspects under investigation, and pastoralists and members of their community were actively involved in the field research activities. Acceptance of the disease prioritisation by the pastoralist as well as an open, accessible forum to discuss the research results and provisional control options have laid the groundwork for a rapid adoption of control strategies once final analysis of all data has been completed. The long-term impacts of the control strategies can be measured through the monthly disease reports from the high-risk areas, which have been clearly identified.

Impact

Livelihood strategies of Maasai and Warusha pastoralists in northern Tanzania range from livestock producers who trade animals for grain, to more integrated crop/livestock farming systems, to livestock dependent households constrained by local conservation laws which only allow minimal cultivation (Homewood and Rodgers, 1991, Homewood et al., 2001, Homewood et al. (2004) *submitted*). Homewood et al. (2001) found that in average only 40% of the Maasai's food requirements could be covered from livestock products and 60% had to be covered by purchase of supplementary food. DANIDA, 1997 (Ereto Project Document) recorded 70% of pastoralist households in Ngorongoro Conservation area as destitute families, with below average livestock units to assure food security. Similar reports are available for Simanjiro District and Monduli District (ELCT, World vision and VETAID reports on pastoralist households in the different districts). These and other more recent studies (Homewood et al, submitted) have produced estimates of production parameters, which can be used as a baseline for impact assessment of the mortality caused by BCT. The approach to household based, as well as key informant and semi-structured interviews can be adopted to allow accurate demographic estimates of livestock survival, offtake and performance.

Since the research activities implemented under the AH R8022 Programme were a direct response to a demand driven request by the pastoralist communities, the interim results of all aspects under investigation have been closely followed by the direct beneficiaries, i.e. pastoralists. Discussions on those results as well as on the success of interim/provisional control options and the severe economic impact of the disease on their livelihoods, will ensure rapid adoption of control strategies once final analysis of all data has been completed. The most worrying aspect of the ormilo problem at this moment is the rapid spread of the disease southwards into the pastoral areas of Morogoro region since 2000-2001. The long-term impacts of control strategies can be measured through the monthly disease reports from the high-risk areas, which have been identified.

Annex / Appendix

- **PEER-REVIEWED Publications**

Nijhof, A.M., B.L.Penzhorn, G.Lynen, J.O.Mollel, P.Morkel, C.P.J.Bekker and **F.Jongejan**. (2003). Babesia bicornis sp.nov. and Theileria bicornis sp. Nov: tick-borne parasites associated with mortality in the black rhinoceros (Diceros bicornis). Journal of Clinical Microbiology, 41(5): 2249-2254.

Jongejan F (2003) Novel opportunities for improved diagnosis and control of ticks and tick-borne diseases in the Sudan. *The Sudan Journal of Veterinary Science and Animal Husbandry* 42:344-345.

- **NOT PEER-REVIEWED**

  - **Oral presentation** (ppt) *Cerebral Theileriosis in African Short-horn Zebu cattle in Tanzania is caused by Theileria taurotragi*. Godelieve Lynen, Marc-Jan Gubbels, Giuseppe di Giulio, Christine Bakuname, Johnson Mollel, Potari Loomu, Cornelis Bekker and Frans Jongejan. International Joint Conference , Society for Tropical Veterinary Medicine and Wildlife Disease Association, Pilansberg, RSA, 22-27th July 2001.

  - **Bovine cerebral Theileriosis in Tanzania**; Report of Participatory rural appraisal in Arusha region, 66pp., submitted in May 2002 and circulated to all relevant parties.

  - **Graphic posters** on the interim analysis and results of the Long-term monitoring of sentinel herds (Sep-01 till Mar-03).

  - **Annual Progress Reports** were submitted to DFID in April 2002/3/4 and circulated to the MWLD.

  - **Oral presentation (ppt)** on Ormilo during the Tanzanian Veterinary Association Annual Scientific Conference, Dec 2002.

  - **Oral presentation (ppt)** *The Pathology of Bovine Cerebral Theileriosis caused by Theileria taurotragi: case studies in Tanzania*. G.M.Lynen, G.Di Giulio, Maria Teresa Capucchio, Deborah Catalano, Laura Tomassone, Carla Grattarola. 6th Veterinary Pathology Symposium C.L.Davis Foundation Esami, Arusha, 28-29 November 2002.

  - **Oral presentation (ppt).** Cerebral Theileriosis in Tanzania is caused by *Theileria taurotragi*. In: Proceedings of the Tanzania Veterinary Association Annual Scientific Conference, 2-4 December 2002.

  - **Poster presentations** on Ormilo, (longitudinal studies, molecular diagnostics and histopathology work) presented at the National Agricultural Show (NaneNane Show), in Mbeya, Morogoro and Arusha town in August 2003.

  - **Oral presentation (ppt).** MT Capucchio, D.Catalano, G.M.Lynen, G.Di Giulio, E.Taracha, D.De Meneghi, E.Cornaglia, F.Valenza. Bovine Cerebral Theileriosis by *T.taurotragi* in Tanzania: pathological investigations. Proceedings 21st Annual

Meeting of European Society of Veterinary pathology, Dublin, 10-13 September 2003, p.60.

- **Lecture material (ppt)** for the EMBO/ICTTD-2 molecular workshop in Pretoria, South Africa, October 2003, which featured as theme Cerebral Theileriosis.

- **Poster presentation** on the molecular diagnostic work carried out for Ormilo was presented at the EMBO/ICTTD-2 molecular workshop in Pretoria, South Africa, October 2003.