"Ormilo disease" a disorder of Zebu cattle in Tanzania: bovine cerebral theileriosis or new protozoan disease?

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Abstract

"Ormilo" disease is a neurological disorder of cattle described by Maasai herders in Tanzania. It is attributed to infection by *Theileria* species, although no detailed data are available in the literature.

The authors describe the macroscopical and histological changes observed in 30 brains of indigenous short-horn zebu cattle from Northern Tanzania, aged 2-9 years, with the characteristic neurological signs of "Ormilo". Moreover the ultrastructural details observed in 14 selected brains samples were reported. Areas of congestion and hemorrhages, associated with the obstruction of the cerebral vessels with large numbers of parasitized lymphoid cells, were observed. Electron microscopy showed the presence of intralymphocytic parasites morphologically comparable to flagellated protozoa, not previously described in the lymphoid cells of cattle, but only reported during the sexual stages within the vector. *Theileria taurotragi* was detected by Polymerase Chain Reaction and Reverse Line Blot in 9 samples. The authors hypothesize that the parasite detected by electron microscopy could be a strain of a *Theileria* endemic to this region till now not investigated, having an intralymphocytic phase and being associated with other *Theileria* spp. infestation. Further studies are needed to better understand the etiology of "Ormilo" disease and to characterize the morphology of the observed parasite, clarifying its role in the disease in Tanzania.

Key words: Bovine Cerebral Theileriosis, Ormilo, pathology, short-horn zebu cattle, Tanzania, Theileria.

Introduction

Theileriae are tick-transmitted intracellular apicomplexan parasites, which infect a wide range of mammals worldwide (Norval et al. 1992; Dobbelaere and McKeever 2002). After the tick bite, *Theileria* sporozoites invade lymphoid cells of the vertebrate host and induce them to proliferate in an unregulated manner. The cells develop into a multinucleate syncytial schizont (macroschizont), later in microschizonts that differentiate into uninucleate merozoites, that are liberated into the blood where they invade the erythrocytes and become piroplasms (Fig. 1).

Several species of *Theileria* are known. *Theileria parva* and *Theileria annulata* are considered the most important pathogens of domestic livestock in the Old World tropical and subtropical regions. *Theileria taurotragi* was first described in 1960 as a benign parasite of the eland (Martin and Brocklesby 1960). Subsequently, in cattle it was demonstrated that this parasite causes a benign parasitosis or fatal infections (De Vos et al. 1981), frequently associated with concurrent presence of *Theileria mutans* and/or *T. parva* (Young et al. 1977; Uilenberg et al. 1982).

T. parva, T. taurotragi, and more rarely *T. annulata* are considered causing agents of the Bovine Cerebral Theileriosis (BCT) (De Vos et al.1981; Saville 2002; Sudan et al. 2012). BCT is described in tropical and subtropical African

regions (Mettam et al.1936; Flanagan et al.1957), but more recently two cases caused by *T. annulata* were reported in Turkey (Dabak et al. 2004) and in India (Sudan et al. 2012*)*.

BCT, or Turning sickness, is a fatal disease of indigenous African short-horn cattle of cattle. It usually occurs in young animals and results in a mild febrile reaction of 1-14 days duration, after an incubation period of 10-21 days. Slight enlargement of the superficial lymph nodes is present, but no other clinical signs are reported. The nervous form of BCT is characterized by uncontrollable movements, sometimes unilateral-bilateral blindness (corneal opacity), circling, head pressing, ataxia, opisthotonous and paralysis. Usually the animals collapse after 2-21 days, but occasionally the cases become chronic and they may live for up to 6 months (Van Amstel 1982; Lawrence et al. 2004). The neuropathological changes include congestion and hemorrhages in the meninges and in the brain, and subacute-chronic areas of malacia. Microscopically, the obstruction of the arteries with a large numbers of parasitized lymphoblasts is the most prominent finding (Bader et al. 1986; Lawrence et al. 2004).

Cases of disease in cattle similar to BCT have been reported from Maasai herders in northern Tanzania (Arusha, Manyara and Tanga regions) since the mid-1980's (Field et al. 1988; Nsengwa 1993). Although its incidence has been increasing, especially in northern Maasai land, detailed pathological studies have never been documented. Local Maasai livestock keepers call the disease "Ormilo" and, from information gathered during a rapid rural in 2001, they consider it as the highest disease priority and, thus, as a severe constraint to livestock production (Lynen, unpublished data).

This paper reports the macroscopical and histological changes observed in 30 brains of indigenous short-horn zebu cattle, ranging from to 2 to 9 years of age, living in Northen Tanzania (Arusha Region, Ngorongoro District, Endulen ward), that presented neurological signs characteristic of "Ormilo". Ultrastructural features and Polymerase Chain Reaction (PCR) investigations are also described.

Materials and methods

The brains of 30 East-African short-horn Zebu cattle aging from 2 to 9 years old, were collected between 2001 and 2003 in Endulen, Ngorongoro Conservation Area, Arusha region, north-eastern Tanzania. In this area, mixed herds of small ruminants and cattle are kept under a traditional pastoralist livestock system.

These animals were selected according to the clinical description by Maasai herders of Endulen ward confirmed by the veterinarians of the Integrated Tick and Tick-borne Disease Control Project. The most frequently reported clinical signs was circling, associated with para-paresis and paralysis, generally followed by recumbency. In one animal, difficulties in chewing and walking were also present. Except in one case, found dead, all the other animals were slaughtered due to the worsening of their clinical signs.

Immediately after death/slaughter, the brains were fixed in 10% neutral buffered formalin and sent to the Department of Veterinary Science of Torino University to perform pathological investigations. Not all the collected samples were suitable for ultrastructural investigations (TEM) due to conservation problems. Therefore, meningeal blood vessels, choroid plexuses and thrombosed brain vessels of 14 animals were fixed in 2.5% glutaraldehyde for TEM.

Tissues were postfixed in 1% osmium tetroxide, deydrated in acetone and embedded in Spurr epoxy resin. Semithin sections (0.90 μm) were cut and stained with toluidine blue. Ultrathin sections of 70 nm were then collected onto 200 mesh copper grids and contrasted by uranyl acetate and lead citrate. The grids were evaluated by Electron Microscopy 109 JD.

Brain tissues from the same 14 animals were also subjected to DNA extraction with the DNeasy tissue kit (QIAGEN, Hilden, Germany). PCR was carried out to amplify the V4 hyper variable region of the 18S rRNA gene of *Theileria* and *Babesia* spp. Species identification was carried out by reverse line blot (RLB) hybridization of the amplified products on a membrane containing oligonucleotides specific for six bovine *Theileria* species and two *Babesia* spp. and a catch-all *Theileria/Babesia* control probe, according to the protocol previously described (Nijhof et al. 2003).

Results

Macroscopically, moderate to severe congestion of the leptomeningeal blood vessels, disseminated sub-meningeal hemorrhages and/or multifocal to diffuse hemorrhages into the brain tissue (Fig. 2a, b, c), disseminated or focal areas of sub-acute/chronic malacia were the most important observed features (Fig. 2d). Histological investigations revealed congestions/hemorrhages of the meningeal vessels, frequently thrombosed and necrotic, hemorrhages and plasmorrhages around the ventricles and especially in the choroid plexuses (Fig. 2e), and focal to multifocal necrotic areas. Severe accumulation of mononuclear cells in the cerebral and meningeal blood vessels was the most significant lesion observed (Fig. 2f). Morphologically large lymphoblastic cells predominated, including sometimes small and medium sized lymphocytes, rare plasma cells and macrophages. Most lymphocytic cells showed a variable number of the schizonts in the cytoplasm. The main involved areas were the cortical meninges and the rostral brainstem, while the pons and medulla were rarely affected by meningeal and parenchymal hemorrhages only.

Ultrastructurally, all the analyzed blood vessels revealed the presence of cytoplasmatic irregular structures, morphologically similar to parasitic forms, in lymphoid cells. The structures observed were considered to be free schizonts in the cytoplasm of the host cells whose nuclei were arranged at their periphery. Moreover, numerous free merozoites were present in the cytoplasm of lymphoid cells and in the lumen of the blood vessel. In all cases, such merozoites showed a flagellum and one cytoplasmatic vacuole containing a small spherical electrondense structure.

These morphological data allowed the authors to propose a developing cycle of the parasite in the lymphoid cells of the cattle as represented in the Fig. 3 (a - f). The first stage observed was a young schizont - spherical in shape, electron dense, with diameters of about 7-9µm characterized by spherical dividing nuclei, numerous mitochondria and many spherical organelles such as vacuoles (Fig 3a). Later the schizont appears as a spherical vesicle with 2-3 nuclei located at the periphery of the cytoplasm, numerous vacuoles and some merozoites. The merozoites are spherical or pear-shaped, measure about 1.6µm in diameter and show some micronemes, vacuoles and numerous microtubules (Fig. 3b). In some cases the microtubules are observed in cross section in both poles of the merozoites, in other cases they are longitudinally sectioned and organized to form a flagellum which is located at the apical pole or sometimes crossing the whole merozoite from one pole to the other. In the following stage the schizont appears as an electron dense vesicle containing only few mithocondria and vacuoles, whereas the merozoites, measuring about 2.1-2.2 µm in diameter, invade the cytoplasm of the host cell (Fig. 3c). Subsequently the schizont seems to degenerate and the merozoites escape from the host cell and are observed free into the lumen of the blood vessel. The merozoites free in the blood measure from about 1.8x2.5 to 3.5x3.7µm and show numerous microtubules and a clear flagellum-like structure. The medium length of these merozoites is of about 4 µm (Fig. 3d, e, f).

Nine DNA extracts from brain tissues were positive to the PCR and specifically hybridised with the *T. taurotragi* oligonucleotide on the RLB membrane (Fig. 4, samples 10 to 14, 34 and 37 to 39). The remaining five samples were negative (samples 9,15,16,35,36).

Discussion

Despite the limited information available, the clinical signs reported by Maasai herders in the cattle of the present study are similar to those described in the literature in cases of BCT (Van Amstel 1982; Lawrence et al. 2004). Contrary to what has generally been observed in BCT, several cases of the present study (n=13 animals) were older than 5 years, reaching 8 and 9 years of age in 6 cases.

Macroscopical and histological lesions resemble those reported by other authors in BCT caused by *Theileria* species (Giles et al.1978; Bader et al. 1986; Lawrence et al. 2004), and particularly the accumulation of parasitized lymphoid cells in the cerebral and meningeal blood vessels represents the pathognomonic feature of these neurological cases as reported in the cerebral form of theileriosis (Giles et al.1978; Bader et al. 1986; Lawrence et al. 2004).

Ultrastructurally, most of the knowledge on developmental stages in the tick and mammalian host cells of theileriae species comes from studies on *T. parva* and, to a lesser extent, *T. annulata*. In the authors' opinion, the morphology of developmental stages of *T. taurotragi* in lymphoid cells is still unknown. Shein and colleagues accurately described by means of electron microscopy, the morphology of the four most important *Theileriae* spp. affecting bovine species (*T.*

parva, *T. annulata*, *T. mutans*, and *T. lawrencei*) during the schizogony (Schein et al. 1978). These authors evaluated the development of a young schizont, spherical in shape with diameters of about 2µm converting into a multinucleate mature schizont (20-50 nuclei) that produces merozoites. The latter, pear-shaped, show a length of about 1 µm and a diameter of 0.6 µm; they show an apical pole where is present a polar ring system at which microtubules are anchored, 3-4 rhoptries, some micronemes and numerous mitochondria. This development destroys the host cell, the merozoites become free and in a position to infect the next host cell, the erythrocyte. Inside the erythrocyte the piroplasms are spherical and variable in size; they may measure from 1.0 to 1.5 µm in *T. parva*, they can reach up to 2.5 µm in *T. mutans* and *T. annulata*, and are even larger in *T. taurotragi* and *T. orientalis* (Schein et al. 1978). When infected erythrocytes are ingested by the tick, lysis of the erythrocytes occurs and many free piroplasms can be seen in gut smears of the ticks. A variable proportion of piroplasms undergo development into sexual stages. In the tick gut, ray bodies are formed by development of ovoid or spherical intra-erythrocytic stages (Mehlhorn and Schein 1984). The ray bodies are spindle-shaped, measuring 8-12 µm in length with a diameter of 0.8 µm. These parasites, in cross section, could show up to four nuclei and up to four flagella-like protrusions with up to 14 tubules within such protrusions.

The samples described in the present paper show mature schizonts in the host cells comparable to those described in the literature in the *Theileria* species cycle. However, they measure 7-9 µm in diameter compared to 2-10 µm reported by Schein et al. (1978). The merozoites observed in the schizont are relatively bigger (about 1.6µm in diameter) compared to the same described by Shein and coll (Schein et al. 1978) (1 x 0.6 µm). The dimensions of the merozoites free in the cytoplasm of the lymphoid cells and in the blood vessels are not reported in the literature and are thus not comparable.

The final structure of the merozoites observed in the present paper is very similar to that described in the literature. They are pear-shaped and limited by a cell membrane. At the apical pole they show two inner membranes which form a polar ring system at which subpellicular microtubules are anchored. Several rhoptries and few micronemes and vacuoles are present. Particularly one cytoplasmatic vacuole is always present containing a spherical structure strongly electrondense (with a diameter of about 0.40µm). Moreover, it is interesting to point out that the merozoites showed in the schizonts or free in the vessels, always show an evident flagellum-like protrusion so far not described in the literature in these phase of the life cycle. This flagellum seems to start from the pole opposite to the apical one; sometimes the microtubules forming the flagellum seem to cross the whole merozoite. Some merozoites show clear microtubules in cross sections in both the poles. A comparable morphology, characterized by the presence of numerous microtubules and flagella-like structures was reported in details only during the sexual stages within the tick gut (Young et al. 1980; Norval et al. 1992), but nobody described it in the final host such as the lymphoid cells of the cattle. These flagellated protozoa were observed in all samples.

Out of 14 tissue samples tested by molecular assays, nine samples were positive and *T. taurotragi* was detected as the unique infectious agent by RLB. Our samples belong to a larger sample of 78 tissues (brain, lymph nodes, spleen) from suspected Ormilo cases from Arusha region tested at the Division of Parasitology and Tropical Veterinary Medicine, Utrecht University, by PCR/RLB. Overall, 59% of the processed samples hybridized exclusively with the *T. taurotragi* oligonucleotide. None hybridized with the *T. parva* oligonucleotide, thereby convincingly excluding *T. parva* as causal agent of the cerebral theileriosis in the study area (Lynen et al., unpublished data).

The neurological signs observed and the neuropathological lesions characteristic of cerebral theileriosis, led the authors to speculate that *Theileria* spp. could be considered the possible etiologic agent in these "Ormilo" disease cases.

Based on the electron microscopy observations, the morphology of the schizonts and the merozoites observed during schizogony is partially different from the morphology of the *Theileria* species described in the literature. Moreover the absence of intra-erithrocitic parasites, not even when numerous merozoites are demonstrated free in the blood, leads the authors to assume that the whole cycle of this parasite is different from the one of the *Theileria* species reported in the literature (*T. parva, T. annulata, T. mutans* and *T. lawrencei*) (Schein et al., 1978). On the basis of the PCR/RLB results *T. taurotragi* could be hypothesized the etiological agent of "Ormilo" cases.

It is also possible that the observed parasites belong to a different strain of *Theileria* genus endemic to this region till now not investigated, with a development cycle different from what is supposed for other species characterized by a distinct morphology. In this case, it is to be assumed that additional factors could determine the simultaneous involvement of the usually benign *T. taurotragi* (Martin and Brocklesby 1960; Young et al. 1977; Grootenhuis 1979; Uilenberg et al. 1982*)* in the cerebral theileriosis pathogenesis, such as the impairment of the immunity by viruses or other concomitant tick-borne infections or heavy tick infestations. Simultaneous occurrence of different tick-borne pathogens within the same host is in fact a frequent feature, but little is known about their possible interactions. Other contributing factors considered for BCT are parasitoses like trypanosomiasis in Kenya (Moll et al. 1985), or *Amblyomma variegatum* tick infestation (Lloyd and Walker 1993).

As the role of the observed parasite in the "Ormilo" syndrome is at the moment unknown, further studies are needed to better understand its morphology and its potential pathogenic importance.

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Conflict of interest statement

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

References

Bader, R., Moll G., Lohding A., 1986. Mophological findings in bovine cerebral theileriosis (BCT). Journal of Veterinary Medicine Series A, 33, 266-285.

Dabak, M., Dabak, D.O., Aktas, M., 2004. Cerebral theileriosis in a Holstein calf. Veterinary Record, 154,533-534.

De Vos, A.J., Bessenger, R., Banting, L.F., 1981. Theileria ? taurotragi: a probable agent of bovine cerebral theileriosis. Onderstepoort Journal of Veterinary Research, 48,177-178.

Dobbelaere, D.A.E. and McKeever, D.J., 2002. Theileria. In: Black SJ, Seed JR (Eds.) World Class Parasites., Kluwer Acad Publishers, Boston.

Field, C.R., Moll, G., OleSonkoi, C., 1988.Ngorongoro conservation and development project. Livestock Development Technical Report No 1.

Flanagan HO, Le Roux JMW. Bovine cerebral theileriosis - A report on two cases occurring in the Union. Onderstepoort Journal of Veterinary Research, 27,453-461,1957.

Giles, N., Davies, F.G., Duffus, W.P.H., Heinonen, R., 1978. Bovine cerebral theileriosis. Veterinary Record, 102,313.

Grootenhuis, J.G., 1979. Characteristics of Theileria species (eland) infections in eland and cattle. Research in Veterinary Science, 27,59-68.

Lawrence, J.A., de Vos, A.J., Irvin, A.D., 2004. Turning sickness. In: Coetzer JAW, Thomson GR, Tustin RC (Eds.) Infectious diseases of livestock. Oxford University Press, Oxford. 331-333.

Lloyd, C.M., Walker, A.R., 1993. The effect of inflammatory and hypersensitivity reactions, in response to the feeding of the tick Amblyomma variegatum, on the progression of the experimentl dermatophilosis infections. Experimental and Applied Acarology, 17,345-356.

Martin, H., Brocklesby, D.W., 1960. A new parasite of eland. Veterinary Record, 72,331-332.

Mehlhorn, H. and Schein, E., 1984. The Piroplasms: life cycle and sexual stages. In: Dawes B (Ed.) Advances in parasitology, vol 3, Academic Press, London. 38-97.

Mettam, R.W.M. and Carmichael, J., 1936. Turning sickness : A protozoan encephalitis of cattle in Uganda. Its relationship with East Coast fever. The Journal Parasitology Research, 28,254-283.

Moll, G., Agan, L., Lohding, A., 1985. Bovine cerebral theileriosis in pure Boran and sahiwalcross cattle immunized against East Coast fever and kept under continuous field challenge. In: Irvin AD (Ed.) Immunization against theileriosis in Africa. International Laboratory for Research on Animal Diseases, Nairobi.

Nijhof, A.M., Penzhorn, B.L., Lynen, G., Mollel, J.O., Morkel, P., Bekker, C.P., Jongejan, F., 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, 41,2249–2254.

Norval, R.A.I., Perry, B.D., Young, A.S., 1992. The epidemiology of Theileriosis in Africa. Academic Press London. 130-154.

Nsengwa, G.R.M., 1993. An epizootic of theileriosis in cattle characterized by a nervous syndrome, in Ngorongoro District, Tanzania. Tanzania Veterinary Journal, 13, 41-44.

Saville, W.J.A., 2002. Cerebral theileriasis. In: Bradford PS (Ed.) Large Animal Internal Medicine, 3rd ed. Mosby, St Louis, Missouri. 917-918.

Schein, E., Mehlhorn, H., Warnecke, M., 1978.Electron microscopic studies on the schizogony of four Theileria species of cattle (T. parva, T lawrencei, T. annulata and T. mutans). Protistologica, 24,337-348.

Sudan, V., Sharma, R.L., Yadav, R., Borah, M.K., 2012. Turning sickness in a cross bred cow naturally infected with Theileria annulata. Journal of parasitic diseases, 36,226-229.

Uilenberg, G., Perié, N.M., Lawrence, J.A., de Vos, A.J., Paling, R.W., Spanjer, A.A., 1982. Causal agents of bovine theileriosis in southern Africa. Tropical Animal Health and Production, 14,127-140.

Van Amstel, S.R., 1982. Bovine cerebral theileriosis, some aspects on its clinical diagnosis. Proceedings 12th World Congress of Diseases in Cattle. Vol II. Amsterdam, The Netherlands, September, 981-985.

Young, A.S., Grootenhuis, J.G., Kimber, C.D., Kanhai, G.K., Stagg, D.A., 1977. Isolation of a Theileria species from eland (Taurotragus oryx) infective for cattle. Tropical Medicine and Parasitology, 28,184-194.

Young, A.S., Grootenhuis, J.G., Leitch, B.L., Schein, E., 1980. The development of Theileria = Cytauxzoon taurotragi (Martin and Brocklesby, 1960) from eland in its tick vector Rhipicephalus appendiculatus. Parasitology, 81,129-144.

Figure captions

Fig. 1 Generalized representation of the typical life cycle of the genus Theileria. Sporozoites are inoculated into a mammalian host when an infected tick takes a meal. The sporozoites invade lymphoid cells, and develop into a multinucleate syncytial schizont or macroschizont (**a**). At the same time, the parasite induces host cell transformation and proliferation (**b**). Then the schizonts differentiates into merozoites and invade erythrocytes (**c**). Ticks become infected by ingesting infected erythrocytes, and gametogenesis and fertilization takes place in the gut lumen (**d**). The resulting zygote invades a gut epithelial cell where it remains during the tick moult cycle and develops into a single motile kinete (**e**). The motile kinete egresses the gut cell and invades the salivary glands (**f**). Tick initiates rapid sporozoite development in the salivary glands, and infective sporozoites that survive in the gut epithelium are transmitted to another mammalian host when the resulting post-moult nymphs or adults feed (**g**) Modified from http://www.theileria.org/ahdw/background.htm [accessed 27-02-2015]

Fig. 2 a Multifocal congestion of the meningeal blood vessels covering cerebral hemispheres and cerebellum. Hemorrhages surrounding the brainstem and the cervical spinal cord. **b** Severe hemorrhages involving the leptomeninges, the IV ventricle, the central canal and the surrounding areas. **c** Punctiform hemorrhages in the cortical white matter. **d** Focus of chronic malacia in the capsula interna area. **e** Cerebellum: hemorrhages involving the leptomeninges and severe congestion of the meningeal vessels. Multifocal hemorrhages and plasmorrhages in the nervous tissue around the IV ventricle (H&E). **f** Severe accumulation of mononuclear cells in the meningeal blood vessels many of them showing irregular round to ovoidal bodies in the cytoplasms corresponding to parasitic schizonts (H&E). (e bar=100 μm, f bar=50 μm)

Fig. 3 Electron microscopy of bovine lymphoid cells with endocellular parasites (**a – f**). **a** Lymphoid cells containing a young schizont (arrow) spherical in shape, electrondense, characterized by spherical dividing nuclei, numerous mitochondria and many spherical organelles such as vacuoles. **b** Lymphoid cells with the cytoplasm occupied by a voluminous schizont (arrow) with two evident nuclei located at the periphery (#), numerous vacuoles and some spherical or pear-shaped merozoites (asterisks). **c** In this following stage the schizont (arrow) appears as an electron dense vesicle containing only some mitochondria–and few vacuoles whereas the merozoites (asterisks) invade the cytoplasm of the host cell. **d** Merozoites free into the lumen of the blood vessel. **e, f** High magnification of the merozoites free into the vessels. They measure from about 1.8x2.5 to 3.5x3.7μm. At the apical pole the merozoites show a polar ring system at which subpellicular microtubules are anchored to form a clear flagellum-like structure

(asterisk). Few rhoptries (long arrows) and micronemes (short arrows) and vacuoles are present. Scale bar 1µm (**a,b,c,d**); 0.2µm (**e,f**)

Fig. 4 Reverse Line Blot results showing species-specific oligonucleotides of the 18S rRNA gene of *Theileria* spp. in the horizontal lanes and PCR products in the vertical lanes. Fourteen brain tissue samples from Ormilo cases from Endulen were analyzed (vertical lanes: 9 to 16 and 34 to 39). Positive *Theileria* spp. and *Babesia spp*. controls (line 1: *T. parva*, 2: *T. taurotragi*, 3: *T. buffeli*, 4: *T. mutans*, 5: *B. bovis*, 6: *B. bigemina*, 7: *T. annulata*) as well as a negative control (water, line 8) are included. Other vertical lanes (17 to 33 and 40) refer to amplified DNA of tissue samples collected by the authors in other study areas in Arusha region.