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An outbreak of East Coast Fever on the Comoros: A consequence of the import of immunised cattle from Tanzania?

R. De Deken [a,b,*], V. Martin [c], A. Saido [d], M. Madder [a], J. Brandt [a], D. Geysen [a]

[a] *Institute of Tropical Medicine Antwerp (ITMA), Nationalestraat 155, B-2000 Antwerp, Belgium*
[b] *Vétérinaires Sans Frontières-Belgique, Avenue P. Deschanel 36, 1030 Brussels, Belgium*
[c] *EMPRES/Department Animal Health, FAO, Rome, Italy*
[d] *Comorian Association of Veterinary Technicians and Assistants, BP 1982, Moroni, Grand Comore*

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Abstract

In 2003 and 2004, a severe epidemic decimated the cattle population on Grand Comore, the largest island of the Union of Comoros. Fatalities started soon after the import of cattle from Tanzania. *Theileria parva* and its vector, *Rhipicephalus appendiculatus*, could be identified as the main culprits of the epidemic. Characterisation by multilocus genotyping revealed that the *T. parva* parasites isolated on the Comoros were identical to the components of the Muguga cocktail vaccine used in Tanzania to immunise cattle. Therefore, it is believed that East Coast Fever reached the Comoros while some of the imported livestock got infected in Tanzania by ticks of which the immature stadia fed on Muguga cocktail vaccinated animals. Since the Comorian government neither has the financial means nor the competent staff to pursue an adequate epidemiosurveillance, the danger exists that without external assistance and in a context of continuing globalisation more transboundary diseases will affect the Comorian livestock sector in the future.

Keywords: *Theileria parva*; Comoros; Molecular epidemiology; East Coast Fever; *Rhipicephalus appendiculatus*; Globalisation

1. Introduction

Increasing worldwide movement of commodities, animals and people is also responsible for the globalisation of pathogens. Early detection and a rapid response to the incursion of these pathogens are often crucial. In this respect the developed world possesses the necessary scientific and technical competence to stop or track the spread of these intruders. Moreover, the developed world has sufficient human and financial resources at its disposal to fight them. On the other hand, some of the poorest third world countries still lack even the most basic provisions to deal with this increased risk. As these countries have no appropriate veterinary legislation and no administrative or financial structures to set up prevention and control systems towards transboundary animal diseases, they are extremely vulnerable to the introduction of these often lethal diseases.

Moreover, due to the social role of livestock, the trade in live animals is still important with increased risk of spread of diseases or infected vectors compared to carcasses or processed foods. At the same time, new trade routes to these countries develop continuously

* Corresponding author at: Institute of Tropical Medicine Antwerp (ITMA), Nationalestraat 155, B-2000 Antwerp, Belgium.
Tel.: +32 3 247 62 70; fax: +32 3 247 62 68.
E-mail address: rddeken@itg.be (R. De Deken).

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increasing the risk of importing diseases, which may be endemic in the exporting country but cause severe epidemics in the importing country. The use of ''live vaccines'' to immunise livestock in the exporting country can even worsen the threat by the development of ''healthy carriers'' of the disease. Consequently, in countries with weak veterinary services, poor legislation and no active disease surveillance transboundary animal diseases can pose a serious national security threat. This paper describes such an epidemic on the island of Grand Comore.

The ''Union of the Comoros'' is a federal republic made up of three autonomous islands (Grand Comore, Anjouan and Moheli), totalling 600,000 inhabitants of which more than 50% live on ''Grand Comore'', the largest island (Fig. 1). More than 80% of the population is working in the agricultural sector. Cattle are socially very important in Grand Comore and often fetch at special occasions (Les Grands Mariages) higher prices than the intrinsic value of meat. Nevertheless, most farmers consider livestock breeding as a secondary, yet profitable activity.

The exact number of cattle on Grand Comore is not known, but estimates range between 15,000 and 25,000 head. They are always tethered and essentially kept on the high plateau.

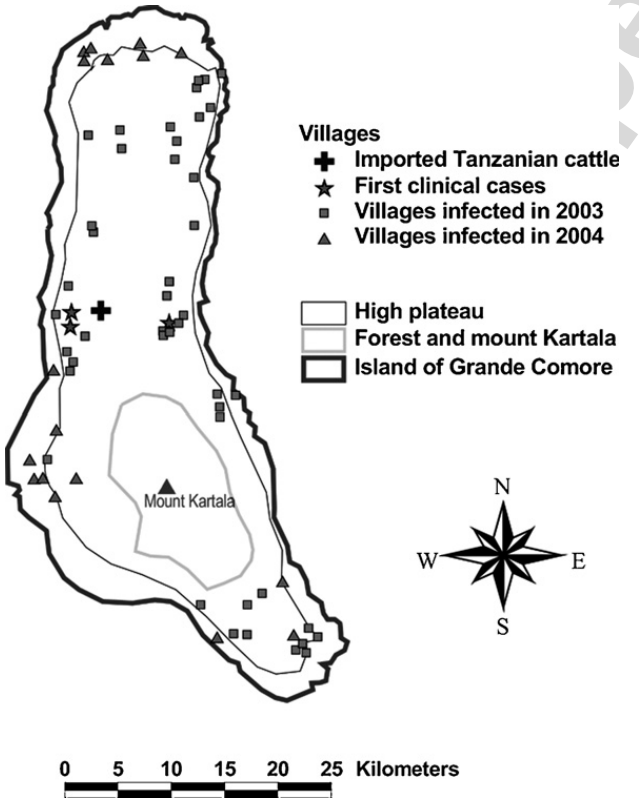

Fig. 1. The spread of theileriosis on Grand Comore from the end of 2002 until May 2004.

Veterinary services on the Comoros are provided by the ''Association of Animal Health Professionals'' (APSA). Members of APSA operate a central pharmacy and several small, poorly equipped private veterinary clinics all over the islands. These services are characterised by a motivated staff with poor management capacity, an irregular supply of veterinary drugs, insufficient transport facilities and inexistent diagnostic facilities.

In November 2002, abnormal high mortality of cattle was reported in the village of Bangahani. Treatment with antibiotics and anthelmintics had no effect on the outcome of the disease and by the end of March 2003 already more than 500 animals had succumbed. A common feature characterising all diseased animals was the sharing of grazing land on the high plateau of Haboho-Itsoundzou with a herd of 300 cattle, recently imported from Tanzania. These animals were introduced into the country without quarantine or any other preliminary veterinary control. Some of these imported animals fell ill after arrival and about 20 of these animals died or had to be culled. At this stage, the national authorities alerted the Emergency Prevention System (EMPRES) of FAO. Because of the urgency of the situation, FAO contacted the non-governmental organisation Comorian Association of Veterinary Technicians and Veterinary Assistants (ACTIV) to carry out a preliminary epidemiological investigation and an expert was sent to the Comoros in June 2003. Although the cause of the epizootic could not be identified at that time, a tentative diagnosis of theileriosis was made.

The objective of this paper is to investigate the cause and origin of the outbreak and to assess the risk of the disease spreading to the island of Anjouan. Hereto two follow-up missions were organised, respectively, in November 2003 by the Emergency Prevention System (EMPRES) of FAO and in May 2004 by the non-governmental organisation (NGO) ''Vétérinaires Sans Frontières'' (VSF).

2. Materials and methods

2.1. Tests carried out subsequent to the mission of November 2003

The representative of EMPRES, Dr V. Martin, visited three villages (Moidja, Ngnadomboni and Mbeni), affected by the disease in November 2003. Twenty-one animals, of which eight presented clinical symptoms, were sampled and an autopsy was performed on three sick animals (two adults and a calf). Clinical symptoms consisted of poor general condition, hyperthermia

(40–41.5 °C), anorexia, weakness, nasal or ocular discharge, ptyalism, haematuria (in two animals), diarrhoea, enlargement of the prescapular lymphatic ganglia and massive tick infestation all over the body. The autopsy revealed an overall satisfactory condition of the three carcasses, no splenomegaly and a moderate hydrothorax. Prescapular lymph nodes were enlarged and sometimes haemorrhagic, the omasum was dry and the kidney of the autopsied calf presented haemorrhagic foci.

Samples (blood, lymph nodes and ticks) were taken from all 21 animals as well as tissue samples from the 3 autopsied animals. These samples were sent by air to Onderstepoort Veterinary Institute, Pretoria, South Africa for further examination. Smears of blood, brain and lymph nodes were examined for parasites. Indirect fluorescent antibody tests for the detection of *Babesia bovis*, *Babesia bigemina*, *Ehrlichia ruminantium* and *Anaplasma* spp. were carried out as well as a card agglutination test for the presence of *Trypanosoma* spp antibodies. Molecular assays using PCR and probes were appplied for the detection of *Theileria parva* parasites (Allsopp et al., 1993).

2.2. Tests carried out following the mission of May 2004

In May 2004, Dr R. De Deken, representing the NGO ''Vétérinaires Sans Frontières'', visited Grand Comore in order to characterize the *T. parva* stock responsible for the epidemic. In Madjeouéni (north-eastern part of Grand Comore), six bovines were sampled. Four of these presented fever and swollen prescapular lymph nodes. One animal had also an enlarged parotid lymph node. Lymph node biopsies were taken together with blood samples from all six animals. Each animal was also examined for the presence of ectoparasites. All samples were brought over to the Institute of Tropical Medicine of Antwerp (ITMA) for further examination.

In Antwerp, molecular diagnosis was carried out using a semi-nested theileria 18S-rDNA based PCR-RFLP on the six samples (Fig. 2). This test will detect all theileria species including *T. parva* as described by Geysen (2000). First round PCR primers were 18SF4 and 18SR4 with an annealing temperature of 56 °C, and second round primers were 18SF4 and 18S nR with an annealing temperature of 55 °C. The restriction enzyme used for RFLP was *Msp*I at a temperature of 50 °C.

T. parva strain characterisation was carried out by multilocus genotyping using three polymorphic antigen loci, p104, PIM and p150 and a PCR-RFLP approach (Geysen et al., 1999) (Figs. 3–5). Positive results were confirmed by RFLP of the amplicons by the appropriate

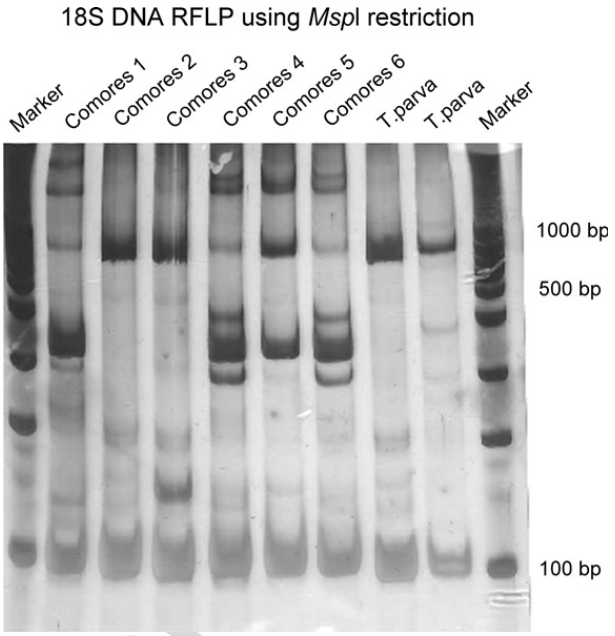

Fig. 2. Species specific RFLP profiles of 18S PCR positive amplicons on silverstained PAGE gel using *Msp*I restriction enzyme. A double band around 100 bp is characteristic for all theileria species. *T. parva* positive profiles (900 bp) are observed in lanes 2, 3 and 5 (Comores isolates) and in the last two lanes (*T. parva* Muguga and Katete controls). Profiles of non-pathogenic *Theileria* spp. were also noticed: *T. velifera* (350 bp) in lanes 1, 4, 5 and 6 and *T. taurotragi* (400 bp and 300 bp) in lanes 4–6. Mixed theileria infections are seen in lanes 4 (*T. velifera* and *T. taurotragi*), 5 (*T. parva* and *T. velifera*) and 6 (*T. velifera* and *T. taurotragi*).

restriction enzymes and profiles compared with the theoretical values.

First round p104 PCR primers were p104 F2 and p104 5 at an annealing temperature of 58 °C, and second round primers were p104 5 and p104 2nF at an annealing temperature of 60 °C. RFLP profiles were obtained by using *AluI* as the restriction enzyme.

First round PIM PCR primers were PIM 1 and PIM R4 with an annealing temperature of 60 °C, while those used in the second round were PIM Fm and PIM R4 with an annealing temperature of 62 °C. The enzyme used was *Bcl I* at a temperature of 50 °C.

The p150 locus was also amplified using first round primers NP150 F and NP150 R at 64 °C and the semi-nested primers NP150 nF and NP150 R at 60 °C. RFLP profiles were obtained by using *Eco571* as the restriction enzyme.

Sequences of all primers used are found in Table 1.

Further characterisation was done using the microsatellite PCR assays MS8 (chrom I), MS3 (I) and MS19 (II) (Fig. 4) and minisatellite ms3 (I) and ms8 (III) PCR assay as described by Oura et al. (2003). The only difference was that poly-acryl amide gel electrophoresis (PAGE) was used.

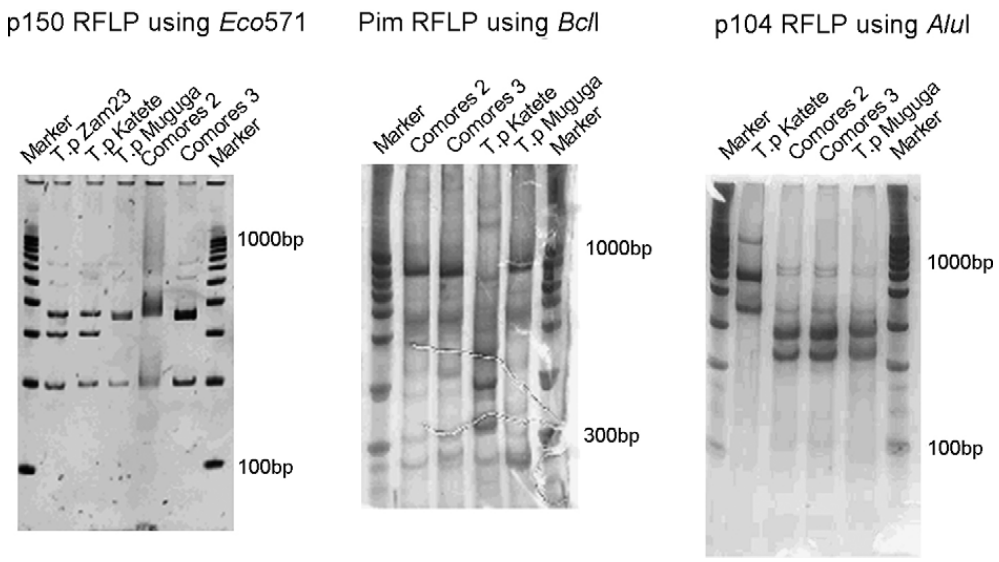

Fig. 3. Isolate specific RFLP profiles on silverstained PAGE gels using three polymorphic antigen loci: p104, PIM and p150. *p150 RFLP profiles*: Muguga and Comores profiles, 372 bp ×2 and 200 bp; Zam23 and Katete profiles, 366, 312 and 207 bp. *Pim RFLP profiles*: Muguga/Comores profile, 900 bp and 320 bp/280 bp; Katete profile, 450–400 and 320 bp. *p104 RFLP profiles*: Muguga/Comores, 281 bp/277 bp and 221 bp; Katete, 498 and 335 bp. *T. parva* Muguga and Katete were included as controls in the PIM and p104 assays. *T. parva* Muguga, *T. parva* Zam23 and Katete were included as controls in the p150 assays.

Molecular diagnosis of *E. ruminantium* was performed using a semi-nested PCR on blood and buffycoat of the sampled animals. The primers used in round 1 were ITM 130 and AB 129 with an annealing temperature of 62 °C. Those used in round 2 were AB 128 and AB 129 with an annealing temperature of 58 °C.

2.3. Tick sampling on the island of Anjouan

In an attempt to assess the risk of ECF spreading to the island of Anjouan and infecting the local herd of dairy cattle, local veterinary technicians sampled ticks in July 2005 and send them to ITMA for identification.

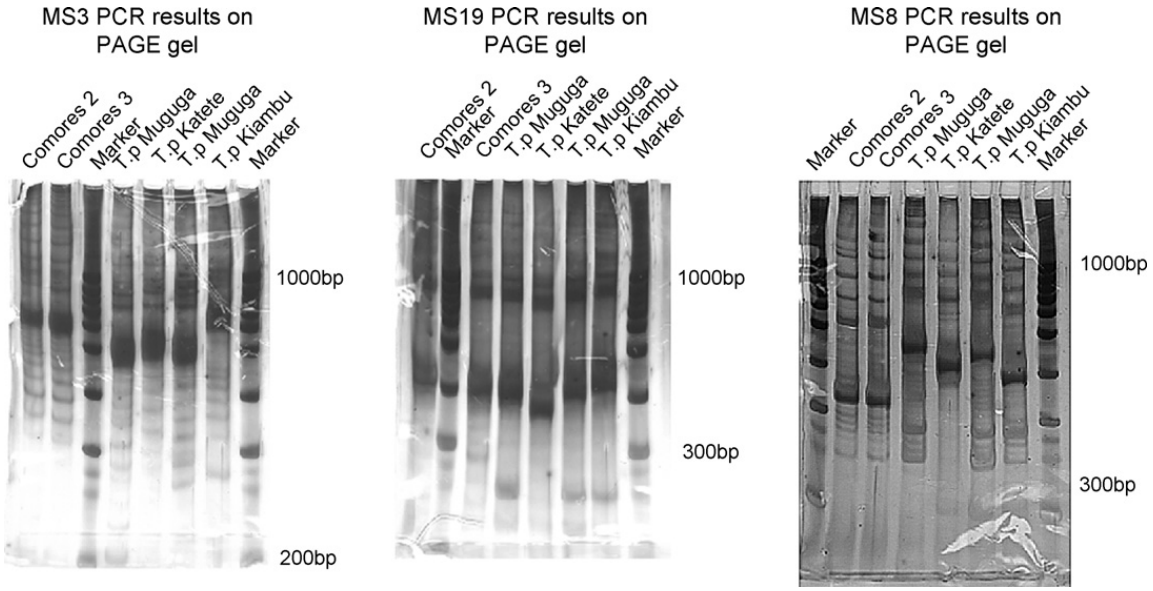

Fig. 4. Isolate specific RFLP profiles on silverstained PAGE gels using the microsatellite PCR assays MS3, MS8 and MS19. *MS3 RFLP profiles*: Kiambu/Comores profile, 600 bp; Muguga, 490 bp; Katete, 510 bp. *MS19 RFLP profiles*: Muguga/Comores profile, 400 bp; Kiambu, 430 bp; Katete, 380 bp. *MS8 RFLP profiles*: Comores profile, 420 bp; Muguga, 550 bp; Kiambu, 490 bp; Katete, 530 bp. *T. parva* Muguga, Kiambu and Katete were included as controls in the MS assays.

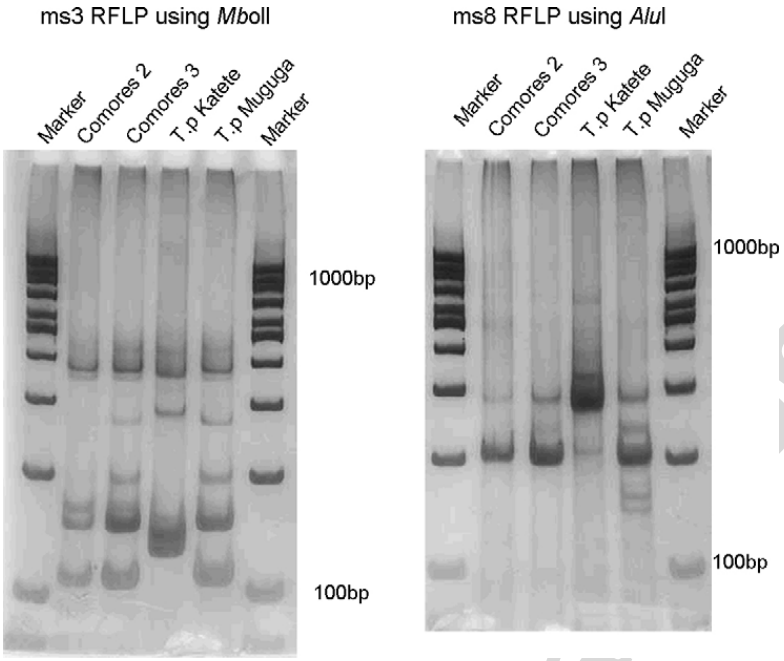

Fig. 5. Isolate specific RFLP profiles on silverstained PAGE gels using minisatellite ms3 (I) and ms8 (III) PCR assay. *ms3 RFLP profiles*: Muguga/Comores profile, 170 and 120 bp; Katete, 150 bp/140 bp. *ms8 RFLP profiles*: Muguga/Comores profile, 200 bp; Katete, 300 bp. *T. parva* Muguga and Katete were included as controls in the minisatellite assays.

3. Results

Members of APSA told the authors to have observed at the start of the outbreak in November 2002, multiple clinical symptoms among the diseased animals in the village of Bangahani. These consisted of fever, agitation, anorexia, mucopurulent nasal flow, salivation, weakness, staggering gait, swollen lymphatic glands, diarrhoea, sometimes haemorrhagic, occasionally nervous symptoms and jaundice. At autopsy splenomegaly, lymphadenitis, ascites, haemorrhages, pulmonary oedema, hydropericard, petechiae on the intestines and heart as well as liver necrosis was observed. The majority of the sick animals had died yet some had recovered. An expert of EMPRES was sent to the Comoros in June 2006 and a tentative diagnosis of theileriosis was made on the basis of clinical symptoms and a therapeutic experiment carried out by ACTIV, i.e. buparvaquone treated herds had shown a significant lower mortality (6% of the animals treated) than those treated with oxytetracycline (42%). However, as treated animals had not been selected randomly over the villages, the test did not allow drawing a final conclusion on the cause of mortalities.

Table 1
Primer sequences of semi-nested PCR assays for the 18S rDNA, p104, p150 and PIM loci of *Theileria parva*

Primers	Sequences	PCR round	Primerpair	Annealing temperature (°C)
18SF4	CGTTTTTACATGGATAACCGTGCTAA	1	18SF4/18SR4	56
18SR4	TAAAAACTGACGACCTCCAATCTCTAGT	2	18SF4/18SnR	55
18SnR	GGCATTGTTTATGGTTAGGA			
P104F2	CCACCATCTCCTAAACCACCGTT	1	P104F2/P104 5	58
P104 5	TAAGATGCCGACTATTAATGACACCACAA	2	P104 5/P104 2nF	60
p140 2nF	AACCACCGTTTGATCCATCATTCA			
NP150R	TTACCATCTTCACCGCGAAC	1	P150F/P150R	64
NP150F	GATATTCCTTTACTTGCTCGAC	2	P150nF/P150R	60
NP150nF	CGACTTGAAGAAGAAGATTACAGT			
PIM1	GTGAATGTTGTGATCTTAATCC	1	PIM 1/PIM R	60
PIMR4	CCCACAACCGTGGAATGGCGTA	2	PIM Fm/PIM R	62
PIMFm	ATTCCACTGGTTCTTCCGATSTA			

Table 2
Overview of laboratory tests carried out on the animals sampled in November 2003

Disease status of the 21 bovines sampled	No. of animals theileriosis-positive in PCR or probe	No. of animals *E. ruminantium* antibodies	No. of animals *Babesia bovis* antibodies	No. of animals *Trypanosoma* spp. antibodies
8 animals with clinical symptoms	6[a]	2	2	2
13 animals without clinical symptoms	2	5	5	1

[a] Three of these animals were also found positive for other pathogens (*E. ruminantium* + *B. bovis*, *B. bovis*, *Trypanosoma* sp.).

Table 3
Overview of laboratory tests carried out on animals sampled in May 2004

Animal	Clinical symptoms	PCV	Schizonts in ln gland	Piroplasms in blood	Molecular diagnosis	Molecular characterisation
1	Yes	35	0	0	–	Not done
2	Yes	31	+	+	*T. parva*	Muguga profile
3	Yes	36	+	+	*T. parva*	Muguga profile
4	No	37	0	0	–	Not done
5	Yes	27	+	+	*T. parva*	–
6	No	36	Not done	0	–	Not done

Slaughter, sales and live imports of cattle into the Union of Comoros had been suspended by a ministerial decision of June 14, 2003 and the Comorian government had informed the population about the epidemic. This had resulted in cattle owners moving their animals away from the outbreak area and as such had assisted in the spreading of the disease.

At the time the second EMPRES representative visited Grand Comore local veterinary services estimated cattle losses due to the disease outbreak at approximately 10% of the total stock on the island. Mortalities were confined to the cattle-breeding area of the island (central and central-west area).

Table 2 gives an overview of the tests carried out at Onderstepoort Veterinary Institute on the samples collected in November 2003. Molecular tests revealed the presence of *T. parva* in 38% ($N = 21$) of the sampled animals. Although they were not always pathognomic for ECF, clinical symptoms were observed in six of these eight animals. One-third of all ($N = 21$) examined animals showed antibodies against *E. ruminantium* and a similar number of animals had antibodies against *B. bovis*. Three animals showed a positive card agglutination test for *Trypanosoma* spp. No antibodies against *B. bigemina* or *Anaplasma* spp. were found. Ticks collected on the animals were identified as *Rhipicephalus appendiculatus*, *Amblyomma variegatum* and *Boophilus microplus*.

In May 2004 veterinary services estimated that since the start of the outbreak approximately 4750 animals died of the disease or had been culled. Meanwhile, more and more owners fled with their animals from the high plateau towards the coast thereby introducing the disease into unaffected zones (Fig. 1). Although cattle owners were willing to pay for treatment, there was an inadequate supply of specific drugs. Neither the private

Table 4
Multilocus profile comparison of the various *T. parva* isolates from the Comores, the Muguga cocktail strains and an exotic Zambian strain

Isolates/locus	PIM	p104	p150	ms3	ms8	MS3	MS19	MS8	Multilocus
Comores 2	A	a	g	k	p	s	d	v	Aagkpsdv
Comores 3	A	a	g	k	p	s	d	v	Aagkpsdv
MugugaCC[a]	A	a	g	k	p	t	d	w	Aagkptdw
KiambuCC[a]	C	a	g	k	p	s	e	y	Cagkpsey
Katete, Zambia	B	c	h	m	r	u	f	x	Bchmrufx
Total allele no.	>20	4	3	5	4	5	5	7	

PIM, Polymorph immunodominant molecule coding locus; p104, polymorphic p104 antigen coding locus; p150, polymorphic p150 antigen coding locus; ms3 and ms8, *T. parva* minisatellites; MS3-MS19-MS8, *T. parva* microsatellites. Nomenclatures of different alleles relate to observed RFLP profiles.

[a] CC: *T. parva* Muguga vaccine cocktail components.

sector nor the Comorian government were willing or able to fund drug purchases. It is also of interest to report that an attempt of Comorian cattle-dealers to export some of the Tanzanian cattle from Grand Comore towards Anjouan could be prevented in time.

Table 3 gives an overview of the tests carried out at ITM, Antwerp on the samples collected in May 2004. *T. parva* parasites were abundant in the lymph node smears of three of the cows. *Theileria p*iroplasms were also found in the red blood cells of these animals. Microscopic examination did not reveal any other blood parasite. Molecular tests confirmed the presence of *T. parva* in these animals (Fig. 2). Further multilocus characterisation of these *T. parva* positive infections was done using three polymorphic antigen genes (p104, PIM and p150) and five different micro/minisatellite markers, mainly on chromosome 1–3 (Table 4). Only two animals were found positive in these single copy PCR assays. All three antigen loci gave profiles identical to the Muguga isolate (Fig. 3). This isolate originating from Central Kenya is part of the sporozoite based live cocktail vaccine, used on the continent in Tanzania. The Comores isolates were identical to each other and showed profiles identical to Muguga in three (ms3-ms8-MS19) and Kiambu (the other component of the cocktail vaccine) in one (MS3) satellite assays (Figs. 4 and 5).

Characterisation of the *T. parva* stocks showed that the multilocus genotype of the parasite stock from the Comores was that of *T. parva* Muguga with part of chromosome 1 of *T. parva* Kiambu (Fig. 4).

A single animal tested positive in the PCR-test for *E. ruminantium* using a large quantity of DNA extract.

Five of the six animals from Grand Comore examined in May 2004 carried *A. variegatum* ticks while *R. appendiculatus* ticks were found on four animals. *Hippobosca rufipes* and *Haematopinus quadripentusus* were also observed.

Three of the four cows from Anjouan sampled in July 2005 carried *Rhipicephalus (Boophilus) microplus*, two carried *A. variegatum* ticks and two were parastised by *H. quadripentusus*, while *R. appendiculatus* was absent on all of them.

4. Discussion

A few decades ago, the Union of Comoros enjoyed a satisfactory sanitary situation due to its isolation and the practice of tethering animals. These factors contributed to low infection risks and almost no spreading of diseases on the island. The situation changed considerably in 1986, when the emergence of bovine spongiform encephalopathy in Europe boosted the import of live animals from Madagascar as an alternative to the import of frozen meat from Europe. However, the import of live animals along this new trade route resulted in the introduction of some diseases in the country. Import of livestock from Madagascar was likely the cause of contagious echtyma in 1999 and of the introduction of blackleg in 1970 and 1995 into the Comoros (Timmermans et al., 2000). The risk of importing devastating diseases (e.g. foot and mouth disease, Rift Valley Fever, rinderpest, theileriosis) increased even more when in 2002 for political reasons the import of live animals from Madagascar was suspended in favour of animals coming from the African mainland. Moreover, the poor status of the Comorian veterinary services, the absence of national contingency plans for animal diseases and of an effective quarantine policy at the borders as well as the poor application of veterinary legislation made the country particularly vulnerable. In addition, an accurate and rapid diagnosis of transboundary diseases is essential in order to take appropriate measures but in the Comorian situation an early diagnosis was problematic due to the lack of experienced laboratory technicians and technical infrastructure.

The good correlation between clinical symptoms and the presence of *T. parva* as detected by molecular techniques (Table 2) and microscopic examination confirms the role of East Coast Fever as the main culprit causing fatalities among cattle on Grand Comore. In some cattle antibodies to *E. ruminantium*, *Trypanosoma* spp. or *B. bovis* were found, yet more frequently in healthy animals than in those with clinical signs (Table 2). Cowdriosis is known to occur on the island of Mayotte (Camus et al., 1998) but the situation on Grand Comore is not known. However, it is unlikely that *E. ruminantium* was the main cause of the disease as neither abnormal mortality nor neurological signs have been reported in small ruminants on this island and no endothelial rickettsias were seen in brain smears of autopsied cattle. Moreover, the animal, positive in a PCR-test for *E. ruminantium* in May 2004, did so only after a larger quantity of DNA extract was used, meaning that the animal was most likely not an acute case but a carrier.

The haematuria or haemoglobinuria, observed in two animals during the EMPRES mission, is more indicative for babesiosis, again no *Babesia* piroplasms were observed in blood nor in brain smears. The presence of antibodies against *Trypanosoma* spp. in Comorian cattle is surprising, as the biological vector does not occur on the island. This could be either a false

positive test due to a lack of specificity of the CATT-test, or positive sera coming from imported cattle, which previously had been in contact with the parasite. It could also be due to a mechanically transmitted trypanosome occurring on the island.

It seems difficult to trace back the multiple clinical symptoms observed at the onset of the outbreak to one single cause, yet it may well be that the effect of other pathogens came to light because of the debilitating effect of theilerioses. Several endemic diseases (e.g. cowdriosis, blackleg, distomatosis and anaplasmosis) have been reported on the islands (Hamers et al., 1993; Camus et al., 1998) and might have increased the mortality and blurred the clinical picture.

In June 2003, the Comorian government alerted the population of the islands about the existence of an epidemic. The warning did however not result in a decrease of losses as owners tended to cull their animals as soon as clinical symptoms, whether or not typical, became apparent, e.g. animals presenting no other signs than haematuria were culled. The effect of the sensibilisation campaign on the spread of the disease resulted in the movement of animals from the epidemic on the high plateau of Haboho-Itsoundzou to more remote pastures on the high plateau. Later on, when the disease became apparent on the entire high plateau, livestock was moved towards the coastal areas (Fig. 1) resulting in spread of the infection over the entire island.

Although no direct cause/effect relation between the import of cattle from Tanzania and the epizootic could be established with certainty, several factors indicate that such relation was indeed the case. Cattle, grazing in the neighbourhood of the imported animals, were the first to show clinical symptoms. Furthermore, characterisation by multilocus genotyping revealed that the *T. parva* parasites isolated on the Comoros are identical to the Muguga or Kiambu stocks, which are two of the three components of the Muguga cocktail vaccine used to immunise cattle on a large scale in Tanzania (Figs. 3–5). The third stock (Serengeti transformed) has been shown to be genetically very similar to the Muguga stock (Bishop et al., 2001; Oura et al., 2004) and gave identical profiles as Muguga in all tests. Therefore, it is probable that some of the animals imported from Tanzania had been immunised in Tanzania with the Muguga cocktail and thus became effective carriers of the disease (Kariuki et al., 1995). In view of the rapid spread of the disease over the whole island *R. appendiculatus* must already have been present on Grand Comore. The tick probably got infected by feeding on the Tanzanian carrier animals and then transmitted the disease to the local cattle. Although cattle immunised in Tanzania by the ''immunisation and treatment method'' are specifically tagged, no such eartags were found on the Tanzanian cattle imported into the Comoros. This does not exclude the role of Muguga cocktail carriers in the Comore epidemic as either the tags could have been removed before exporting the animals, or the animals or some of them got infected while still in Tanzania by ticks carrying one of the strains of the Muguga cocktail after they fed on Muguga cocktail vaccinated animals.

5. Conclusion

The fact that local authorities and private veterinary services were unable to identify the cause of the epizootic illustrates the urgent need for a veterinary diagnostic laboratory and trained personnel to carry out basic analyses and follow-up the health status of the Comorian livestock. The Comorian authorities need to work out a control strategy against the disease and its vector in order to alleviate the problem. In addition, strict regulations on the import and export of livestock will have to be implemented in order to prevent the occurrence of similar problems and the spread of the infection into unaffected areas.

Whether *R. appendiculatus* can establish itself on the other islands of the Comoros, is as yet unknown. Analysis of recently sampled ectoparasites from cattle in Anjouan did not reveal the presence of the tick but the number of sampled animals was low and the sampling was carried out only once. We can conclude that there is a sereious risk that *R. appendiculatus* could establish itself on the other islands of the Comoros, and would sooner or later introduce East Coast Fever unless appropriate measures are taken.

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