



## Trypanosoma evansi: Recent outbreaks in Europe

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### ABSTRACT

Here, two recent outbreaks of *Trypanosoma evansi* infection in mainland France and Spain associated with the importation of dromedary camels from the Canary Islands, are reported. The disease is well-known on the Archipelago since 1997 and many efforts have been made towards control and eventual eradication, but some areas still remain affected. Both mainland outbreaks were controlled by means of massive treatments and monthly serological, parasitological and molecular (PCR) evaluations carried out by Valencian Regional Animal Health laboratory and by CIRAD, Montpellier, respectively. Possible causes for the persistence of the parasite in a small area of the Canaries are discussed. *T. evansi* must be included among the animal health conditions for international trade within the European Union as well as many other countries. Moreover, procedures including diagnosis, curative or preventive treatment and quarantine should be established to insure the status of the animals moving from a country to another.

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### 1. Introduction

*Trypanosoma evansi* is the most widely distributed of the pathogenic African trypanosomes of animals, affecting domestic livestock and wildlife in Asia, Africa and Latin America (Luckins and Dwinger, 2004). *T. evansi* originated in Africa and spread to South America and Asia with the exportation of animal hosts from Africa, including camels, horses and mules (Hoare, 1972). The parasite causes a disease known as surra, and is mechanically transmitted by biting flies. The vampire bat is a vector in South America. It affects a number of domestic animals and the principal host species varies according to geographical location. Buffalo, cattle, camels and horses are particularly susceptible, although other animals, including wildlife, can also be infected (OIE, 2008).

Dromedary camels (*Camelus dromedarius*) were introduced into the Canary Islands six centuries ago from the near West African coast for labor, but are currently raised for tourism. With an approximate census of 2000 camels, the Canary Islands harbour the most important camel population in Europe. Since exportation of camels out of Africa is very difficult due to health regulations, many camels imported to European and South America during the last decade came from the Canary Islands. However, *T. evansi* has been known to be present in the Canary Islands since 1997, when the first case was diagnosed in a dromedary camel imported from Mauritania (Gutierrez et al., 2000). Despite this published evidence, *T. evansi* has not been included in the animal health conditions for international trade within the European Union and other countries. This failure to recognize *T. evansi* as endemic within the European Union has caused two recent outbreaks of *T. evansi* infection, one in metropolitan France in 2006 (Desquesnes et al., 2008) and another in continental Spain in 2008 (Tamarit et al., 2010). Both outbreaks were associated with

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the import of camels from the Canary Islands without prior checking for potential *T. evansi* infection. In this article we summarize these recent surra outbreaks observed on mainland Europe.

## 2. *Trypanosoma evansi* in the Canary Islands

*T. evansi* was diagnosed for the first time in the Canary Islands in 1997 in a dromedary camel presenting the chronic stage of the disease (Gutierrez et al., 2000). Afterwards, in collaboration with the Institute of Tropical Medicine (Antwerp, Belgium), 745 camels were examined by serological (card agglutination test (CATT/*T. evansi*)) and parasitological methods (micro-hematocrit centrifugation technique and mouse inoculation) (Verloo et al., 1998; Woo, 1969). Thirty-six camels were CATT/*T. evansi* positive, indicating presence of circulating *T. evansi* specific antibodies. The parasite was identified in 7 of these. A further seroprevalence study was carried out using an Ab detection ELISA. Four hundred and forty-four camels were randomly selected from Lanzarote, Fuerteventura, Gran Canaria and Tenerife. Forty animals were positive in ELISA, resulting in a seroprevalence of 9% (Molina et al., 2000). Following these observations, a control program was established to avoid the spread of the disease among the different islands and to eventually eradicate *T. evansi* from the Canary Islands. To this aim, melarsomine (0.25 mg/kg body weight; Cymelarsan®, Merial, France) was administered to all camels with clinical signs of surra as well as to all asymptomatic animals that were positive by parasitological and/or serological testing. Annual serological surveys with CATT/*T. evansi* showed that the seroprevalence progressively decreased and seroprevalence reached 0% on the islands of Tenerife, Fuerteventura and Lanzarote in 2003 and has remained such as of 2008. The only currently affected island is Gran Canaria, where it has not been possible to control the disease in a limited area. Although the entire camel population in this area (around 200 heads) has been treated twice (0.25 and 0.5 mg/kg b.w.) with Cymelarsan®, about 5% of the animals has remained parasitologically positive at micro-hematocrit centrifugation technique (Woo, 1969). Two possibilities may explain this persistence: lack of treatment efficacy and subsequent relapse of infection or the presence of a reservoir in the affected area and subsequent re-infection. To assess the latter possibility, horses and small ruminants living on farms located within the affected area are currently being examined in order to detect *T. evansi* infection. Only seropositive animals (by CATT/*T. evansi*) have been observed at present and infection has not been confirmed by parasitological techniques. However, this does not rule out a reservoir since goats are susceptible to experimental infection with the Canarian strain of *T. evansi* (Gutierrez et al., 2004). Small ruminants like goats are often raised close to camel habitats in the Canary Islands and in many other regions of the world and may be an important reservoir of *T. evansi*. Other possible reservoirs include wild rodents, particularly rabbits, rats and mice that are present in the *T. evansi*-affected areas in Gran Canaria.

## 3. *Trypanosoma evansi* in mainland Spain

*T. evansi* was observed in mainland Spain in February 2008 in a dromedary camel that presented clinical symptoms including progressive weight loss, severe anemia and weakness (Tamarit et al., 2010). The animal belonged to an equine and camel farm located in Alicante province and had been imported from Gran Canaria about 6 months earlier. Trypomastigote forms were observed in stained blood smears and were identified morphologically as *T. evansi*. The animal was treated with Cymelarsan® (0.25 mg/kg b.w.) and showed clinical improvement within two weeks. After this first case, a prevalence survey was carried out on all camels and horses present in the farm. According to the official report of Spain to the Organisation Internationale des Epizooties (OIE), of 21 camels (*C. dromedarius*) tested, 12 were positive and of 67 equines tested, 2 were positive (OIE-WAHID, 2008). All serologically and parasitologically positive animals were isolated until melarsomine treatment was administered (Cymelarsan®, 0.5 mg/kg b.w.), and were kept isolated for 2 months. Serological and parasitological evaluations are being carried out on all camels and equines with consistently negative results. Thus, the sanitary measures taken by the Regional Veterinary Services, Valencian Government (Generalitat Valenciana) appear to have controlled the outbreak.

## 4. *Trypanosoma evansi* in mainland France

*T. evansi* was detected for the first time in metropolitan France in 2006 on a sheep and camel farm in the Aveyron Department (Desquesnes et al., 2008). Eight camels were already present on the farm before the outbreak, some of which had been imported from Gran Canaria in 1995 and which did not present any health problem. In June 2006, 5 more camels were imported from Gran Canaria. Three-and-a-half months later, one of them developed weakness, weight loss and anemia leading to death. Post-mortem blood samples were taken and *T. evansi* was detected by smear examination. Blood was taken from the 12 remaining camels, and parasitemia was evidenced in 5 of them. Of these 5, 2 camels had been recently imported, but 3 were previously present on the farm. It is likely that the recently imported camels were responsible for the transmission of the parasite among the other camels through tabanids and stable flies. The surrounding areas were surveyed by sampling livestock and dogs. Serologically positive sheep were found and sacrificed, while parasitemia was not detected in any animal.

When the first cases were observed, all camels were treated with melarsomine (Cymelarsan®, 0.25 mg/kg IM, October–November 2006) and later with quinapyramine sulphate/chloride (Triquin®, 3.75 mg/kg IM, December 2006) and followed-up monthly by parasitological, PCR and serological examination (CATT/*T. evansi* and ELISA/*T. evansi*). Treatment was efficient in 11 camels that remained negative to all tests up to date. One camel however relapsed 8 months later. Interestingly, a complete history revealed that this animal had been underdosed, due to an incorrect evaluation of its body weight (Desquesnes et al., 2009). In August 2007, this animal was retreated with a higher dose

(5.25 mg/kg IM) of Triquin<sup>®</sup> where after trypanosomes disappeared from its blood. At the same moment, all the other animals were retreated with Triquin<sup>®</sup> at 3.75 mg/kg IM. In October 2007, all animals were retreated with a higher dose of Cymelarsan<sup>®</sup> (0.5 mg/kg IM). Follow-up indicated that the relapsed animal was definitively cured since it has remained negative to all tests for over 20 months to date. Periodical assessment indicates that the sanitary measures taken by the French National Veterinary Services and collaborators should be adequate.

### 5. Features of the *Trypanosoma evansi* Canarian strain

Many camels have been imported or have travelled from the Canaries to the European mainland (zoos, circuses, etc.) without any previous examination to detect *T. evansi* infection. Based on the Canarian Animal Health Department's information, approximately 220 dromedaries have been exported to European countries during the last 10 years. Thus, it is important to be aware of the features of the Canarian strain of *T. evansi* in order to detect the appearance of the disease as soon as possible.

The Canarian strain usually causes the chronic form of the disease, with progressive weight loss, oedema (normally of the prepuce and hind limbs), abortions (coinciding with elevated parasitemia in pregnant females), and emaciation, with fatal consequences if not treated. Natural infection can be transmitted to equines living in close contact with infected camels, and weight loss and edema are also the main clinical signs seen in affected equines. Goats have been experimentally infected, and they usually present with the sub-clinical form of the disease, although arthritis has been observed in some cases. Hematofagous insects, particularly *Stomoxys calcitrans*, are commonly involved in the transmission of the disease in the Archipelago. Reliable diagnostic tools include the micro-hematocrit centrifugation technique (Woo, 1969) for microscopic identification of the parasite and CATT/*T. evansi* for serological testing. These are both widely used given their high sensitivity and ease of application in the field. In cases of seropositive animals without parasitemia, more sensitive parasitological or molecular techniques such as inoculation in mice and PCR (pMURT, Njiru et al., 2004; 18S rDNA and RoTat 1.2, Deborggraeve et al., 2008) should be performed. However, if the parasite cannot be detected, seropositive animals should be re-evaluated periodically in order to confirm/exclude infection. Finally, the use of trypanocides such as melarsamine or quinapyramine, both commercially available, is highly effective and parasites are commonly cleared from the blood by 24 h after treatment.

### 6. Discussion

The occasional introduction of *T. evansi* into countries free of the parasite has most often resulted in the parasite becoming endemic (Hoare, 1972). The introduction of *T. evansi* into the Canary Islands, and later onto mainland Europe, is clearly the result of lack of control measures.

These should now be adopted urgently by the different European countries in order to face the current situation. The Agriculture and Livestock Council, Regional Government of the Canary Islands, has implemented veterinary services for *T. evansi* control on camel farms and surrounding areas. Thanks to this service, the disease is currently considered controlled and has likely been eradicated from the islands, where seroprevalence has been 0% for over 5 years. Furthermore, movement of camels among the islands is only permitted when *T. evansi* infection has been ruled out and only within those areas where *T. evansi* is absent. However, it should be kept in mind that no single diagnostic test is 100% sensitive and 100% specific and therefore combined use of different tests (serological, parasitological and molecular) is recommended to detect the presence of *T. evansi*. Furthermore, national veterinary services should establish continuous surveillance measures, because re-infection of animals from an unknown reservoir cannot be ruled out. For areas still affected in Gran Canaria, the dissemination of the disease is controlled by treating the affected camels, assessing the animals periodically and controlling biting insect populations with insecticides.

Compared to the persistent infections observed in the past (Hoare, 1972), it should be emphasized that progress has been made in diagnosis, with the development of sensitive and specific techniques recommended by the OIE (2008), including CATT/*T. evansi*, ELISA or PCR. Progress has also been made in the treatment of surra with the use of melarsamine (Cymelarsan<sup>®</sup>) that is a highly effective trypanocide against *T. evansi* which is thought to cross the blood brain barrier to some extent. Proper use of these new techniques and drugs should contribute to the control of this disease.

Concerning importation procedures, *T. evansi* should be absent not only in the country of origin, but also in the country of destination. Quarantine and evaluation of animals must be carried out before they can be relocated to a surra-free area. That the exporting country be officially free of *T. evansi* is a logical expectation, but in our opinion it is not enough. *T. evansi* may be present in a country that has not declared infection status to the OIE or other animal health organizations. However, simple serological testing of animals that will be imported to surra-free areas with CATT/*T. evansi*, as recommended by OIE (2008), would be sufficient. We hypothesize that if these diagnostic measures had been established the outbreaks in continental France and Spain probably would not have occurred.

Until recently, countries were obligated to declare to the OIE surra outbreaks only in equine species, while infection in other animal species was excluded. Recently, this limitation was modified at the OIE Executive Committee meeting held in May 2008: surra is now considered an "OIE listed disease – multi-species" – to be reported to the OIE in the same way as the previously listed trypanosomes (Dourine, surra in horses, tsetse transmitted trypanosomoses) (see: OIE Manual of Diagnostics for terrestrial animals, edit. 17 July 2008, online).

Finally, it is important to note that *T. evansi* probably exists in all countries where the camel, particularly the dromedary camel, is present with the exception of Australia. For this reason, procedures including diagnosis,

curative or preventive treatment and quarantine should be established to insure the status of the animals moving from one country to another.

### Conflict interest statement

The authors declare that they have no conflict of interest with the contents of this paper in any respect.

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