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Paper

Outbreaks of African horse sickness in Senegal, and methods of control of the 2007 epidemic

N. D. Diouf, E. Etter, M. M. Lo, M. Lo, A. J. Akakpo

Since first being detected in Nigeria in January 2007, African horse sickness virus serotype 2 (AHSV-2) has spread throughout the northern hemisphere, and was first reported in Senegal. A retrospective study was conducted from December 2009 to April 2010 using data collected in the field combined with information available at the Direction of Veterinary Services. The epidemic started in the Dakar region with two outbreaks in March and June 2007, respectively, and spread in several parts of the country between July and November 2007. During this period, 232 outbreaks and 1137 horse deaths were reported. The epidemic was controlled by mass vaccination using a polyvalent-attenuated vaccine. This retrospective study was conducted with various assumptions of AHSV-2 introduction, and provides recommendations for implementing an early warning surveillance system for African horse sickness in Senegal.

Introduction

African horse sickness (AHS) is caused by an orbivirus (family Reoviridae) that is mainly transmitted by adult *Culicoides* midges (Diptera, Ceratopogonidae) (Venter and others 2009, Wilson and others 2009). Nine serotypes of the AHS virus have been described (Howell 1962). The virus is endemic in sub-Saharan Africa, particularly eastern and southern Africa (Leforban and others 1983, Blackburn and Swanepoel 1988, Mellor and Hamblin 2004), but outbreaks have occasionally been reported in North Africa (Pilo-Moron and others 1967), the Middle East (Yasarol 1962, Maurer and Cully 1963, Parker 1974, Hassanain and others 1990), and southern Europe (Rodriguez and others 1992, Portas and others 1999). Senegal is a coastal country in West Africa between the Atlantic Ocean, Mauritania, Mali, Republic of Guinea and Guinea Bissau. The disease was first described in this country in 1942 (Mornet 1949). In the late 1980s, there have been repeated outbreaks in several parts of Senegal with more than 100 reported cases of AHS (Sarr and others 1988). AHS virus serotype 9 (AHSV-9) was identified as the only serotype circulating in West Africa (Adeyefa and Hamblin 1995, Sailleau and others 2000). Then in January 2007, AHSV-2 was reported in Nigeria, and it was the first such report in the northern hemisphere (Folorunso and others 2008, Anon 2010). The most

recent epidemic occurred in Senegal in 2007. During this outbreak, AHSV-2 was isolated for the first time. Numerous areas were infected. Starting in Dakar region in March and June 2007, the disease spread to several parts of the country between July and November 2007. The introduction and the spread of this new serotype within the country have not been well investigated. Therefore, a retrospective study was conducted with various assumptions. This study will focus on the understanding of mechanisms through which the disease spread to better prepare control measures against another AHS virus threat.

In this paper, details of the 2007 outbreaks in Senegal are described with the control methods adopted by the government to stop the disease spread. Also, recommendations are provided for implementing an early warning surveillance system for AHS in Senegal.

Materials and methods

Until 2007, Senegal was administratively divided into 11 regions, and additionally partitioned into 34 departments divided into rural communities. By 2008, it was again reorganised into 14 regions. However, during this study, we will refer to the former Senegal's 11 regions as they existed in 2007 at the beginning of the outbreak. During the Senegal AHS outbreaks in August 2007, data have been collected by the Direction of Veterinary Services through the Regional Veterinary Services Inspections. By this method, data collection was performed in real time with status reports on the epidemic.

This retrospective study took place between December 2009 and April 2010. Data collected by the Regional Veterinary Services Inspections were combined with those collected by the Direction of Veterinary Services, and several national veterinarian and livestock agencies were analysed for this study. Regions where horse deaths were reported, were visited and investigations conducted with horse sellers and owners in all large commercial horse markets (Mpal, Dahra, Dinguiraye, Birkelane, Sagatta Guet, Touba, Toubatoul) within the country. Interviews were also organised with the Director of Veterinary Services and its team, the Director of the Livestock department, the Director of the Equine Breeding department and its team, each of the Regional Veterinary Services Inspectors whose region was affected by AHS outbreaks, a group of veterinary practitioners and heads of commercial horse markets.

ArcGIS V.9.3 software was used for mapping.

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Results

Description of the outbreaks

A first outbreak of AHS occurred from March 16 to April 6, 2007. The disease was reported in five stud farms in Niaga belonging to Rufisque department located in Dakar region where 15 horses out of 214 died (7 per cent of the population). The horses that died during this epidemic were thoroughbreds and crossbreds. The fatalities due to AHS brought into doubt the quality of the monovalent vaccine against AHSV-9 produced by the National Laboratory (LNERV) of the Senegalese Research Institute in Agriculture, and used each year for the national vaccination campaign organised within the country.

Between May 21 and June 1, 2007, the disease reappeared in two stud farms in Petit Mbao, Dakar region. During this period, eight horses died, followed by seven other horses, putting the number of deaths at 15 horses out of 65 exposed horses. Those fatalities included 12 horses of exotic breeds, and three mares of local breeds. Sick horses presented hyperthermia, anorexia, supraorbital oedema, frothy nasal exudate, conjunctivitis. Postmortems revealed pulmonary conjunctiva and oedema, pleural extrusion, petechiae on trachea with curds, cerebral haemorrhage, spleen conjunctiva, hepatomegaly, splenomegaly. These signs strongly implicated AHS.

It is important to note that there are three clinicopathologic syndromes associated with AHS virus infection in horses. These different forms of AHS (pulmonary, cardiac and fever forms) vary in the organs affected, the severity of lesions, time of onset of clinical signs and mortality rates (Laegreid and others 1993).

As soon as AHS was confirmed, an emergency plan was made by the veterinary administration. Horses within a defined risk zone were not allowed to leave the department of Rufisque (Dakar region). Within the department, leisure activities with horses, such as horse racing, were forbidden. These two measures remained in place until 40 days after the last case reported in Niaga.

The Ministry of Livestock decided to send whole blood, sera and organ samples to independent laboratories to confirm the biological diagnostic result of the LNERV by performing serotyping. Samples were sent to the World Organisation of Animal Health (OIE) reference laboratories for AHS virus and Bluetongue virus (BTV) in Pirbright (UK) and Onderstepoort (South Africa). Samples of the monovalent serotype 9 vaccine were also sent to those laboratories for quality control.

The results of the serologic controls carried out on horses in stud farms in Dakar, led to two worrisome discoveries. First, the tests revealed that horses that had been inoculated and had enough antibodies to AHSV type 9, had died, nonetheless. Secondly, a number of horses inoculated with the type 9 vaccine (15–25 per cent depending on the stud farm) had very low antibody counts against the same serotype. These two discoveries cast doubts on the quality of the vaccine and raised questions about the vaccination process. Other attempts to explain the discoveries revolve around serum clutter in the cases of the horses that died in spite of having sufficient antibodies (titre 80). Another possibility is the introduction of a new serotype, because AHSV was detected by the lab (MacLachlan and Guthrie 2010).

Laboratory results produced by OIE Reference laboratories for AHSV and BTV (Pirbright and Onderstepoort) confirmed the good quality of the monovalent vaccine against serotype 9 produced by LNERV. These laboratories also found that spleen, liver and blood samples contained no traces of AHSV-9. This finding attested the introduction of AHS serotype 2 in the country.

Course of the epidemic outside of Dakar

In spite of the containment of all horses within the borders of Rufisque (Dakar region), the disease continued to spread throughout the department. Between early July and November 2007, several outbreaks occurred in 23 out of 30 departments outside of Dakar (Table 1). The disease consistently hit horses of local breeds, and (officially) caused 1137 horse deaths (Fig 1).

African horse sickness virus isolation

The OIE Reference laboratory in Pirbright isolated AHSV serotype 2 (AHSV-2) from spleen samples of two stallions from Diourbel region.

The samples were taken between August 26 and September 2, 2007. The same serotype was isolated from blood samples collected from 12 horses on July 5, 2007 in Diarere, department of Fatick (Fatick region). In those horses, clinical signs, such as heightened rectal temperature, serous nasal discharge, slight tearing and extra-orbital oedema were observed.

The OIE Reference laboratory (Pirbright) also isolated AHSV-2 from a lung sample taken from a mare in Lerane Sambou, department of Foundiougne (Fatick region) on August 24, 2007.

None of the samples had traces of serotype 7, although a high seroprevalence of this serotype was observed in healthy horses during another study in the Senegal river Delta area in August 2007 (ND Diouf, E Etter, O Ndiaye, S Lecollinet, S Zientara, AJ, Akakpo, unpublished data). The serotype 7 was isolated by the OIE Reference laboratory in Pirbright in September 2007 in dead horse samples from Guede Bousso village, Diourbel region (Mertens and others 2010). This lab had already isolated AHS virus serotype 2 in horse blood, spleen and lung from Fatick and Diourbel regions in July, August and September 2007.

Phylogenetic analysis of AHS virus type 2 isolates from South Africa, Botswana and Nigeria showed that the Senegal isolate is almost identical to the AHSV-2 isolates from all these countries (Anon 2010).

Control strategies during the epidemic

First AHS epidemic in Niaga (Dakar region)

On March 16, 2007, samples were sent to the LNERV of Dakar, and on March 19, 2007, the government created a departmental task force to monitor and fight epidemics. In the following days, preventive health measures, such as movement restrictions, the burying of corpses, and vector-control methods including spraying, cleaning, disinfection and disinfection of stables and the woods of Petit Mbao, were set in place. On March 20, 2007, sera collected from sick horses were tested by the LNERV in Virus Neutralisation Test. Out of an estimated 6910 horses, 5938 horses (85.9% per cent) living in the area of Dakar were vaccinated with the monovalent AHS vaccine with serotype 9 produced by the LNERV without cost to the owners.

Second AHS epidemic in Petit Mbao (Dakar region)

The balance at this point shows 15 dead horses. The government declared suspected AHS in the area of Petit Mbao on May 23, 2007. Samples were sent to the LNERV on May 29, 2007 to confirm AHS and to check the quality of the monovalent AHSV-9 vaccine. Next, calls for offers for an order of monovalent vaccine against AHSV-9 were sent to South Africa, Sudan and Egypt. On May 30, 2007, samples were mailed to OIE Reference laboratories for AHSV and BTV (Pirbright and Onderstepoort). On June 5, 2007, samples of the monovalent AHSV-9 vaccine produced in Senegal were mailed to Pirbright for quality control, and 10,000 doses of monovalent AHSV-9 vaccine were ordered for the second time in Egypt and Sudan. On June 30, 2007, OIE Reference laboratories for AHSV and BTV isolated serotype 2, and thus confirmed the diagnosis of AHS and the good quality of the monovalent serotype 9 AHS vaccine produced in Senegal. This diagnosis led to a further order of 10,000 doses of polyvalent AHS vaccine in Egypt instead of the 10,000 doses of monovalent vaccine against AHSV-9. The vaccine was delivered on August 26, 2007.

TABLE 1: Global situation of the 2007 AHS epidemic in Senegal

Infected regions	Date of first outbreak	Date of last outbreak	Number of outbreaks
Kaolack	August 1-7	September 20	52
Tambacounda	–	–	18
Louga	August 17	October 29	38
Diourbel	August 17	October 1	29
Fatick	July 5	October 20	43
Thies	August 29	October 30	17
Matam	August 10	October 24	21
St-Louis	September 11	October 17	14
AHS African horse sickness			

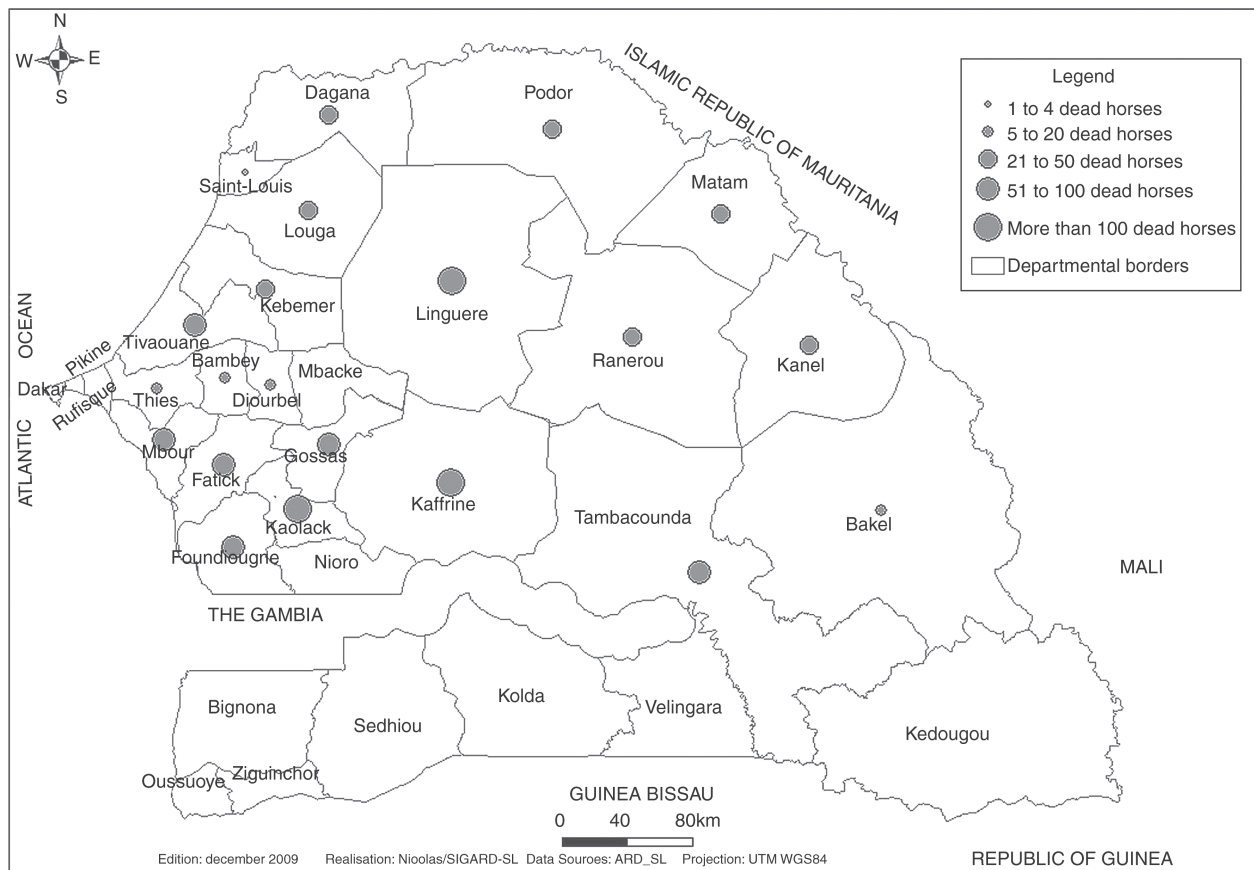


FIG 1: Number of horse deaths from African horse sickness, by department, during the epidemic in Senegal, 2007

Outbreaks of AHS out of Dakar

The Director of the Livestock department of Senegal visited the affected regions (Louga, Diourbel, Fatick, Kaolack and Tambacounda) between August 24 and August 26, 2007. Regional veterinary services of those regions received 5000 doses of a polyvalent AHS vaccine. On August 30, 2007, a letter addressed to the governors installed a monitoring system that gave real-time status reports on the AHS epidemic. The status report included data on geographic and temporal distribution of the afflicted horses and made it possible to quickly bring the epidemic under control. On September 1, 2007, veterinary services of affected areas met with the LNERV and received another 5000 doses of the polyvalent AHS vaccine with serotypes 1, 2, 3, 4, 5, 7, 8 and 9, since AHSV-9 can immunise against the AHSV-6. On September 3, 2007, veterinary officers in the field were warned to control further spreading of the disease.

Under the Emergency vaccination programme, 230,000 doses of the polyvalent AHS vaccine were ordered: 120,000 doses from the LNERV and 110,000 doses from Egypt. On September 6, 2007, the veterinary services addressed a request to Food and Agriculture Organisation to fund an investigation into the different AHS serotypes circulating in Senegal.

The scientific committee for the monitoring of diseases composed of veterinary services, public and private structures with a vested interest and professional organisations, was created on September 7, 2007. Also on this day, the LNERV diagnosed AHS and identified serotypes 2 and 9. On September 10, 2007, regional veterinary services received 11,000 doses of the polyvalent AHS vaccine (10,000 doses originating from Egypt and 1000 doses produced by the LNERV) and 2250 litres of fuel to support the vaccination campaign.

On September 19, 2007, regional veterinary services received 40,000 doses of the polyvalent AHS vaccine. On October 2, 2007, they received another 41,830 doses of the polyvalent AHS vaccine, fuel, conservation materials (freezers, ice-boxes) and communication/publicity aids (t-shirts, caps, posters and flyers).

On October 9, 2007, the LNERV is requested to produce and deliver a solvent to dilute the vaccine to avoid use of unsafe diluents by field veterinarians.

On October 17, 2007, the Livestock Direction, the Forestry Direction and the Hygiene services worked together to bury horse cadavers in the protected forest of Petit Mbao. The next day, regional veterinary services received 17,170 doses of polyvalent AHS vaccine, and another 32,830 doses on October 25, 2007.

Results of the vaccination campaign

A total of 175,300 horses (33.8 per cent) out of 518,212 horses were vaccinated with the polyvalent vaccine from August to December 2007 in eight regions. Let us point out that there were no horse deaths reported in the following seven departments which were free from AHS outbreaks: Kolda, Sedhiou, Velingara, Bignona, Oussouye, Ziguinchor and Kedougou. However, the peak of the epidemic was observed in Fatick region during the last week of August 2007 (Fig 2b), while it occurred during the first and the second week of September 2007, respectively, in Thies (Fig 2c) and Diourbel regions (Fig 2a). The incidence of the disease decreased in all these three regions after a mass vaccination programme was started.

Under the urgent AHS vaccination programme, regional veterinary services received polyvalent vaccines, fuel, compensations, conservation materials (freezers, ice-boxes).

In spite of all these efforts, 1137 horses were officially reported to have died during the 2007 epidemic. The outbreaks occurred in nearly four months, with a morbidity of 0.26 per cent and a mortality of 0.23 per cent. This means that the global assessment of the disease cost was 1.4 million euros, with 55.60 per cent for disease cost (morbidity, opportunity cost, mortality), and 44.40 per cent for control measures (Akakpo and others 2011).

To avoid these high losses, we should improve epidemiosurveillance networks for main livestock infectious diseases and adapt vaccines used with serotypes circulating in the different regions (Akakpo and others 2011).

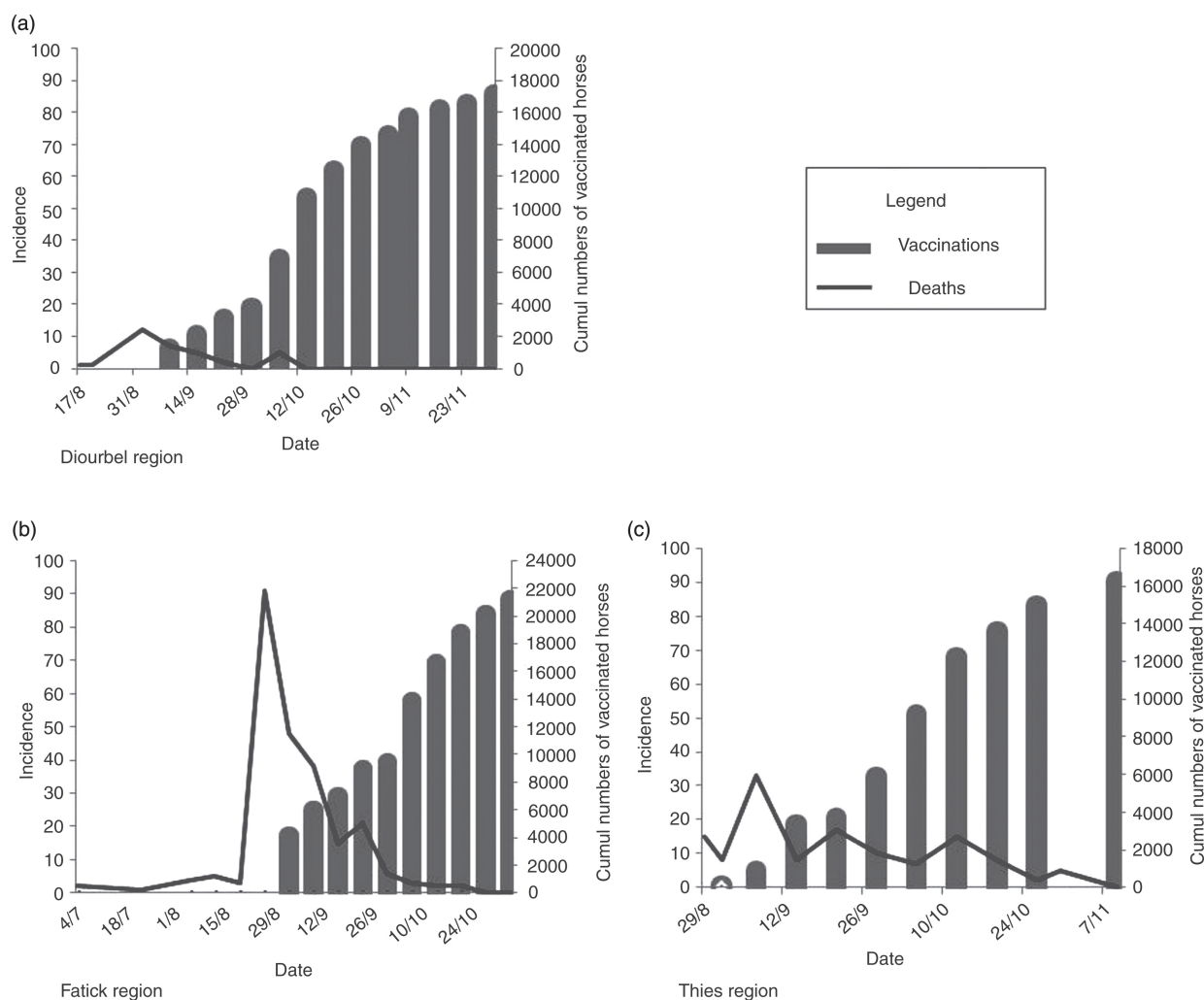


FIG 2: Epidemic curves and vaccine-induced immunity for African horse sickness in Diourbel (a), in Fatick (b) and Thies (c), Senegal, 2007. The polygon represents new deaths, by week, of the epidemic. The vertical columns represent the number of vaccinated horses with the polyvalent vaccine during the emergency vaccination campaign

The set goal to vaccinate 300,000 horses by the end of December 2007 was not reached.

Discussions

Hypothesis of AHSV-2 introduction in Senegal

The first hypothesis of AHSV-2 introduction sustains the possibility that it might be due to a fraudulent import of horses into Senegal from countries where the serotype 2 virus was present, most likely South Africa or Nigeria. In this case, the infected horse would be the direct origin of the outbreaks in the Dakar region, and particularly, the outbreak in Niaga in March 2007.

There are no records of horses having been imported from South Africa or Nigeria before the outbreaks in Niaga and Mbao. Neither the veterinary services at Dakar port, nor the services at Dakar airport registered incoming horses from those countries. From 2005 to 2008, a total of 57 horses had been registered as imported from Brazil and France.

The second hypothesis of AHSV-2 considers an illegal or fraudulent movement of horses from neighbouring countries. Taking into account that antibodies to AHSV-2 were found in healthy horses in the Senegal river delta (St Louis region) between August 12 and 19, 2007 (ND Diouf, E Etter, O Ndiaye, S Lecollinet, S Zientara, AJ, Akakpo, unpublished data), thus, before the introduction of the polyvalent vaccine against AHS in Senegal. It is likely that those wild-type strains of AHSV-2 had been circulating discreetly before the outbreaks in Niaga and Mbao (Dakar region), as was the case in Corsica during the outbreak of BTV (BTV-4) in 2003 (Gerbier and others 2008). This phenomenon can be explained in the following possible scenario: one or more of Senegal's neighbours, such as Mali or Mauritania (Wilson

and others 2009) might have imported horses from countries affected by serotype 2 before 2007. The virus could subsequently have circulated within the horse population of these neighbouring countries, including horses of local breeds. Horses carrying the virus could then have been sold in weekly markets near the border, and then be sold again in major markets in Fatick and Kaolack regions (Fig 3). The new serotype was thus carried to regions where carters make seasonal treks to Dakar at the end of the rainy season. Following this train of thought, AHSV-2 would have been circulating discreetly among local breed horses in Dakar as early as December 2006. Only in March 2007 were the first outbreaks among exotic breeds registered in the Dakar suburbs. In affecting the exotic breeds, it may be possible that the virus gained virulence (or mutated), and then became fatal to local breeds. When the carters moved back into the country from Dakar before the rainy season, they carried the virus to the different locations where, later, there would be outbreaks. Indeed, the first suspicion of AHS outside of Dakar was reported on July 5, 2007 in the region of Fatick, in Diarere. The carters of this village had stayed in Dakar during the dry season, and had been home for the rainy season for less than a month.

It is important to note that the first clinical cases of AHS in the St Louis region were reported on September 11, 2007 in the department of Dagana, and on September 24 in the department of St Louis.

The exotic horses that died between March 2007 and June 2007 in the Dakar suburbs were probably only the tip of the iceberg – being more sensitive than local breeds – and their death was indicative of the virus circulation.

It should be noted that the different AHS serotypes supposedly originating from the outbreak points in the area of Dakar seem more

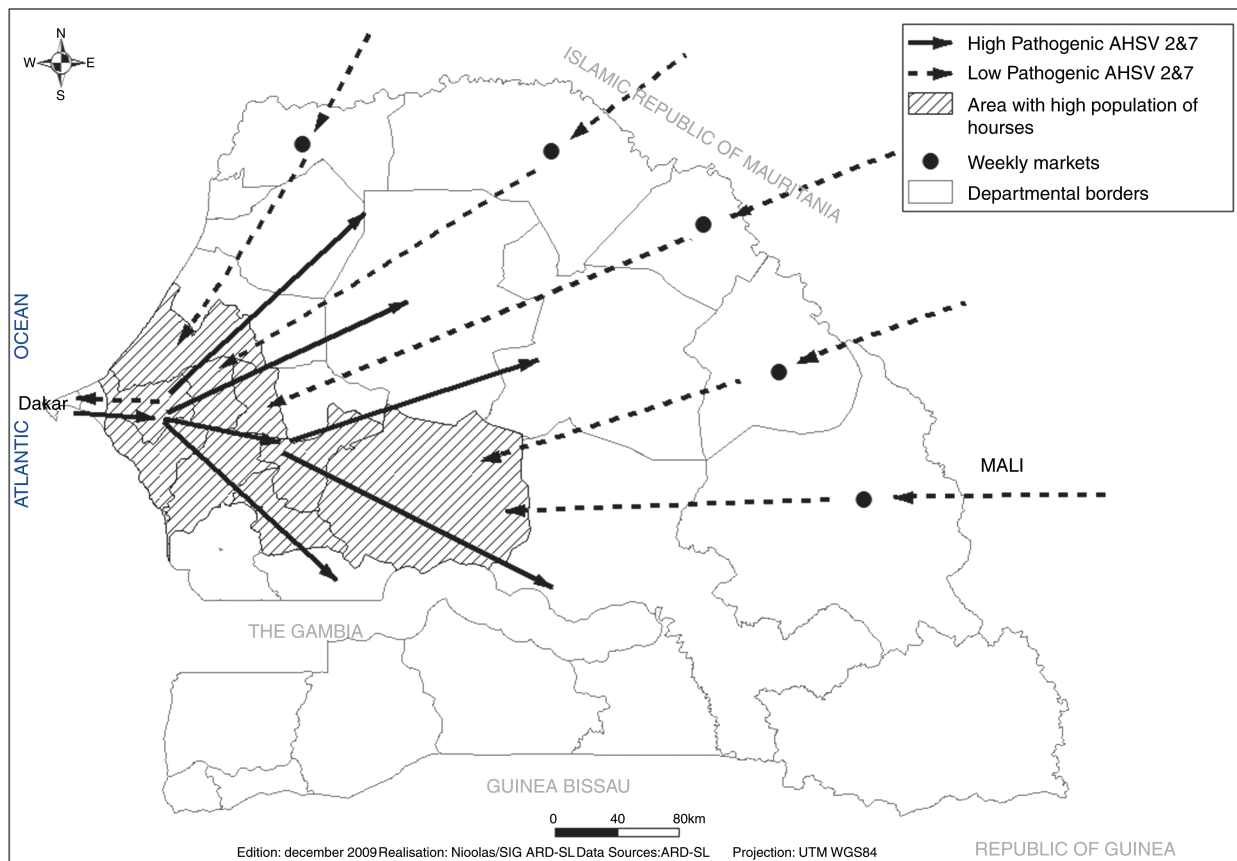


FIG 3: Possible route of introduction and spread of African horse sickness virus serotypes in the 2007 epidemic

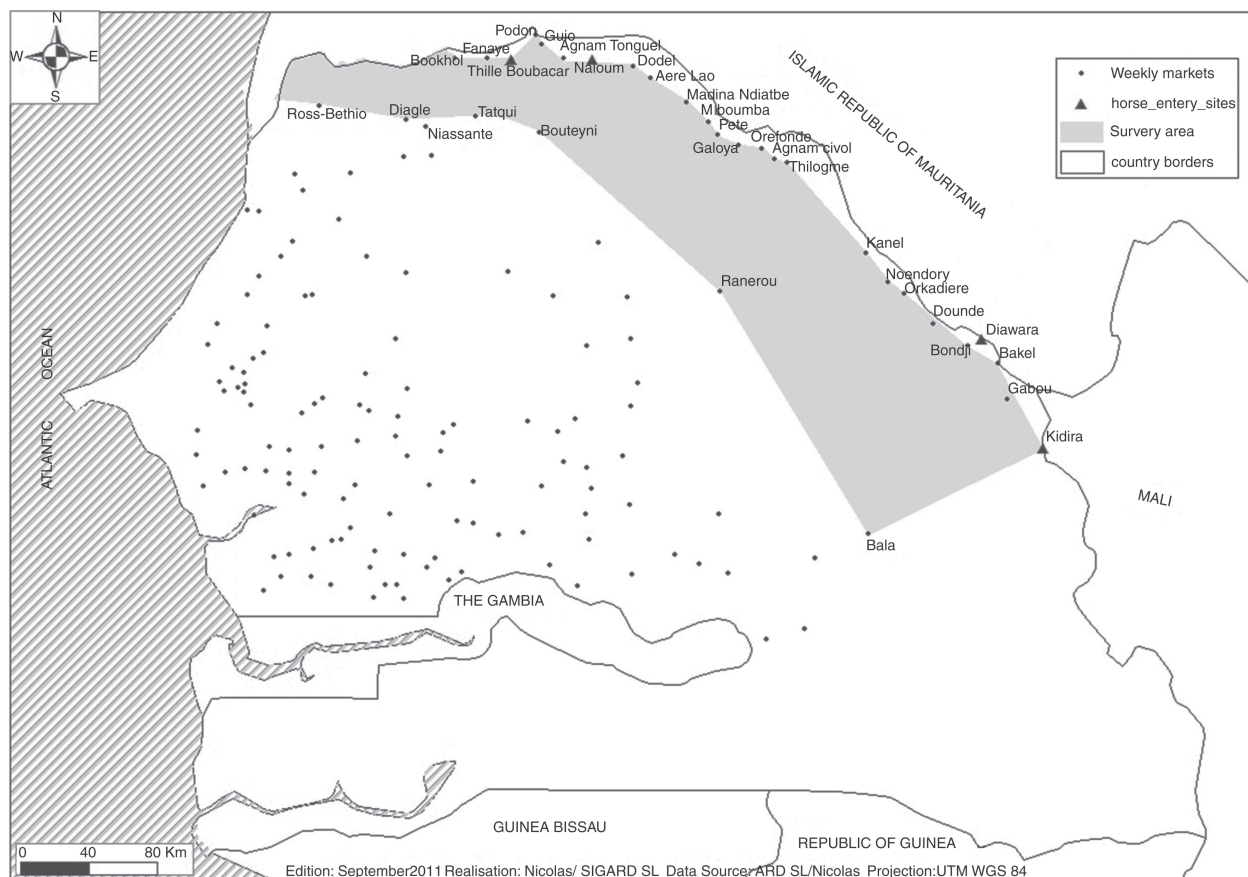


FIG 4: Early detection survey area for African horse sickness virus new serotypes and other horse diseases. Diawara, Kidira, Thille Boubacar and Ndoum are entrances for horses from Mauritania and Mali. Active surveillance in the epidemiological area would allow the detection of viruses before they reach the network of weekly markets

virulent and more lethal to local breeds than those that were found in St Louis prior to the outbreaks.

This virus caused many equine fatalities which helped to detect the virus circulation expecting the virus' virulence to increase while it moved from horse to horse.

A third hypothesis considers that the virus was introduced by infected *Culicoides* vectors that reached the country travelling by ship, plane, wind or other modes of transportation.

The second hypothesis seems to be the most plausible. The first hypothesis is problematic because the time frame between import and outbreak is too long. Clinical signs are usually observed after seven to 14 days of infection, and no horse imports were reported in the days before the outbreaks. It might also be a fraudulent importation of horses from Nigeria by Senegalese horse traders or owners (Folorunso and others 2008) using cars for transportation. The third hypothesis seems problematic because planes have strict disinfection guidelines. The journey by ship or car is generally too long for vectors to survive. It is thus unlikely that those vectors could introduce new serotypes. However, an introduction of AHSV-2-infected vector by wind might be considered if the sources of infection are the neighbouring countries. On the other hand, if Nigeria is considered to be the source of AHSV-2, most of the West African countries located between Nigeria and Senegal should be infected before the Senegalese outbreaks.

Before the introduction of AHSV-2 in Senegal, a monovalent vaccine against AHSV-9 was routinely used for yearly vaccination campaign, but since the 2007 outbreak, polyvalent vaccine with seven valences was used, later eight serotypes were included in the polyvalent vaccine used in Senegal taking into account the cross-reactivity between AHSV-6 and AHSV-9. These vaccines are all live attenuated formulations.

An advantage in the use of inactivated vaccines is that tests based upon one or other of the non-structural proteins of the virus can be developed that will enable differentiation between naturally infected and vaccinated animals (Laviada and others 1995).

Vaccination may have greatly contributed to the control of the AHS outbreaks in Senegal in 2007, but it needs, therefore, further research to evaluate its role by estimating the basic reproduction number (R_0) in the early phases (Lord and others 1996). The effective reproduction number (R_e) should also be assessed to consider the effect of increasing immunity throughout the epidemic and the vaccination (Cowled and others 2009). However, the number of vaccinated horses is negatively correlated to incidence in Fatick, Thies and Diourbel regions, respectively, -0.34 , -0.60 and -0.61 . Other control measures, such as movement restrictions, were imposed but not respected by owners in the field; the vector control (disinfection and disinfection of stables) was just done in some places in Dakar region. Lack of laboratory diagnosis confirmation for each horse death might have led to an overestimation of the number of outbreaks. However, under-reporting of outbreaks might have compensated for this overestimation.

Recommendations

An early warning surveillance system for AHS should be implemented in Senegal by doing a sero-monitoring in donkeys which are usually not vaccinated against AHSV, in order to detect the circulation of wild-type strains of AHS virus (Fig 4). Indeed, donkeys are not active in the introduction of AHS virus because there is no trade business from borders, but they might play a role in keeping and spreading the virus as a reservoir. Other preventive measures can include intense vigilance near the Malian and Mauritanian borders, particularly around weekly markets, an active surveillance of AHSV circulation with a raised level of involvement of veterinary officers, veterinarians and horse owners. It is necessary to implement a network based on serological and virological tests, not only for the reporting of clinical signs, but also in order to detect in real time the spread of a new serotype. Since AHSV is endemic in Senegal and occasionally re-emerges, environmental and meteorological factors contributing to amplify potential epidemics should be identified. Data, such as annual temperature (Ward 2005), rainfall, framework should be available for veterinary services. On the other hand, horse densities and vector dynamics in the vigilance zone should also be known. All

this information can be used to reduce the potential impact of future epidemics.

The lessons learned from the 2007 epidemic are first that, restrictions on horse movement should be respected by owners because it might be an effective way to reduce the disease impact (Perez and others 2004); secondly, a mass vaccination campaign involving other subregional countries, such as Mali, Mauritania, The Gambia and Ghana, might be an effective control strategy against the AHSV epidemic, and a successful way for its eradication (Portas and others 1999); thirdly, a vaccination programme should be based on the adapted vaccines against serotypes circulating in the area.

The northeastern part of Senegal should be considered as a risk zone because it is the only side in which introduction of the AHS virus can take place. Senegal is a coastal country with the Atlantic Ocean in the western part, while the south and southeast are infested by Tsetse flies which can transmit Trypanosomiasis to horses. Horses can barely live in that area at the time of this writing.

The current study shows that a new AHSV serotype can reach an endemic area, but be free from this serotype without immediately inducing clinical signs in horses. Further investigations should be conducted in Nigeria, Mali, The Gambia and Mauritania to know the real number of AHSV serotypes which are circulating in these countries. Such investigations can also be done in all West African countries, such as Ghana, where the AHS virus 2 was found in 2010. The recent spread of new serotypes of AHSV into the northern part of sub-Saharan Africa is problematic because recent experiences with BTV show that once the viruses reach North Africa they may spread within the Mediterranean basin because of the wide distribution and movement of *Culicoides* midges. The incursion of AHSV into European countries would be economically devastating.

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