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REGULAR ARTICLES

Sero-surveillance of emerging viral diseases in camels and cattle in Nouakchott, Mauritania: an abattoir study

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Abstract

This study reports the monitoring of several emerging viral pathogens in Mauritania, which was carried out by the analysis of bovine and camel samples taken at the slaughterhouse of Nouakchott. Blood and serum were collected by random sampling from 159 camels and 118 cattle in March 2013 at the large animals abattoir in Nouakchott. Serological tests for Rift Valley Fever (RVF), Peste des Petits Ruminants (PPR), West Nile disease (WND), epizootic haemorrhagic disease (EHD) and African horse sickness (AHS) were carried out using commercial ELISA kits. The samples, which resulted positives for PPR, WND and AHS, were tested with the confirmatory virus neutralization test (VNT). According to ELISA results, serological prevalence of RVF was 45% (95% CI 52.3–37.7) in camels and 16% (95% CI 22.6–9.4) in cattle. The difference between the observed prevalences in camels and in cattle was significant (p value ≤ 0.01). PPR was absent in camels and had 12% prevalence (95% CI, 17.86–6.14) in cattle. Furthermore, camels showed 92% (95% CI, 96.1–87.9) prevalence of WNV, 73% (95% CI, 82.3–63.64) of EHD and 3% (95% CI, 5.6–0.4) of AHS. This data are of relevance since provided useful feedbacks on the circulation of the pathogens in field. Moreover, this survey provided new information on the susceptibility of camels to several emerging pathogens and on the possible use of this species as sentinel animal.

Keywords Mauritania · Abattoir survey · Camel · Bovine · Rift Valley Fever (RVF) · West Nile disease (WND) · Epizootic haemorrhagic disease (EHD) · African horse sickness (AHS) · Peste des Petits Ruminants (PPR)

Introduction

During the last decades, viral diseases such as Rift Valley Fever, Peste des Petits Ruminants (PPR), West Nile (WN), epizootic haemorrhagic disease (EHD) and African horse sickness (AHS) have extended their geographic distribution and emerged in new regions of Africa.

Rift Valley fever virus (RVFV; family *Bunyaviridae*, genus *Phlebovirus*) is an enveloped RNA virus transmitted mainly by mosquitoes. This virus causes severe disease in humans and animals. Although direct transmission through contact with infected tissue might occur and could play a major role in human infection (Chevalier et al. 2010), mosquitoes still represent the most common way for virus spread. In Mauritania, RVF firstly occurred in 1987, after the building of the Diama dam (Digoutte and Peters 1989). Since then, disease outbreaks have been reported repeatedly in the country from 1993 to 2016 (OIE). The review on RVF seroprevalences in livestock, wildlife and human in African countries was carried out by Clark and coworkers (Clark et al. 2018). Camels have been regularly involved in RVF epidemics; however, clinical disease is unusual in adults, and abortion is the only observed sign. Recently El Mamy et al. (2011) reported an unprecedented outbreak of RVF in north Mauritania with high mortality rates and severe clinical signs observed among dromedary camels.

Peste des Petits Ruminants (PPR) is a contagious disease of sheep and goat which has recently emerged, and it is now widely distributed in through large part of Africa, the Middle East and Asia. The disease is caused by the Peste des Petits Ruminants virus (PPRV), which belongs to the *Morbillivirus* genus of the

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Paramyxovirus family. The disease is characterised by severe pyrexia, anorexia, ulcerative stomatitis, diarrhoea, respiratory distress and high mortality. Susceptibility of camels to PPRV infection was firstly reported by Ismail et al. (1992); clinical PPRV infection in camels has been frequently reported from endemic regions in Africa (Rahman et al. 2020). PPR is endemic in Mauritania; a recent study reported high seroprevalences among small ruminants in different provinces (El Arbi et al. 2014).

Epizootic haemorrhagic disease (EHD) is a non-contagious, arthropod transmitted viral disease affecting wild and domestic ruminants. The receptivity of camels to epizootic haemorrhagic disease (EHD) deserves further investigation. The EHD is caused by epizootic haemorrhagic disease virus (EHDV), a species of the genus *Orbivirus* of the *Reoviridae* family that is related to African horse sickness (AHS) virus and bluetongue virus (BTV). EHD has not been reported in Mauritania; however, the circulation of the agent is reported to be widespread in the Maghreb region (Madani et al. 2011).

West Nile virus (WNV) is a mosquito-borne flavivirus (family *Flaviviridae*) found throughout the world. WNV is maintained in nature trough passages between avian hosts and mosquito vectors; the spill over from the enzootic may affect mammals, as dead end hosts. Horse is the animal species most frequently affected by clinical disease, together with human. WND was not reported in Mauritania, despite the virus is considered endemic across sub-Saharan Africa. Camels are susceptible to WNV (Joseph et al. 2016). Antibody survey in camels provided evidence of the circulation of the disease (Olaleye et al. 1990).

Mauritania's economy is mainly based on livestock farming with almost 70% of agricultural GDP and 14% of the national GDP, provided by livestock-related activities (Hatfield and Davies 2007). Animal breeding is of fundamental importance in poverty reduction because of the following: (i) its weight in the rural value added; (ii) the highly redistributive nature of this value added; (iii) traditional social solidarity mechanisms which are attached to it; and (iv) the major role played by animal breeding in food security of rural households (African Development Bank Group 2005). The national herd is estimated at 15.9 million sheep and goats, 1.4 million cattle and 1.4 million camels (Beekhuis et al. 2006). Transboundary animal diseases have the potential to threaten food security, through serious loss of animal, and increase poverty levels particularly in poor communities that have a high incidence dependence on livestock farming for sustenance. Due to the lack of adequate resources, surveillance does not cover these infectious diseases, and therefore the movement of pathogens in animals and the environment is likely to be seriously underestimated.

Abattoir surveillance is an important strategy, for detection of disease cases, and represents a relatively inexpensive way to collect samples which can provide essential information from field that can be utilized for research and disease control purposes. This study reports the monitoring of emerging viral pathogens, which was carried out by the analysis of bovine and dromedary samples collected at the slaughterhouse of Nouakchott. This activity was in the frame of a scientific and technical collaboration between the National Office for Research and Development of Livestock (ONARDEL) of Nouakchott, Mauritania, and the Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise (IZSAM), the Italian Reference Centre for Foreign Diseases of Livestock.

Materials and methods

The study

The study was carried out in March 2013 at the large animals abattoir in Nouakchott. All animals were adults and they looked healthy at the ante mortem inspection. Blood samples were collected from the jugular vein from 159 camels and 118 cattle, before the animals were slaughtered. The animals to sample were selected randomly during 5 days of activity of the slaughterhouse, within a period of 2 weeks. At the time of the study, around 150 camels and 150 cattle were slaughtered every working day at the large animals slaughterhouse in Nouakchott, and the plant was active around 3 days per week. The animals were from the live animals market, close to the slaughterhouse, where the animal traders bring them for sell from different regions of the country.

Blood and serum samples were properly packaged and shipped at the laboratory of IZSAM where laboratory tests were carried out.

ELISA test

In order to detect the presence of an immune response against the emerging viral diseases object to this study, the collected sera were screened by several commercial ELISA kits: ID Screen® Rift Valley Fever Competition Multi-species (IDvet, Montpellier, France) for RVF, ID Screen® PPR Competition (IDvet, Montpellier, France) for PPRV, ID Screen® West Nile Competition Multi-species, (IDVet, Montpellier, France) for WNV, INgezim AHSV Compac Plus (Eurofins Technologies, Ingenasa, Madrid, Spain) for AHS and LSI VET EHDV BLOCKING (LSI, Lyon, France) for EHD. ELISA kits use plates pre-coated with recombinant antigens and measure the interference caused by antibodies eventually which present in the animal serum tested with the expected signal of a monoclonal anti-antigen antibody conjugated to horseradish peroxidase (HRP).

The results of the competitive ELISA kits for RVF, PPR and WNV are expressed as competition percentage (S/N), which is calculated from optical density (OD) of the sample, recorded at a wavelength of 450 nm, as follows: S/N = OD sample / OD Negative Control × 100. The following

thresholds are applied: (i) ID Screen® Rift Valley Fever Competition Multi-species (less or equal to 40% is considered positive, greater than 50% is considered negative, and less than or equal to 50% and greater than 40% is considered doubtful); (ii) ID Screen® PPR Competition (less or equal to 50% is considered positive, greater than 60% is considered negative, less than or equal to 60%, and greater than 50% is considered doubtful); (iii) ID Screen West Nile Competition Multi-species (less or equal to 40% is considered positive, greater than 50% is considered negative, and less than or equal to 50% and greater than 40% is considered doubtful).

The results of the blocking ELISA kits for AHS, INgezim AHSV Compac Plus, are expressed as the blocking percentage (BP) of each sample which is calculated from the OD recorded at a wavelength of 405 nm, as follows : $BP = [\text{OD (negative control)} - \text{OD (sample)}]/ [\text{OD (negative control)} - \text{OD (positive control)}] \text{ X } 100$. A sample showing BP value lower than 45% is considered negative, greater than 50% is considered positive, and BP value between 45% and 50% is considered doubtful.

The results of the blocking ELISA kits for EHD, LSI VET EHDV, are based on the value of the inhibition percentage (inh%); value lower than 55% is considered negative, greater than 60% is considered positive, and inh% value between 55% and 60% is considered doubtful. LSI VET EHDV kit is no longer available in the market, since the production was discontinued in 2018.

The ELISA kits used in this study are widespread for laboratory diagnosis of RVF, PPR, AHS, WNV and EHD, because of elevated diagnostic performances. In particular, (i) ID Screen® Rift Valley Fever Competition Multi-species has 100% sensitivity (91.24–100%) and 100% specificity according to Comtet et al. (2010); (ii) ID Screen® PPR Competition kit demonstrated 94.5% sensitivity and 99.4% specificity compared to virus neutralisation test (VNT) (Libeau et al. 1995); (iii) ID Screen West Nile Competition Multi-species exhibits 100% sensitivity and 80–96% specificity as evaluated by Sotelo et al. (2011); and (iv) INgezim AHSV Compac Plus is one of the serological tests prescribed for the control of movement and importations to the European Union, according to requirements of Directive 2009/156/EC . The test sensitivity is 98.4% (95% CI: 95.3–99.7) and 100% specificity (95% CI: 98.9–100) (Durán-Ferrer et al. 2019).

Virus neutralisation test

The samples, which resulted positives for PPR, WND and AHS, were tested by VNT for confirmation of the presence of neutralizing antibodies. Neutralizing antibody titres were determined by the micro-method of VNT in 96-well plates according to OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (2019). Prior to the test, the antigens were titrated in a cytopathic effect (CPE) TCID50 assay using African green monkey kidney—vero cells for AHS and WNV. Vero cells constitutively expressing canine SLAM (VerodogSLAMtag cells) (von Messling et al. 2003) are used for PPRV. Serial 10-fold dilutions of the antigen were made. After 6 days, the titres were determined by using the Reed and Muench formula. The serum samples were inactivated at 56 °C for 30 min. Starting from 1:5, serial 2-fold dilutions were made from serum samples in microtitre plates, and 100 $TCID_{50}$ units of antigen were added to each dilution. Thereafter, the mixtures were incubated at 37 °C for 1 h, and 10^5 cells were added to the wells. Plates were read after 6 days of incubation at 37 °C. The antibody titre was defined as the reciprocal of the highest dilution of the test serum sample, which showed at least 90% neutralization. Samples showing neutralising titre equal or higher than 1:10 are considered positives. Positive and negative control sera were included in each plate.

Data analysis

The disease prevalences were calculated from the ELISA test results. Ninety-five per cent confidence intervals were calculated applying the following formula, which take into account the sample size (Bottarelli 1998):

$$CI = p \pm 1.96 * \sqrt{\frac{p*(1-p)}{n}}$$

p = prevalence
n = number of animal tested

The prevalences of antibodies against RVF respectively in camels and cattle were compared by the Chi-square test, and the difference was considered significant at a probability level ≤ 0.01.

Results

Ninety-three percent of the camel sera (148 out of 159) were positives for at least one of the diseases under study, as well as the 74% (87 out of 118) of the bovine sera. The serological survey revealed wide circulation of RVF, PPR, WNV, AHS and EHD (Tables 1, S1 and S2). The prevalence of RVF was 45% (95% CI 52.3–37.7) in camels and 16% (95% CI 22.6–9.4) in cattle. The difference between the observed prevalences in camels and in cattle was significant (p value ≤ 0.01). PPR was absent in camels and had 12% prevalence (95% CI, 17.86–6.14) in cattle. Furthermore, camels showed 92% (95% CI, 96.1–87.9) prevalence of WNV, 73% (95% CI, 82.3–63.64) of EHD and 3% (95% CI, 5.6–0.4) of AHS. The ELISA positives sera for PPR, WNV and AHS were analysed with VNT for a further confirmation. Fifteen cattle sera analysed with VNT for PPR were positives with neutralising antibody titres ranging from

Table 1 Seroprevalences of Rift Valley fever (RVF), West Nile disease (WND), epizootic haemorrhagic disease (EHD), African horse sickness (AHS) and Peste des Petits Ruminants (PPR) in camels and cattle based on the results of the Elisa tests

Camels				
	Tested	Positive	% of prevalence	95% CI
RVF	159	72	45	52.3–37.7*
PPR	159	0	0	0
WNV	159	146	92	96.1–87.9
AHS	159	5	3	5.6–0.4
EHD	86	63	73	82.3–63.64
Cattle				
	Tested	Positive	% of prevalence	95% CI
RVF	118	19	16	22.6–9.4*
PPR	118	15	12	17.86–6.14

*p value ≤ 0.01

1:40 to > 1:640 (Table S2). Forty-one camel sera out of 146 were confirmed to be WNV positives by VNT. None was confirmed for AHS (Table S1).

Concomitant presence of antibodies against different diseases were observed in the majority of the camel sera. Notably, antibody response against two pathogens was detected in around 43% of the samples (67 out of 159); particularly, the association of antibodies against WNV and RVF, or WNV and EHD, was observed. Twenty-four percent of the samples were positives for 3 (35 out of 159) or 4 (2 out of 159) diseases. Concomitant serological reactions against PPR and RVF were observed only in in 2 out 118 (1.7%) of the bovine sera. These results are summarised in Table 2 and reported in details in Tables S1 and S2.

Discussion

Mauritania's economy is mainly based on livestock farming with almost 70% of agricultural GDP and 14% of the national GDP, provided by livestock-related activities. The animal population (cattle, dromedaries and small ruminants) is mostly concentrated in the arid zone of the south close to the border with Senegal. Nouakchott, as an important animal market, gets livestock from southern Mauritania, as well as from eastern Mauritania (Apolloni et al. 2018).

During September–October 2010, El Mamy and coworkers (El Mamy et al. 2011) reported severe outbreaks of RVF activated by exceptional rainfall which created highly favourable conditions for colonization and multiplication of competent vectors. The high mortality rates and severe clinical signs observed among dromedary camels suggested that these animals played a major role in the epidemiology of this outbreak. In this study, we observed high serological prevalence of RVF in camels (45% (95% CI 52.3–37.7) and cattle (16% (95% CI 22.6–9.4)), sampled at the Nouakchott abattoir in March 2013, indicating the occurrence of an active circulation of the virus in the field, thus providing a warning on the severe risk for livestock and human health represented by the eventual occurrence of climatic conditions, which are able to amplify the virus load in the environment, triggering the development of disease outbreaks. Furthermore, this result confirmed that camels are highly susceptible to RVFV, even remaining clinically asymptomatic, and suggest that they may contribute to the dissemination of RVF. Intense dromedary movement reported from Mauritania to South Morocco (Apolloni et al. 2018) may indicate a possible pathway for RVF to spread to North Africa (Selmi et al. 2020).

The global distribution of EHDV infection still need to be well characterised (Maclachlan et al. 2015). From 2004 to 2007, disease outbreaks were reported among cattle in the Mediterranean basin, notably in Morocco (in 2004 and in 2006) in Algeria, in Tunisia, in Israel (2006) and in Turkey (2007) (Savini et al. 2011). EHD was never officially reported in Mauritania. In our study, we observed 73% serologic prevalence of EHD in camels. This is an unprecedented finding, since camel was not considered an usual host of EHDV (Savini et al. 2011). This result indicates that EHD is endemic

Table 2 Concomitant presence of antibodies against different diseases in camels, ELISA results

AHS ELISA	PPR ELISA	RVF ELISA	WNV ELISA	EHD ELISA	No. positive/no. tested (% positive)
-	-	-	-	-	11/159 (7)
-	-	-	+	-	43/159 (27)
-	-	+	+	-	37/159 (23)
-	-	-	+	+	29/159 (18)
-	-	+	-	+	1/159 (0,6)
+	-	+	+	-	2/159 (1,2)
+	-	-	+	+	1/159 (0,6)
-	-	+	+	+	33/159 (20)
+	-	+	+	+	2/159 (1,2)

in Mauritania and it suggests that most of the infected animals survive infection with the virus and experience only subclinical infections. Further study would be necessary to characterise the disease in camels and to evaluate the role of this species in the epidemiology of EHD.

Antibody prevalence against flaviviruses was 92% (95% CI, 96.1–87.9) detected by c-ELISA test; around 28% of the flavivirus positive samples were confirmed to be WNV positives by VN test (Di Gennaro et al. 2014). Susceptibility of camels to flaviviruses is known according to previous study (Touil et al. 2012; Hassine et al. 2017; Selim and Abdelhady 2020). However, seropositive results, especially with serological tests, such as ELISA, should be interpreted with care due to frequent cross-reactions with other flaviviruses (Beck et al. 2017). Indeed, WNV and the related flaviviruses Bagaza (BAG) were both reported in Mauritania (Diallo et al. 2005). Infection by such flaviviruses has been shown to induce antibodies that generate positive results in rapid serological diagnostic tests (Beck et al. 2017). We cannot exclude that any other WNV-related flaviviruses are currently circulating in the country and are still undetected.

Antibodies against AHS were detected in 5 out of 159 camel sera. This result was not confirmed by VN test which failed to detect neutralising antibodies in the sera of the ELISA positive animals. This finding may be due to a past exposure where the titres of the neutralising antibody decreased under the test threshold (Sande et al. 2013) or they may be true ELISA false negatives. Finally, antibodies against PPRV virus were detected in 12% of the bovine sera, confirmed by VN thus indicating the circulation of the virus in field.

The present study reports information acquired during the survey for emerging viral disease at the Nouakchott abattoir. This data are of relevance because provided useful feedbacks on the pathogen circulation on field. With this respect, a better knowledge and understanding of the livestock healthy situation could provide essential information to enhance the national animal diseases surveillance system and the development of control measures for animal and zoonotic diseases as well as to improve the effort coordination at a regional level.

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Declarations

Human and animal rights and informed consent The manuscript does not contain clinical studies or patient data.

Conflict of interest The authors declare no competing interests.

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