Identification of the first cases of Peste des Petits Ruminants and Rift Valley Fever viruses in Koure's *Giraffa camelopardalis peralta* of Niger Republic.

Abdou Alassane[1], Issa Ibrahim Abdoulkarim[2*], Souley Kouato Bachir[3], Haladou Gagara[1], Bangana Ibrahima[4], Phillippe Chardonnet[5].

Abstract

PPR and RVF are two known diseases in domestic ruminants. An annual vaccination Campaign is being done on the first while in 2016 an epidemic outbreak affected both animals and humans in Niger on the second.

The aim of this investigation is to assess the circulation of these two diseases in another wild ruminant, specifically *Giraffa camelopardalis peralta*.

Having an average annual increase rate of 10% together with conservation, the giraffe population increases from 56 individuals in 1996 to 607 in 2017. In order to reduce the pressure of this species on its current habitat, a translocation operation was conducted in November 2018 where ten (10) individuals were transferred to the Gadebédji Biosphere Reserve. Blood, sera, swabs and tissues samples were collected, and sera were tested for RVF virus nucleoprotéin (NP) antibodies and for anti-PPR antibodies using competitive ELISA test. One serum out the ten (1/10) tested positive for PPR and another one tested positive for RVF virus. The preliminary results of the study reported herein suggest RVF and PPR viruses are probably maintained in those giraffes. This call for a large surveillance of those diseases in giraffes, knowing the public health importance of the former and the economic impact of the later in domestic animals.

Key words: Giraffe, PPR, RVF, c-ELISA, Republic of Niger

Résumé

Identification de la circulation des premiers cas du virus de la Peste des petits Ruminants (PPR) et la Fièvre de la Vallée de Rift (FVR) chez les girafes (*Giraffa camelopardalis peralta*) de Kouré au Niger.

La PPR et la FVR sont deux maladies connus chez les ruminants domestiques. La PPR fait l'objet de campagne de vaccination annuelle alors que la FVR a connu une flambée épidémique en 2016 au Niger aussi bien chez les animaux que chez les humains.

L'objectif de cette étude est de mener une investigation sur la circulation de ces deux maladies chez un autre ruminant, cette fois ci sauvage, à savoir *Giraffa camelopardalis peralta*. En effet, les efforts de conservations ont vu croire la population des girafes qui était de 56 individus en 1996 à 607en 2017 avec un taux moyen annuel d'accroissement de 10%. Aussi, pour réduire la pression de cette espèce sur son habitat actuel, une opération de translocation a été conduite en Novembre 2018 où dix (10) individus, furent transférés dans la réserve de Biosphère de Gadebédji. Des prélèvements de sérums, de sang, de poils et des tiques furent réalisés sur ses girafes capturées après une anesthésie. Les sérums obtenus ont été analysés au LABOCEL par la technique c-ELISA pour la recherche des anticorps dirigés contre la PPR et la FVR. Les résultats obtenus démontrent que 1/10 possèdent des anticorps respectivement pour la PPR et la FRV. Les résultats préliminaires de cette investigation, démontrent que ces deux virus sont maintenus dans la population des girafes. Il s'avère nécessaire d'entreprendre une surveillance de ces deux (2) maladies chez les girafes au vu de l'impact économique de la PPR chez les animaux domestiques et l'importance en santé publique de la FVR qui est une zoonose.

Mots clés en Français : Girafe, PPR, FVR, c-ELISA, République du Niger.

[1]Laboratoire Central de l'élevage de Niamey BP 485, Niamey Niger :vetabdou@yahoo.fr.
[2]Université Boubacar BA de Tillabéri, BP 175, Tillabéri, Niger :karimlebelge@yahoo.fr.
[3]Institut National de la Recherche Agronomique du Niger (INRAN) : souleyb@yahoo.fr.
[4]Ministère de l'Agriculture et de l'Elevage, BP : Niamey, Niger : ibangana@yahoo.fr.
[5]IGF Foundation & François Sommer Foundation, 58 Rue Beaubourg, 75003 Paris, France:pchardonnet@yahoo.fr.
*To whom correspondence should be addressed. Tél : (00227) 96 57 30 21.

INTRODUCTION

Peste des Petits Ruminants (PPR) and Rift Valley Fever (RVF) are two known infectious diseases in domestic ruminants. PPR caused by peste des petits ruminants virus (PPRV), which belongs to the genus *Morbillivirus* in the *Paramyxoviridae's* family. It primarily affects sheep and goats but can also infect a wide range of domestic and nondomestic species. Studies in Niger have confirmed the circulation of the virus in domestic animals (Tounkara et al., 2018, Souley et al., 2019). For the control of the disease, an annual vaccination campaign is regularly carried out in Niger.

The Rift Valley fever virus (RVFV) is an arbovirus of the genus *Phlebovirus* of the family *Bunyaviridae*, and replicates in mosquitoes and in vertebrates (Ellis et al., 1988). The virus causes Rift Valley fever (RVF), an acute mosquito-borne zoonotic disease affecting animals and humans. In domestic ruminants, RVF causes high mortality in young animals and sudden onset of abortions in pregnant animals. In humans, uncomplicated RVF cases may present as an acute febrile illness, although more serious complications do occur (ranging from fatal hemorrhagic disease, meningoencephalitis, renal failure, and blindness) and in some cases death. In 2016, several outbreaks of RVF were reported in humans in the north-west part of Niger.

Neither serological nor clinical reports of PPRV and RVFV infections in wildlife in Niger. The paucity of this information led to this study on giraffes which are one of the West African giraffe species only present in Niger and considered, by the International Union for Conservation of Nature (INCU), as an endangered species. However, because of the conservation efforts, the population of this specie of giraffe (*Giraffa camelopardalis peralta)* increases considerably from 56 individuals in 1996 to 607 in 2017(Halilou & Laouel, 2018). In order to reduce the demographic pressure of this species on its current habitat, which is the Kouré's reserve, a translocation operation was conducted in November 2018 where ten (10) individuals were moved from Kouré to the Gadebédji's biosphere reserve. This paper presents the results of serological investigation for PPR and RVF viruses' circulation in the ten giraffes before being transferred in their new habitat.

MATERIEL AND METHODS

Study area

This present study was carried out in Kouré (13° 18′ 38″ Nord, 2° 34′ 34″ Est) located in Tillabéri's region (Figure 1). Kouré is located at 60 km of Niamey, which is the capital of the country. The region is well known due to the presence of the giraffe population in this area.

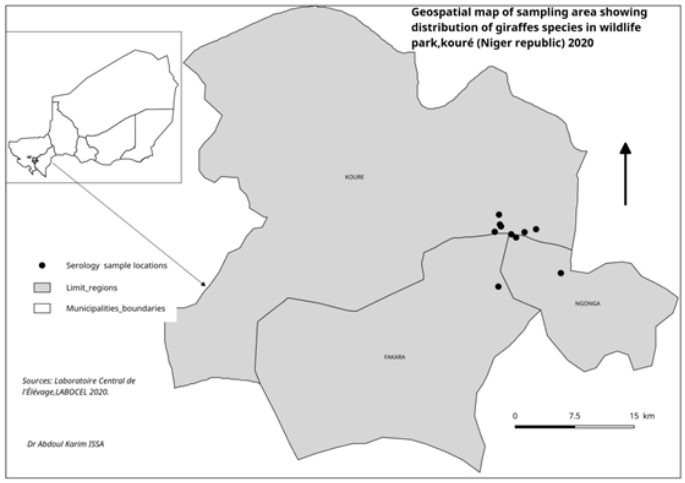

Figure 1: Study area

Study populations and sampling

Niger giraffes are the last specie in West Africa. They are found in the Kouré's reserve, a village located around 60 kilometers away from Niamey. Blood samples from jugular vein, were collected following immobilization of individual with chemical anesthetic agents (Xylazine, Etorphine.). Other samples, such as swabs, feces, pieces of ear-tissues were also taken from sampled animals. The samples were conditioned and transported to Central Veterinary Laboratory of Niger (LABOCEL).

Serological Analysis

A total of 10 sera, were screened for PPR and RVF virus antibody using a competitive ELISA test.

PPR c-ELISA was performed using competitive ELISA kit provided by The French Agricultural Research Centre for International Development (CIRAD-EMVT).

PPR antigen was diluted in coating buffer (PBS-0.01 M, pH 7.4), each well of microtiter plate was charged with 50ul diluted antigen followed by 1 h incubation at 37°C on an orbital shaker. After 3 washing with washing buffer and blot dry, 45ul of blocking buffer (PBS +0.05% tween 20 + 0.5 negative lamb serum) was added to all wells. According to the manufacturer instructions, these chemicals were added: 5ul of blocking buffer to monoclonal control wells, 55ul of blocking buffer to the conjugate control wells and 5ul of test, strong positive, weak positive and negative control sera were added to the corresponding wells. 50ul of mAB (diluted 1/100 in blocking buffer) was added to all the wells except the conjugate control ones followed by incubation of the plate for 1 hour at 37°C on orbital shaker. After 3 washings and blot dryness, 50ul of anti-mouse conjugate were added to all wells. After 1 hour incubation and 3 washings, 50ul of the chromogen/substrate mixture (OPD/H2O2) were added to all wells. After 10 minutes incubation at room temperature, Colour development was stopped by adding 50ul of stop solution (H2SO4, 1M) to all wells. Optival density (OD) values were read at 492nm with ELISA plate reader (Immunoskan BDSL, Thermo Lab. Systems, Finland).

The absorbance was converted to percentage inhibition (PI) using the formula: PI = 100 – [Absorbance of the test wells/ Absorbance of the mAb control wells] x 100. The test serum samples showing PI value of 50 or above were taken as positive for PPR antibodies.

Serological assays of all samples were carried out using anti-RVF nucleoprotein (NP) IgG antibodies using ID screen® RVF competition multispecies ELISA (ID-Vet Innovative Diagnostics, Montpellier, France) according to the manufacturer's instructions. After adding the stop solution, the optical density (OD) was read at room temperature using an ELISA reader (Immunoskan BDSL, Thermo Lab. Systems, Finland) at 450nm.

For the validity of each plate, the mean value of the two negative controls (ODNC) was calculated and the plate was considered valid when ODNC > 0.7. For a valid plate, the mean value of the two positive controls divided by ODNC was < 0.3. For each sample the competition percentage (S/N%) was calculated by dividing (OD sample/ODNC) x 100. Sample was considered positive if the value was equal to or less than 40%. A value greater than 50% was a considered as negative and values between 40 and 50% indicated a doubtful result.

RESULTS

Using the competitive ELISA test, one (10%), out of the ten tested sera was positive for the presence of nucleoprotein (NP) antibodies against RVFV. Similarly, one other serum sample was tested positive for anti-PPR antibodies using competitive ELISA test.

Table 1: Serological result

Sample ID	Sexe	Date of collection	Results	
			FVR	**PPR**
1	Female	02.11.2018	Negative	Negative
2	Female	03.11.2018	Negative	Negative
3	Male	03.11.2018	Negative	Negative
4	**Female**	04.11.2018	**Positive**	Négatif
5	Female	04.11.2018	Negative	Negative
6	Female	04.11.2018	Negative	Negative
7	Male	05.11.2018	Negative	Negative
8	**Female**	05.11.2018	Negative	**Positive**
9	Female	05.11.2018	Negative	Negative
10	Female	05.11.2018	Negative	Negative

DISCUSSION

Rift Valley fever is a vector-borne tropical zoonotic disease. The disease affects ruminants and humans and severely impacts the health and economy of affected countries (Turell et al., 2010). In Niger, a serological study revealed that domestic ruminants were exposed to the rift valley fever virus in the seven administrative regions of the country at the time, although the authors had reported no exposure of humans with the virus (Bada, 1986).

In August 2016, the Ministry of Public Health in Niger reported to WHO of unexplained deaths in humans and livestock in the region of Tahoua, located in the north-east part of country. Human and animal specimens were tested and were reported positive for Rift Valley Fever (RVF) virus by PCR and for specific IgM antibodies confirming the first epizootic and epidemic outbreak of RVF in the country (Doutchi et al., 2017; Adamou Hama et al., 2019). Serological studies conducted in 1991 in Niger revealed exposure of animals to the virus, practically in all the seven regions of the county(Akakpo et al., 1991). No serological and clinical reports of PPRV and RVF infection in wildlife was reported in Niger, though seropositivity was recorded in Uganda, Ethiopia, and other West and Central African countries (Beechler et al., 2015; LaBeaud et al., 2011; Leylabadlo et al., 2016; Mahapatra et al., 2015).This study, for the first time to our knowledge, described the presence and circulation of RVFV in wildlife in Niger as it was previously reported in some studies elsewhere(Bird et al., 2008; Britch et al., 2013;).Similar investigation done in Kenya concerning 81 individuals revealed no positivity against RVF (Evans et al., 2008).Since contacts between domestic and wild animals are frequent, further investigation on the circulation of RVFV in the giraffe population, together with measures to strengthen the "one health" approach, are necessary to prevent RVF outbreak in Niger.

PPR is an important infectious disease affecting small ruminants with consequences on the livelihood of farmers around the world (FAO, 2016). This lead the joint OIE/FAO division to set a world PPR strategic eradication plan, adapted in 2014 at Nepal by member states (FAO/OIE, 2015). In Niger, small ruminants mass vaccination campaign against PPR, is one of the pillars of the National Strategic Plan (NSP) for PPR eradication. PPR outbreak in Gazelle and Ibex was reported from Mongolia in 2018, where almost 85% of the population has been affected (Pruvot et al., 2020). No epidemiologically confirmed outbreaks of PPR in free-ranging wildlife have been reported from West, Central and East Africa to date but suspected unconfirmed outbreaks were noticed in Sudan 2017 in dorcas gazelle (Asil et al., 2019). Since eradication program concerns PPR, taking into consideration wildlife could contribute to destroy the virus from any potential reservoir. The above result revealed one serum positive out of the ten tested, confirming the importance of taking wildlife into consideration in the eradication plan.

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