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Short communication

Experimental infection of dromedary camels with virulent virus of Peste des Petits Ruminants

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ABSTRACT

Peste des Petits Ruminants Virus (PPRV) causes a severe contagious disease of sheep and goats and has spread extensively in last years through Asia and Africa. PPRV, known to infect exclusively small ruminants, has been recently reported in camels in Iran and Sudan. Reported clinical symptoms are similar to those observed in small ruminants, fatality rate still unknown. However most of the authors reported seropositive camels without clinical signs. Camel sensitivity to PPRV is still controversial and more investigation need to be performed. In this study, we tested camel susceptibility by an experimental infection using a virulent PPRV strain belonging to lineage IV. Young dromedary camels were infected intravenously and observed one month for clinical symptoms. Viraemia and virus secretion charge in swabs were evaluated by PCR. Seroconversion was assessed by ELISA and virus neutralisation test. Infected animals did not manifest any clinical symptoms of the disease and no virus was detected in secretions. Seroconversion was observed from day 14 post infection.

1. Introduction

Peste des petits ruminants (PPR) is a highly contagious disease, widespread in western, central, eastern, southern and northern Africa, Middle East, Arabian Peninsula, and wide parts of Asia (EFSA AHAW Panel, 2015; Parida et al., 2015). Four distinct lineages (I–IV) of PPRV are detected, the first two lineages are mainly circulating in West and Central Africa, lineage III in East Africa and Middle East, while lineage IV is present in Asia, Middle East and Africa (Kwiatek et al., 2011; Parida et al., 2015).

The causative agent, a *morbillivirus*, recently named as small ruminant morbillivirus by the International Committee on Taxonomy of Viruses (ICTV), infect mainly domestic small ruminant, the natural hosts. The disease is reported to be one of the major limitations of small ruminant farming (Brown, 2011; Albina et al., 2013; Parida et al., 2015). Wildlife may also be affected (Lefèvre and Diallo, 1990; Bidjeh et al., 1995; Couacy-Hymann et al., 2005; Balamurugan et al., 2012). Clinical manifestations varies between species and breeds.

Dromedary camel has been reported sensitive with clinical signs to PPR in Saudia Arabia (El-Hakim, 2006), Sudan (Khalafalla et al., 2010) and Iran (Zakian et al., 2016). Only positive serology, with low rate (0–3%), has been reported in other countries. Dromerdary camel susceptibility is controversial despite antibody presence because no typical disease observed in the field in most of endemic regions.

Camels cross the desert and link different regions together and may have a role in virus dissemination. The Global Strategy For Control and Eradication of PPR launched by OIE & FAO in 2015 target PPR global eradication by 2030 (FAO and OIE, 2015). A good knowledge of species sensitivity and other epidemiological parameters is essential for the success of the program.

In this study, we carried out an experimental infection using a PPR virulent strain to determine sensitivity of dromedary camels to PPRV by evaluation of clinical symptoms, virus excretion and antibody response.

2. Material and methods

2.1. Virus strain

The PPR Moroccan virulent isolate, belonging to lineage IV, was used for the experimental infection. This strain was isolated during the

Abbreviations: PPR, peste des petits ruminants; VNT, virus neutralization test; bELISA, blocking enzyme-linked immunosorbent assay; PCR, polymerase chain reaction

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2015 outbreak in Morocco from a lamb showing characteristic clinical signs of PPR. The strain was characterized by molecular biology and confirmed highly pathogen on goats with typical symptoms and lesions (Fakri et al., 2016).

The viral material used for the challenge infection on camels is a virus challenge stock previously produced. Infected lung tissue and mesenteric node were collected from goats infected experimentally by the 2015 PPRV isolate of lamb origin. Alpine goats known to be sensitive to the disease were used (Fakri et al., 2017). Tissues were homogenized, pooled together (lumb tissue and mesenteric nodes) and centrifuged at 2000 rpm for 20 min at 4 °C. The supernatant of the mashed tissues represented the viral material and presenting a Ct of 20.2 in qPCR. The virus material was considered as a virus challenge stock after pathogenicity evaluation on goats. This virus challenge stock is routinely used for challenge of PPR vaccine in our laboratories.

2.2. Animals

Five dromedary camels (*Camelus dromedarius*) of two years, 2 females and 3 males, were tested negative for PPRV specific antibody by virus neutralization test (VNT) and b-ELISA. The experiment was carried out according to international guidelines described for the care and handling of experimental animals, chapter 7.8 of the Terrestrial Animal Health Code and Directive 2010/63/UE of the European commission (EU Commission, 2010; OIE and Terrestrial Animal Health Code, 2016). The protocol was submitted and approved by the internal Laboratory Committee.

2.3. Experimental infection, sampling and monitoring

Animals were infected by intravenous route (5 mL) according to the protocol previously described (El Harrak et al., 2012).

Monitoring was based on daily observations of hyperthermia, clinical symptoms and general behavior. Blood samples were collected from jugular vein by using sterile needles and vacutainer tubes which were placed into two separate tubes, one tube containing EDTA for qPCR analysis and another tube without anticoagulant for serological testing. Samples were kept in cold boxes at 4 °C and then transferred to the laboratory for centrifugation and storage at −20 °C until use. Conjunctival, nasal and rectal swabs were collected in 2 ml PBS supplemented with 2% antibiotic-antimycotic solution.

EDTA blood samples and swabs were collected every three days post infection (dpi) for qPCR testing (Batten et al., 2011) to monitor viraemia and viral excretion. RNA extraction was carried out using an RNA extraction kit (Bioline BIO-52075, isolate II RNA Mini kit). Superscript III Platinum R one step qRT-PCR system kit (Invitrogen) was used for qPCR.

Serological testing was carried out every 7 days until 42 dpi by VNT using the described method in the OIE Terrestrial Manual (Chapter 2.7.11) and b-ELISA (OIE, 2013; Bodjo et al., 2018).

3. Results

3.1. Observation of clinical signs of PPR

No hyperthermia was noticed on infected animals, the average temperature stay between 37.5 °C and 38.4 °C. As reported by El Allali et al., in the absence of heat stress and with free access to water, normal rectal temperature presents an amplitude of about 2 °C, the animal being then a perfect thermoregulator or homeotherm (El Allali et al., 2013).

No clinical signs were detected on infected camels, only a slight congestion of ocular mucosa was observed. Animals did not show any change in general behavior or feeding apetite remaining in a good health status during the observation period of 6 weeks pi.

Table 1
Neutralizing PPRV antibody titers (log10) after experimental infection of dromedary camels with PPR Moroccan virulent isolate.

	Days post infection						
Camel identification	0	7	14	21	28	35	42
1	0	0	1,0	1,9	2,2	2,2	1,9
2	0	0	0	0	1,0	1,0	1,0
3	0	0	1,0	1,3	1,0	1,0	1,0
4	0	0	1,0	1,9	2,2	2,5	2,5
5	0	0	0	0,8	1,0	1,0	1,0

3.2. Detection of PPRV nucleic acid

No nucleic acid of the PPRV was detected by qPCR on lacrymal, nasal and rectal swabs, indicating absence of viral excretion in lachrymal, respiratory and digestive tractus of infected animals. No PPRV nucleic acid was detected by qPCR in blood, indicating absence of viraemia.

3.3. Detection of specific anti-PPR antibodies

Three animals among five seroconverted at day14 pi and the two others were positive at day 28 pi by both VNT and b-ELISA. Two camels presented high values of antibody response with an average of 2.2 $\log_{10}$ neutralizing antibody titer at 42 dpi. For the three other camels, the neutralizing antibody titer was low, not exceeding 1.0 $\log_{10}$ (Table 1).

4. Discussion

This work was justified by the contradictory reports on dromedary camels sensitivity to PPRV. The geographical distribution of dromedary camel from India to Morocco represent an endemic area of PPR in small ruminants. Very often herds are mixed between goats and camels in semi-desertic space mainly. Those criteria increase exposition of camel to infection from small ruminants.

A great number of serological surveys have been performed in different countries giving evidence of presence of antibody against PPRV in dromedary camels (Table 2). Prevalence reported in Ethiopia was 3% (Abraham et al., 2005), Turkey 0% (Albayrak and Gür, 2010), Tanzania 2.1% (Swai et al., 2011), Morocco 0% (Touil et al., 2012), Nigeria 3.3% (Woma et al., 2015) and Sudan 2.1% (Saeed et al., 2017). However one author from Libya reported a seroprevalence of 22.8% in camels after a survey in 492 samples (El-Dakhly, 2015), this high percentage cannot be explained in a country who never notified the disease to the OIE.

Serological results have a limited predictive value, as they confirm only whether or not the animal has been in contact with the virus and produced antibodies. The published percentages in different regions are low if compared to small ruminant prevalence (up to 48.2% recently reported in India (Hota et al., 2018)) suggesting that camels are less sensitive to this virus.

The PPRV primarily affects small ruminants, the natural hosts, and occasionally wildlife susceptible species (Wohlsein and Saliki, 2006; Munir, 2014). Reports described clinical signs in camels have emerged from Ethiopia (Roger et al., 2000; Abraham et al., 2005), Sudan (Khalafalla et al., 2010; Kwiatek, 2011) and Iran (Zakian et al., 2016). Report from Ethiopia described a contagious acute respiratory disease, characterized by respiratory distress, lacrimation, and fever with over 90% morbidity and 5% mortality. Authors from Sudan described a sudden death, fever, oral erosion, diarrhea, pneumonia and respiratory distress, enlargement of lymph node, severe dehydration, dermatitis, ulcerative keratitis, and conjunctivitis. In Iran, identical clinical signs have been described in affected animals by Zakian et al. (2016), with no specific indication on the field morbidity and mortality rates, phylogenetic similarity with other isolates. In both Sudan and Iran, authors

Table 2
PPR serological survey performed in different countries.

Country	Year of publication	Sera quantity	Reported prevalence	Authors
Morocco	2012	1392	0 %	(Touil et al., 2012)
Libya	2013	492	22,8%	(El-Dakhly, 2015)
Egypt	1992	142	4,2%	(Ismael et al., 1992)
Nigeria	1997	250	4%	(Daneji et al., 1997)
	2008	136	0%	(Ibu et al., 2008)
	2014	1517	3,36%	(Woma et al., 2015)
Sudan	2002	100	7%	(Haroun et al., 2002)
	2010	392	0,3%	(Saeed et al., 2010)
	2017	1988	2,1%	(Saeed et al., 2017)
Ethiopia	2001	90	7% - 7,8%	(Roger et al., 2000)
	2005	628	3%	(Abraham et al., 2005)
	2010	400	1,5%	(Megersa, 2010)
Tanzania	2011	2010	2,6%	(Swai et al., 2011)

confirmed PPR by Ic-ELISA and qPCR (Khalafalla et al., 2010; Saeed et al., 2010, 2015, 2017; Zakian et al., 2016), isolation on cells was reported in Sudan only (Khalafalla et al., 2010; Saeed et al., 2017).

Experimental infections performed by El-Hakim (2006), report only subclinical infections or mild respiratory disease with coughing, nasal discharge and fever. Wernery (2011) inoculated a camel bull with a lineage IV strain without inducing any clinical signs. Our experiment also showed that dromedary camels are not sensitive to PPR after infection by a highly virulent isolate (Morocco 2015). No symptoms observed, no hyperthermia registered and no change in the animal behavior during 6 weeks of observation. The used strain caused death of goats after 7–10 days with typical PPR symptoms (Fakri et al., 2016).

Our results indicates that camels can replicate the virus but does not shed or transmit it as QPCR results are negative in conjunctival, nasal and rectal swabs. Banyard et al (2010) reported that pigs and cattle are not able to excrete the virus but they produce specific antibodies against PPR. Serology results confirm replication of the virus and antibody response at level considered low if compared to small ruminant (Fakri et al., 2015). Zakian et al. (2016) observed also an antibody response after camel vaccination with Nigeria 75 live vaccine from day 7 post vaccination.

These results suggest that dromedary camel is unlikely to be susceptible to PPRV and do not play a significant role in the epidemiology of PPRV as carriers.

However, more investigations are needed to clarify the situation regarding camel sensitivity. Alternative routes of challenge (subcutaneous and intranasal routes) could be evaluated.

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Availability of data and materials

All the data supporting our findings is contained within the manuscript

Authors' contributions

FZF carried out the experimental infection, laboratory tests, data analysis and interpretations and drafted the manuscript. ZB conducted the experimental infection and sample collection. MJ carried out PCR tests. KT participated in the design and the follow up of the study. ME participated in the design of the study, manuscript drafting, data analysis and interpretations. All authors read and approved the final manuscript.

Consent to publish

Not applicable.

Ethics approval and consent to participate

All procedures were followed in accordance with the international guidelines for care and handling of experimental animals and approved by the internal ethic committee for animal experiment, MCI santé animale.

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