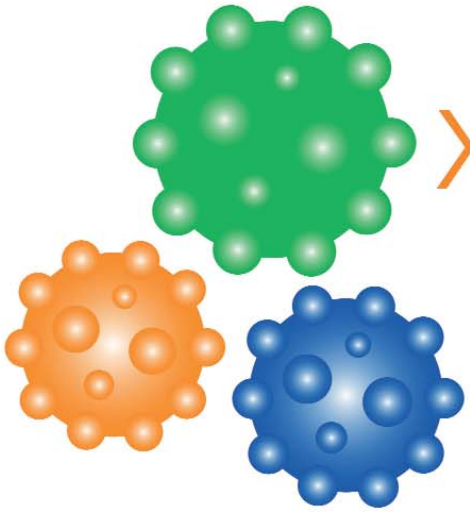

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ders and died within 6 days. The histopathology revealed an acute rhombencephalitis and ganglionitis of the trigeminal ganglion with massive neuronal necrosis in brain and ganglion. The relevant bacterial and viral infections of cattle were routinely excluded.

A brain sample from the cow was analyzed using metagenomic next generation sequencing and a bovine astrovirus (BoAstV) was identified. To confirm these findings a specific BoAstV RT- qPCR as well as a pan-reactive Mamastrovirus (MAstV) RT-qPCR for screening purposes were developed.

The genome of the novel bovine astrovirus (BoAstV), belonging to the family of *Astroviridae* in the genus *Mamastrovirus*, had a length of 6443 bp and showed only 71% sequence identity to a sheep astrovirus and 69% sequence identity to two newly described bovine astroviruses from the USA and Switzerland, which also caused encephalitis in cattle. The new virus was found in different brain sections using a specific quantitative BoAstV RT-PCR. Screening of the goat herd, in which the cow had been kept, revealed that no further animals were infected.

Astroviruses are mainly connected to gastroenteritis and are common in many animals and humans. Although, recently astrovirus caused encephalitis was described in a few human cases and also twice in cattle. However, the source of infection remains obscure and work on this newly discovered bovine astrovirus will be extended.

Peste des petits ruminants in Spanish sheep breed: protection study and experimental infection using NIG75/1 vaccine strain and MOR/08 field isolate

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Introduction and objectives: Peste des petits ruminants virus (PPRV), classified in the genus Morbillivirus, within the family *Paramyxoviridae*, causes an acute transboundary animal disease that affects mainly small ruminants, whereas cattle undergo a subclinical infection. PPR is considered to be highly contagious and spreads rapidly by direct contact through excretions/secretions from sick animals. This ability to spread, together with its socio-economic importance in developing countries made it one of the notifiable diseases listed by the OIE[1].

The severity of the disease depends on diverse factors such as host species and/or breed as well as on the virus strain involved. The acute clinical pattern is characterized by high fever, nasal and ocular discharge, oral lesions, bronchopneumonia, evidenced by coughing, dyspnoea and often diarrhoea. Affected animals may die within 5–10 days after the onset of clinical signs, reaching up to 90 % of mortality and morbidity in naïve populations. A subacute form is known in which clinical signs are very mild, the animals usually recovering within a week of the onset of symptoms.

PPR is endemic in most of Africa, the Middle East, South Asia and China. The most effective way of controlling PPR is by vaccination. Attenuated vaccines, mainly Nigeria 75/1 strain, have been commonly used in different endemic zones, inducing a reported life-long protective immunity in sheep and goats against all genetically defined lineages. Due to the epidemiological and economic significance, the international animal health organizations have given priority to the establishment of effective vaccination programs, control of animal movements and availability of efficient and rapid diagnosis.

Recently, PPRV strains belonging to genetic lineage IV have undergone a major expansion in close proximity to the Southern European borders, increasing the perception of risk associated with its introduction in this continent. Consequently, preparedness plans were implemented in Southern European countries to enable an earlier response in case of PPR emergence in their territories. In the framework of these plans, a project was implemented in Spain on the effect of PPRV in local sheep breeds, which included an evaluation of the efficacy of the vaccines available. The study presented here aimed at evaluating the protection conferred by the immunization of a Spanish native sheep breed with the most widely used live attenuated PPRV vaccine (Nigeria75/1 vaccine, lineage II) against a challenge with a pathogenic strain of PPRV isolated in Morocco in 2008 (Mor/08), belonging

to lineage IV. A second objective was to study the disease pattern produced in this Spanish sheep breed by the same pathogenic PPRV strain.

Methodology: PPRV Nigeria75/1 attenuated vaccine was inoculated subcutaneously to Spanish "Colmenareña" sheep breed (n=6) at a dose of 10^3 TCID50. At day 21 post-vaccination, four out of the six vaccinated sheep were challenged intravenously with a virulent PPRV Moroccan strain (Mor/08) at a dose of 10^4TCID50 and housed in contact with two naïve sheep (Group A). In parallel, in a different housing physically separated from the above group, another group of naïve sheep (n: 4) were infected intravenously with identical dose and strain, and housed together in contact with two naïve sheep and two previously vaccinated sheep (Group B).

Samples consisting of whole blood, serum, faeces, ocular, pharyngeal and nasal swabs, were collected at different days post-infection (dpi) and/or post-vaccination (dpv). Clinical signs using a clinical score system and rectal temperature (ºC) were recorded daily. Selected tissues were collected at post-mortem examination from all sheep used in the experiment.

Total RNA was extracted using QIAamp®cador Pathogen Kit (Qiagen®), adding an exogenous internal positive control (IPC). A real-time qRT-PCR reference protocol2 was adapted as a duplex method in order to incorporate IPC primers and probe. In order to detect specifically the vaccine RNA (Nig75/1), a DIVA qRT-PCR was developed. Duplicates of serum samples were tested for detecting PPRV-specific antibodies with a competitive ELISA[3], according to the manufacturer´s instructions.

Results:

Clinical follow-up: Sheep remained healthy during the vaccination stage. Group A (vaccinated and in-contact control sheep) remained asymptomatic after intravenous challenge. However, Group B presented a varying degree of symptoms: while the naïve infected sheep and in-contact sheep developed mild and discontinuous clinical signs consistent with the milder form of PPR disease, in- contact vaccinated sheep did not show any symptomatology. Necropsies displayed unspecific lesions in most of the animals. Only mild lesions were observed in symptomatically affected sheep.

Viral load estimated by qRT-PCR: Neither viral load in blood nor fecal virus shedding was detected by qRT-PCR in vaccinated animals prior to challenge. Only low levels of vaccine RNA were occasionally detected in swabs in 3 out of 6 individuals, gradually disappearing.

After intravenous challenge with the virulent strain, Group A remained RT-PCR-negative at any time in all samples examined, evidencing a sterilizing protective response on immunized sheep, and showing that no cross-infection between challenged and contact sheep occurred, which demonstrated an absence of PPRV circulation in the animal setting hosting Group A.

Regarding Group B, PPRV RNA was detectable by qRT-PCR as early as 2 dpi in whole blood in intravenously inoculated sheep before the onset of the first clinical signs, peaking at 7 dpi on average, and rapidly decreased at 10 dpi, but persisting for up to 21 dpi in all sheep and later showing up intermittently in some sheep up to the end of the experiment (up to 37 dpi). Virus excretion/secretion in collected swabs and faeces samples was first detected at 4 dpi, with a peak observed at 7 dpi on average, and then gradually declined. On the other hand, transmission of PPRV from infected to in-contact animals, including both naïve control and vaccinated sheep, was observed, with a kinetics of viral load in blood (peaking at 17 dpi) and virus shedding (peaking at 21 dpi) in the unvaccinated animals consistent with the acquisition of the infection from the experimentally infected sheep housed together. Lower virus loads in swabs and faeces and lack of viremia throughout the experiment were observed in the vaccinated contact sheep.

Serology: Vaccinated sheep developed detectable antibodies by c-ELISA at 7 dpv peaking at 10 dpv on average, remaining positive until the end of the vaccination phase.

During the second stage of the study, vaccinated sheep of Group A remained seropositive after challenge up to the end of the experiment, while the absence of antibodies in naïve contact controls confirmed that there was no cross-infection in this setting.

Similarly, in Group B, animals infected with MOR/08 strain showed antibodies from 4-7 dpi, rising on 7-10 dpi and remaining high during the period of the study. Within Group B, in-contact naïve and in-contact vaccinated

sheep showed different results: the first seroconverted between 14 and 21 dpi, while in the vaccinated group, one individual maintained the antibody titre acquired in the vaccination phase, but the other one showed a gradual decrease in its humoral response raised by vaccination to become undetectable after 10 dpi until the end of the experiment (30 dpi).

Vaccine genome detection by DIVA qRT-PCR: Tested at necropsy, in Group A, PPRV vaccine genome was present mainly in lymph nodes and spleen in vaccinated sheep but not detectable in unvaccinated control sheep. Notably, only vaccine RNA was detected in vaccinated sheep from Group B (kept in contact with infected sheep), but to a much lesser extent than in vaccinated sheep in Group A. Naïve control and infected sheep from Group B showed no vaccine RNA but a high level of challenge virus RNA in almost all selected tissues.

Conclusions: Spanish sheep breed "Colmenareña" inoculated with the pathogenic strain Mor/08 showed generally mild clinical signs, suggesting that this sheep breed is not particularly susceptible to PPR disease, at least in the conditions of this study. Vaccination with live attenuated vaccine PPRV Nigeria75/1 strain protected these animals against direct intravenous challenge with the pathogenic strain PPRV Mor/08. Conversely, the results evidenced the ability of Moroccan PPRV to spread by direct contact from excretion/secretion sites to contact sheep, including vaccinated ones. Absence of viremia and clinical signs in these vaccinated sheep that became infected, probably through contact, suggests the activation of a kind of immune response conferring partial protection. The role this process plays in the epidemiology of PPR as generator of potential asymptomatic carriers is as yet unknown. Interestingly, the loss of antibodies observed in one of these sheep upon challenge might indicate a weakness of the humoral antibody response elicited by vaccination in this particular sheep. Whether this loss is permanent or transitory and could be recovered after 30 dpi (monitoring period in the experiment) is not known.

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Novel insights in experimental peste des petits ruminants virus infection, transmission, pathogenesis and propagation

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The recent rapid spread of PPRV lineage 4 (L4) to Northern African and in Asian countries bordering Europe poses a high risk of PPRV emergence to the naïve population of susceptible European animal species. PPRV-pathogenesis in goats that are highly susceptible to PPRV infection has been thoroughly investigated. However, the maximal duration of excretion of infectious PPRV after experimental infection and the possibility of oral PPRV-infection remain to be investigated. Whether other Artiodactyla species might contribute to PPRV-spread is scarcely understood, but is important for the design of effective vaccination strategies with regard to the discussed plans about the eradication of PPRV.

Therefore, various animal trials were conducted to further illuminate these gaps in knowledge:

1) The minimum infectious dose (MID) of a highly virulent PPRV-lineage 4 strain from Kurdistan (2011) was investigated in 4 groups of 4 goats and compared to virus isolation in a Vero cell line expressing CD150 (SLAM). Four goats in each group were intranasally infected with any of the undiluted or tenfold diluted (10^{-1} to 10^{-3}) PPRV preparations. In four goats that showed mild clinical signs the maximum duration of PPRV excretion was monitored until PPRV-RNA excretion has ceased according to real-time RT-PCR analysis at 90 dpi.

2) Three cattle and three pigs were intranasally infected with PPRV Kurdistan/2011 in two independent experiments. Two days after infection (dpi), two goats were housed together with the cattle and pigs, respectively.