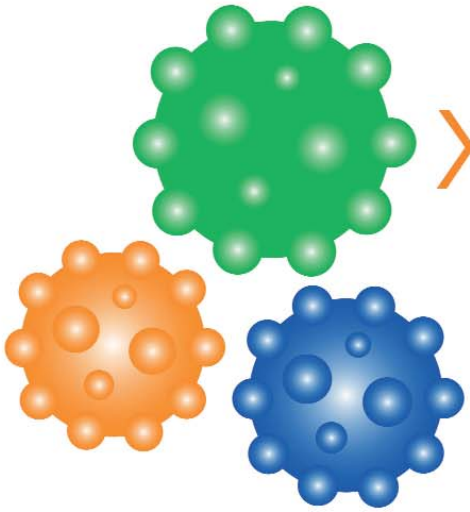

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fa domesticus) is of increasing concern in many European countries. In Serbia, the wild boar is one of the most important big game species. However, information on the prevalence and distribution of potentially important infectious disease agents among wild boar populations in Serbia is currently very limited, or doesn't exist. The aim of the current study was to investigate the presence of selected viral pathogens in wild boars populations in Serbia, and to assess possible role of wild boars in the epidemiology as reservoirs of these viruses for domestic pigs and other domestic and wild animals and for human population in Serbia.

Methods: Blood samples from 381 wild boars from 53 hunting grounds and 13 out of 25 counties in Serbia, that are 3.66% of predicted number of wild boars (10 409) on the observed territory, and 1.91% of predicted number of wild boars (19 908) from all 12 districts and 142 hunting grounds of Serbia, were collected during the hunting season from October 2011 until March 2012. Blood samples were taken by hunters or by veterinarians from the heart after the wild boars had been shot. Collected blood sera were tested by commercial enzyme-linked immunosorbent assays (ELISAs) for the presence of antibodies against Aujeszky's disease virus (ADV), H1N1 and H3N2 swine influenza viruses (SIV), and hemagglutination inhibition (HI) test was used for detection of antibodies against porcine parvovirus (PPV).

Results: Out of 381 analyzed blood sera samples, antibodies against ADV, SIV H1N1, SIV H3N2 and PPV were detected in 27.03% (103), 4.73% (18), 5.51% (21), and 61.68% (125) samples, respectively. The prevalence of seropositive wild boars to ADV (102/32.38%); SIV H1N1 (18/5.71%); SIV H3N2 (20/6.35%); and PPV (209/66.35%) from 34 hunting grounds on the northern part of the country (315 samples from Vojvodina province) was higher than those found among wild boars (66) from 19 hunting grounds from south part of the country (ADV (1/1.52%); SIV H1N1 (0/all negative); SIV H3N2 (1/1.52%); and PPV (26/39.39%)). Anti-PPV antibodies were detected in wild boars originated from all counties from where the samples were collected. Seropositive wild boars to ADV were detected in all (6 out of 6) tested counties on the northern part and in just one out of 7 counties on the southern part of Serbia. Seropositive wild boars to SIV H1N1 were found just on the northern part of the country in 4 and 3 out of 6 examined counties, and seropositive wild boars to SIV H3N2 were found in 3 out of 6 and in 1 out of 7 examined counties on the northern and southern part of Serbia, respectively.

Conclusion: Our results indicate that wild boar populations throughout the Republic of Serbia are exposed to PPV. Also, our results show that ADV is highly prevalent, especially among wild boars from northern part of Serbia, the area of higher density and intensive pig production. In addition, our results indicate presence of both H1N1 and H3N2 swine influenza virus infections that are more prevalent at the northern part of the country. This is the first comprehensive serologic study on selected viral diseases in wild boars in Republic of Serbia. Our results provide information on the current disease exposure to selected viruses and health status of wild boars in Serbia. The obtained results point on the possibility that wild boars in Serbia may play a significant role in the epidemiology of studied viral diseases and act as a potential reservoir and source of infection for domestic, especially free range pigs, and other animals as well as humans. Further and more comprehensive research is needed including testing of wild boar samples from the whole country and from a few hunting seasons on antibody and virus presence to obtain more conclusive results on presence and role of examined viruses in wild boars on the epidemiology of disease in Serbia.

Keywords: ADV, SIV (H1N1/H3N2), PPV, wild boar, seroprevalence, Serbia

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Is Ornithodoros erraticus able to transmit the Georgia2007/1 African Swine Fever virus isolate to domestic pigs?

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Objective: African swine fever, one of the most devastating diseases affecting wild and domestic swine, is due to a large DNA virus, only member of the *Asfarviridae* family. After ASF introduction in Georgia in 2007, the disease became endemic in the Caucasian region of the Russian Federation and spread towards the Western regions of Europe entering in EU Members States at the beginning of 2014. As no vaccine or antiviral are available to fight against this infection, the only tools to control it are preventive measures based on early detection and actual knowledge of the epidemiological risks. In African sub-Saharan countries, ASF persistence is described to be related to different and complex epidemiological scenarii involving domestic and wild suids and soft tick vectors of the genus *Ornithodoros*. In EU, one species of *Ornithodoros*, *O. erraticus*, is known to be able to maintain and/or transmit some ASFV isolates classified in the genotype I. Recently, the Pirbright Institute also demonstrated that *O. erraticus* was able to amplify the Georgia2007/1 ASFV (genotype II), at least during 3 months. The objective of the current study was to evaluate the in- vitro and in-vivo transmissibility of the Georgia 2007/1 ASFV by infected *O. erraticus* ticks.

Methods: The Georgia 2007/1 ASFV strain belonging to the genotype II, kindly provided by L. Dixon (OIE reference lab), was grown on porcine pulmonary alveolar macrophages to the titre of $10^6$to 10^7 HAD_{50} , then diluted 100-fold into pig blood for tick infection or 1000-fold in medium for intradermal inoculation to pigs.

Ticks were captured in Portugal by F. Boinas and mass reared at CIRAD for one year and a half to obtain a stable and mature population. During this period, several techniques of artificial feeding were tested to optimize the method. In December 2014, 60 adults or last nymphal stages -group A- coming both from field and 1st generation laboratory were artificially engorged on pig blood supplemented with Georgia 2007/1 at a final titre of $10^{4.5}$ HAD_{50}/mL blood. Two other groups of ASFV-free ticks –group B with 60 individuals and group C with 30 individuals- were reared to be used for a second infection directly on infected pigs (group B) and as control group (group C), respectively. Moreover to confirm the possibility to infect ticks through artificial blood meal, another group of 10 ticks was also engorged and tested for virus multiplication three months later. Fifteen other females were also infected and secondarily engorged on ASFV-free pig blood to test in-vitro transmission through virus isolation on second blood meal. Considering that it is difficult to obtain ASFV titres with in-vitro cultivation as high as in infected pigs developing ASFV clinical signs, it seems important to compare ASFV transmissibility between ticks artificially infected in laboratory and ticks directly infected on ASFV-infected pigs and conclude on possible dose effect. In March 2015, 18 Large-White pigs obtained from a high sanitary level field herd were distributed to 4 groups at Anses-Ploufragan high security facilities. Two negative control groups of 3 pigs were either intra-dermally inoculated with MEM or bitten by group C of 30 healthy ticks. One group of 6 pigs was intra-dermally inoculated with 10^3 HAD_{50} ASFV while the last group of 6 pigs was bitten by group A of 60 ticks previously infected through artificial blood meal and dispatched in 10 ticks/pig. Pots of 10 ticks were placed on one ear held there with adhesive tape, then removed after 3 hours. After removal, ticks were numbered in two batches: engorged and unengorged ticks. Finally, as soon as the 6 pigs intra-dermally inoculated with ASFV showed fever and high viremiae, group B of 60 ASFV-free ticks were proposed to engorge on their opposite ear. These ticks would be proposed to secondarily engorge on membrane feeding or healthy pigs three months later.

Post tick feeding or intradermal inoculation, clinical examination and rectal temperatures were recorded daily, until the animals were euthanized or for a minimum period of 18 days. Except on D1 pi, serum and EDTA blood samples were daily collected from all the pigs during the first week pi, then twice a week during the 2 following weeks, and at the day of euthanasia for virological and serological assays. Organ samples were collected during necropsy. The animal experiment protocol was approved by the French national ethics committee ComEth Anses/ENVA/UPEC (10/03/15-9).

Results: Ten ticks from the original batch of ticks that were artificially fed on infectious blood were tested by virus titration. Out of them, 8 were positive with a titre ranging from 10^2 to $10^{4.2}$ HAD_{50}/tick and 2 ticks clearly amplified the virus regarding the estimated amount of virus originally ingested (minimum of 1 log superior). After feeding on pigs, the mean level of engorged ticks was of 62%, whatever the group of pigs.

The experiment, currently running, confirmed the high virulence of the Georgia strain as all the 6 intra-dermally inoculated pigs displayed typical symptoms of ASF including lost of appetite and hyperthermia from D3 pi. The 6 pigs were euthanized from D5 to D7. The group of the 6 pigs bitten by the infected ticks was still healthy at 18 days post feeding, as well as the two negative control groups. However, among the 15 female ticks secondarily

engorged on ASFV-free pig blood, no heamadsorption effect was observed after two passages on alveolar macrophage culture using blood-meal leftovers. Further investigations are needed to confirm the presence of ASF Virus . The final experimental infection of pigs through tick bite using ticks previously engorged on viremic pigs should allow concluding on the ability of *O. erraticus* to transmit Georgia2007/1 and a possible dose effect on this transmissibility. The results will be presented and discuss during the symposium.

Conclusion: The objective of this study was to experimentally assess the ability of the European *O. erraticus* tick to transmit the Georgia 2007/1 ASFV currently circulating in North-Eastern EU. First results showed that ticks artificially infected in laboratory did not induce ASF clinical signs in pigs by biting. However, virus titration in ticks seems to show that the virus is present in the arthropod. Further in-vitro and in-vivo investigations are running to explore the hypothesis of a dose effect. The expected results should clarify the potential epidemiological role of *O. erraticus* ticks in transmission and re-emergence of ASFV in the field, in case of the spread of current active foci from North-Eastern EU.

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A multidisciplinary approach to combat wildlife diseases: Vaccination with hematophagous arthropods as "living syringes"

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In a different approach for combating insect-borne zoonoses, we propose that hematophagous arthropods could be used to carry no longer a pathogen but a vaccine. Using vectors as "living syringes" provides a unique way of reaching in accessible wild host populations. This in order to protect endangered wild species directly, but it could also reduce risk of zoonoses (to humans, cattle…) while protecting the wildlife species that acts as a disease reservoir.

Our research focuses on the protection of the European Wild Rabbit *Oryctolagus cuniculus* (L.) against 2 fatal viral diseases: myxomatosis and RHD (Rabbit Hemorrhagic Disease), using a specific rabbit flea species. There are two major research areas in our project: entomology and virology.

Entomology studies have involved (i) selection of a suitable vector, (ii) laboratory studies to develop an efficient mass rearing, (iii) verification that it has no side-impact on the ecosystems where it is introduced and (iv) definition of a field release strategy that promotes host-parasite contact while minimizing insect loss.

Virology involves development of an efficient vaccine against the 2 diseases that can be transmitted by the insect-vector. Among the various natural strains of myxomatosis, the most suitable according to us is a virus of very low virulence (grade IV), which causes the formation of antibodies in rabbits without mortality, yet protects them against further acute disease. This attenuated strain also maintains a good natural disease resistance among rabbit populations. Regarding RHD, as virus culture is impossible, it was necessary to use a viral recombination RHD/myxomatosis as a potential vaccine against both diseases.
Finally, at the interface between entomology and virology, we are working on a method for introducing sufficient vaccine onto the mouth-parts of mass-reared fleas to assure its transmission to rabbits.

Key words: wildlife vaccination, vectorisation, insect vector, flea, siphonaptera, *Xenopsyllacunicularis*, european wild rabbit, *Oryctolagus cuniculus*, myxomatosis, RHD, recombinant vaccine, mass-rearing, release strategy, biological control