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Development of micro-amount of virion enrichment technique (MiVET) and application to transboundary animal diseases countermeasures

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Background
Transboundary Animal Diseases (TADs), such as African swine fever (ASF), foot-and-mouth-disease (FMD) etc cause vast economic losses throughout the world. Despite of the importance of identifying sources/propagation route of the spread, it is often confounded due to incomplete epidemiological evidence. Isolation/detection of the micro-amount of virion from environmental samples is rarely successful due to very low contamination level in the environmental samples. We have recently developed a "micro-amount of virion enrichment technique" (MiVET) in combination with the complex of polyclonal Ab and protein G-coated magnetic beads (pG-MB), and simple SDBS (sodium dodecyl benzenesulfonate) elusion. In this study, we have evaluated the performance of MiVET system.

Methods
Two avian influenza viruses (AIVs), consists of H5N2 and H7N7 subtypes, were artificially spiked into 1mL of 50% duck fecal supernatants and 50mL of PBS, respectively. IMB (immuno-magnetic beads, complex of commercially available anti-fluA polyclonal Ab and pG-MB) were added into the samples. Then, the mixture was incubated at 37 degree for 15 min. The tube was set onto a magnetic stand for 2 min, and then, supernatant was removed. After washing the IMB with PBS at 1 time, the supernatant was removed again. The IMB capturing virions were eluted 2 µL (for 1 mL of fecal supernatant) or 8 µL (for 50mL of PBS) of SDBS. The resulting all supernatant was used as template RNAs for real-time RT-PCR assay.

Results
The MiVET system successfully concentrated AIVs in fecal and PBS samples at least 10 and 100 times, respectively, in comparison to conventional column kit extraction. The MiVET system required less than 30 min from the beginning of incubation to the final RNA elution.

Conclusion
The MiVET system appears to be valuable in providing useful information to control programs for various animal diseases such as ASF, FMD etc, as well as identifying the source/propagation route of the spread.

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Widening the scope of PPR diagnostic: adaptation and development to target atypical host species and field situations

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Background
Peste-des-petits ruminants (PPR) is a highly contagious and devastating viral disease affecting sheep, goats, and a large number of species within the order Artiodactyla. Robust commercial serological and virological diagnostic kits are available to detect PPR infection, but they were mainly developed for domestic small ruminants (goat and sheep) and for high quality, invasive samples sometimes hard to obtain in the field. New tools are needed to detect PPR infection in atypical hosts (e.g. camels, wildlife) and in complex field situations. Here we present adaptation of existing methods and new diagnostic tools to resolve some of these issues. In some regions, farmers are reluctant to have their animals handled and tested for PPR infection. As well, in the case of wildlife survey, animal capture is very costly and demands complicated logistics. Detection of PPR virus in non-invasive samples (feces) can be extremely useful in such cases.

Methods and results
We adapted methods of RNA extraction, RT-PCR, RT-QPCR and antigen capture ELISA to detect PPR viral particles or genetic material from fecal samples. The methods were validated with samples collected during an infection experiment. Our protocol was then used with fecal samples obtained in the field in Tanzania in 2015, and compared to results obtained from ocular swab samples taken from the same animals. Another issue is that existing LFD tests used in the field do not allow for direct confirmation by PCR. Here we present a new rapid penside test, produced and distributed by IDvet (France), which can be performed without any lab equipment. Lastly, we also tackled the issue of PPR antibody detection from camelid sera, usually suboptimal because of their particular antibody structure.

Results
Our results show that virus particles can be detected in fecal samples from 4 days post infection (dpi) until at least 14 dpi. Sensitivity of RT-QPCR from fecal samples was similar to RT-QPCR and lateral flow device (LFD) on ocular swab samples. The penside test was as sensitive as the antigen capture ELISA test. Once dried, positive strips can be stored and used later on for confirmation by RT-QPCR or RT-PCR. A simple modification of the protocol of a commercially available competitive ELISA for PPR antibody detection (IDvet) increased the sensitivity of the test on camelid serum.

Conclusion
These tools will be extremely useful to unravel important questions that still remain about PPR epidemiology, notably the role of atypical host in PPR transmission dynamics.