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The role of digital PCR in veterinary and environmental diagnostic: one health concept

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INTRODUCTION

The coronavirus disease 2019 (COVID-19) pandemic has put the one health concept in a first plan with the contribution of the veterinary world in the massive SARS-CoV-2 testing and with the monitoring of environmental samples like wastewater. Through this crisis it appears clearly that pandemic pathogens might come of an animal to Human transmission. It seems crucial to monitor the pathogens presence in animals. As well as the monitoring of wastewater during the COVID-19, controlling the breeding environment should be a great alternative to the individual testing.
The real-time polymerase chain reaction (qPCR) is widely used for the detection and quantification of micro-organism in the veterinary diagnostic. It allows to detect and quantify pathogens rapidly with a high sensitivity and specificity. However, environmental samples like wastewater, environmental wipes or bedding material sampling may contains very few pathogens quantity and a lot of inhibitor. Thus, the digital PCR (dPCR) appears as a new solution. Its extreme sensitivity, huge quantification reliability and a high inhibitor resistance has been put forward in the literature. All these benefits could be gamechanger for veterinary and environmental diagnostic.

MATERIAL & METHODS

We evaluate the dPCR potential on 3 key features for veterinary and environmental testing: sensitivity, reliability of quantification and inhibitor resistance. The sensitivity, as well as the quantification repeatability, has been evaluated on wastewater for the SARS-CoV-2 quantification. The dPCR has also been tested for avian influenza virus detection in strongly inhibited samples. All analysis has been realized both in dPCR and in qPCR on a Mic qPCR cycler (Bio Molecular Systems, Australia).

RESULTS

About 40 wastewater samples have been tested for the E gene of Sarbecoviruses and N gene of SARS-CoV-2 both on digital PCR and on Mic qPCR cycler with the Bio-T kit® Environmental SARS-CoV-2. Similar sensitivities have been observed on those samples for both methods. Moreover, the dPCR seem to improve the reliability of quantification on low concentration samples.
Environmental samples and organs found inhibited on Mic qPCR cycler for the detection of avian influenza virus, are also found inhibited in dPCR. Moreover, the level of inhibition is lower, and most samples can be quantified.

DISCUSSION AND CONCLUSION

The close sensitivity observed between the qPCR and the dPCR did not demonstrate an important impact of this technology for the requirements of veterinary and environmental diagnostic. However, a better inhibitor resilience and the capacity of quantifying pathogens without the need of calibration curve could have an interest. Furthermore, the dPCR allows the analysis of multiple replicates as one whole sample. It increases the analysis cost but could improve the sensitivity. Thus, in order to monitor a whole breeding and reduce analysis cost, the digital PCR would be interesting for an absolute quantification of pathogens in breeding inhibited environmental samples.