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Influenza D pseudotyped lentiviruses: production, neutralisation assay and serological surveillance

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Influenza D virus (IDV) has been reported in many animal species and potentially humans worldwide. Cattle are considered the major reservoir. There are currently three main lineages based on the haemagglutinin-esterase (HEF) gene: D/OK, D/660 and D/Japan. We performed pilot surveillance for IDV by using pseudotyped lentivirus (PVs) to generate a cell-based test to identify prior-exposure to IDV in animals. The expression plasmids of the HEF genes, D/swine/Italy/2015, D/bovine/France/2014, and D/bovine/Ibaraki/2016, were constructed. The HEF plasmid was co-transfected with lentiviral vector plasmid expressing luciferase, lentiviral Gag-Pol plasmid, and HAT protease plasmid in producer cells (HEK293T/17). Three days post-transfection, supernatants were collected and used for titration on various cell lines and in micro-neutralisation tests. Sera from pigs vaccinated with D/swine/Italy/2015 and D/swine/Oklahoma/2011 were used to undertake a preliminary validation of the micro-neutralisation assay. All pig sera have neutralising activity to influenza D (Italy) pseudotyped lentiviruses. Cow and sheep sera, 145 and 114 specimens, respectively, collected from UK farms were screened using the micro-neutralisation test. We found 97 bovine sera (66.9%) were influenza D antibody positive. Collectively, pseudotyped lentivirus technology opens up opportunities for serological surveillance of influenza D viruses.

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