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Title: Susceptibility of camelids (alpacas) to experimental infection with influenza C and D virus

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Background:

Influenza D virus (IDV), a member of the influenzavirus D genus within the *Orthomyxoviridae* family, was initially detected in 2011 in pigs and subsequently in cattle. IDV has a similar genome organisation to influenza C virus (ICV) and both encode a single hemagglutinin-esterase-fusion (HEF) envelope glycoprotein. Influenza A (IAV), C and D viruses are all maintained in non-human mammalian host species, but unlike IAV and ICV, IDV has not been conclusively associated with human infection. Although camelid species have not been extensively studied as potential reservoir hosts for zoonotic viruses, we have previously reported the detection of ICV- and IDV-reactive antibodies during a serosurvey of dromedary camels in Kenya (Salem et al, 2017). To gain further insight into camelid susceptibility to ICV and IDV, alpacas (*Lama pacos*) were experimentally inoculated with ICV (C/Victoria/1/2011) or IDV (D/bovine/France/5920/2014) and infection parameters were monitored longitudinally.

Methods:

Groups of seven seronegative alpacas were inoculated intranasally with ICV or IDV. Clinical signs were monitored daily and nasal swabs were obtained until 14 days post-infection (dpi) to monitor shedding of viral RNA. Serum samples were obtained pre-inoculation and on 3, 7, 14 and 21dpi. *Post-mortem* examination (PME) was planned for three alpacas per group on 3dpi and for two alpacas per group on 7 and 21dpi.

Results:

Infection with either ICV or IDV resulted in a mild clinical presentation for most animals. Elevated temperature and slight diarrhoea were observed in some animals in the ICV group, although one alpaca was humanely euthanised at 14dpi with a pyrexial response. Gross pathology and histology findings were unremarkable for all animals at PME. For both viruses, shedding of viral RNA was detected in nasal swabs between 1-7dpi and peaked at 2-4dpi. ICV and IDV RNA was detected in upper respiratory tract (URT) tissues at 3 and 7dpi, indicating active viral replication. Host responses to infection were monitored and seroconversion to both viruses was detected by 21dpi.

Conclusions:

We have demonstrated that alpacas can be experimentally infected with ICV or IDV. Subclinical or mild infection of the URT was detected, suggesting the potential for virus transmission by infected animals. These findings accord with our previously reported detection of ICV and IDV antibody positive samples during a serosurvey of camelids. Further studies are warranted to assess a putative role for camelids as a reservoir host species capable of supporting ICV and IDV circulation.