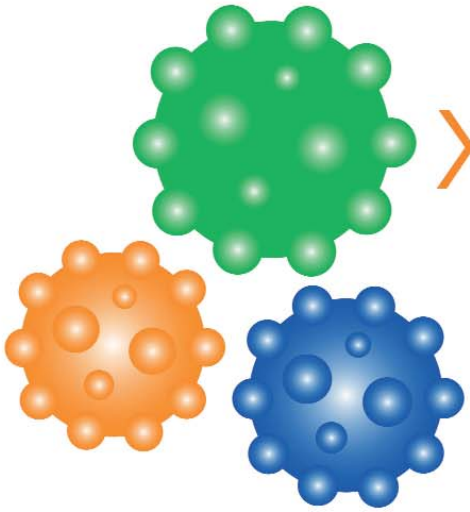

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measuring transcript levels by RT-qPCR. BTV-4 infection led to enhance transcription of IFN-γ, TNF-α, IL-6, IL-12-p40, and IL-1β mRNA in thymus, spleen and lung and the increase of cytokine induction correlated with the level of virus replication in these tissues.

Conclusions: IFNAR(-/-) mice are susceptible to the infection of BTV-4 MOR2009/09. After infection, BTV infected mice show clinical signs characterized by ocular discharges, apathy and the disease progression led to animal death. Infectious virus is recovered from the spleen, lung, thymus, and blood. Disease progression and pathogenesis (induction of inflammatory modulators, apoptosis, and pro-inflammatory cytokines) closely mimic hallmarks of bluetongue disease in ruminants. IFNAR(-/-) mice are a good choice to facilitate a faster advance in the field of orbiviruses.

Bluetongue and epizootic haemorrhagic disease viruses in Reunion Island

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Objective: Bluetongue (BT) and epizootic haemorrhagic disease (EHD) are arthropod-borne diseases of wild and domestic ruminants caused respectively by viruses belonging to the species Bluetongue virus (BTV) and *Epizootic haemorrhagic disease virus* (EHDV) within the genus *Orbivirus* of the *Reoviridae*. The viruses are transmitted between ruminants by biting midges of the genus *Culicoides* (Diptera: Ceratopogonidae). BTV went undetected in Reunion Island between its first documented emergence in 1979 and two other serious outbreaks with both BTV-3/ EHDV-6 in 2003, and both BTV-2/EHDV-6 in 2009. In these outbreaks, infected animals developed symptoms including hyperthermia, anorexia, congestion, prostration and nasal discharge.

In order to get an overview of the circulation of BT/EHD in Reunion island, an assessment of the prevalence in ruminants native to Reunion Island by a cross-sectional study was undertaken in2011on 67 farms, including a total of 276 cattle, 142 sheep and 71 goats with a total of 489 ruminant samples. Data concerning farm characteristics, type of production, and number of animals were collected through farmer questionnaires for an evaluation of the associated risk factors. In addition, investigation of clinical cases based on the observation of clinical signs was also performed in order to get BTV/EHDV isolates with the aim to track the origins of the circulating strains.

Methods:

Risk factors analysis

Data concerning farm characteristics, type of production, number of animals, closeness to another farm and sugar cane fields, presence of organic and other waste on the farm, exposure to wind, distance to a permanent water point, type of animal housing, presence of ticks on animals, use of treatment against ectoparasites and insects, animal's contacts with other animals or humans, grazing practice, spreading of manure on pastures, presence of *Tenrece caudatus*, rodent control, number of abortions in the herd in the last 12 months, purchasing behaviour, quarantine of newly purchased animals, other biosecurity factors like hygienic precautions taken by the staff or other people entering the farm (truck driver, vets and other visitors) were taken from a questionnaire which was filled in during an interview with the farmers. This questionnaire was pre-tested on five farms in a preliminary study. The final questionnaire comprised 40 questions of which 75% were closed-ended.

Serological assays

Specific anti-BTV antibodies were tested in serum samples with a group-specific competitive ELISA based on the VP7 protein using a commercial kit (LSIVet™ Ruminant BT Advanced II- Serum, Life technologies, France). Specific anti-EHDV antibodies were tested using a blocking commercial kit (LSIVet™ Ruminant EHDV-Serum

ELISA kit, Life technologies, France). A Sunrise ELISA reader was used for reading at 450 nm (Tecan, France). Optical density values were converted to percentage inhibition (PI). According to the cut-off value of the test, test samples with PI values ≥ 40% for BT and ≥ 60% for EHD were considered as positive.

BTV/EHDV genome detection

For the BTV group specific real-time RT-PCR, 6 µl of denatured double-stranded RNA prepared with the EZ1 robot and EZ1® Virus Mini Kit v2.0 (Qiagen, France) were reverse transcribed (RT) and amplified using the one-step QuantiTect Probe RT-PCRkit (Qiagen, France) based on segment 1 developed by Toussaint et al. 2007. For the EHDV group specific real-time RT-PCR, 5 µl of denatured double-stranded RNA were reverse transcribed (RT) and amplified using the commercial TaqVet™ EHDV (Life technologies, France).The subgroup-specific EHDV RT-PCR based on segment 2 was performed according to Sailleau et al., 2012.Embryonated chicken eggs (ECE) were each inoculated as previously described in Sailleau et al., 2012

Sequence analysis, alignment and phylogenetic analysis

To identify the genetic relatedness of the detected virus, phylogenetic analyses were performed with published *EHDV* sequences. Sixteen full-length VP2 gene sequences were cleaned by hand from the results of several BLAST nucleotide searches as well as direct references from available up-to-date literature and then aligned using the ClustalW translation alignment tool in MEGA (Ver. 5.05). Phylogenetic analysis was performed using the neighbour-joining method using distance measures generated by the p-distance algorithm running 1, 000 iterations with Geneious® Pro.

Statistics

A Fisher exact test was used to compare differences in prevalence between diseases and species. All statistical procedures were performed using R.3.0.1. A value of $P < 0.05$ was considered significant. The prevalence rates were estimated as the overall mean and 95% confidence interval (CI).

Results:

The observed EHD prevalence rate in cattle was 63.77% (95% CI [57.99–69.55]), 5.63% (95% CI [0.03–10.99]) in goats, and 3.70% (95% CI [0.05–6.88]) in sheep, suggesting that EHD occurs more often in cattle than in goats and sheep. These findings were supported by a significant statistical difference in the EHD prevalence rate between species (Fisher exact test, P <<2.2e-16).

The observed BT prevalence rate in cattle was 79.62% (95% CI [74.77– 84.47]), 50.70% in goats (95% CI [39.08–62.33]) and 21.48% in sheep (95% CI [14.55–28.40]) with a significant difference in BT prevalence between species (Fisher exact test, P = 4.367e-10).

Additionally, three suspected outbreaks occurred during the 2011 study period, one BTV/EHDV negative, one BTV specific and one combined BTV/EHDV outbreak. In total, 14 EHDV positive cases and 1 BTV/EHDV co - infection case were identified. Two further suspected outbreaks were confirmed to involve EHDV and BTV/EHDV. Isolations of EHDV were successful resulting in the identification of the Reunion -specific EHDV-1 serotype. Phylogenetic analyses of segment 2 showed that the Reunion isolate 6010 _2011 belongs to the group C (hypothesised in Anthony et al. 2009 together with EHDV-1 strains from Australia, 1995, Nigeria, 1967, French Guyana, 2011 and New Jersey, USA, 2011). In January 2014, once more suspected outbreaks occurred on cattle with observed clinical signs such as hyperthermia, congestion and nasal discharge. Virus isolations were successful and led us to identify a new EHDV serotype for Reunion island, the EHDV-7 serotype**.**

Conclusion: Our results confirm that the prevalence of both BT and EHD is high and that both are likely currently circulating. A high risk of BTV and EHDV infections was associated with the introduction of ruminants from neighbouring farms without quarantine, the presence of organic and other waste on the farm, and treatment against ectoparasites and insects. New circulating EHDV serotype 1 and serotype 7 of unknown origin were isolated in 2011 and 2014 respectively. The mechanisms involved in the introduction, maintenance, and perpetuation of both BTV and EHDV orbiviruses in Reunion Island need to be further investigated. How and when the EHDV serotypes were introduced onto the island are unknown, the most likely being the introduction of infected animals from eastern and southern Africa, Madagascar or Australia over a period of many years. The introduction of Malagasy breeds, which could be considered as orbivirus susceptible breeds many decades ago, is one possible hypothesis. Since 1976, importation of domestic ruminants from these countries has stopped. Until 2008, imports were only from mainland France. The maintenance of both viruses in the livestock population could also be due to the presence of reservoirs such as deer as was the case in many places including southern California between 1990 and 2007 (Roug et al., 2012). Pathogens can easily be shared between wildlife and

domestic ruminants which has implications for both the animal production industry and wildlife health. Whether animal reservoirs such as Rusa deer *Cervus timorensis rusa* imported from Mauritius Island and now present in Reunion Island play a role in EHDV epidemiology need to be investigated. The same species of Rusa deer was introduced on the island of Mauritius in 1639 and serological evidence of both EHDV and BTV circulation is documented. Since 1992, in accordance with European Union regulations, importation of live deer from Mauritius to Reunion Island is forbidden. The intermittent detection of certain serotypes and the occasional appearance of new serotypes suggest that, in the past, regular but separate introductions of BTV/EHDV may have also taken place from Madagascar, and from Southeast Asia including Mauritius via windborne *Culicoides*. Although it exists, the observed herd immunity in Reunion Island is not high enough to prevent the maintenance of an enzootic cycle, which could also be related to the abundance and activity of *Culicoides* throughout the year. The findings reported here provide additional hypotheses regarding the ecological characteristics of bluetongue and epizootic haemorrhagic disease and other vector -borne livestock diseases. Sentinel surveillance programmes are a useful way of documenting regionalization zones for diseases, which can be of great importance when securing livestock international markets.

First detection of porcine epidemic diarrhea virus in Slovenia, 2015

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Objective: Porcine epidemic diarrhea (PED) is a viral disease that affects swine of all ages, often leading to high piglet mortality rates. Limited data about the prevalence are known for European countries and no data were available for Slovenia.

Methods: In this study a total 63 samples were collected from 10 herds, where diarrhea was main clinical symptom for suspicion, but without increased mortality in piglets. Feces samples were collected between December 2014 and February 2015. After RNA extraction, PEDV nucleic acids was detected by commercial real-time RT-PCR (virotype® PEDV/TGEV).

Results: First PEDV positive results were identified from six samples collected on January 6 2015 on a pig farm. PEDV was detected by real-time RT-PCR method also from feces samples collected one and two weeks after first positive results on the same farm. The obtained cycle threshold values for positive samples were between 16.3 and 23.9, confirming high viral load in a feces on infected farm. The second PEDV positive farm was confirmed in February 2015, located in the same geographic region as the first pig farm. Three representative positive samples were amplified by conventional RT-PCR and sequenced for phylogenetic analysis. Based on a phylogenetic comparison of 390 nucleotides of the RNA polymerase gene, the detected PEDV strain showed 99.7% nucleotide identity to the closest sequence GER/L00719/2014 detected in Germany in 2014 and 99.2 to 99.7% to strains detected between 2013 and 2014 in USA and China. Preliminary results of commercial ELISA for 92 randomly selected samples, collected between November 2014 and February 2015 from 14 different pig farms, showed that antibodies against PEDV were detected in 51% of tested pigs and in 78% of tested farms.

Conclusion: The origin of this new virus on territory of Slovenia is still unclear, but may be a result of one or more imports of live PEDV positive pigs and then virus was spread rapidly through many swine farms.

Identification and genetic characterization of Aichivirus (porcine kobuvirus) in pig farms in Slovakia

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