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Review

New viruses in veterinary medicine, detected by metagenomic approaches

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ABSTRACT

In our world, which is faced today with exceptional environmental changes and dramatically intensifying globalisation, we are encountering challenges due to many new factors, including the emergence or re-emergence of novel, so far "unknown" infectious diseases. Although a broad arsenal of diagnostic methods is at our disposal, the majority of the conventional diagnostic tests is highly virus-specific or is targeted entirely towards a limited group of infectious agents. This specificity complicates or even hinders the detection of new or unexpected pathogens, such as new, emerging or re-emerging viruses or novel viral variants. The recently developed approaches of viral metagenomics provide an effective novel way to screen samples and detect viruses without previous knowledge of the infectious agent, thereby enabling a better diagnosis and disease control, in line with the "One World, One Health" principles (www.oneworldonehealth.org). Using metagenomic approaches, we have recently identified a broad variety of new viruses, such as novel bocaviruses, Torque Teno viruses, astroviruses, rotaviruses and kobuviruses in porcine disease syndromes, new virus variants in honeybee populations, as well as a range of other infectious agents in further host species. These findings indicate that the metagenomic detection of viral pathogens is becoming now a powerful, cultivation-independent, and useful novel diagnostic tool in veterinary diagnostic virology.

Contents

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1. Introduction

Due to intensive globalisation, climatic changes, and viral evolution, among other factors, the emergence of viruses and new viral diseases has increased in the last decades. In this situation, it is crucial to apply powerful methods for the broad-range detection and identification of the emerging viruses. In combination with classical methods, the molecular-based techniques provide sensitive and rapid means of virus detection and identification (Belák et al., 2009). However, most of the conventional diagnostic tests are designed to be virus-specific or aimed at a limited group of infectious agents. This makes them unsuitable for the detection of unexpected and/or completely new viruses, as well as novel viral variants. In contrast, the novel viral metagenomic approaches allow unbiased detection of a very wide range of infectious agents in a culture-independent manner and hold the promise to significantly improve diagnosis and disease control, in line with the "One World, One Health" principles (www.oneworldonehealth.org). Our group at the OIE CC in Uppsala has established state-of-the art facilities and practical skills for next-generation sequencing (NGS)-based metagenomic detection of pathogens, including "unknown, new viruses" which have a high degree of divergence from other known infectious agents. In addition to the use of established sequencing technologies, such as the 454 sequencing platform (Roche), we are following the ongoing development of further novel high-throughput methodologies, such as the Ion Torrent technology (Life Technologies). In contrast to earlier sequencing by using synthesis methods, Ion Torrent relies on a microchip-based array of semiconductor sensors for reading the incorporation of nucleotides onto the synthesis strand, rather than fluorescence signalling (Rothberg et al., 2011). By this way, it opens a new, rapid and more affordable path in the costly approaches of metagenomics. Herewith we briefly present some technical experiences and summaries of several recent investigations, which were published, or will be reported in separate specific articles, reporting on the application of viral metagenomics to detect "unknown, new viruses" in veterinary medicine.

2. Materials and methods

2.1. Specimens

Samples were collected in various disease syndrome groups in different animal species in three European countries and analysed at our OIE CC in Uppsala by metagenomic approaches. The cases were briefly the following (see also Table 1).

Pigs with postweaning multisystemic wasting syndrome (PMWS): This disease complex is considered to be a multifactorial disorder. Lymph nodes were obtained from 36 animals with PMWS and from 24 individuals, who were not displaying clinical symptoms. The samples, collected in Sweden between the years 2003 and 2007, originated from animals of 26 herds. Using an initial metagenomic approach, two of the collected clinical specimens were investigated for possible viral co-infections. For verification and prevalence estimates of identified viruses, all samples were analysed.

Shaking mink syndrome (SMS): This neurological disorder affected farmed mink kids in Denmark, as observed in 2000. Brain homogenates from minks affected by SMS were used to reproduce the disease in three healthy individuals. The experimentally infected animals developed the disease, however, the applied conventional methods were unable to detect any infectious agent (Gavier-Widén et al., 2004). These brain samples were then subjected to metagenomic analysis. Specimens from six healthy individuals and three naturally infected animals were investigated simultaneously, to enable confirmation of results.

Honeybees with unspecified symptoms: Approximately 50 adult worker honeybees, *Apis mellifera*, were sampled in 2010 from one colony with unusual depopulation belonging to a commercial apiary of 25 hives in the northern part of Spain. A homogenate prepared from 20 whole bees were found to be positive for Israeli acute paralysis virus (IAPV) by a RT-PCR assay (Palacios et al., 2008). Since IAPV has been linked to colony collapse disorder (CCD) (Cox-Foster et al., 2007), which is considered to be multifactorial in nature (José Manuel Sánchez-Vizcaíno, personal communication), we have further investigated these bee samples, by using metagenomics.

Nursery and weaned pigs with diarrhoea: Small intestines from a total of 21 piglets (1–2 weeks old) were collected at nine different locations in Hungary (János Benyeda and Ádám Bálint, personal communication). The samples were examined for viral etiological agents at the OIE CC in Uppsala, by using a two-step approach. First, all samples were pooled and screened for viral sequences using 454-sequencing. The result was then used to identify individuals carrying novel viruses by PCR and each of these samples was deep-sequenced using Ion Torrent technology.