REVIEW

Next-generation sequencing technologies: Tool to study avian virus diversity

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Summary. – Increased globalisation, climatic changes and wildlife-livestock interface led to emergence of novel viral pathogens or zoonoses that have become serious concern to avian, animal and human health. High biodiversity and bird migration facilitate spread of the pathogen and provide reservoirs for emerging infectious diseases. Current classical diagnostic methods designed to be virus-specific or aim to be limited to group of viral agents, hinder identifying of novel viruses or viral variants. Recently developed approaches of next-generation sequencing (NGS) provide culture-independent methods that are useful for understanding viral diversity and discovery of novel virus, thereby enabling a better diagnosis and disease control. This review discusses the different possible steps of a NGS study utilizing sequence-independent amplification, high-throughput sequencing and bioinformatics approaches to identify novel avian viruses and their diversity. NGS lead to the identification of a wide range of new viruses such as picobirnavirus, picornavirus, orthoreovirus and avian gamma coronavirus associated with fulminating disease in guinea fowl and is also used in describing viral diversity among avian species. The review also briefly discusses areas of viral-host interaction and disease associated causalities with newly identified avian viruses.

Keywords: next-generation sequencing; viral metagenomics; viral transcriptomics; virus diversity; DNA; RNA; genome; gene

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Abbreviations: AIV = avian influenza virus; AGV 2 = avian glycoprotein 2; aHEV = avian hepatitis E virus; APMV-1 = avian paramyxovirus 1; ILTV = infectious laryngotracheitis virus; ITV = Israel turkey meningoencephalitis virus; LPDV = lymphoproliferative disease virus; MDV = Marek’s disease virus; NGS = next-generation sequencing; PPMV-1 = pigeon paramyxovirus 1; USUV = Usutu virus; WNV = West Nile virus

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1. Introduction

Globalisation, climate change, wildlife-livestock interface, changes in agricultural practices (e.g. intensive farming), growth in live animal market are some of the drivers responsible for the emergence of novel pathogens and zoonoses (Jones et al., 2013). Among the animal species, wild birds are well-known reservoirs of emerging infectious diseases such as avian influenza (AI) and West Nile (WN) in humans.
discovery and downstream analysis of viruses with other
diagnostic methods, including electron microscopy, serology
and PCR. These other methods accompanied by use of virus
culturing have been reported in identification of chicken
proventricular necrosis virus (CPNV) and adenoviral gizz-
vard erosion in broiler chickens (Guy et al., 2011; Schade
et al., 2013). Currently, a variety of singleplex serological and
molecular assays are used for avian viral disease diagnosis.
Multiplex assays can be more useful in differential diagnosis.
Recently, multiplex fluorescent microsphere immunoassay
has been developed for simultaneous detection of avian in-
fluenza virus (AIV) antibodies against (H1-H16) subtypes
and avian paramyxovirus serotype 1 (APMV-1) (Pinette
et al., 2014). A wide range of genetic methods have been es-

tablished for multiplex detection of various poultry viruses
causing enteric and respiratory syndromes (Nguyen et al.,
2013). Even higher levels of multiplexing can be obtained
with DNA microarrays and panviral microarrays that can
comprise millions of individual probes to identify or dis-
cover divergent avian viruses (Kistler et al., 2008; Maughan
et al., 2014).

Sequencing of gene or genome is playing an important
role in virus characterisation, epidemiological studies and
has also allowed the discovery of new viruses. Novel RNA se-
quences of an avian calicivirus were revealed from intestinal
contents of poultry (Wolf et al., 2012). Novel avian picorna-
virus species (turdivirus 1 and 2) from wild birds (Woo et al.,
2010), pigeon picornaviruses (Kofstad and Jonassen, 2011),
quail picornavirus (Pankovics et al., 2012) and two novel
picornaviruses (Galliviruses) were discovered (Farkas et al.,
2012). Aalivirus, a novel duck origin picornavirus has been
reported in China (Wang et al., 2014b). The picobirnaviruses
are recently described group of viruses in several mammal
species that contain dsRNA, bi-segmented genomes and
are classified into two distinct genogroups (I or II). Avian
picobirnaviruses (AvPBV) genogroup I in Brazilian broiler
chickens and distinct group of turkey origin picobirnavirus
have been identified (Ribeiro Silva et al., 2014; Day and Zsak,
2014). A novel avian hepatitis E virus (aHEV) genotype IV
has been identified in Hungary and Taiwan (Banyai et al.,
2012; Hsu and Tsai, 2014). CPNV associated with transmis-
sible viral proventriculitis in broiler chicken was identified as
a novel avian birnavirus, representing a distinct genetic clus-
ter within the Birnaviridae family (Guy et al., 2011). Novel
psittacid herpesvirus 3 and phasianid herpesvirus have been
identified with respiratory disease in Bourke’s parrots and
pheasants with fatal infection, respectively (Shivaprasad and
Phalen 2012; Seimon et al., 2012). An existence of a distinct
avian papillomavirus group has been reported in various
birds (Johne et al., 2009). A novel wood pigeon and feral
pigeon astrovirus were identified in Norway (Kofstad and
Jonassen, 2011). Rubenstroth et al. (2014) discovered a new
avian bornavirus genotype in estrildid finches (Estrildidae)
in Germany. Emerging novel orthomyxovirus, named as

2. Classical methods for study of virus diversity

Cell culture has been the gold standard method for virus
discovery and downstream analysis of viruses with other
Wellfleet Bay virus has been reported in eider ducks (Pello and Olsen, 2013). A novel siadenovirus has been identified from polar seabird (Park et al., 2012). During the surveillance for arboviral encephalitis, Travassos de Rosa et al. (2002) identified rhadoviruses from wild birds.

A wide variety of genetic and antigenic variants of novel avian coronaviruses have been reported in poultry (Jackwood and Handel, 2012). Interspecies transmission of avian coronaviruses and emergence of a novel gamma and delta coronavirus were identified in wild aquatic birds (Woo et al., 2009). A wide genetic diversity of novel avian astroviruses detected in different species of wild birds which showed different groups of astroviruses had different host ranges (Chu et al., 2012). Role of recombination has been documented to be a contributing factor in emergence of turkey coronavirus (TCoV); the S gene of infectious bronchitis virus (IBV) recombined with an unknown virus (likely of avian origin), which resulted in a host change (chicken to turkey) and a tropism switch (respiratory to enteric) (Jackwood et al., 2010). Sarker et al. (2014) reported quasispecies variants and recombination events in an outbreak of emerging beak and feather disease virus (BFDV) that caused psittacine beak and feather disease. Chicken anemia virus (CAV) was the only known representative of the Gyrovirus genus, novel avian gyrovirus 2 (AGV2) and genetic variants of AGV2 were reported in chickens (Rijsewijk et al., 2011; Dos Santos et al., 2012). Avian orthoreoviruses have been associated with a variety of diseases in chickens. Dandar et al. (2013) characterized divergent avian reovirus strain from a broiler chicken with central nervous system disease. For the first time, Beserra et al. (2014) reported the occurrence of rotavirus group F in broiler from Brazil. Gyuranecz et al. (2013) established worldwide phylogenetic relationship of avian poxviruses and provided novel insights into the complex relationship between avian poxviruses and their hosts.

Evidence for intercontinental spread of APMV-1 class II genotype I was provided in migratory wild birds (Ramey et al., 2013). A distinct class II genotype VI consisting of a glo

3. NGS technologies

Several commercially available high-throughput sequencing platforms exist that vary by way of their sequencing principle, sequencing speed, expense, and read length. NGS essentially entails the following steps: sample preparation, sequence-independent amplification, high-throughput sequencing, and bioinformatics analysis as outlined in Fig. 1. Due to low abundance of viral sequences relative to total host nucleic acids, viral enrichment and concentration procedure requires to remove non-viral nucleic acid. To enrich viral nucleic acid, several measures have been taken such as homogenization, filtration, chloroform treatment, ultracentrifugation, nuclease treatment or combinations of these methods prior to viral nucleic acid extraction (Hall et al., 2014).

The amount of total nucleic acids isolated from viral particles is often too low for sequencing and may require amplifying of viral nucleic acid. The amplification has been aided by the development of sequence independent methodologies which simultaneously amplify several highly divergent viral genomes (Rossel et al., 2013). Sequence-independent single-primer amplification (SISPA), random PCR (rPCR), multiple displacement amplification (MDA) or virus discovery cDNA-amplified fragment length polymorphism (VIDISCA) are commonly used sequence-independent
amplification methods (Johne et al., 2009; de Vries et al., 2011; Sujayanont et al., 2014).

NGS platforms require the viral genomic sequences in the samples to be converted into sequencing libraries and later their amplification. Sequencing libraries are constructed by fragmenting the DNA (or cDNA) sample and ligating adapter sequences onto the ends of the DNA fragments and clonal amplification for sequencing. NGS technologies are based either on sequencing by synthesis or sequencing by ligation reaction controlled by polymerase or ligase. 454 pyrosequencing (Roche) is based on emulsion PCR (emPCR), in combination with a sequencing-by-synthesis approach. SOLiD (Applied Biosystems) also uses emPCR for clonal DNA amplification on beads to allow sequencing by ligation. Illumina (Illumina Inc.) is based on bridge-PCR on the surface of glass flow cell and uses sequencing-by-synthesis approach. Ion Torrent (Life Technologies) is sequencing-by-synthesis method that also uses emPCR. Ion Torrent detection relies on ion-sensitive, field-effect transistor-based sensors to measure hydrogen ions released during polymerase-mediated incorporation. PacBio RS (Pacific Biosciences) is able to sequence single DNA molecule in real time without the need for clonal amplification. Currently, Nanopore sequencers (Oxford Nanopore Technologies) are being developed, which are based on the concept that single DNA molecule can be identified when passing through engineered nanopore protein immobilised on a solid surface. A detailed comparison and performance of various NGS instruments have been reviewed elsewhere (Mardis, 2013; Ku and Rouks, 2013).

The applications of NGS technologies in avian virology with examples are listed in Table 1.

3.1 Genome characterization and virus diversity

Molecular epidemiology using nucleotide or full genome sequence data is widely used to monitor transboundary movements of avian viruses which clarify our understanding of the genetic diversity and viral evolution. Moreover, genomic sequences of viral strains can be used to identify the molecular determinants that underpin important phenotypic traits such as virulence and pathogenicity and prediction of novel genes and viral features that are important for viral replication or pathogenesis. NGS techniques have been used to sequence and conduct comparative full genome analysis of district genotype of infectious laryngotracheitis virus (ILTV) (Spatz et al., 2012). Complete genomes of fowl adenovirus A to E species (Marek et al., 2013) and the non-chicken aviadenovirus species has been reported (Kajan et al., 2010), which provided phylogeny and evolution of the genus Aviadenovirus. Recently, Marek et al. (2014) revealed the existence of two unknown lineages and common evolutionary origin of turkey adenovirus 5 (TAdV-5) and fowl adenovirus 1 (FAdV-1). Bodewes et al. (2013) identified novel gull adenovirus and demonstrated that birds of the Laridae family are infected by family-specific adenoviruses.

Many RNA viruses exhibit extreme evolutionary dynamics and genetic diversity due to the high mutation rates and short generation times (Beerenwinkel et al., 2012). APMVs are composed of the antigenically and genetically distinct avian paramyxovirus serotypes APMV 1 to 9 and two more APMV-10 and APMV-11 serotypes have been suggested (Miller et al., 2010; Briand et al., 2012). Based on antigenic and genetic characterization, Terregino et al. (2013) proposed new APMV-12 group. Forrester et al. (2013) revealed ancestral lineage of APMV 1 class II genotype XII isolated from Indonesia and provided an insight into historic Newcastle disease virus (NDV) evolution. Van Borm et al. (2013) identified genome sequence variability in PPMV-1 isolates from Belgium. Three APMV's, two different APMV-4 and one APMV-6 were identified (Rosseel...

![Workflow for virus detection using next-generation sequencing](image)
et al., 2011) and their genetic diversity within the serotypes was demonstrated. NGS approaches have been approved for use in routine diagnostics to monitor the genomic diversity of AIV, early emergences and transmission of these viruses from waterfowl to domestic poultry (Croville et al., 2012). Based on ultra-deep sequencing approaches, evidence of emerging HPAIV from a LPAIV progenitor (H7N1) has been provided (Monne et al., 2014). USUV and WNV are members of Flavivirus genus belonging to the Japanese encephalitis antigenic complex and are phylogenetically closely related. First epizootic emergence of USUV in wild and captive birds in Germany and WNV genetic variability has been reported from birds in USA (Becker et al., 2012; Grinev et al., 2013). Divergent avian origin picornaviruses genera such as Avisivirus, Megriviruses and Mesiviruses have been identified from turkeys and chickens (Ng et al., 2013; Boros et al., 2014). Two novel picornaviruses (mesivirus 1 and 2) were identified from wild pigeons (Pham et al., 2013).

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<td>Genomic characterization and gene discovery</td>
<td>Revealed the unique genome feature like internal ribosomal entry site and aphythovirus 2A-like sequence with 'ribosome-skipping' sites in avian picornaviruses</td>
<td>Boros et al. (2014)</td>
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<td>Molecular determinants associated with virulence</td>
<td>Identified a novel small-RNA population encoded from exogenous virus-specific region E (XSR) element of avian leuakosis virus subgroup 1 (ALV-J)</td>
<td>Yao et al. (2014)</td>
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<td>Novel pathogen discovery</td>
<td>Identification of emergence of virulence-associated E627K substitution in polymerase basic protein of H7N7 as adaptation marker</td>
<td>Jonges et al. (2014)</td>
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<td>Characterization of viral quasispecies</td>
<td>Identification of US10 gene and UL41 gene associated with reversion to virulence of low and high passages of tissue culture origin and chicken embryo origin infectious laryngotracheitis vaccine strains respectively</td>
<td>Garcia et al. (2013)</td>
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<td>Novel pathogen discovery</td>
<td>Discovery of a novel orthomyxovirus, designated Cygnet river virus, within genus quarjavirus in Muscovy ducks</td>
<td>Kessell et al. (2012)</td>
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<td>Novel pathogen discovery</td>
<td>Discovery of Farmington virus as a new species within the family Rhabdoviridae isolated from a wild bird</td>
<td>Palacios et al. (2013)</td>
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<td>Novel pathogen discovery</td>
<td>Discovery of Tvarminne avian virus as a candidate new species within the genus ortohereovirus in a crow</td>
<td>Dandar et al. (2014)</td>
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<td>Viral metagenomics</td>
<td>Metagenomic identification of several viral families, such as Picornaviridae, Caliciviridae and Picobirnaviridae from turkey gut</td>
<td>Day et al. (2010)</td>
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<td>Virus associate with disease of unknown etiology</td>
<td>Identification of a novel avian gammacoronavirus associated with Fulminating disease (X disease) of guinea fowl</td>
<td>Liass et al. (2014)</td>
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<td>Immune escape</td>
<td>Investigation of the emergence of escape mutants during serial in-vitro passaging of H5N1 AIV under immune pressure</td>
<td>Hooper et al. (2012)</td>
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<td>Quality control of live attenuated viral vaccines</td>
<td>Detection of nucleic acids from endogenous avian leuakosis virus in live attenuated viral vaccines derived from chicken embryo fibroblasts</td>
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<td>Discovery of viral non-coding RNA</td>
<td>Identification of role of viral encoded microRNAs in tumour transformation and latency in Marek’s disease virus</td>
<td>Hicks and Liu (2013)</td>
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<td>Discovery of viral non-coding RNA</td>
<td>Identification of novel miRNAs encoded by duck enteritis in regards to their genomic positions</td>
<td>Yao et al. (2012)</td>
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<td>Host-pathogen interaction</td>
<td>Discovery of novel genes and signalling pathways associated with disease resistance to avian influenza virus infection in chickens</td>
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<td>Chromatin immunoprecipitation sequencing</td>
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<td>Subramaniam et al. (2013)</td>
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Most recently, Bullman et al. (2014) identified picornavirus (Sicinivirus) from chickens which form a monophyletic group with Passerivirus (formerly turdivirus 1) and proposed novel genus within Picornaviridae family. The highly variable mixture of closely related genomes within a given host is referred as quasispecies. NGS technologies which sequence viruses with ultra-deep coverage (‘ultra-deep sequencing’) are able to track genomic changes over time and are high enough to capture rare variants. NGS approaches have significantly improved our understanding of the intra-host viral population dynamics. The feasibility of using ultra-deep sequencing to reveal the genomic diversity of PPMV-1 and AIV quasispecies has been demonstrated (Ramakrishna et al., 2009; Van Borm et al., 2013).

Using ultra-deep sequencing, Jonges et al. (2014) investigated the viral quasispecies dynamics associated with AIV subtype H7N7 infection and identified emergence of virulence-associated E627K substitution in polymerase basic protein of H7N7 as adaptation marker. To detect the evolution of HPAI virus from LPAI virus within the infected host, Iqbal et al. (2014) reported ultra deep sequencing of hemagglutinin (HA) gene polybasic cleavage site and failed to detect any evidence of HPAI virus signatures. New evidence obtained from the analysis of whole genome sequences of ILTV vaccine and field strains indicated that recombination between attenuated vaccines belonging to different genotypic lineages in the field resulted in the emergence of novel virulent strains of ILTV (Lee et al., 2013). Evidence of interspecies transmission and reassortment among avian group A rotaviruses has been reported (Schumann et al., 2009). NGS has been employed to characterise an avian group A rotavirus genome containing a novel viral protein (VP) 4 gene which is closely related to mammalian rotaviruses representing an avian/mammalian rotavirus reassortment (Trojan et al., 2013).

3.2 Virus discovery and viral metagenomics

The introduction of culture-independent NGS technologies, termed as viral metagenomics (community genomics), made powerful diagnostic technology to investigate the complete viral genetic populations of a sample. Viral metagenomics is nowadays routinely used for viral detection, novel virus discovery, and divergent virus genome recovery. This approach has been reported in detection of AGV2, avirulent NDV co-infection in chickens with neurological symptoms and high mortality, avipolyomavirus and avipoxvirus co-infection in periocular skin lesion of gery butcherbird (Abolnik and Wandrang, 2014; Bennett and Gillett, 2014). Based on metagenomics data, Belak et al. (2013) reported interstitial nephritis associated with a variant of NDV-V4 vaccine strain. To explore the role of live poultry markets in the origin of the novel human H7N9 virus, Yu et al. (2014) provided direct evidence of coexistence of influenza H7N9 and H9N2 in poultry linked to human H7N9 infection. Reuter et al. (2014) identified novel group of unclassified single-stranded circular DNA viruses called stool-associated circular virus in a faecal sample from a domestic turkey. Tvarminne avian virus (TVAV), avian origin reovirus was identified in a crow showing neurological disorders. Dan dar et al. (2014) reported TVAV as a candidate new species within the genus Orthoreovirus. A novel duck specific avian coronavirus genetically closer to some coronaviruses circulating in wild water fowl has been identified (Chen et al., 2013). A novel delta coronavirus and astrovirus were identified in shorebird samples from the Delaware Bay, an important feeding ground for thousands of migratory birds (Honkavuori et al., 2014). Durham and Farmington viruses as new species from wild birds (Allison et al., 2011; Palacios et al., 2013) and novel Sunguru virus within Rhabdoviridae family have been discovered from domestic chickens in Uganda (Ledermann et al., 2014). Khurdun virus a new representative of the Orthobunyaviruses has been identified from coot (Alkovskhovskii et al., 2013).

Metagenomics have the capacity to detect viruses either as single agents or as syndromes to identify the etiologic agents. Honkavuori et al. (2008) discovered two strains of a novel Borna viruses in psittacine birds with proventriculus dilatation syndrome (PDS) characterised by gastrointestinal dysfunction and neurological signs. To identify a candidate of etiologic agent for turkey viral hepatitis (TVH), Honkavuori et al. (2011) reported picornavirus in liver samples from diseased turkey poults. Fulminating disease (X disease) of guinea fowl is acute enteritis characterised by intense prostration and a very high mortality. Although its viral origin was previously suspected, virus remained unknown. Liais et al. (2014) identified a novel avian gamma coronavirus associated with this disease which was distantly related to TCoVs. To decipher the RNA virus community from turkey flocks experiencing enteric disease, Day et al. (2010) analyzed turkey gut RNA virus metagenomics and demonstrated the presence of turkey-origin members of the Picornavirales, and the genus Caliciviridae, and the turkey picobirnaviruses. In order to better understand the viruses shed by pigeons, Phan et al. (2013) characterized enteric virome in droppings from wild pigeons and identified sequences of novel aviparvovirus, picornaviruses and described group G rotavirus from pigeons.

4. Transcriptomics and virus-host interaction

Initially, microarrays have been used to quantify gene expression. Introduction of NGS technologies revolutionized transcriptome profiling, an approach referred to as RNA sequencing (RNA-seq). NGS offers an opportunity
for detailed examination of transcriptomics at both host and pathogen level during an infection, thereby, elucidating the mechanisms of disease and pinpointing the functional pathways involved in the host response to infection. Comprehensive analysis of AIV-infected lung tissue suggested that several microRNA (miRNAs) and mRNAs were likely to be involved in regulating the host response (Wang et al., 2012). Allele-specific expression (SNPs, genes and pathways) that was associated with transcriptional response to Marek’s disease virus (MDV) infection was identified to underlay genetic basis for disease resistance to MDV infection in both broilers and layers (Perumbakkam et al., 2013). Gene expression patterns of normal and duck hepatitis A virus genotype C (DHAV-C) infected duck livers were explored and compared, and new insights into the molecular mechanism of host-DHAV-C interaction were provided (Tang et al., 2013). Huang et al. (2013) performed deep transcriptome analysis responsive to AIV using the lung of control ducks and ducks infected with highly pathogenic and weakly pathogenic H5N1 virus. The analysis revealed that β-defensin and butyrophilin-like gene repertoires were involved in host immune response. The information gained by using RNA-seq to obtain an overview of pathogen-induced host responses or to follow temporal expression changes during the infectious cycle can be exploited to design better control strategies as well as help in identification of genetic markers for disease resistance.

Chromatin immunoprecipitation (ChIP) was first developed to discover DNA-protein binding sites. ChIP coupled with high-throughput sequencing (ChIP-Seq) has been applied to quantitatively analyze binding sites of DNA associated proteins across the entire genome. ChIP-Seq has been reported to identify the binding sites in host genomes or transcription factors involved in virus infection and pathogenicity. Using chromatin immunoprecipitation - sequencing analysis, Subramaniam et al. (2013) identified binding sites occupied by MDV oncoprotein, (Meq) and/or c-Jun within the chicken genome and found enriched Meq binding sites in the promoter regions of upregulated genes. These technologies will contribute in achieving a comprehensive understanding of the DNA-binding profiles and epigenetic modification patterns associated with immune responses to both pathogens and vaccines.

5. Evidence of causal virus-disease relationship

NGS has provided a powerful tool for novel virus discovery but, it is only the first step to determine the etiology of a disease. To assign etiological agent, attempts should be made to accomplish Koch’s postulates, which require the agent to be isolated in culture, or River’s modifications, which recognize the added presence of neutralizing antibodies to an agent in evidence of infection or relationship between the presence of viral sequences and disease (Lipkin, 2013). Moreover, in vitro culture assays may be performed to assess the host range, cell tropism and entry receptors. Recent successes in identifying avian viruses and associating them with disease include DTMUV associated with severe egg drop syndrome (Huang et al., 2013). In case of unculturable viruses, the ability to construct infectious clones of novel virus, directly from clinical samples using either NGS de-novo sequencing or by standard methods such as primer walking can be helpful in identifying etiological agents and characterization of the virus. For example, due to the lack of an efficient cell culture system for aHEV, Kwon et al. (2011) developed an infectious cDNA clone of aHEV strain recovered and assessed causality, pathogenicity and seroconversion in SPF chickens. To prove etiological agent using metagenomics, the metagenomic Koch’s postulates have previously been introduced. They focus on the identification of metagenomic traits in diseased subjects (Mokili et al., 2012). However, this adaptation still requires the introduction of disease to prove causal virus-disease relationship (Bibby, 2013). Complicating disease association studies are the extent of viral genetic diversity and co-infections with other agents may influence clinical outcome. Deciphering such complex interactions will require viral metagenomics that query all virus families or genotypes in such cases, therefore, number of samples and repeatability in independent studies should be considered (Delwart, 2012).

6. Conclusions and perspectives

NGS platforms have been recognized as important tools in veterinary medicine which have enabled the discovery of viruses, viral diversity and development of viral metagenomics. Although adoption of NGS in routine clinical laboratory has enormous challenges due to the high costs of instruments and supplies, and requirements of skills and expert personnel to analyse the large datasets and their interpretation. As the cost and complexity of sequencing platforms are declining the routine use of NGS technology in the microbiology laboratory particularly in diagnostic clinical microbiology will become a routine practice. The discovery of novel viruses has been reported from wild and domestic birds. The discovery of these viruses will enable the development of diagnostic assays for the future detection or epidemiological investigation of viruses. Based on viral metagenomics approaches, complex gut viral community related to enteric syndrome in poultry and avian related gyroviruses were identified on human skin or in diarrheal samples. However, the identification of the etiological agents which are not proven yet, requires isolation of agent to fulfil Koch’s postulates.
References


