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MOLECULAR TOOLS AVAILABLE FOR THE DETECTION OF *SCHMALLENBERG-SIMBU* GROUP VIRUSES

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Schmallenberg virus (SBV) is the first Simbu serogroup virus reported in Europe. Despite his recent identification by metagenomic analysis (November, 2011), SBV was found throughout Europe, and in less than four years almost all European countries reported seropositive prevalence. Therefore, several teams of researchers developed and proposed molecular and/or immunological tools for diagnosis, designed to detect directly or indirectly the presence of SBV or Simbu serogrup viruses in livestock. In some European countries the serological prevalence of SBV or Simbu sero-grup viruses in livestock is above 90%, while in Romania the preliminary reports revealed a prevalence around 22% in cattle and 5% in small ruminants. The aim of study was meta-analyses of the molecular tools available for the detection of Schmallenberg-Simbu sero-group viruses. Based on 20 original papers, articles and reviews, the protocols of PCR assays were analyzed. Despite several types PCR assays described, only one type of PCR assay has been extensively used in Europe for SBV surveillance or diagnostic: a real-time quantitative reverse transcription PCR (RT-qPCR) test, developed by "Friedrich-Loeffler" Institute. As a conclusion the molecular tools available for the detection of Schmallenberg-Simbu group viruses allow both, the detection of the Simbu sero-group and SBV.

Key words: Schmallenberg virus, livestock pathology, emerging diseases, PCR.

INTRODUCTION

Schmallenberg virus (SBV) is the first Simbu sero-group virus reported in Europe[1], which has typical orthobunyavirus genetic organization (a tripartite single-stranded, negative-sense RNA)[2] and a possible phylogenetic origin in the reassortament of Sathuperi (SATV M RNA segment) and Shamonda (SHAV S and L RNA segments) viruses[3]. The virus was identified by fullgenome sequencing combined with metagenome analysis in November, 2011, at Friedrich-Loeffler-Institut (Greifswald–Insel Riems, Germany)[1].

SBV affect mainly domestic ruminants (cattle, sheep, and goats), but infections have been reported in dogs[4,5] alpacas (*Vicugna pacos*)[6], Anatolian water buffalo (*Bubalus bubalis*)[7], elk (*Alces alces*)[8], bison (*Bison bonasus*)[8], red deer (*Cervus elaphus*)[9-13], fallow deer (*Dama dama*)[10,13], roe deer (*Capreolus capreolus*)[9,10,13], sika deer (*Cervus nippon*)[13], muntjac (*Muntiacus reevesi*)[10], chamois (*Rupicapra rupicapra*)[11], European mouflon (*Ovis orientalis musimon*)[13], and wild boar (*Sus scrofa*)[13].

In less than four years after the first report of SBV[1], almost all European countries reported seropositive animals[9,14-20], and at least 11 countries infected adult animals[17].

SBV spread mainly by biting midges of the *Culicoides* genus, which are considered the major source of infection[14]. Also, the presence of SBV in semen of bulls suggest that the insemination of dams is another route of infection[16].

Hoffmann *et al*. (2012) identified SBV in adult cattles with unspecific and transitory fever, decreased milk production, and diarrhea[1]. Also, malformed newborn and foetuse of all domestic ruminants proved to be SBV-positive[20,21]. Newborns seem normal at birth but with behavioral abnormalities, blindness, ataxia, amaurosis, recumbency, inability to suck and even convulsions. Foetuse were with ankylosis, arthrogryposis, severe torticollis, brachygnathia, kyphosis, lordosis and scoliosis[17,20,21].

Afonso *et al*. (2014) used the preliminary clinical and lesional data collected from clinical cases of SBV infection to formulate different case definitions in correlation with the age of animals[17]:

- Foetuses and neonates with suspicion of SBN infection: *"Arthrogryposis hydranencephaly syndrome (AHS) in ruminants (stillbirths, premature births, mummified foetuses, and dysfunctions or deformities of foetuses or neonates with two or more of the following: arthrogryposis, hydranencephaly, ataxia, paralysed limbs, muscle atrophy, joint malformations, torticollis, kyphosis, scoliosis, brachygnatia inferior, behavioural abnormalities and blindness)"*[17].

- Adult animals: *"Ruminants with transient fever (>40◦C), diarrhoea, anorexia and reduced milk production (that is not attributed to a known cause)"*[17].

However, the majority of reports support the subclinical evolution of infection as the main characteristic of infection[4-20]. In all cases of clinical suspicion of SBV-infection, the confirmation can be done by RT-qPCR, virus isolation or detection of antibodies (immunoenzymatic assay, viral neutralization test, immunofluorescence)[17].

Data supplied by several teams of investigations have been structured for case detection and the estimation of infection/disease prevalence is difficult[9,14-20]. In the light of these data, several teams of researchers developed and proposed molecular and/or immunological tools for diagnosis, designed to detect directly or indirectly the presence of SBV or Simbu sero-grup viruses in livestock.

In some European countries the serological prevalence of SBV or Simbu sero-grup viruses in livestock is above 90%, while in Romania the preliminary reports revealed a prevalence around 22% in cattle and 5% in small ruminants[18].

These data suggest that SBV infection should be included in active surveillance programs of cattle, goat and sheep herds or in the differential diagnosis of ruminant diseases mainly after the vector activity period[18,19].

After the emergence of this novel *Orthobunyavirus*, several molecular methods were developed for the detection of SBV RNA in various biological samples collected from domestic and wild animals. In this context, we aim a meta-analysis of molecular tools designed to detect RNA of SBV and Simbu sero-group viruses.

MATERIAL AND METHODS

Based on 20 original papers, articles and reviews, the protocols of PCR assays were analysed[1-3,15,16,21-35].

The meta-analysis is focused on (1) selection of suitable biological samples for the confirmation of SBV infection and methods used for extraction of SBV RNA from this matrices; (2) metods used for reverse transcription and cDNA amplification; and (3) the SBV RNA segments and primers and probes used in PCR assays.

RESULTS AND DISCUSSIONS

Several RT-qPCR assays were developed for the detection of different RNA segments of SBV: small (S), medium (M), and large (L) [1,21-24,29,35]. However, one type of PCR assay has been extensively used in Europe both in SBV active surveillance and diagnostic: a real-time quantitative reverse transcription PCR (RT-qPCR) test, developed by Friedrich-Loeffler-Institut (Greifswald–Insel Riems, Germany). The assay targets the L or S segments, and has been often used in European studies of SBV or Simbu sero-grup viruses[1-3,15,16,21-35]. However, the SBV S segment-specific RT-qPCR has a slightly higher sensitivity than the L segment-specific assay[24].

Inter-laboratory comparison of SBV real-time RT-PCR protocols revealed that all methods of extraction were robust and produced positive results for almost all SBV RNA-positive matrices[29]. The biological samples collected from adult cattle, sheep, and goats with suspicion of SBV infection were whole blood, serum, spleen and mesenteric lymph nodes[1,28,29,34], while in fetuses the best samples are cerebrum, brainstem, amniotic fluid, spinal and umbilical cords[21,27,31].

QIAamp viral RNA mini kit (Qiagen, Germany) seems to be the most widely used commercial kit for extraction of SBV RNA from whole blood, serum and tissue homogenates[1-3,15,16,21-35], but both magnetic bead and membrane-based extractions revealed true-positive PCR results[29].

In breeding males, the higest interest present the quality of semen and the risk for venerian transmision of SBV infection[22,25,26]. The inter-laboratory trials revealed that the method of extraction used in SBV real-time RT-PCR protocols is critical for SBV RNA detection in semen[29]. The extraction methods that included a pretreatment with Ambion TRIzol LS reagent (Life Technologies, NY, USA) proved to be the best option for semen samples[25,26,29].

Extraction of SBV RNA from *Culicoides* used several commercial kits: QIAampH All Nucleic Acid MDx Kit (Qiagen, UK)[23], NucleoSpin RNA Virus (Macherey Nagel, Germany)[30], BioSprint 96 One-For-All Vet Kit (Qiagen, Valencia, CA)[30], EZ1 virus mini kit v2.0 (Qiagen, California, USA)[30]. SBV RNA was amplified using commercial PCR kits[1-3,15,16,21-35]: Applied Biosystems AgPath-ID one-step RT-PCR kit, (Life Technologies, Grand Island, NY)[29,33]; *virotype* SBV RT-PCR kit (Qiagen Inc., Valencia, CA)[29]; Taq Vet Schmallenberg virus S gene 50 kit (Laboratoire Service International, LSI, France)[30]; reverse transcription with Superscript III (Invitrogen) and cDNA amplification with Advantage 2 PCR Kit (Clontech)[27].

Primers and probes used by Friedrich-Loeffler-Institut targeted SBV-S segment [SBV-S-382F: 5' - TCA GAT TGT CAT GCC CCT TGC - 3' (Genome position 382–402); SBV-S-469R: 5' - TTC GGC CCC AGG TGC AAA TC - 3' (Genome position: 450–469); SBV-S-408FAM: FAM-TTA AGG GAT GCA CCT GGG CCG ATG GT-BHQ1 (Genome position: 408–433)][1,21-23], and SBV-L segment [SBV-L1-11F: 5' - TTG CCG TTT GAT TTT GAA GTT GTG - 3'; SBV-L1-155R: 5' – TCA GGG ATC GCA AAT TAA AGA ACC - 3'; SBV-L1-36: FAM - 5' – TCA TCC GTG CTG ACC CTC TGC GAG - 3' - BHQ1][32], while Fischer *et al.* (2013) proposed other three real-time RT-PCR assays which targeted SBV-M1 segment (genome position: 1690-1827) [SBV-M1-213F: 5′ - TCA ATT CAG CAA GTA ACA TAC AAT GG - 3′; SBV-M1-350R**:** 5′ - CGT GGT CTG TCT TGG TTG ATG - 3′; SBV-M1-

240FAM: 5′ - FAM-AAG CAC TGG CCC GAA GTT TCA CCT-BHQ1 - 3′], SBV-L1 segment (genome position: 367-511) [SBV-L1-11 F: 5′ - TTG CCG TTT GAT TTT GAA GTT GTG - 3′; SBV-L1-155R: 5′ - TCA GGG ATC GCA AAT TAA AGA ACC - 3′; SBV-L1-36FAM: 5′ - FAM-TCA TCC GTG CTG ACC CTC TGC GAG-BHQ1 - 3′], and SBV-L1.4 segment genome position: 361-468) [SBV-L1.2 F: 5′ - TCA GAA TTG CCG TTT GAT TTT GAA G - 3′; SBV-L1.4R: 5′ - GTT GAG CGG CCC AAA TAT TTC C - 3′; SBV-L1-36FAM: 5′ - FAM-TCA TCC GTG CTG ACC CTC TGC GAG-BHQ1 - 3′][24]. Another appreciated PCR protocol was designed for the detection of the Simbu sero-group and use pan-Simbu primers in a generic real-time PCR for amplification of L-Segment [panOBV-L-2959 F: 5′ - TTG GAG ART ATG ARG CTA ARA TGT G - 3’ (genome position: 2888-3167); panOBV-L-3274R: 5′ - TGA GCA CTC CAT TTN GAC ATR TC-’ (genome position: 2888-3167)][24].

CONCLUSIONS

The molecular tools available for the detection of Schmallenberg-Simbu group viruses allow both, the detection of the Simbu sero-group and SBV.

PCR assays could be useful tools to investigate the emerged microorganisms in various ecosystems[1,23,24,30,35,36] or in the active surveillance of such events together with or as an alternative to methods of SBV-antibodies detection, when only tissues homogenates are available.

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