Veterinary Microbiology 196 (2016) 23–26

Contents lists available at ScienceDirect

Veterinary Microbiology

journal homepage: www.elsevier.com/locate/vetmic

Short communication

Effective interference between Simbu serogroup orthobunyaviruses in mammalian cells

Kerstin Wernike[a,*], Emiliana Brocchi[b], Martin Beer[a]

[a] Institute of Diagnostic Virology, Friedrich-Loeffler-Institut (FLI), Suedufer 10, 17493 Greifswald – Insel Riems, Germany
[b] Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, Via Bianchi 7, 25125 Brescia, Italy

ARTICLE INFO

Article history:
Received 31 July 2016
Received in revised form 5 October 2016
Accepted 8 October 2016

Keywords:
Orthobunyavirus
Schmallenberg virus
Interference
Interaction
Reassortment
Coinfection

ABSTRACT

The Simbu serogroup of orthobunyaviruses comprises a wide range of viruses with different medical and veterinary relevance. These viruses are known to reassort, and coinfection of the same cell is one of the prerequisites for reassortment. Here, a mammalian cell line was infected with various members of this virus group, inoculated after several time points with a second Simbu serogroup virus, and analyzed by strain or species specific immunofluorescence staining. Different virus species or different strains of the same virus species were able to co-infect mammalian cells, but only for a limited time frame. After a few hours, the replication of the first virus led to a gradual inhibition of a second virus until a complete resistance to superinfection after 24 h regardless whether it is another strain of the same virus species or a distinct member of the serogroup.

1. Introduction

The family *Bunyaviridae,* one of the largest and most diverse RNA virus families, is divided into the five genera *Hantavirus*, *Nairovirus*, *Phlebovirus*, *Tospovirus*, and *Orthobunyavirus*. The genus *Orthobunyavirus* comprises more than 170 different viruses (Plyusnin et al., 2012) currently divided into 18 serogroups. One of them is the Simbu serogroup, which contains insect-transmitted viruses predominantly responsible for diseases in livestock but also in humans. In late 2011, Schmallenberg virus (SBV), a novel member of this serogroup, was discovered in Europe (Hoffmann et al., 2012). Like other Simbu serogroup viruses such as Akabane virus (AKAV) or Aino virus (AINOV), SBV is responsible for severe congenital malformation, stillbirth or premature birth, when pregnant ruminants are infected during a vulnerable phase of gestation (Conraths et al., 2013; Wernike et al., 2014). The closest relatives of SBV are the Australian Douglas virus (DOUV), Sathuperi virus (SATV), and based on two of the three unique segments of single stranded RNA the Shamonda virus (SHAV), which was suggested to be a reassortant containing the S- and L-segments of SBV and the M-segment from an unclassified virus (Goller et al., 2012). More distantly related, but to some extent neutralized by anti-SBV antibodies are e.g. Aino virus (AINOV), Peaton virus (PEAV), and for the serogroup eponymous Simbu virus (SIMV). No serological cross-reactivity was e.g. observed between SBV and Sabo virus (SABOV) (Goller et al., 2012).

As in other viruses with segmented genomes, natural genetic reassortment is widespread among orthobunyaviruses (Bowen et al., 2001; Collao et al., 2010; Ding et al., 2013; Nunes et al., 2005; Saeed et al., 2001; Yanase et al., 2006) and bares the risk of the emergence of new reassortant viruses with altered biological properties such as an increase in pathogenicity compared to the parenteral viruses, the introduction into a new host or changes of the vector competence (Briese et al., 2006; Burt et al., 2009; Elliott, 2014).

The basis for such reassortment events is the co-replication of two closely related viruses within an insect vector or the mammalian host. It has been e.g. shown previously that mosquitoes can became dually infected with different mutants of LaCrosse virus (LACV) or LACV and snowshoe hare virus (SSHV), both members of the California serogroup of orthobunyaviruses (Beaty et al., 1985; Borucki et al., 1999).

Data about Simbu serogroup viruses, however, are scarce. Furthermore, the vast majority of studies on co-infections of bunyaviruses were performed in insects or insect cell lines. To evaluate whether viruses of that family, especially members of the Simbu group, are able to co-infect mammalian cells in vitro and if potential inhibition of a second infection is dependent on the genetic relationship of the viruses, baby hamster kidney (BHK) cells were infected with two strains of the same virus, very closely

* Corresponding author.
E-mail address: kerstin.wernike@fli.de (K. Wernike).

http://dx.doi.org/10.1016/j.vetmic.2016.10.007
0378-1135/

related viruses or two more distantly related members of the Simbu serogroup.

2. Methods, results and discussion

BHK cells were inoculated with SBV strains D495/12-1 and D495/12-2, SATV, DOUV, SHAV, SIMV, SABOV, PEAV or AINOV at a multiplicity of infection (MOI) of 1.5. After adsorption for 1 h in serum-free medium the virus solution was removed and Minimum Essential Medium (MEM) with 5% fetal calf serum was added. At 0, 2, 4, 6, 8, 16, 24, and 48 h after the first infection the cells were incubated with SBV strain BH80/11-4 (MOI 1.5). After 24 h the medium was removed, the cells were washed twice with Tris-buffered saline with 0.1% Tween-20 (TBST), fixed using heat treatment (2 h at 80 °C), and stained with a mAb specific for SBV strain BH80/11-4 (please see Fig. 1A for an example of the specificity) and a fluorescein isothiocyanate (FITC-) conjugated goat anti-mouse IgG (Sigma-Aldrich Co.) as secondary antibody.

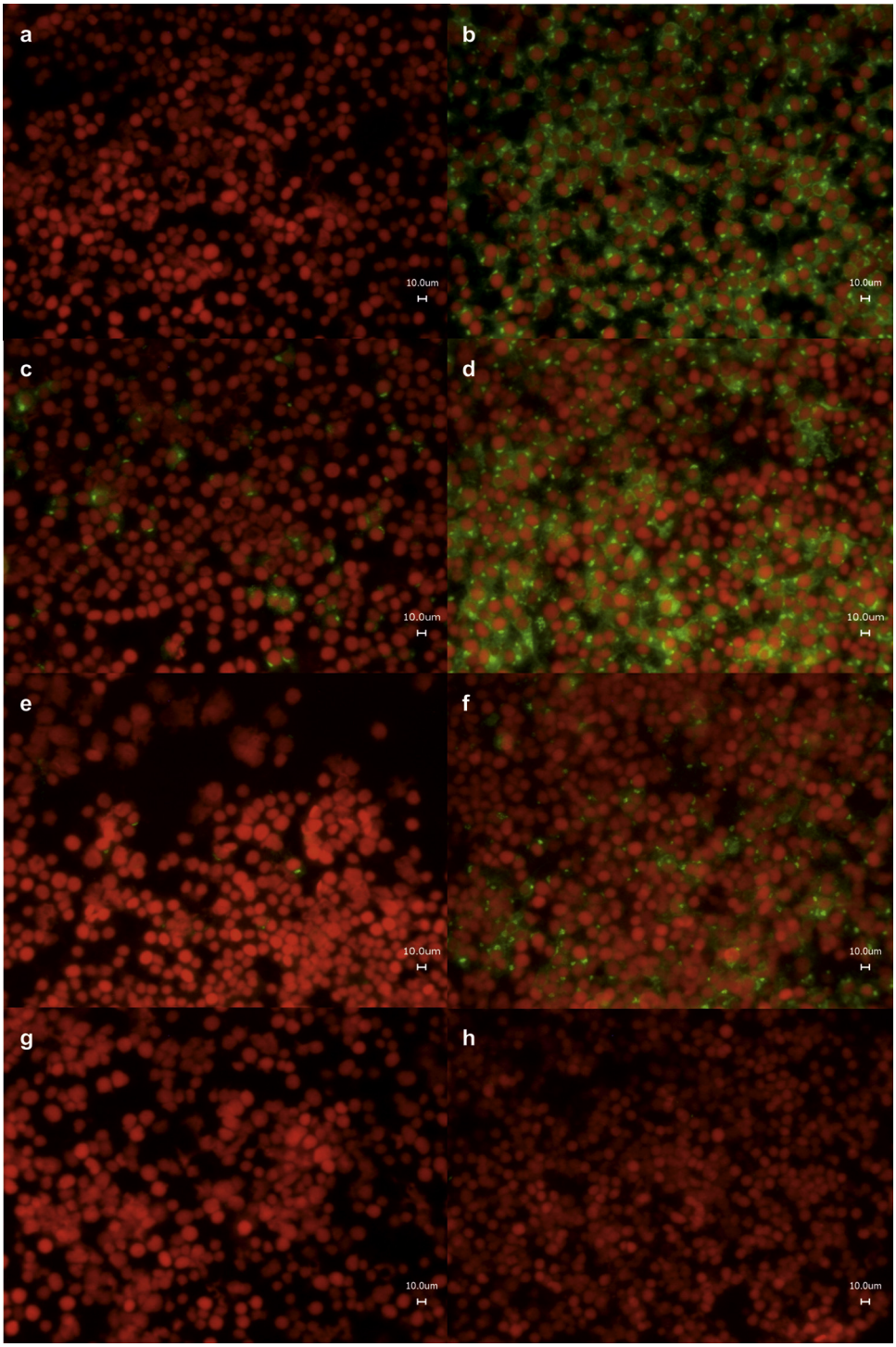

Fig 1. Immunofluorescence analyses of Schmallenberg virus (SBV) isolates D 495/12-1 (A) and BH80/11-4 (B-H) stained with a monoclonal antibody specific for SBV BH80/11-4. BHK cells were infected with SBV BH80/11-4 2 h post infection (p.i.) with Sathuperi virus (B), 8 h p.i. with SBV strain D495/12-1 (C), Peaton virus (PEAV) (D) or Shamonda virus (SHAV) (E), 16 h p.i. with PEAV (F) or SHAV (G), or 24 h p.i. with PEAV (H).