Journal of Virological Methods 248 (2017) 26–30

Contents lists available at ScienceDirect

Journal of Virological Methods

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

Short communication

A novel highly sensitive, rapid and safe Rift Valley fever virus neutralization test

Paul J. Wichgers Schreur[a,*], Janusz T. Paweska[b,c], Jet Kant[a], Jeroen Kortekaas[a]

[a] Wageningen Bioveterinary Research, Department of Virology, Lelystad, The Netherlands
[b] National Institute for Communicable Diseases of the National Health Laboratory Service, Center for Emerging and Zoonotic Diseases, Sandringham 2131, South Africa
[c] School of Pathology, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg 2193, South Africa

ARTICLE INFO

Keywords:
Rift Valley fever virus
4-Segmented
Virus neutralization test
GFP
Ruminants

ABSTRACT

Antibodies specific for Rift Valley fever virus (RVFV) can be detected by diverse methods, including ezyme-linked immunosortbent assay (ELISA) and virus neutralization test (VNT). The VNT is superior in sensitivity and specificity and is therefore considered the gold standard serological assay. Classical VNTs make use of virulent RVFV and therefore have to be performed in biosafety level 3 laboratories. Here, we report the development of a novel VNT that is based on an avirulent RVFV expressing the enhanced green fluorescent protein (eGFP), which can be performed safely outside level 3 biocontainment facilities. Evaluation with a broad panel of experimental sera and field sera demonstrated that this novel VNT is faster and more sensitive than the classical VNT.

Rift Valley fever virus (RVFV) belongs to the family *Bunyaviridae*, genus *Phlebovirus*, and causes severe disease in ruminants and occasionally in humans. The virus is endemic to Africa, the Arabian Peninsula and several islands located off the coast of southern Africa. Domesticated ruminants are highly susceptible to the virus, which is transmitted by mosquito vectors. Epizootics are characterized by massive abortions and fatal disease among newborns. RVFV contains a tri-segmented negative-sense RNA genome consisting of a small (S) medium (M) and large (L) segment. Although virus isolation and PCR can be used for diagnostics, detection of the virus can be challenging due to the relatively short viremic period in animal hosts. Indirect detection of the virus via antibodies is therefore most reliable. Two ELISAs are commercialized by the company IDvet (France) and various promising experimental ELISAs have been developed that detect antibodies against the immunodominant nucleocapsid protein (Fafetine et al., 2007; Jansen van Vuren and Paweska, 2009; Jansen van Vuren et al., 2007; Paweska et al., 2005; Paweska et al., 2007; Paweska et al., 2002; Paweska et al., 2008). Additionally, an experimental ELISA targeting glycoprotein Gn has been developed (Jackel et al., 2013). Although ELISAs are convenient diagnostic tools, the virus neutralization test (VNT) is superior in sensitivity and specificity and is therefore regarded the gold standard serological assay. The classical VNT is based on the incubation of a standard amount of RVFV with serial dilutions of sera followed by addition of cells. The induction of cytopathic effect (CPE) after 4–7 days is used as the readout. In this manuscript we report the development of a novel VNT that makes use of a recently developed avirulent RVFV that is faster and considerably more sensitive than the classical VNT. Moreover, this novel VNT can be performed safely outside bio-safety level 3 facilities.

The wildtype RVFV, as used in the classical VNT, comprises a genome consisting of a small (S), medium (M) and large (L) RNA segment. The virus that is used in the novel VNT, was created by splitting the M genome segment, normally encoding the structural glycoproteins Gn and Gc from a single gene, into two genome segments encoding either the Gn or Gc protein. The resulting four-segmented RVFV (RVFV-4s) virus was shown to be completely innocuous in mice (Wichgers Schreur et al., 2014), lambs (Wichgers Schreur et al., 2015) and pregnant ewes (Wichgers Schreur et al., 2017). As mice, young lambs and pregnant ewes are much more susceptible to disease than humans, it is plausible to assume that RVFV–4s is completely avirulent in humans as well. Consequently, RVFV–4s may be handled safely outside biosafety level 3 facilities. Here, we investigated the ability to use a RVFV–4s variant expressing enhanced green fluorescent protein (eGFP) from the NSs locus of the S-segment (RVFV-4s$_{eGFP}$) as the antigen in a novel RVFV VNT.

We compared the use of the avirulent RVFV-4s$_{eGFP}$ virus with a tri-segmented eGFP variant (RVFV$_{eGFP}$), also expressing eGFP from the NSs locus of the S-segment, and with a virulent recombinant virus (strain 35/74). Briefly, in a 96-wells plate format, serial dilutions (50 µl) of heat-inactivated sera (2 h 56 °C) were incubated with a standard amount (between 30 and 300 50% tissue culture infective dose [$TCID_{50}$] in 50 µl) of recombinant wildtype RVFV, RVFV$_{eGFP}$ or RVFV-

* Corresponding author.
E-mail address: paul.wichgersschreur@wur.nl (P.J.W. Schreur).

http://dx.doi.org/10.1016/j.jviromet.2017.06.001
Received 20 January 2017; Received in revised form 8 May 2017; Accepted 1 June 2017
Available online 03 June 2017
0166-0934/

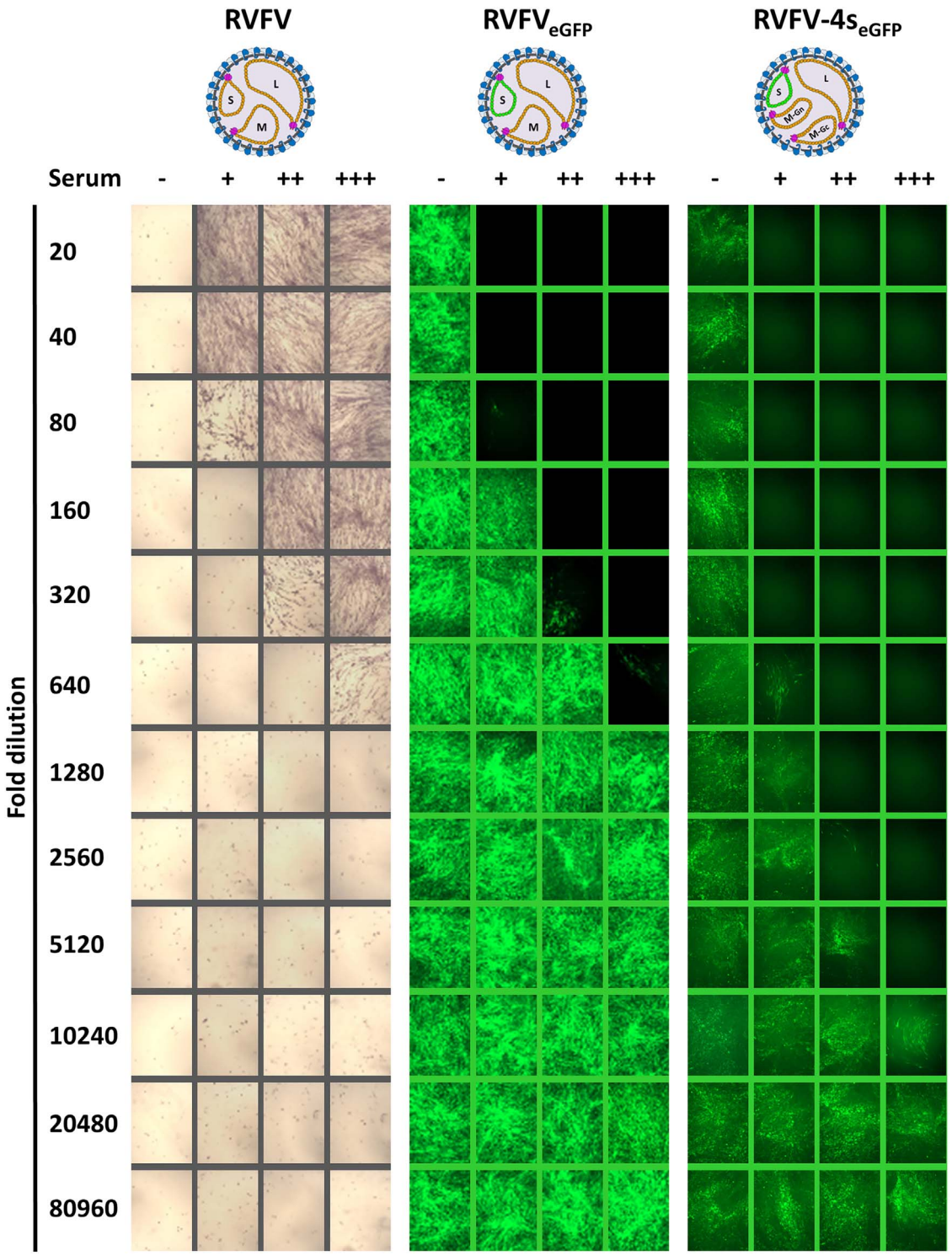

Fig. 1. Comparative analysis of RVFV, RVFV$_{eGFP}$ and RVFV-4s$_{eGFP}$ based VNTs using sheep sera. A negative (−), weak positive (+), moderately positive (++) and highly positive (+++) sheep serum were serially diluted and mixed with RVFV, RVFV$_{eGFP}$ or RVFV-4s$_{eGFP}$ as described. At 5, respectively 2 days post infection, CPE or GFP expression was assessed. For optimal presentation of the results of the classical VNT, cells were stained using Amido Black. GFP expression was detected by an EVOS-FL microscope. From each well a representative picture was taken.

4s$_{eGFP}$ for 1.5 h. Subsequently, 20,000 BHK-21 cells (in 50 µl) were added to each well. All dilutions and suspensions were made in complete growth medium consisting of Glasgow Minimum Essential Medium (GMEM; Invitrogen, Bleiswijk, The Netherlands) supplemented with 4% tryptose phosphate broth (Invitrogen), 1% non-essential amino acids (Invitrogen), 5% fetal bovine serum (Bodinco, Alkmaar, The Netherlands) and 1% antibiotic/antimycotic (Invitrogen). VNT plates containing wildtype RVFV were subsequently incubated for 5 days, whereas VNT plates containing RVFV$_{eGFP}$ or RVFV-4s$_{eGFP}$ were incubated for 2 days, at 37 °C and 5% CO_2. The VNT that makes use of