Virus Research 210 (2015) 279–282

Contents lists available at ScienceDirect

Virus Research

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

Short communication

Antibodies for strain 2117-like vesiviruses (caliciviruses) in humans

Barbara Di Martino[a,*], Federica Di Profio[a], Gianvito Lanave[b], Simona De Grazia[c], Giovanni M. Giammanco[c], Antonio Lavazza[d], Canio Buonavoglia[b], Fulvio Marsilio[a], Krisztián Bányai[e], Vito Martella[b]

[a] Faculty of Veterinary Medicine, Università degli Studi di Teramo, Italy
[b] Faculty of Veterinary Medicine, Università Aldo Moro di Bari, Valenzano, Italy
[c] Department of Scienze per la Promozione della Salute "G. D'Alessandro", Università degli Studi di Palermo, Italy
[d] Centro di Referenza Nazionale per le Malattie Virali dei Lagomorfi, Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, Italy
[e] Institute for Veterinary Medical Research, Centre for Agricultural Research, Hungarian Academy of Sciences, Budapest, Hungary

ARTICLE INFO

Article history:
Received 22 June 2015
Received in revised form 13 August 2015
Accepted 21 August 2015
Available online 28 August 2015

Keywords:
Caliciviruses
Vesivirus strain 2117
Antibodies
Humans

ABSTRACT

The vesivirus strain 2117 has been identified as contaminant of bioreactors used for production of human drugs, due to possible contamination of the reagents used for cell cultivation. Using an ELISA assay, antibodies specific for 2117-like viruses were detected in 32/410 (7.8%) human sera, indicating exposure to these viruses.

© 2015 Elsevier B.V. All rights reserved.

The *Caliciviridae* are small non-enveloped viruses approximately of 35 nm in diameter with single-stranded, positive-polarity RNA genomes of 7.4–8.3 kb. The family includes the genera *Vesivirus*, *Lagovirus*, *Norovirus*, *Sapovirus* and *Nebovirus* (Carstens, 2010). In addition, a number of unclassified caliciviruses have been identified in mammalians, avians and fishes (Cubitt and Barrett, 1985; Farkas et al., 2008; L'Homme et al., 2009; Wolf et al., 2011; Liao et al., 2014; Mikalsen et al., 2014)

In 2003, a novel calicivirus, the vesivirus (VeV) strain 2117, was identified incidentally as a contaminant in Chinese Hamster Ovary (CHO) cell cultures by a German pharmaceutical company (Oehmig et al., 2003). Possible sources of contamination included reagents commonly used for cell cultivation, such as porcine-derived trypsin or fetal bovine serum. Interestingly, strain 2117 showed about 72% nucleotide (nt) identity in the full-length genome to the prototype canine calicivirus strain 48 identified in Japan 1990 in a 2-month-old pup with intermittent watery diarrhea (Mochizuki et al., 1993).

Contamination of CHO cell cultures by the VeV strain 2117 was documented in three additional episodes, in 2008 and 2009 in Allston, Massachusetts, USA, and in 2008 in Geel, Belgium. The 2008–2009 contamination in the USA was estimated to cost $100–300 million in loss of revenues to the biotech company Genzyme, due to interruption of the production of Cerezyme (imiglucerase) and Fabrazyme (agalsidase β), i.e. drugs for extremely rare and life-threatening diseases, Gaucher's disease and Fabry's disease, respectively (Allison, 2010). In addition, Genzyme's contamination raised concerns about potential exposure of humans to these novel viruses, as VeVs are able to cross easily the host species barrier (Smith et al., 1998b). However, there was no evidence that the contaminant virus harmed people and the only inconvenience was the diminished productivity of the CHO cell lines. More recently, screening of faecal specimens from household and shelter dogs in Europe has led to the identification of novel canine VeVs (Martella et al., 2015) more genetically similar in their genome (89–90% nt) to the VeV strain 2117, than to the prototype canine strain 48 (71% nt). The 2117-like VeVs were detected at high prevalence (64.8%) in the rectal swabs of healthy dogs housed in shelters. Accordingly, as these viruses appear to be common in dogs, and shed trough the faeces, it may be hypothesized that faecal contamination of canine origin may also be a source of exposure to these VeVs for humans.

In order to assess if humans are exposed to 2117-like VeVs, we screened an age-stratified collection of human sera ($n = 410$) collected between 2010 and 2011 by using an ELISA assay based on the recombinant capsid protein (VP1) of a 2117-like virus expressed in baculovirus system. Human sera were collected

* Corresponding author. Fax: +39 0861 266885.
E-mail address: bdimartino@unite.it (B. Di Martino).

http://dx.doi.org/10.1016/j.virusres.2015.08.016
0168-1702/© 2015 Elsevier B.V. All rights reserved.

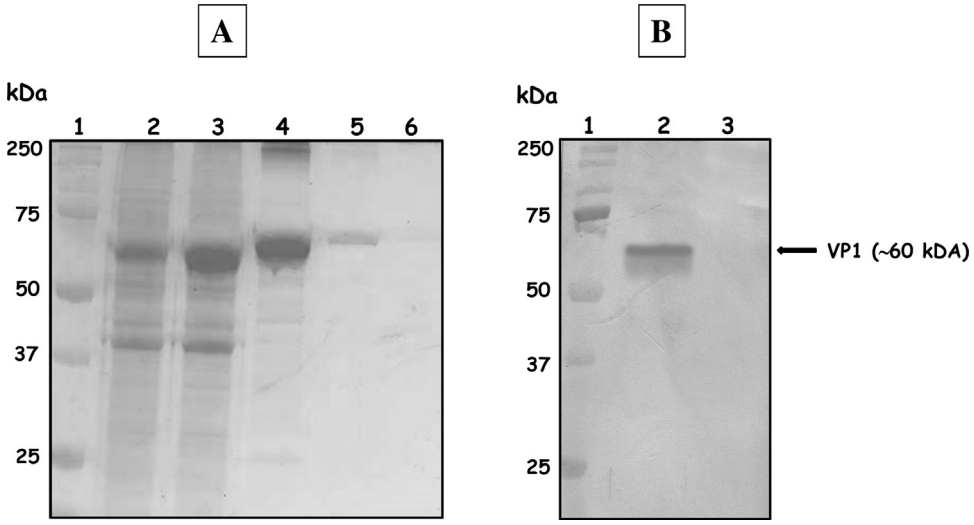

Fig. 1. A)SDS-10% PAGE of the recombinant VP1 capsid protein of the of the 2117-like VeV strain Bari/212/07/ITA compared to serial dilution of BSA. Line1: Precision Plus protein Standards (Bio-Rad, Italy); line 2 and line 3: VeV VP1 at final concentration of 100–500 μg/ml by spectrophotometer analysis (OD280); lines 4–6: aliquots of BSA at different concentrations (line 4: 100 μg/ml; line 5: 10 μg/ml and line 6: 1 μg/ml). B)Western blotting analysis of the VeV VP1 protein using a serum sample at dilution of 1:100 collected from a dog infected by a 2117-like VeV. Line1: Precision Plus protein Standards (Bio-Rad, Italy); line 2: wild-type baculovirus *Sf9* insect cells; line 3: VeV VP1 purified from the supernatants of *Sf9*insect cells.

randomly from both inpatients and outpatients presenting with various pathologies, at the Microbiology Unit of the University Hospital "P. Giaccone" of Palermo (Italy), between September 2010 and June 2011. Risk factors were not considered when collecting the human sera. All the patients were enrolled in the study after giving informed consent. Also, a total of 611 serum samples collected in 2011 from pigs of various age and of different Italian geographic regions were included in the screening, to address the hypothesis that contamination of CHO was originated from cell culture reagents, such as porcine-derived trypsin.

The recombinant baculovirus carrying the gene for the viral capsid protein (VP1) of the canine strain Bari/212/07/ITA (GenBank accession number JN204722) (Martella et al., 2015) was obtained as previously described (Di Martino et al., 2010). Briefly, the full-length VP1 (ORF2) was cloned into the pRN16 vector (kindly provided by Prof. Polly Roy) and transfection was performed with triple cut linearized AcNPV DNA (BD Biosciences, San Diego, CA). The recombinant baculovirus was selected using X-Gal blue/white screening and plaque purified on *Spodoptera frugiperda* (*Sf21*) cells. For large-scale production of the antigen, 100 ml *Sf9* cell (1×10^6 cell/ml) suspension culture were infected with the recombinant baculovirus at a multiplicity of infection of three plaque forming units/cell. The VP1 protein was isolated from the culture medium of infected cells at 48 h post-infection by centrifugation at 4000 rpm for 20 min. After purification by rate-zonal centrifugation on a discontinuous 20–60% (wt/vol) sucrose gradient, the collected fractions were dialyzed against PBS, and the protein concentration was determined by measuring the optical density at 280 nm (OD_{280}) and visually by running aliquots on SDS-10% PAGE containing bovine serum albumin (BSA) standards (Fig. 1A). The antigenicity of the recombinant VP1 was confirmed by Western blotting (WB), using field sera collected from dogs infected by a 2117-like VeV (Martella et al., 2015) (Fig. 1B). Antigenic cross-reactivity between VeV antigen and other human or porcine caliciviruses was ruled out in WB using specific rabbit hyperimmune sera raised against GIV.2 NoV and St-Valerien-like virus (SVLV) virus-like particles (VLPs) and by an antigen-ELISA kit (Ideia Norovirus, Oxoid, Basingstroke, UK) specific for human NoVs of genogroups GI and GII. The GIV.2 and the SVLVs-specific rabbit antisera did not show reactivity in WB analysis using antigen concentrations of 1 μg/ml to 100 μg/ml whilst the VeV VP1 was not detected by the commercial antigen-ELISA kit at concentrations as high as 100 μg of protein per ml. For the development of the antibody-detection ELISA, mock infected cells and VeV VP1 antigen (final concentration of 1 μg/ml) were coated onto 96 well EIA plates (Costar, Italy) at 100 μl per well in carbonate–bicarbonate buffer (0.05 M, pH 9.6) and incubated at 4 °C overnight (Di Martino et al., 2012, 2014). After blocking with 2% BSA in phosphate buffered saline (PBS) buffer at room temperature for two hours, the antigen-coated microplates were incubated with 100 μl of human or swine serum samples respectively diluted to 1:100 and 1:50 in 1% dried milk (Blotto)-PBS, at 37 °C for 1 h. Plates were washed five times in PBS with 0.1% tween 20 (PBS-T) and then incubated with horseradish peroxidase-conjugated goat anti-human immunoglobulin G (IgG) (Sigma–Aldrich, Italy) or with horseradish peroxidase-conjugated goat anti-swine IgG (Sigma–Aldrich, Italy) at 1:5,000 dilution, for 30 min at 37 °C. The reaction was developed with the addition of 100 μl per well of 2,2′-azino-di-(3-ethylbenzthiazoline-6-sulfonate) (ABTS) substrate for 15 min and stopped adding an equal volume of 1 M phosphoric acid. Absorbance was measured at 405 nm using a multiskan automatic plate reader (ThermoLabsystems, Finland). The cut-off point of the tests ($OD_{405} \geq 0.5$) was established as the mean of the OD_{405} readings of 50 serum samples negative in WB for the presence of VeV-specific antibodies, plus 2 standard deviations. For each tested sample, a positive/negative ratio (OD_{405} of VeV antigen/OD_{405} of mock infected cells) ≥2.0 was used to evaluate the background binding.

Out of 410 human sera, a total of 32 sera (7.8%) reacted with the VeV antigen at a dilution of 1:100 with an OD_{405} ranging from 0.5 to 1.2 (mean OD_{405} of 0.8). The prevalence of 2117-like VeV-specific antibodies was 5.3% in persons <1–20 years of age and it increased to 8.0% in the 21–40 year age class. This rate tended to be stable in the 41–60 year (8.6%) and in the 61–80 year age class (8.2%), declining to 6.8% in persons >80 years of age (Fig. 2). There was no statistically significant difference comparing the various age classes by Chi Square Test analysis. Interestingly, none of the tested porcine sera showed reactivity to the VeV antigen at dilution of 1:50.

Overall, these findings seem to indicate that humans are exposed to 2117-like VeVs. This group of VeVs is genetically distant from feline caliciviruses and VeVs of marine mammals (Martella et al., 2015), although antigenic cross-reactivity has been docu-

Download English Version:

https://daneshyari.com/en/article/3428070

Download Persian Version:

https://daneshyari.com/article/3428070

Daneshyari.com