Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
Human bocavirus 1 (HBoV1), a human parvovirus, belongs to the genus Bocavirus of the Parvoviridae family. It causes wheezing in young children with acute respiratory tract infections. HBoV1 has been shown to infect polarized human airway epithelium (HAE) made in house, and induces airway epithelial damage. In this study, two commercially available HAE cultures, EpiAirway and MucilAir HAE, were examined for HBoV1 infection. Both HAE cultures support fully productive HBoV1 infection. Infected EpiAirway and MucilAir HAE cultures showed loss of cilia, disruption of the tight junction barrier, and a significant decrease in transepithelial electrical resistance. Notably, HBoV1 persistent infection was demonstrated by maintaining HBoV1-infected EpiAirway HAE for as long as 50 days. After 2 days post-infection, progeny virus was produced consistently daily at a level of over 10^8 viral genome copies per culture (0.6 cm^2). This study is the first to use commercial sources of HAE cultures for HBoV1 infection. The availability of these cultures will enable a wide range of laboratories to study HBoV1 infection.

© 2013 Elsevier B.V. All rights reserved.
only from the basolateral surface, e.g., adenovirus (Zabner et al., 1997). In 2008, Dijkman et al. demonstrated that HBoV1 infects apically and replicates in HAE made in house (Dijkman et al., 2009). In 2012, an infectious clone of HBoV1 was established, which generates HBoV1 progeny virions from HEK293 cells transfected with this clone. Moreover, these HBoV1 virions infected HAE made in house productively, from the apical surface (Huang et al., 2012) as well as from the basolateral surface (Deng et al., 2013), which leads to airway epithelial damage.

In this report, two commercially available HAE cultures, i.e., EpiAirway and MucilAir HAE purchased from MatTek Co. (MA, USA) and Epithelix SàRL (Geneva, Switzerland), respectively, were tested for HBoV1 infection. Both HAE cultures can be infected by HBoV1 and caused hallmarks of airway epithelial damage.

2. Materials and methods

2.1. Polarized primary HAE cultures

EpiAirway HAE, which was purchased from MatTek (Ashland, MA, USA), was cultured in a Millicell insert of 0.6 cm² (Millipore, Billerica, MA, USA). MucilAir HAE was obtained from Epithelix SàRL (Geneva, Switzerland), and was maintained in a Costar Transwell insert of 0.33 cm² (Corning, NY, USA). Both HAE cultures were derived from healthy human primary tracheobronchial epithelial cells (derived from individual donors) cultured in an air-liquid interphase (ALI) with their respective property media.

2.2. Virus and infection

HBoV1 virions were collected from apical washes of HBoV1-infected primary B-HAE (Huang et al., 2012), and were used for infection as described previously (Deng et al., 2013). For apical infection of EpiAirway HAE, HBoV1 virions were diluted in 200 μl of EpiAirway medium to achieve an MOI of 100 viral genomic copy numbers (gc)/cell, and were applied to the apical chamber. For apical infection of MucilAir HAE, HBoV1 was diluted in 100 μl of MucilAir medium to reach an MOI of 100 gc/cell. After incubation for 2 h, the apical chamber of the infected HAE ALI was washed three times with 400 μl and 200 μl of PBS for EpiAirway and MucilAir HAE, respectively. The cultures were maintained continuously at an ALI. To determine virus release kinetics, 200 μl and 150 μl aliquots of PBS, for EpiAirway and MucilAir HAE, respectively, were added to the apical chamber of the HAE culture at various time points, incubated for 15 min at 37°C, and were harvested as apical washes. Meanwhile, an aliquot of 100 μl of medium from the basolateral chamber of the HAE culture was collected, followed by replacement with 100 μl of fresh medium. All the harvested aliquots were stored at 4°C for quantification of viral DNA as gc.

For basolateral infection, HBoV1 virions were diluted in the ALI medium (1 ml and 0.5 ml for EpiAirway and MucilAir, respectively) in the basolateral chamber of the HAE cultures. The cultures were incubated at 37°C and 5% CO₂ for 2 h, and then the basolateral inoculums were removed and washed three times with PBS (1 ml and 0.5 ml for EpiAirway and MucilAir, respectively), followed by supplementation of fresh media. Progeny virus release was monitored daily by quantification of viral gc in samples collected from the basolateral chamber at a volume of 100 μl.

2.3. Real time quantitative PCR (qPCR) analysis of HBoV1 genome copy numbers

Aliquots of 100 μl of apical sample and 50 μl of basolateral sample, respectively, were incubated with 25 units of benzonase (Sigma, St. Louis, MO, USA) for 2 h at 37°C. The treated samples were digested with 20 μl of proteinase K (15 mg/ml; Amresco, Solon, OH, USA) at 56°C for 10 min. Viral DNA was extracted using the QIAamp blood mini kit (Qiagen, Valencia, CA, USA), and eluted in 100 μl and 50 μl of deionized H₂O for apical and basolateral samples, respectively. The extracted DNA samples were quantified with respect to the numbers of HBoV1 gc by the qPCR method described previously (Deng et al., 2013; Huang et al., 2012; Lin et al., 2007).

2.4. Immunofluorescence analysis

Immunofluorescence analysis of HBoV1-infected HAE was performed as described previously (Deng et al., 2013; Huang et al., 2012). A rat anti-HBoV1 NS1 polyclonal antibody (Chen et al., 2010) was developed previously in-house (Chen et al., 2010). Anti-ZO-1 (Invitrogen, Grand Island, NY, USA) and anti-β-tubulin IV (Sigma, St. Louis, MO, USA) antibodies were used for detecting the tight junction and cilia, respectively.

2.5. Measurement of the transepithelial electrical resistance (TEER)

The TEER of both mock- and HBoV1-infected HAE cultures was measured using an epithelial voltm-ohm meter (Millipore, Billerica, MA, USA) at the indicated days p.i. as described previously (Deng et al., 2013; Huang et al., 2012).

3. Results

3.1. HBoV1 infects EpiAirway HAE persistently

EpiAirway HAE was first infected with the apically washed HBoV1 virions at a multiplicity of infection (MOI) of 100 gc/cell. Progeny virions released from the apical surface at 1 × 10⁹ gc/μl at 3 days post-infection (p.i.), and remained as a plateau along with the infection for 50 days (Fig. 1A). Virus was not detected in mock-infected EpiAirway HAE. Since 200 μl of phosphate buffered saline (pH 7.4; PBS) was used to wash the progeny virions from the apical surface every day, the total virus release from one HAE culture was approximately 2 × 10⁸ gc per day. Virus was also released from the basolateral surface, but at a level of ~1–2 log lower than that from the apical surface (Fig. 1A).

Consistent with the virus release kinetics, the transepithelial electrical resistance (TEER) of HBoV1-infected HAE started to drop off at 4 days p.i., gradually lowering to a level of 390 Ω cm² at 12 days p.i., 306 Ω cm² at 28 days p.i., and to a level close to 200 Ω cm² at 48 days p.i. (Fig. 1B). Compared with the mock-infected counterpart, the TEER decreased ~3.5-fold by the end of infection (at 48 days p.i., Fig. 1B).

Of note, a few of the epithelial cells were washed off in the apical chamber of HBoV1-infected HAE, and many of which were NS1-positive (Fig. 1C), suggesting that HBoV1 infection induces epithelial cell death. By the end of the infection (at 50 days p.i.), β-tubulin IV (a marker of cilia (Matrosovich et al., 2004; Villenave et al., 2012)), the tight junction protein zona occludens-1 (ZO-1) (Gonzalez-Mariscal et al., 2003), as well as HBoV1 NS1 expression, was detected. Although only one third of the cells in the HBoV1-infected EpiAirway HAE expressed NS1, the infected HAE showed no expression of β-tubulin IV and a disassociation of the ZO-1 (Fig. 1D).

In addition, EpiAirway HAE was infected at the basolateral surface. A gradual virus release from both the apical and basolateral surface was observed from 1 to 11 days p.i. Virus release from apical infection reached a plateau (~2 × 10⁸ gc/μl) at 12 days p.i.; however, there was ~1–1.5 log less virus released from the basolateral surface during infection between 10 and 26 days p.i. (Fig. 2A).
Fig. 1. HBoV1 infects EpiAirway HAE productively and persistently. EpiAirway HAE was infected with the apically washed HBoV1 at an MOI of 100 gc/μl. (A) At the indicated days p.i., HBoV1 virions were collected from both the apical and basolateral chambers and quantified by qPCR. Averages and standard deviations of the viral gc/μl are shown. (B) The TEER of HBoV1-infected EpiAirway HAE was monitored at the indicated days p.i. Averages and standard deviations of the TEER are shown. (C) At 11 days p.i., the apical chambers of three infected EpiAirway HAE cultures were washed with 200 μl of PBS to collect the apically washed cells. A total of 600 μl of the sample was concentrated to 200 μl, which was then cytospun onto a slide followed by immunofluorescence analysis with an anti-HBoV1 NS1 antibody. Confocal images were taken with an Eclipse C1 Plus confocal microscope (Nikon, Melville, NY, USA) controlled by Nikon EZ-C1 software, at a magnification of 40× as indicated. (D) At 50 days p.i., insert membranes of the infected EpiAirway HAE were fixed and analyzed by an immunofluorescence assay with an anti-HBoV1 NS1 antibody with co-staining of anti-β-tubulin IV or anti-ZO1 antibody. Confocal images were taken at a magnification of 40×. Nuclei were stained with DAPI (blue).
Fig. 2. Analyses of EpiAirway HAE infected with HBoV1 basolaterally. EpiAirway HAE was infected with HBoV1 at an MOI of 100 gc/μl from the basolateral surface. (A) At the indicated days p.i., both apically washed and basolaterally collected samples were quantified by qPCR as gc/μl, which are plotted to the days p.i. and shown as averages and standard deviations. (B) The TEER of HBoV1-infected HAE was monitored at the indicated days p.i. Averages and standard deviations are shown. (C) At the end of the infection, insert membranes of the HAE cultures were fixed and stained with anti-HBoV1 NS1 and anti-β-tubulin IV or anti-ZO1 antibodies. Nuclei were stained with DAPI (blue). The cells were visualized by confocal microscopy at a magnification of 40×.
Basolateral infection also caused significant epithelial damage represented by the gradual decrease in TEER (Fig. 2B), cilia loss and destruction of the tight junction (Fig. 2C).

Taken together, these results suggest that HBoV1 infects EpiAirway HAE both apically and basolaterally, and that the infection is persistent and causes airway epithelial damage.

3.2. HBoV1 infects MucilAir HAE cultures

MucilAir HAE was infected with HBoV1 either apically or basolaterally at an MOI of 100 gc/cell. Virus production after infection was monitored by collecting samples from both the apical and basolateral chambers of the infected HAE.

During apical infection, progeny virions were released apically at a level of $2 \times 10^4$ gc/µl at 3 days p.i., reached a peak of $7 \times 10^6$ gc/µl at 9 days p.i., and gradually decreased to $2 \times 10^5$ gc/µl at 26 days p.i. (Fig. 3A). Virus was also released from the basolateral surface, but at a level of 1–2 log less than that from the apical surface (Fig. 3A). Mock-infected HAE did not release any virions from either the apical or basolateral surfaces. During basolateral infection, virions were released much more slowly in early infection (at 1–10 days p.i.), reached a peak ($1 \times 10^6$ and $1 \times 10^5$ gc/µl, respectively, from the apical and basolateral surfaces) at 12–13 days p.i., and gradually decreased to levels of $8 \times 10^4$ and $5 \times 10^3$ gc/µl by the end of infection (Fig. 3B).

The TEER was detected to monitor the epithelial barrier function of the HAE affected by HBoV1 infection. Although the TEER of the MucilAir HAE was initially low (300–400 Ω cm$^2$), after HBoV1 infection, it decreased further by ∼2- to 3-fold, gradually to levels of 130 and 180 Ω cm$^2$ during apical and basolateral infection, respectively, at 26 days p.i. (Fig. 3C), which is consistent with the virus release kinetics (Fig. 3A and B).

At 26 days p.i., at least one third of the cells in the infected HAE expressed HBoV1 NS1 (Fig. 4A). Either apically- or basolaterally infected MucilAir HAE lost cilia and the tight junction, as shown by staining with anti-β-tubulin IV (Fig. 4B) and anti-ZO1 (Fig. 4C).
Fig. 4. HBoV1 infection of MucilAir HAE causes airway epithelial damage. At the end of the infection (26 days p.i.), insert membranes of the infected MucilAir HAE cultures were fixed and stained with an anti-HBoV1 NS1 antibody (A), or co-stained with anti-HBoV1 NS1 and anti-β-tubulin IV antibody (B) and with anti-HBoV1 NS1 and anti-ZO-1 antibodies (C). Nuclei were stained with DAPI (blue). The stained cells were visualized by confocal microscopy at magnifications of 40× and 100×, as indicated.
antibodies, respectively, and showed an obvious nucleus enlargement (Fig. 4B and C, DAPI), strongly suggesting airway epithelial damage.

4. Discussion

Pseudostratified and polarized HAE cultures have been reported for productive HBoV1 infection (Deng et al., 2013; Dijkman et al., 2009; Huang et al., 2012), but they were made in house, and thus were not available for most of the researchers in the field. In this report, HBoV1 infection was studied in polarized primary HAE cultures, MucAir and EpiAirway HAE, purchased from MatTek Co. (Ashland, MA, USA) and Epithelix SàRL (Geneva, Switzerland), respectively. HBoV1 progeny virions were released from both infected MucAir and EpiAirway HAE cultures, but are at an average of ~1–2 × 10^8 gc per insert. These results suggest that the commercially available HAE cultures support productive HBoV1 infection. Thus, our study provides valuable information to culture HBoV1 and study HBoV1 infection in primary HAE.

Notably, HBoV1 infection can be persistent in EpiAirway HAE for at least 50 days p.i. Previous studies have identified the presence of episomal covalently closed circular (ccc) viral DNA in patients infected with HBoV1-3 (Kang et al., 2009; Kapoor et al., 2011; Windisch et al., 2013; Zhao et al., 2012). However, whether the cccDNA is the viral genomes persisting in HBoV1-infected HAE cultures from the manufacturers. Previously, primary B-HAE cultures made in house was used, which had a TEER of >1000 Ω cm^2 (Huang et al., 2012). However, both MucAir and EpiAirway HAE had a low TEER (400 and 800 Ω cm^2, respectively) when we received them. Nevertheless, HBoV1 infection disrupts the polarity of both the MucAir and EpiAirway HAE cultures infected with HBoV1. The TEER of the infected MucAir and EpiAirway HAE decreased by 2– to 3-fold, similar to the decrease in TEER in HBoV1-infected HAE made in house (Deng et al., 2013). Notably, in the HAE cultures we tested, there was a higher expression level of cili on MucAir HAE than in EpiAirway HAE (Figs. 2C and 4B, compare the β-tubulin). Apparently, the high expression level of cilia did not contribute a high level of virus production from infected MucAir HAE. Interestingly, by the end of infection, all the cilia in the infected HAE of both types were lost, although only one third of the cells showed NS1 expression, suggesting that HBoV1 infection may release cytotoxic cytokines into the cultures as do RNA respiratory viruses (Vareille et al., 2011), which warrants further investigation.

Because of the nature of the MucAir and EpiAirway HAE insert membrane materials, further histology analyses of the airway epithelia were not successful in either frozen section or paraffin-embedded section (data not shown). However, for the MucAir HAE, the polyethylene terephthalate (PET) membrane of the Transwell insert provides a better cell visualization that allowed us to take confocal images of directly fixed infected cells on the insert membrane at a high magnification (Fig. 4B and C), which show much clearer nucleus enlargement of the cells and destruction of the epithelial tight junction in the infected HAE. MucAir HAE has been used to infect influenza virus (Brookes et al., 2011), respiratory syncytial virus and human rhinovirus (Huang et al., 2011), and EpiAirway HAE has been reported to infect influenza virus (Triana-Baltzer et al., 2010), parainfluenza virus (Moscona et al., 2010; Palermo et al., 2009; Palmer et al., 2012), human rhinovirus (Sharma et al., 2010), and respiratory syncytial virus (Donalasio et al., 2012). MucAir HAE was cultured in a Transwell insert of 0.33 cm² (Costar), while EpiAirway HAE was cultured in a Millicell insert of 0.6 cm² (Millipore). Both HAE cultures are suitable model systems to study HBoV1 for many laboratories, which may lack experience to make primary HAE in house.

In conclusion, this study supports strongly that HBoV1 is a highly infectious respiratory virus. Two sources of HAE cultures, MucAir and EpiAirway HAE, are commercially available for productive HBoV1 infection, which will facilitate the study of HBoV1 worldwide. More importantly, the airway barrier function was destroyed in both HBoV1-infected MucAir and EpiAirway HAE. For the first time, HBoV1 infection was demonstrated to be persistent, for as long as 50 days p.i., in an in vitro model of human airway epithelia.

Acknowledgements

We thank Dr. Sarah Tague for help in taking confocal microscopy images and members of the Qiu lab for valuable discussion.

References

Allander, T., Jartti, T., Gupta, S., Nieters, H.G., Lehtinen, P., Osterback, R., Vuorinen, T., Waris, M., Bjerkner, A., Tiveljung-Lindell, A., van den Hoogen, B.C., Hyypia, T., Rusku, O., 2007. Human bocavirus and acute wheezing in children. Clin. Infect. Dis. 44, 904–910.

Allander, T., Tammi, M.T., Eriksson, M., Bjerkner, A., Tiveljung-Lindell, A., Andersson, B., 2005. Cloning of a human parvovirus by molecular screening of respiratory tract samples. Proc. Natl. Acad. Sci. U.S.A. 102, 12891–12896.

Arthur, J.L., Higgins, G.D., Davidson, G.P., Givney, R.C., Ratcliff, R.M., 2009. A novel bocavirus associated with acute gastroenteritis in Australian children. PLoS Pathog. 5, e1000391.

Brodzinski, H., Ruddy, R.M., 2009. Review of new and newly discovered respiratory tract viruses in children. Pediatr. Emerg. Care 25, 352–360.

Brookes, D.W., Mahi, S., Lackenby, A., Hartgroves, L., Barclay, W.S., 2011. Pandemic H1N1 2009 influenza virus with the H275Y neuraminidase resistance neuraminidase mutation shows a small compromise in enzyme activity and viral fitness. J. Antimicrob. Chemother. 66, 466–470.

Chen, A.Y., Cheng, F., Lou, S., Luo, Y., Liu, Z., Delwart, E., Pintel, D., Qiu, J., 2010. Characterization of the gene expression profile of human bocavirus. Virology 403, 145–154.

Chen, K.C., Shull, R.C., Moses, E.A., Lederman, M., Stout, E.R., Bates, R.C., 1986. Complete nucleotide sequence and genome organization of bovine parvovirus. J. Virol. 60, 1085–1097.

Christensen, A., Døllner, H., Shankie, L.H., Krokkstad, S., Moe, N., Nordba, S.A., 2013. Detection of spliced mRNA from human bocavirus 1 in clinical samples from children with respiratory tract infections. Emerg. Infect. Dis. 19, 574–580.

Christensen, A., Nordba, S.A., Krokkstad, S., Rognsen, A.G., Døllner, H., 2010. Human bocavirus in children: mono-detection, high viral load and viraemia are associated with respiratory tract infection. J. Clin. Virol. 49, 158–162.

Deng, Y., Yan, Z., Luo, Y., Xu, J., Cheng, Y., Li, Y., Engelhardt, J., Qiu, J., 2013. In vitro modeling of human bocavirus 1 infection of polarized primary human airway epithelia. J. Virol. 87, 4097–4102.

Deng, Y., Gu, X., Zhao, X., Luo, Z., Wang, L., Fu, Z., Yang, X., Liu, E., 2012. High viral load of human bocavirus correlates with duration of wheezing in children with severe lower respiratory tract infection. PLoS One 7, e43453.

Dijkman, R., Roekkoeck, S.M., Molkenkamp, R., Schildgen, D., van der Hoek, L., 2009. Human bocavirus can be cultured in differentiated human airway epithelial cells. J. Virol. 83, 7739–7748.

Don, M., Soderlund-Venermo, M., Valent, F. Lehtinen, A., Hedman, C., Lanciani, M., Hedman, K., Korppi, M., 2010. Serologically verified human bocavirus pneumonia in children. Pediatr. Pulmonol. 45, 120–126.

Donalasio, M., Rusnati, M., Cagno, V., Civa, A., Bugatti, A., Giuliani, A., Pirri, G., Volante, M., Papotti, M., Landolfo, S., Lombo, D., 2012. Inhibition of human respiratory syncytial virus infectivity by a dendrimeric heparan sulfate-binding peptide. Antimicrob. Agents Chemother. 56, 5278–5288.

Edner, N., Castillo-Rodas, P., Falk, L., Hedman, K., Soderlund-Venermo, M., Allander, T., 2011. Life-threatening respiratory tract disease with human bocavirus-1 infection in a four-year-old child. J. Clin. Microbiol. 50, 531–532.

Garcia-Garcia, M.L., Calvo, C., Falcon, A., Pozo, F., Perez-Brena, P., De Cea, J.M., Casas, I., 2010. Role of emerging respiratory viruses in children with severe acute wheezing. Pediatr. Pulmonol. 45, 585–593.

Gendrel, D., Gueld, R., Pons-Catalan, C., Enamiran, A., Raymond, J., Rozenberg, F., Lebon, P., 2007. Human bocavirus in children with acute asthma. Clin. Infect. Dis. 45, 404–405.

Gonzalez, V., Betanzos, A., Nava, P., Jaramillo, B.E., 2003. Tight junction proteins. Prog. Biophys. Mol. Biol. 81, 1–44.

Hao, W., Bernard, K., Patel, N., Ulbricht, N., Feng, H., Svabeck, C., Wilson, S., Stracener, C., Wang, K., Suzich, J., Blair, W., Zhu, Q., 2012. Infection and propagation of human rhinovirus C in human airway epithelial cells. J. Virol. 86, 13524–13532.

Huang, Q., Deng, Y., Yan, Z., Cheng, F., Luo, Y., Shen, W., Leu-Butters, D.C., Chen, A.Y., Li, Y., Tang, L., Soderlund-Venermo, M., Engelhardt, J.F., Qiu, J., 2012. Establishment...
of a reverse genetics system for studying human bocavirus in human airway epithelial 3D cell models. PLoS Pathog. 6, e1000829.

Huang, S., Wiszniewski, L., Constant, S., 2011. The use of In Vitro 3D cell models in drug development for respiratory diseases. In: Kapetanovic, I. (Ed.), Drug Development and Discovery — Present and Future. InTech, Rijeka, Croatia, pp. 107–116.

Ilyushina, N.A., Bovin, N.V., Webster, R.G., 2012. Decreased neuraminidase activity is important for the adaptation of H5N1 influenza virus to human airway epithelium. J. Virol. 86, 4724–4733.

Jartti, T., Hedman, K., Jartti, L., Ruuskanen, O., Allander, T., Soderlund-Venermo, M., 2011. Human bocavirus—the first 5 years. Rev. Med. Virol. 21, 46–64.

Kahn, J., 2008. Human bocavirus: clinical significance and implications. Curr. Opin. Infect. Dis. 21, 62–66.

Kang, W., Wang, L., Harrell, H., Liu, J., Thomas, D.L., Mayfield, T.L., Scotti, M.M., Ye, G.J., 2008. Human bocavirus: clinical significance and implications. Curr. Opin. Infect. Dis. 21, 62–66.

Kapetanovic, I. (Ed.), Drug Development and Discovery — Present and Future. InTech, Rijeka, Croatia, pp. 107–116.

Kapoor, A., Aschenbrenner, L.M., Triana-Baltzer, G.B., Babizki, M., Chan, M.C., Wong, A.C., Aschenbrenner, L.M., Piccoli, R., 2011. Human bocavirus: passenger or pathogen in acute respiratory tract infections? Clin. Microbiol. Rev. 21, 229–232.

Kapoor, A., Aschenbrenner, L.M., Triana-Baltzer, G.B., Babizki, M., Chan, M.C., Wong, A.C., Aschenbrenner, L.M., Piccoli, R., 2011. Human bocavirus—its clinical significance and implications. Curr. Opin. Infect. Dis. 21, 62–66.

Kapoor, A., Aschenbrenner, L.M., Triana-Baltzer, G.B., Babizki, M., Chan, M.C., Wong, A.C., Aschenbrenner, L.M., Piccoli, R., 2011. Human bocavirus—the first 5 years. Rev. Med. Virol. 21, 46–64.

Koshevar, M., Kieninger, E., Edwards, M.R., Regamey, N., 2011. The airway epithelium: soldier in the fight against respiratory viruses. Clin. Microbiol. Rev. 24, 210–229.

Koshevar, M., Kieninger, E., Edwards, M.R., Regamey, N., 2011. The airway epithelium: soldier in the fight against respiratory viruses. Clin. Microbiol. Rev. 24, 210–229.

Koshevar, M., Kieninger, E., Edwards, M.R., Regamey, N., 2011. The airway epithelium: soldier in the fight against respiratory viruses. Clin. Microbiol. Rev. 24, 210–229.

Koshevar, M., Kieninger, E., Edwards, M.R., Regamey, N., 2011. The airway epithelium: soldier in the fight against respiratory viruses. Clin. Microbiol. Rev. 24, 210–229.

Koshevar, M., Kieninger, E., Edwards, M.R., Regamey, N., 2011. The airway epithelium: soldier in the fight against respiratory viruses. Clin. Microbiol. Rev. 24, 210–229.

Koshevar, M., Kieninger, E., Edwards, M.R., Regamey, N., 2011. The airway epithelium: soldier in the fight against respiratory viruses. Clin. Microbiol. Rev. 24, 210–229.

Koshevar, M., Kieninger, E., Edwards, M.R., Regamey, N., 2011. The airway epithelium: soldier in the fight against respiratory viruses. Clin. Microbiol. Rev. 24, 210–229.