

Variation in pestivirus growth in testicle primary cell culture is more dependent on the individual cell donor than cattle breed

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Abstract The causes of bovine respiratory disease complex (BRDC) are multifactorial and include infection with both viral and bacterial pathogens. Host factors are also involved as different breeds of cattle appear to have different susceptibilities to BRDC. Infection with bovine pestiviruses, including bovine viral diarrhea virus 1 (BVDV1), BVDV2 and ‘HoBi’-like viruses, is linked to the development of BRDC. The aim of the present study was to compare the growth of different bovine pestiviruses in primary testicle cell cultures obtained from taurine, indicine and mixed taurine and indicine cattle breeds. Primary cells strains, derived from testicular tissue, were generated from three animals from each breed. Bovine pestivirus strains used were from BVDV-1a, BVDV-1b, BVDV-2a and ‘HoBi’-like virus. Growth was compared by determining virus titers after one passage in primary cells. All tests were run in triplicate. Virus titers were determined by endpoint dilution and RT-qPCR. Statistical analysis was performed using one way analysis of variance (ANOVA) followed by the Tukey’s Multiple Comparison Test (P 0.05).

Significant differences in virus growth did not correlate with cattle breed. However, significant differences were observed between cells derived from different individuals regardless of breed. Variation in the replication of virus in primary cell strains may reflect a genetic predisposition that favors virus replication.

Keywords Pestivirus · Bovine · Taurine · Indicine · Cell culture

Introduction

Bovine viral diarrhea viruses (BVDV) are segregated into two species, BVDV-1 and BVDV-2, within the genus *Pestivirus* of the family *Flaviviridae* (Simmonds et al. 2011). Infection with an emerging species within the pestivirus genus, ‘HoBi’-like viruses (Schirrneier et al. 2004), results in clinical symptoms in cattle that are indistinguishable from those caused by BVDV-1 and BVDV-2 infections including reproductive, respiratory and digestive disorders (Decaro et al. 2011; Weber et al. 2016b). Pestiviruses can cross species barriers and infect a variety different hosts within the order Artiodactyla (Lies and Moennig 1990; Nettleton 1990; Becher et al. 1997; Krametter-Froetscher et al. 2010). However, not all pestivirus strains grow to the same efficiency in cells derived from different species (Roehle and Edwards 1994; Liang et al. 2003). This suggests that there are selective host factors involved in the control of viral growth.

Cattle belong to the *Bos taurus* species (Order Artiodactyla, Family Bovidae, Subfamily Bovinae). There are three subspecies within this species: *Bos taurus primigenius* (the extinct aurochs), *Bos taurus indicus* (indicine cattle) and *Bos taurus taurus* (taurine cattle) (Grubb 2005). While taurine cattle have greater growth rates in the absence of stressful situations, indicine exhibit

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higher heat tolerance and resistance to some internal and external parasites (Frisch and Vercoe 1984; Léger et al. 2013) and pathogens such as bovine tuberculosis (Ameni et al. 2007).

Bovine respiratory disease complex (BRDC) causes significant economic losses to beef and dairy producers worldwide. The development of BRDC is the result of the interaction of multiple factors including infection with pathogens and physiological or physical stressors. While the heritability of BRDC is low, breed differences have been observed (Snowder et al. 2005; Taylor et al. 2010). A study comparing the incidence of BRDC in several breeds of *Bos taurus* to the incidence in two different breeds of *Bos indicus* found the taurine cattle to be at a greater risk for BRDC than indicine cattle (Cusack et al. 2007). As stated above, infection with viral and/or bacterial pathogens has been associated with the development of BRDC. In particular, infection with BVDV is often suspected as an initiating event for BRDC (Fulton et al. 2000). Consequently variation in BVDV replication could translate into variation in susceptibility to BRDC.

Castration is a common practice in beef production. Primary cell strains are easily generated from the testicles that are removed during castration. Replication of virus in testicular tissue occurs during natural infections (Givens et al. 2009) and commonly used protocols for BVDV viral amplification use testicle cell lines (OIE 2008). The aim of the present study is to compare growth of different bovine pestivirus species and subtypes in testicle cell cultures obtained from different individuals of taurine, indicine and mixed taurine and indicine breeds.

Materials and methods

Virus propagation and titration

Madin-Darby bovine kidney (MDBK) cells (CCL-22; ATCC) were used to propagate and titrate three cytopathic bovine pestivirus strains: BVDV-1a strain NADL (GenBank accession number AJ133738.1), BVDV-1b strain TGAC (Z54175.1) and BVDV-2a strain 296c (AF268172.1). All three strains were isolated from US cattle suffering from mucosal disease. The cytopathic 'HoBi'-like virus strain used was a clone of Italy-1/10-1 (Decaro et al. 2011) kindly provided by Dr. Nicola Decaro (Faculty of Veterinary Medicine of Bari, Bari, Italy). This strain was cloned by limiting dilution and a clone, selected, based on faster and clearer expression of cytopathic effect, was used for the studies detailed here. The MDBK cells were grown in minimal essential medium (MEM), supplemented with L-glutamine (1.4 mM), gentamicin (50 mg/L), and 10 % fetal bovine serum (FBS). Cells were confirmed free of pestivirus based on RT-PCR, and FBS was confirmed free of pestivirus and antibodies against pestiviruses by RT-PCR and virus neutralization test (VNT), respectively (Bauermann et al. 2014b). For virus propagation, 75-

cm² flasks containing 70 % confluent MDBK cell monolayers were inoculated with one of the four pestivirus strains and incubated at 37 °C for 72 to 96 h. Following one freeze-thaw cycle, the suspension was centrifuged for 10 min at 1000 x g. Supernatants were collected, aliquoted, and stored at -80 °C until use. The virus stocks were titrated in 96-well microtiter plates by endpoint dilution. Titers were calculated and expressed as median tissue culture infective doses (TCID₅₀) (Reed and Muench 1938).

Bovine testicle (BTe) cells preparation, virus inoculation and titration

Testicle were collected, following castration, from three animals each from the following breed: Angus (taurine cattle) (#B.TAURUS 314c, #B.TAURUS 352c and B.TAURUS 392c), Brahman (indicine cattle) (#B.INDICUS 524c, #B.INDICUS 528c and #B.INDICUS 578c) and mixed Nelore and Angus (mixed taurine/indicine cattle) (#MIXED 299c, #MIXED 442c and #MIXED 461). Aseptically, the epididymis and serosa were removed, and the primary BTe cell cultures were prepared (Burlinson et al. 1992). Cells were verified to be free of pestivirus by RT-PCR (Bauermann et al. 2014b).

The resulting nine cell strains were passed once every four days. No differences were observed among the nine strains in the replication rate or cell morphology. At passage seven, cells were plated into a 12-well plate and, when approximately 70 % confluent, three wells were each infected with one of the four bovine pestivirus strains, at a multiplicity of infection (MOI) of 0.1 and incubated for seven days. At harvest cultures (including cell monolayer and culture fluid of 2.0 mL/well) were frozen at -80 °C for 24 h. The resulting lysates were then thawed, transferred to a centrifuge tube and centrifuged for 10 min at 1000xg. Supernatants were collected, aliquoted, and stored at -80 °C until testing. Virus titrations were performed in MDBK by endpoint dilution as described above.

RNA isolation and quantitative RT-PCR (RT-qPCR)

Total RNA was prepared using 140 µL of sample from the supernatants generated as described above. A robotic workstation (Qiacube, Qiagen, Hilden, Germany) was used for automated RNA purification by a spin-column system (QIAamp Viral RNA Mini Kit, Qiagen) according to the manufacturer recommendation. The extracted RNA was stored at -80 °C.

VetMax-Gold-bovine virus diarrhea RNA test kit (Life Technologies, Austin, TX, USA) was employed for RNA quantification. Samples were run in triplicate and each time samples were run NADL, TGAC, 296c or Italy-cp positive samples were run as controls. The 25-µL reaction mixture used for the test consisted of 12.5 µL of 2X RT-PCR buffer, 1 µL of 25X BVDV primer-probe mix, 1 µL of 25X RT-PCR enzyme mix, and 8 µL of extracted RNA. Quantification of virus RNA was

performed using a standard curve based on ten-fold serial dilutions (10^{-1} to 10^{-4}) of a positive control of known titer.

Sequencing

In order to rule out cross-contamination between infected BTe cultures, the sequence of passed virus was compared to the sequence of inoculation virus as follows. Total RNA was isolated from cell lysates, and RT-PCR was performed followed by DNA sequencing of the amplicon. Briefly, RNA isolation was performed as described above, and RT-PCR used the primers 324 and 326 (Vilcek et al. 1994). Amplification product was purified and concentrated using QIAquick® PCR Purification Kit (Qiagen) according to the manufacturer's instructions followed by quantification using the Qubit® 2.0 Fluorometer for dsDNA (Life Technologies). The appropriate amount of dsDNA was labeled in both directions using Big Dye terminator chemistries (Applied Biosystems Inc., Foster City, CA, USA) according to the manufacturer's instructions. The labeled products were sequenced using an ABI 3100 genetic analyzer (Applied Biosystems Inc.). The sequences of all samples were confirmed by sequencing both strands in duplicate.

Statistical analysis

Correlation of virus load obtained using titration by endpoint dilution and RT-qPCR was evaluated using the Pearson's correlation coefficient. For statistical comparison, one way analysis of variance (ANOVA) followed by the Tukey's Multiple Comparison Test (MCT) were used. Growth of each viral strain in BTe cells originating from different bovine breeds, growth of different viral strains in BTe cell originating from the same breed and growth of different viral strains in the different animals from the same breed were compared using the results obtained in virus titration by endpoint dilution. *P*-values less than 0.05 were considered significant. Graphs were drawn using the GraphPad Prism 5 Software (GraphPad Software Inc., San Diego, USA).

Results

Bovine testicle cells from different individuals of taurine, indicine and mixed taurine and indicine breeds were prepared and inoculated with different bovine pestivirus species and subtypes. Based on time to reach confluency, no difference in cell growth rate was observed. Viral titration by endpoint dilution and RT-qPCR for the different animals within the taurine, indicine and mixed cattle breed BTe cells are shown in Fig. 1a and b, respectively. The results obtained by virus titration using both methods in each animal showed negative

correlation using the Pearson's correlation coefficient ($r = -0.5186$; $P = 0.0012$; 95 % CI: -0.7239 to -0.2290).

ANOVA was used to evaluate differences in of virus growth between different bovine breeds, between animals within each breed group and between the different virus strains in cells from same donor. Titration by endpoint dilution showed no significant difference ($P = 0.8889$) in growth of each virus based on breed (Fig. 2a). However, there were significant differences in growth rate among animals within each breed group (Fig. 2a). Significant differences were also observed in growth rate of NADL and TGAC in cells derived from different animals within breed groups (Fig. 1a). The differences between the triplicates in each animal expressed as the standard error of the means (SEM) were generally low, and null in some cases (Fig. 1). While variation in viral growth was observed between cells derived from individual animals, there was no one individual who cells grew all viruses tested to universally higher or lower levels (Fig. 1a).

The same analyses performed using the RT-qPCR yielded similar results. No significant difference was observed when the growth of each virus in BTe cells originated from the different bovine breeds when measured by RT-qPCR ($P = 0.2618$) (Fig. 2b). As observed in the statistical analysis with the endpoint dilution, there were significant differences for the viral growth rate between animals within the breed groups (Fig. 2b) and between strains NADL and TGAC in different individuals within same bovine breeds (Fig. 1b).

DNA sequencing confirmed that the virus titrated in BTe cell cultures matched the inoculation virus confirming absence of cross contamination.

Discussion

In the present study, cattle ($n = 3$) of taurine, indicine or taurine/indicine cross breed, of similar age and from the same location were castrated and their testicles used to prepare primary cell cultures. These primary cell cultures were then inoculated with the different pestivirus strains and viral replication compared. The virus titers revealed differences in viral replication between individuals, but that these differences are not associated with breed. Previously differences in the viral load have also been reported in persistently infected (PI) cattle generated using the same pestivirus strain (Bauermann et al. 2014a) and that viral titer can vary over time (Arenhart et al. 2009). Variation has also been observed in the width and breath of the viral swarms circulating in persistently infected cattle (Ridpath et al. 2015; Weber et al. 2016a; Weber et al. 2017). Cattle infected in the same outbreak can have greatly different outcomes (Carman et al. 1998). It has been proposed

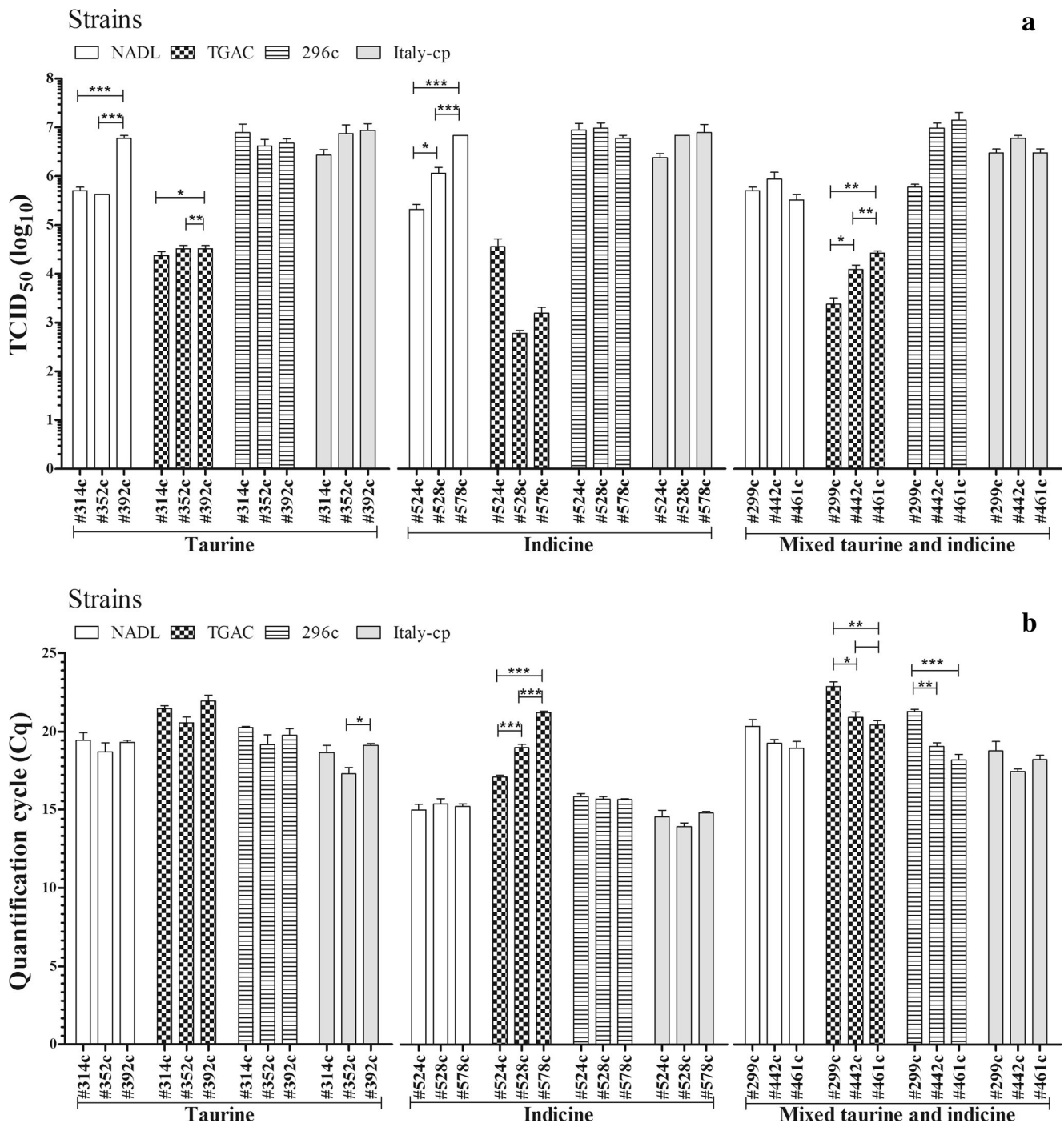


Fig. 1 Virus load (vertical axis) obtained by endpoint dilution (A) and RT-qPCR (B) of bovine testicle (BTe) cells obtained from different taurine, indicine and mixed breed cattle infected with different bovine pestivirus strains (horizontal axis). All assays were performed in triplicate. Data are presented as means with standard errors indicated

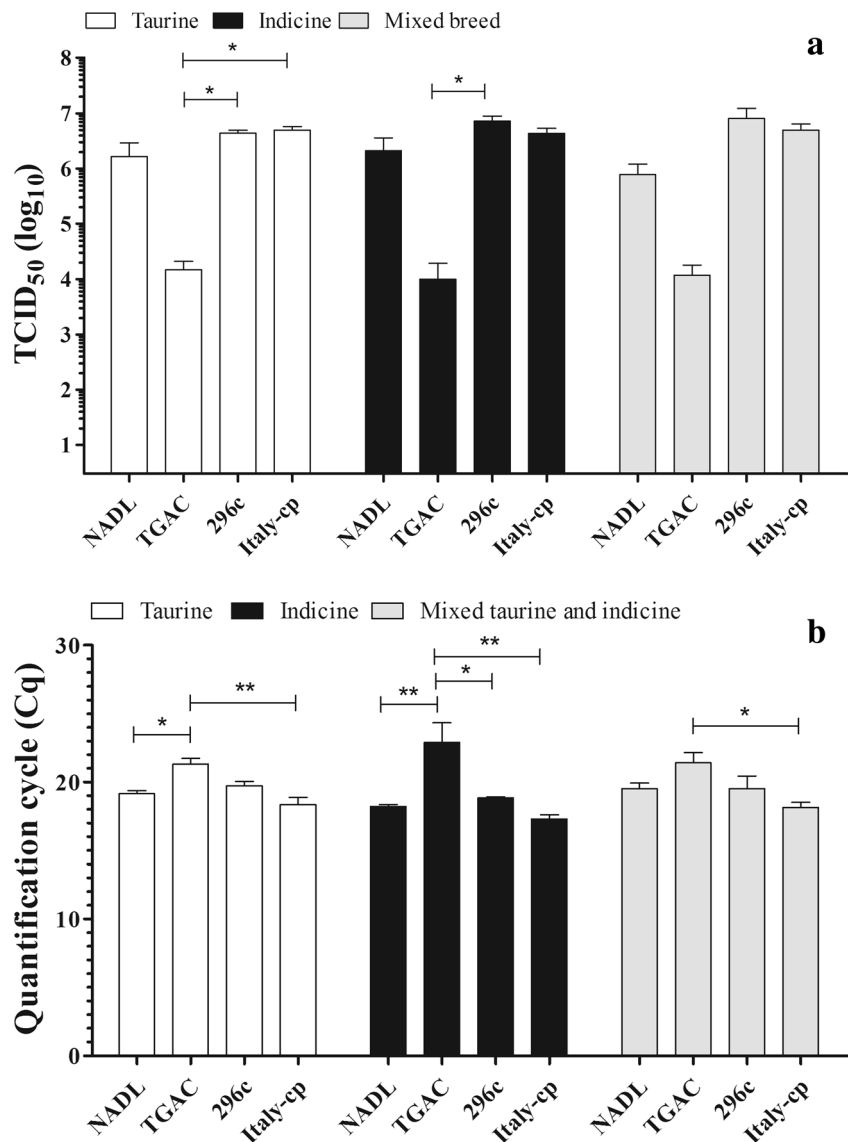
that resulted for the triplicate combination of each individual cell donor. Comparisons of the growth of different viruses in the different animals from same breed were made by ANOVA, followed by Tukey's MCT. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

that these differences are associated with host factors, particularly host factors that control immune response (Peterhans and Schweizer 2013; Lussi and Schweizer 2016). Immune response was not a factor in the in vitro system used in the present study, thus variation observed in viral replication are

due to host factors that directly impact viral uptake, replication, assembly or release.

Previously published research has indicated that pestiviruses replicate at different rates in host cell from different species (Lies and Moennig 1990; Roehle and Edwards 1994; Liang

Fig. 2 Bovine pestiviruses titer in bovine testicle (BTe) cells obtained from taurine, indicine and mixed cattle breeds obtained by endpoint dilution (A) and RT-qPCR (B). Vertical axis represents the mean of the virus titers obtained by the triplicate of the three different animals that compose each breed group. Horizontal axis represents the pestivirus strain used to infect each BTe cell group. Data represents means \pm standard errors that resulted for the combination of all the triplicates of individuals within each cattle breed. Comparisons of the growth of different viruses in the different animals from same breed were made by ANOVA, followed by Tukey's MCT. * P 0.05, ** P 0.01, *** P 0.001



et al. 2003). In this study, differences in replication were not associated with differences in host subspecies (taurine and indicine cattle). However, differences in viral replication were observed in cells derived from different animals belonging to the same breed, implying that individual animal traits may impact on viral replication. These findings are supported by the low SEM observed in the triplicates in two different tests from each individual within same cattle breed. The virus strains used in this study did not universally grow to higher titers in any one of the cell strains derived from the individual animals. Instead, individual viral strains grew to different titers in cells derived from different animals. Thus, not only are host factors involved but there is an interplay between viral and host factors. Clinical presentation observed in both acute and persistent BVDV infections are highly variable. The results of the current study suggest that viral strain and host factors may

both play a part in viral replication and could lead to differences in clinical presentation.

Bovine pestiviruses are able to infected and induce disease in swine and ruminant species other than cattle (Nettleton 1990; Decaro et al. 2012; Tao et al. 2013; Bauermann et al. 2015; Wolff et al. 2016). The present study lacks the evaluation of the viral growth on testicular cells of other animal species that can allow comparison of in vitro observations with in vivo health outcomes. This evaluation can be the topic of future studies.

It is important to note that while the present work reveals differences between individuals in viral growth it does not reveal the reasons/factors behind them. Further study is needed in order to elucidate the mechanisms of adaptation of pestiviruses in different animals to the better understand the host and viral mechanisms involved. However, the model developed in this study, which employs cell strains generated

from the testicular tissue of castrated cattle, would allow comparison of in vitro observations with in vivo health outcomes during beef production.

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Compliance with ethical standards

Conflict of interest The authors whose names are listed below certify that they have no affiliations with or involvement in any organization or entity with any financial interest (such as honoraria, educational grants, participation in speakers' bureaus, membership employment, consultancies, stock ownership, or other equity interest, and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript. We have no conflict of interest.

Statement on the welfare of animals All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Informed consent Informed consent was obtained from all individual participants included in the study.

References

- Ameni G, Aseffa A, Engers H et al (2007) High prevalence and increased severity of pathology of bovine tuberculosis in holsteins compared to zebu breeds under field cattle husbandry in Central Ethiopia. *Clin Vaccine Immunol* 14:1356–1361. doi:10.1128/CVI.00205-07
- Arenhart S, Bauermann FV, Oliveira SAM et al (2009) Excreção e transmissão do vírus da diarréia viral bovina por bezerros persistentemente infectados. *Pesqui Vet Bras* 29:736–742. doi:10.1590/S0100-736X2009000900010
- Bauermann FV, Falkenberg SM, Vander Ley B et al (2014a) Generation of calves persistently infected with HoBi-like pestivirus and comparison of methods for detection of these persistent infections. *J Clin Microbiol* 52:3845–3852. doi:10.1128/JCM.01563-14
- Bauermann FV, Flores EF, Falkenberg SM et al (2014b) Lack of evidence for the presence of emerging HoBi-like viruses in north American fetal bovine serum lots. *J Vet Diagn Investig* 26:10–17. doi:10.1177/1040638713518208
- Bauermann FV, Falkenberg SM, Decaro N et al (2015) Experimental infection of calves, sheep, goats and pigs with HoBi-like viruses by direct inoculation or exposure to persistently infected calves. *Vet Microbiol* 181:289–293. doi:10.1016/j.vetmic.2015.10.011
- Becher P, Orlich M, Shannon AD et al (1997) Phylogenetic analysis of pestiviruses from domestic and wild ruminants. *J Gen Virol* 78:1357–1366. doi:10.1099/0022-1317-78-6-1357
- Burleson FG, Chambers TM, Wiedbrauk DL (1992) Primary cell cultures. In: Burleson FG, Chambers TM, Wiedbrauk DL (eds) *Virology: a laboratory manual*, 1st edn. Academic Press, San Diego, pp. 25–35
- Carman S, Van DT, Ridpath J et al (1998) Severe acute bovine viral diarrhoea in Ontario, 1993–1995. *J Vet Diagn Investig* 10:27–35
- Cusack PMV, McMeniman NP, Lean IJ (2007) Feedlot entry characteristics and climate: their relationship with cattle growth rate, bovine respiratory disease and mortality. *Aust Vet J* 85:311–316. doi:10.1111/j.1751-0813.2007.00184.x
- Decaro N, Lucente MS, Mari V et al (2011) Atypical pestivirus and severe respiratory disease in calves, Europe. *Emerg Infect Dis* 17:1549–1552. doi:10.3201/eid1708.101447
- Decaro N, Mari V, Lucente MS et al (2012) Experimental infection of cattle, sheep and pigs with “Hobi”-like pestivirus. *Vet Microbiol* 155:165–171. doi:10.1016/j.vetmic.2011.08.030
- Frisch JE, Vercoe JE (1984) An analysis of growth of different cattle genotypes reared in different environments. *J Agric Sci* 103:137–153. doi:10.1017/S0021859600043409
- Fulton RW, Purdy CW, Confer AW et al (2000) Bovine viral diarrhoea viral infections in feeder calves with respiratory disease: interactions with *Pasteurella* spp., parainfluenza-3 virus, and bovine respiratory syncytial virus. *Can J Vet Res* 64:151–159
- Givens MD, Riddell KP, Edmondson MA, et al. (2009) Epidemiology of prolonged testicular infections with bovine viral diarrhoea virus. *Vet Microbiol* 139:42–51. doi: 10.1016/j.vetmic.2009.04.029
- Grubb P (2005) Order Artiodactyla. In: Wilson DE, Reeder DM (eds) *Mammal species of the world: a taxonomic and geographic reference*, 3rd edn. The Johns Hopkins University Press, Baltimore, pp. 637–722
- Krametter-Froetscher R, Duenser M, Preyler B et al (2010) Pestivirus infection in sheep and goats in West Austria. *Aust Vet J* 186:342–346. doi:10.1016/j.tvjl.2009.09.006
- Léger E, Vourc'h G, Vial L et al (2013) Changing distributions of ticks: causes and consequences. *Exp Appl Acarol* 59:219–244. doi:10.1007/s10493-012-9615-0
- Liang D, Sainz IF, Ansari IH et al (2003) The envelope glycoprotein E2 is a determinant of cell culture tropism in ruminant pestiviruses. *J Gen Virol* 84:1269–1274. doi:10.1099/vir.0.18557-0
- Liess B, Moennig V (1990) Ruminant pestivirus in pigs. *Rev Sci Tech* 9:151–161
- Lussi C, Schweizer M (2016) What can pestiviral endonucleases teach us about innate immunotolerance? *Cytokine Growth Factor Rev* 29:53–62. doi:10.1016/j.cytogfr.2016.03.003
- Nettleton PF (1990) Pestivirus infections in ruminants other than cattle. *Rev Sci Tech* 9:131–150
- OIE (2008) Bovine viral diarrhoea. In: *Manual of diagnostic tests and vaccines for terrestrial animals*, 6th edn. Office International des Epizooties, Paris, pp. 698–711
- Peterhans E, Schweizer M (2013) BVDV: a pestivirus inducing tolerance of the innate immune response. *Biologicals* 41:39–51. doi:10.1016/j.biologicals.2012.07.006
- Reed L, Muench H (1938) A simple method of estimating fifty per cent endpoints. *Am J Epidemiol* 27:493–497
- Ridpath JF, Bayles DO, Neill JD et al (2015) Comparison of the breadth and complexity of bovine viral diarrhoea (BVDV) populations circulating in 34 persistently infected cattle generated in one outbreak. *Virology* 485:297–304. doi:10.1016/j.virol.2015.07.022
- Roehe PM, Edwards S (1994) Comparison of pestivirus multiplication in cells of different species. *Res Vet Sci* 57:210–214. doi:10.1016/0034-5288(94)90059-0
- Schirmmeier H, Strebelow G, Depner K et al (2004) Genetic and antigenic characterization of an atypical pestivirus isolate, a putative member of a novel pestivirus species. *J Gen Virol* 85:3647–3652. doi:10.1099/vir.0.80238-0

- Simmonds P, Becher P, Collet MS et al (2011) Flaviviridae. In: King AMQ, Adams MJ, Carstens EB, Lefkowitz EJ (eds) Virus taxonomy: ninth report of the international committee on taxonomy of viruses. Academic Press, San Diego, pp. 1003–1020
- Snowder GD, Van Vleck LD, Cundiff LV, Bennett GL (2005) Influence of breed, heterozygosity, and disease incidence on estimates of variance components of respiratory disease in preweaned beef calves. *J Anim Sci* 83:1247–1261
- Tao J, Liao J, Wang Y et al (2013) Bovine viral diarrhea virus (BVDV) infections in pigs. *Vet Microbiol* 165:185–189. doi:[10.1016/j.vetmic.2013.03.010](https://doi.org/10.1016/j.vetmic.2013.03.010)
- Taylor JD, Fulton RW, Lehenbauer TW et al (2010) The epidemiology of bovine respiratory disease: what is the evidence for predisposing factors? *Can Vet J* 51:1095–1102
- Vilcek S, Herring AJ, Herring JA et al (1994) Pestiviruses isolated from pigs, cattle and sheep can be allocated into at least three genogroups using polymerase chain reaction and restriction endonuclease analysis. *Arch Virol* 136:309–323. doi:[10.1007/BF01321060](https://doi.org/10.1007/BF01321060)
- Weber MN, Bauermann FV, Bayles DO et al (2016a) Comparison of “HoBi”-like viral populations among persistent infected calves generated under experimental conditions and to inoculum virus. *Virology* 492:225–231. doi:[10.1016/j.virol.2016.03.001](https://doi.org/10.1016/j.virol.2016.03.001)
- Weber MN, Mósena ACS, Simões SVD et al (2016b) Clinical presentation resembling mucosal disease associated with “HoBi”-like pestivirus in a field outbreak. *Transbound Emerg Dis* 63:92–100. doi:[10.1111/tbed.12223](https://doi.org/10.1111/tbed.12223)
- Weber MN, Bauermann FV, Canal CW et al (2017) Temporal dynamics of “HoBi”-like pestivirus quasispecies in persistently infected calves generated under experimental conditions. *Virus Res* 227:23–33. doi:[10.1016/j.virusres.2016.09.018](https://doi.org/10.1016/j.virusres.2016.09.018)
- Wolff PL, Schroeder C, McAdoo C et al (2016) Evidence of bovine viral diarrhea virus infection in three species of sympatric wild ungulates in Nevada: life history strategies may maintain endemic infections in wild populations. *Front Microbiol* 7:792. doi:[10.3389/fmicb.2016.00292](https://doi.org/10.3389/fmicb.2016.00292)