VIRUS Reviews and Research

XXVII Congresso Brasileiro XI Encontro de Virologia do Mercosul 18 a 21 de setembro de 2016 PIRENÓPOLIS • GO POUSADA DOS PIRENEUS RESORT s b v . o r g . br / c o n g r e s s o











Vol 20 (2), August-December 2016

JOURNAL OF THE BRAZILIAN SOCIETY FOR VIROLOGY



of chicks at one day before infection and at 1, 5 and 11 days postinfection (dpi). The levels of anti IBV IgG were measured in tear and serum samples and the levels of antiIBV IgA were measured only in tear samples, using the sandwichELISAconcanavalin A technique. The chicks vaccinated with live attenuated vaccine followed by vaccination with the nanoparticles vaccine (L+Nano) or oil adjuvant (L+Oil) developed higher levels of anti-IBV IgG antibodies in serum and tear samples, either in pre and postchallenge periods, and there was a marked increase in IgG levels at 11 dpi compared to other groups. The tear antiIBV IgA levels were higher at 5 dpi in chicks vaccinated with nanoparticle vaccine (Nano) compared to L+Oil and NC chicks groups. Additionally, IgA reached the highest levels at 11 dpi in PC, Nano, L+Nano and L+Oil groups when compared to the NC group. In conclusion, the nanoparticle vaccine administered by oculonasal route was capable to induce high levels of mucosal and systemic antibody responses and the nanoparticles of chitosan prove to be a potent mucosal adjuvant for mass use in veterinary vaccines due to their easier administration and non invasiveness.

VV74 - GENOMIC CHARACTERIZATION OF A NOVEL HUMAN INFLUENZA A(H1N2) VARIANT DETECTED IN BRAZIL

RESENDE, P.C.; Born, P.S.; Matos, A.R.; Motta, F.C.; Caetano, B.C.; Debur, M.C.; Riediger, I.; Brown, D.; Siqueira, M.M.

Influenza A(H1N2) virus has been described to infect human, avian and especially swine populations over the years. In contrast to the widespread circulation of seasonal H1N1 and H3N2 influenza A viruses, the H1N2 subtype has been observed sporadically in humans. In this study, we report the detection and characterization of a H1N2 variant (H1N2v) strain with a genomic combination not previously reported in humans. The virus A/Parana/720/2015 (H1N2v) was identified from a nasopharyngeal aspirate collected on November 26th, 2015, from a 16 years old female patient from a rural area from Castro city, Paraná, located in the Southern region of Brazil. Castro has approximately 67,000 inhabitants and a strong agricultural center for dairy cattle, poultry and pigs. The patient did not present any risk factor for influenza and had influenza like illness with an onset of symptoms on November 23rd, 2015. Direct contact with

pigs was not reported in the epidemiological investigation form. She did not receive previous antiinfluenza vaccine, her clinical outcome was uneventful and no antiviral treatment was necessary. Basic Local Alignment Search Tool (BLAST) was performed for each gene segment sequenced and revealed strong identity with an H1N2 genome detected in swine in Brazilian Santa Catarina Southern State, in 2011 (9799%). The human viruses with more identity with this novel H1N2v were a 2003 H1N2 human lineage for HA gene (95%), a 1998 H3N2 human seasonal lineage for NA (93%), and H1N1pdm09 lineage for the other genes (9899%). Phylogenetic reconstructions strengthens the BLAST findings and suggests a recent human introduction of this Brazilian H1N2v strain, from swine, once these similar swine strains were detected around 300 kilometers distance where the human case occurred. Regarding analyses of genetic markers associated to antivirals resistance, this novel virus presented the S31N marker in M2 protein, which confers resistance to adamantane antiviral class, as H1N1pdm09 viruses. To date, no further H1N2 human cases have been detected, however other samples from this region and period are being investigated to verify their occurrence. This finding highlights the importance of influenza surveillance in humans and animals and their interface, especially during influenza season when infectivity is high. Surveillance should be focused on geographical areas where humananimal contact is frequent to ensure early detection of influenza variants.

80 - GENETIC CHARACTERIZATION OF INFLUENZA VIRUSES CIRCULATING WITHIN BRAZILIAN SWINE BETWEEN 2009 AND 2016

Schaefer, R.; Gava, D.; Nelson, M.I; Haach, V.; Ciacci-Zanella, J.R.; Cantão, M.E.

Although Brazil has one of the largest pig populations in the world (~ 41 million pigs), very few and scattered information about influenza A virus (FLUAV) infection in pigs prior 2009 is available. Since 2009, with the introduction of H1N1 pandemic (H1N1/2009) virus in pig farms, influenza virus diversity has increased via reassortment between cocirculating viruses, including H1N1/2009. As a result of the increased influenza surveillance efforts in pigs, we have found that H1N1/2009, humanlike H1N2 and H3N2 FLUAVs are widespread in Brazilian pig herds. From 2009 to



2016 (July), a total of 1952 nasal swabs and 1871 sera collected from nursery and growing pigs, and 165 lung tissue samples collected from suckling, nursery and fattening pigs from 171 pig farms located in the southern, midwest and southeast regions of Brazil were submitted to ELISA, HI assay, RTqPCR, virus isolation and genomic sequencing. Swine from all tested farms had antibodies to FLUAV. Seventyfive percent (75.2%) of sera tested by ELISA were positive for FLUAV antibodies. The HI analysis revealed specific antibodies for H1N1/2009, H1N2 and H3N2/2015 in pig sera from 24 out of 48 of the tested pig farms. Antibodies against two or more influenza virus subtypes were detected in pigs in seven of those 24 farms. Influenza A virus was detected by RTqPCR in 306 (14.45%) of the 2117 tested samples (nasal swabs and lungs). Virus isolation of the influenza positive samples by RTqPCR was performed by the inoculation of lung tissue supernatant or nasal swab samples into MDCK cells or into SPF embryonated chicken eggs and resulted in 162 virus isolates. Complete and partial sequences of 58 FLUAVs were obtained by genetic sequencing and together with RTPCR subtyping results, revealed 23 H1N1/2009, 15 H1N2 and seven H3N2 FLUAVs. The sequence analysis showed that the HA genes of subtypes H3N2 and H1N2 are most closely related to human seasonal H3N2 and H1N2 viruses that circulated in humans in the 1990s and early 2000s, respectively. A novel N1 gene closely related to a human influenza virus that circulated in 2007 was detected in three H1N1 viruses isolated in 2014 and 2015. These findings highlight the importance of humantoswine transmission in the evolution of influenza virus diversity in swine in Brazil and represent a challenge for the design of effective crossprotective vaccines.

VV85 - NEONATAL PIG MORTALITY ASSOCIATED WITH SENECAVIRUS A

Gava, D.; Lorenzett, M.P.; Haach, V. Driemeier, D.; Joshi, L.R.; Mohr, K.A.; Diel, D.G.; Caron, L.; Morés, N.; Morés, M. A. Z.; Schaefer, R.

Senecavirus A (SVA) is an emerging picornavirus that has been associated with outbreaks of vesicular disease in swine. In 2015, neonatal mortality affecting piglets of 07 days of age correlated with SVA, was reported in Brazil. Here, we present an investigation carried on during 20152016 in five farrowtofinish swine operations in Southern Brazil showing an increased neonatal mortality and also vesicular disease that have been associated to SVA infection. Piglets were lethargic and had a watery diarrhea. The mortality rate increased in 23% and in some littermates a 100% of mortality was observed. Despite of a relatively fast onset of wasting syndrome progressing to mortality, all herds recovered to baseline mortality levels within 410 days. Piglets were necropsied and tissue samples were collected for histopathology, RTPCR for SVA detection targeting the VP1 VP3 region, and for viral isolation in H1299 cell culture. Genome sequences of VP1 gene of five SVA isolates were compared to other SVA sequences available on GenBank. Necropsy of six piglets revealed empty stomach and mesocolonic edema. In general, it was observed enlargement and edema of inguinal lymph nodes, pulmonary edema, ascites and ulcerative lesions on the snout and coronary band. Microscopic lesions were characterized by necrotic epidermitis and dermatitis of coronary band, mild enteritis with villus degeneration on small intestine, marked mesocolon edema and multifocal hemorrhage with lung edema. Senecavirus A was detected by RTPCR in tonsil, lung, liver, intestine and coronary band. SVA was isolated in cell culture from tonsil, lung, intestine and coronary band from piglets of all farms. Sequence comparisons based on a region of the VP1 gene (541 base pairs) revealed that the Brazilian isolates characterized here share 96-99% of nucleotide (nt) identity with contemporary Brazilian isolates, 9598% nt identity with US and 90-93% nt identity with the prototype strain SVV001. SVA was associated with neonatal mortality based on RT-PCR, virus isolation and sequencing results. The genetic analysis shows the diversity of the Brazilian SVA isolates and that more studies are needed to demonstrate if there are differences between SVA from neonatal mortality and vesicular cases. SVA is clinically and economically important due to its resemblance with vesicular diseases, so the diagnosis tools are critical to confirm the initial investigation.