Original Article

Fostera™ PRRS modified live vaccine efficacy against a Canadian heterologous virulent field strain of porcine reproductive and respiratory syndrome virus (PRRSV)

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Abstract

Vaccination is a useful option to control PRRSV infection and several PRRSV modified live vaccines have been developed. These vaccines have shown some efficacy in reducing clinical disease, as well as the duration of viremia and virus shedding but have failed to provide sterilizing immunity. Efficacy of modified live virus vaccines is greater against a homologous strain compared to heterologous PRRSV strains. The objective of this study was to evaluate the efficacy of Fostera™ PRRS modified live vaccine to protect against challenge with a heterologous field strain widely circulating in Eastern Canada swine herds. Forty-six piglets were divided into four groups: non-vaccinated/non-challenged, non-vaccinated/challenged, vaccinated/challenged and vaccinated/non-challenged. Animals were vaccinated at 23 days of age with Fostera™ PRRS and they were challenged with a heterologous field strain (FMV12-1425619) 23 days following vaccination. Overall, the vaccine has shown some beneficial effects in PRRS challenged animals by reducing clinical signs and viral load. A significant difference between non-vaccinated and vaccinated animals was detected for some parameters starting at 11-13 days post-challenge suggesting that cell-mediated immune response or other delayed responses could play more important role than the pre-existing PRRSV antibodies in vaccinated animals within the context of heterologous vaccine protection.

Introduction

Porcine reproductive and respiratory syndrome (PRRS) represents one of the most economically important viral disease of swine industry in North America, causing losses estimated at US $664 million annually (1). In sows, PRRS virus (PRRSV) is responsible for reproductive failure, characterized by late-term abortions, increased numbers of stillborn fetuses, and/or premature and weak pigs. PRRSV is also responsible for increased morbidity and mortality in growing and finishing pigs as a result of severe respiratory disease and poor growth performance (2, 3). Its etiological agent, PRRSV, is an enveloped, single-strand, positive-sense RNA virus belonging to the *Arteriviridae* viral family, which includes lactate dehydrogenase-elevating virus (LDV) of mice, simian hemorrhagic fever virus (SHFV) and equine arteritis virus (EAV) (4). The PRRSV ~15 kb RNA genome is composed of at least 10 ORFs coding for at least 7 structural proteins and 14 non-structural proteins (5). Like many RNA viruses, PRRSV genome heterogeneity represents the main hurdle to effective prevention and control of the disease through vaccination (6). PRRSV strains have been classified in two main genotypes: genotype I (previously named European) and genotype II (previously named North American) (7). Genotype II strains circulating in North America can be classified in several subgenotypes (7-10). Interestingly, several subgenotype II strains circulating in the United States (USA) have not been yet reported in Canada (8-10), suggesting that some subgenotypes are geographically restricted. The two main genotypes possess between 50-60% viral genomic nucleotides homology to one another and are normally not cross-neutralized by antibodies raised against each other even though some level of cross reactivity has been previously reported (11, 12). Moreover, it has also been shown

that genetic and antigenic diversity exists within each genotype and negatively affects the efficient cross protection among different viruses (13-15).

Vaccination is an important tool used to control PRRSV infection. Up to now, many PRRSV vaccines have been developed, including products that contain live virus derived from cell culture attenuation of virulent field isolates, inactivated preparations of attenuated PRRSV strains, inactivated preparations of virulent isolates expanded by *in vitro* cell culture for use as an autogenous vaccine, inactivated preparation of multiple virulent isolates enriched with viral antigens, and subunit vaccines expressing selected viral proteins (16). Modified live (or attenuated) vaccines have been widely used and have shown some efficacy in reducing clinical disease and severity, as well as viremia duration and virus shedding but have failed to provide complete sterilizing immunity (6). However, the efficacy of modified live vaccines is greater for homologous strains and can decline dramatically when facing genetically unrelated heterologous PRRSV strains. The use of the new PRRS live attenuated vaccine Fostera™ PRRS has been approved in USA and Canada. This vaccine has just been shown to reduce the levels of viremia and nasal shedding, and severity of PRRSV-induced lesions following experimental infection with a Korean heterologous strain (17). Unfortunately, most of the wild-type strains circulating in Canada are not from the same lineage in which the Fostera™ PRRS vaccinal strain is reported to be. Therefore, the objective of this study was to evaluate the efficacy of modified live Fostera™ PRRS vaccine to protect against challenge with a heterologous virulent field strain widely circulating in Eastern Canada swine herds.

Materials and Methods

Animals

Animal care procedures followed the guidelines of the Canadian Council on Animal Care and the protocol was approved by the Institutional Animal Care Committee (Protocol 12-Rech-1669). Forty-six conventional piglets of 16 days old were used in this study; they were obtained from a single farm with a common genetic and health background. The farm was negative for PRRSV, *Mycoplasma hyopneumoniae*, swine influenza virus with no previous report associated with post-weaning multisystemic wasting syndrome disease related to porcine circovirus type 2 infection. Animals were randomly divided into four homogeneous groups, non-vaccinated/non-challenged (n=7), non-vaccinated/challenged (n=15), vaccinated/challenged (n=15) and vaccinated/non-challenged (n=9) and housed in separate rooms with ad libitum access to feed and water.

Vaccination

After a 1 week acclimation period, 23-day old animals were vaccinated intramuscularly (im) with Fostera^{TM} PRRS vaccine (lot #A282040A) as recommended by the manufacturer (Zoetis Canada, Kirkland, QC, Canada). A placebo (PBS solution) was given im to non-vaccinated animals. Animals were weighed the day before vaccination to assure that the experimental groups were homogenous. No significant difference in body weight was found between experimental groups with a one-way ANOVA model using the parametric Tukey test (P>0.05) (data not shown).

PRRSV challenge strain and experimental infection

Sera from pig farms experiencing an acute outbreak of PRRSV were collected and ORF5 gene of PRRSV was subsequently sequenced to select a virulent heterologous PRRSV strain. Based on ORF5 phylogenic analyses, the selected PRRSV strain (FMV12-1425619, Genbank accession number KJ888950) was classified within a cluster of lineage 1 of type II genotype frequently found in Quebec over the past 2 years and often associated with clinical signs (unpublished data). PRRSV ORF5 amino acid identities between the selected field and vaccine strains were analyzed using SIM alignment tool for protein sequences on bioinformatics resource portal ExPASY (http://web.expasy.org/sim/). Several attempts to isolate the virus in MARC-145, SJPL and PAM cells have failed. Thus, the viral inoculum used to challenge animals was a lung tissue homogenate obtained from a piglet infected with 3 mL of PRRSV FMV12-1425619 positive serum. PRRSV concentration in the filtrated lung tissue homogenate was determined to be 1.5 X 10^4 $TCID_{50}$/mL using a previously described RT-qPCR method (18). A pilot study with four piglets confirmed the capacity of lung tissue homogenate challenge strain to induce PRRSV-specific clinical signs, viremia, and lung lesions in infected animals (data not shown). It was determined by PCR that the homogenate used for challenge was negative for bacteria (with a 16S gene PCR diagnostic assay), swine influenza virus, porcine parvovirus and porcine circovirus. The challenge was done at 23 days post-vaccination. Non-vaccinated/challenged (n=15) and vaccinated/challenged (n=15) groups were inoculated with 1 mL of tissue homogenate of 1.5 x 10^4 $TCID_{50}$ PRRSV im and 1 mL of the same inoculum in each nostril. Non-vaccinated/non-challenged (n=7) and vaccinated/non-challenged (n=9) groups were mock-inoculated with PBS.

Clinical signs and blood sampling

During challenge period (i.e. 28 days), body temperature and body weight were daily monitored. Average daily gain (ADG) was calculated over different periods of time: 1) from time of vaccination to day of challenge (post-vaccination period); 2) during the first 13 days post-challenge (day 0 to day 13 pc); 3) during the 27 days pc (post-challenge period); and 4) during the entire period of the experiment (post-vaccination and post-challenge periods). Average daily gain was calculated by subtracting the initial body weight from the final body weight and divided by the number of days for different periods. Also, a growth rate was calculated to take into account the initial weight of animals on challenge day since the weight of the experimental groups were not uniform on that day. It was calculated by dividing the weight gain over the period and dividing it by the initial weight at the beginning of the period. Fever was defined as body temperature higher than 40˚C for two consecutive days. Clinical signs were scored daily using the scoring index presented in Table 1. Blood samples were collected on -3, 3, 7, 10, 13-14, 21, 27-28 days pc to determine viremia by RT-qPCR. At 14 days pc (i.e. 37 days post-vaccination), 3, 7, 9, and 4 pigs were sacrificed in non-vaccinated/non-challenged, vaccinated/challenged, non-vaccinated/challenged and vaccinated/non-challenged experimental groups, respectively. The remaining animals were sacrificed at 28 days pc (i.e. 51 days post-vaccination).

Macroscopic and microscopic lung lesions

Macroscopic lung lesion were scored as previously described (19). Apex of cranial lung lobes, intermediate dorsal sections of both right and left diaphragmatic lung lobes and

tracheobronchial lymph nodes were collected for each animal and were fixed in 10% neutral buffered formalin for evaluation of specific microscopic lesions. Subsamples of those lung sections and tracheobronchial lymph nodes were also collected and stored at -20˚C until tested by RT-qPCR to determine viral load. Histopathological lesions were scored for their interstitial pneumonia severity as follows: 0 = normal, 1 = mild, 2 = moderate, 3 = severe, and 4 = severe with alveolar disappearance. Presence of leucocytes, serum, or necrotic debris in alveolar exsudate were also scored as follows: 0 = normal, 0.5 = rare, 1 = mild, 2 = moderate, 3 = important, and 4 = severe. Finally, lymphoid follicular hyperplasia was scored as follows: 0 = normal, 1 = mild, 2 = moderate, and 3 = severe.

PRRSV quantification by RT-qPCR

PRRSV viremia and viral load in tissues were determined using RT-qPCR assay as previously described (18) . Briefly, QIAamp Viral RNA kit (Qiagen, Mississauga, ON, Canada) was used to isolate viral RNA from serum samples and lung homogenates as described in the manufacturer's instructions. A commercial PRRSV RT-qPCR diagnostic kit (NextGen, Tetracore Inc., Gaithersburg, MD, USA) was used for PRRSV quantification as recommended by the manufacturer. The quantification of PRRSV was determined by comparing the sample results with a standard curve based on the amount of serially diluted PRRSV IAF-Klop strain which was produced in MARC-145 cells and subsequently titrated as $TCID_{50}$/mL of viral particles in the MARC-145-infected cell culture supernatant (18). The PRRSV RT-qPCR results were expressed in $TCID_{50}$/mL of serum or g of tissue.

179 ***PRRSV specific antibodies***

180 Sera were tested using Herdchek PRRS X3 diagnostic ELISA kits (IDEXX Laboratories,
181 Portland, ME, USA). Sera were diluted 1/40 in diluents supplied by the manufacturer and
182 the assays were performed following the manufacturer's instructions. A sample-to-
183 positive (S:P) ratio equal to or greater than 0.4 was considered positive.

184 ***Statistical analyses***

185 All statistical analyses were performed using GraphPad Prism software (version 5.03,
186 GraphPad Prism software Inc., San Diego, CA). Parametric data (growth rate, rectal
187 temperature, clinical sign score and viral titer) were analysed by a two-way ANOVA for
188 repeated measures with Bonferroni multiple comparison test. ADG was analysed by a
189 one-way ANOVA with Tukey multiple comparison test. Non parametric data (antibody
190 S:P ratios, lungs lesions scores) were analysed by using one-way ANOVA with the
191 Kruskal-Wallis test. In some instances, vaccinated/challenged versus non-
192 vaccinated/challenged animals were compared by applying Student′s unpaired ′t′ test. $P <$
193 0.05 was considered to reflect a statistically significant differences.

194

195

Results

Animal exclusion

One animal in the vaccinated/challenged group died at day 8 post-vaccination. No macroscopic or histopathological findings other than changes compatible with post mortem modifications were observed at necropsy. Its lungs were PRRSV PCR positive. Another animal in the vaccinated/challenged group was sacrificed at day 8 pc due to excessive weight loss (> 10% of total weight on a 2-day period). This animal had shown whitish nasal discharges prior to challenge. Consequently, nasal swabs were collected from several animals in each experimental group and tested for the presence of respiratory pathogens (such as influenza A virus, porcine circovirus type 2, PRRSV, *Mycoplasma hyorhinis*, *Mycoplasma hyopneumoniae* and *Streptococcus suis*). Analyses showed that the sacrificed animal was PCR positive for PRRSV, as expected and for *Mycoplasma hyorhinis* and *Streptococcus suis*. However, these two pathogens were also detected in animals of all other experimental groups (data not shown). Macroscopically, interstitial pneumonia covering several regions of the lung tissue was observed as well as a large emphysema lesion on the right diaphragmatic lobe. This type of lesion has no direct cause-effect relationship with PRRSV infection and it is also a rare finding in swine. Overall, the macroscopic lesions were estimated to affect 48% of the lung tissue, but after removing the emphysema lesion, the lung lesion score of this pig was established to be 36%. Microscopic lung lesions were related to PRRSV infection, such as interstitial pneumonia and BALT hyperplasia. Nonetheless, as stated previously, data from this pig were removed from all analyses mainly because of the unexpected and marked emphysema lesion. At 20 days pc, one pig in the vaccinated/non-challenged

group died during blood collection. Although a small hemorrhage was observed at the blood collection site, no other macroscopic lesions were found at necropsy. No histopathological findings related to PRRSV infection were found. Interestingly, several multifocal hemorrhages were found in the lung tissue but no direct link could be done with the sudden death of the animal associated with blood collection procedure. Finally, one animal in the non-vaccinated/non-challenged group died suddenly at day 9 pc. On arrival, this animal was cachectic and had locomotor problems, but still was kept in the experiment even though its weight was significantly lower than that of other animals from the same group. No macroscopic lung lesions and no histopathological findings related to PRRSV infection were found. All PRRSV PCR assay results were negative for this animal. For all the excluded animals, no histopathological findings were found within the examined tissues other than lungs, such as spleen, kidney and liver. All the data related to animals described within this section were entirely removed.

Vaccine and challenge strain identities

ORF5 genomic analyses demonstrated that the amino acid homology between the Fostera^{TM}PRRS vaccine strain and the challenge strain was 86.4% (Figure 1).

Antibody responses

At day 1 post-vaccination, all animals were serologically negative against PRRSV (data not shown). Two animals, one in each of the vaccinated groups were negative for specific PRRSV antibodies at 20 days post-vaccination. The antibody response against PRRSV at 20 days post-vaccination in both vaccinated groups was similar ($P>0.05$) but was significantly higher when compared to non-vaccinated groups ($P<0.01$) (Figure 2A),

indicating that control animals were naive in regards to PRRSV infection. This result indicates that a PRRSV specific immune response was initiated following vaccination. All non-vaccinated animals challenged with the virulent field strain had developed a PRRSV specific antibody response by day 13 pc (Figure 2B), but this response was significantly lower than that developed by vaccinated/challenged animals at the same day.

Growth performance and clinical signs

Average daily gain (ADG) was calculated over different periods of time (Figure 3). On day 20 post-vaccination, only the ADG of the vaccinated/challenged experimental group was significantly different ($P<0.05$) from the non-vaccinated/challenged group (Figure 3A). These two experimental groups were not significantly different from non-vaccinated/non-challenged and vaccinated/non-challenged animals. No differences were identified between vaccinated/challenged and non-vaccinated/challenged experimental groups for any of the three other periods ($P>0.05$), suggesting that the ADG of vaccinated/challenged has improved over time compared to the earlier part of the experiment (i.e. at 20 days post-vaccination). The results on growth rate (Figure 3B) also supported that and suggested that the growth of vaccinated/challenged animals improved over time. This latter was significantly higher compared to non-vaccinated/challenged animals from 24 days pc until the end of the experiment ($P<0.05$). The ADG of both challenged groups, vaccinated and non-vaccinated, were significantly lower compared to both non-challenged groups, vaccinated and non-vaccinated, ($P<0.05$) (Figure 3A). In addition, vaccination alone did not significantly affect the growth rate, since the ADG of

263 vaccinated/non-challenged group was not significantly different from the non-
264 vaccinated/non-challenged group (Figure 3A).

265 Earlier in the post-challenge period, challenged animals, both vaccinated and non-
266 vaccinated, had higher body temperature compared to non-challenged animals, but from
267 11 to 18 days pc, the difference was no more significant for the vaccinated/challenged
268 animals, suggesting a protective effect of the vaccine. Later on, body temperature of non-
269 vaccinated/challenged animals was similar to other experimental groups. In regards to
270 clinical signs during post-challenge period, significant higher clinical sign scores were
271 identified at days 3, 11 and 15 pc for non-vaccinated/challenged animals compared to
272 vaccinated/challenged animals($P<0.05$) (Figure 3D). Overall, the clinical sign scores
273 tended to by higher for non-vaccinated/challenged compared to vaccinated/challenged
274 animals between 11 to 22 days pc.

275 ***Virological parameters***

276 Several PRRSV RT-PCR diagnostic assays (NextGen, Tetracore Inc., Gaithersburg, MD,
277 USA) were conducted to establish the impact of vaccination on the level and duration of
278 PRRSV viremia and on the persistence of PRRSV in tissues, such as lungs and
279 tracheobronchial lymph nodes (Figure 4). The duration of viremia in regards to the
280 vaccine strain was established using data from animals that were vaccinated but not
281 challenged. At 36 days post-vaccination, only one of eight animals was still viremic,
282 albeit at a very low PRRSV titer (Figure 4B). After this time point, PRRSV vaccine strain
283 could not be detected in vaccinated animals, indicating that these animals were no longer
284 viremic in regards to the vaccine strain by day 44 post-vaccination (Figures 4C and 4D).

285 At day 13 pc, PRRSV viremia of vaccinated/challenged animals was significantly lower
286 (P<0.001) compared to non-vaccinated/challenged animals (Figure 4B). At day 21 pc,
287 PRRSV viremia of non-vaccinated/challenged animals was significantly higher (P<0.05)
288 compared to both non-challenged groups (Figure 4C). However, no significant
289 differences were observed between vaccinated/challenged and non-vaccinated/challenged
290 groups (Figure 4C). At 27 days pc, no significant difference was observed between all
291 experimental groups (Figure 4D). Nonetheless, several animal from both challenged
292 groups (3 out of 6 vaccinated and 4 out of 6 non-vaccinated animals) were still viremic at
293 27 days pc but with very low titers.

294 At necropsy, dorsal middle sections of both left and right diaphragmatic lung lobes and
295 tracheobronchial lymph nodes were collected from all animals for PRRSV RT-qPCR
296 quantification. Two vaccinated/non-challenged animals were positive for PRRSV in lung
297 tissues at 14 days pc (Figure 5A) but no animals were positive at 27-28 days pc (Figure
298 5B), indicating that the vaccine strain was no longer persisting in lungs after 50 days
299 post-vaccination. However, the vaccine strain was still persisting in lymph nodes of
300 vaccinated animals at 50 days post-vaccination (Figure 5D). Interestingly, lung viral load
301 was significantly higher in non-vaccinated/challenged animals compared to
302 vaccinated/challenged animals at 14 and 28 days pc, respectively (P<0.05) (Figures 5A
303 and 5B), suggesting that vaccination has an impact on the lung viral load. In regards to
304 tracheobronchial lymph nodes, the viral load was similar between non-
305 vaccinated/challenged animals and vaccinated/challenged animals at 14 and 28 days pc,
306 respectively (P>0.05) (Figures 5C and 5D).

307 ***Viral shedding***

Nasal swabs were collected at 7, 13, 21 and 27 days pc. Viruses were detected at very low titers at 7 days pc in vaccinated, non-challenged animals (4 animals out of 5 were positive) (Figure 6A). At that time, the viral load in nasal swabs was higher in non-vaccinated/challenged animals compared to non-challenged animals ($P<0.05$) but was not statistically different from vaccinated/challenged animals (Figure 6A). At later time points, 13, 21 and 27 days pc, respectively, all nasal swabs of vaccinated, non-challenged animals were PRRSV negative (Figures 5B, 5C and data not shown). At 13 days pc, viral load in nasal swabs was lower in vaccinated/challenged animals compared to non-vaccinated/challenged animals but differences between both experimental groups were not found to be statistically significant ($P=0.11$) (Figure 6B). Still, nasal viral load was higher in non-vaccinated/challenged animals compared to non-challenged animals ($P<0.05$). At day 27 pc, all tested nasal swabs were negative (data not shown).

Macroscopic and microscopic lung lesions

No significant differences in regards to macroscopic lungs lesions scores between vaccinated/challenged and non-vaccinated/challenged animals were observed ($P>0.05$) (Figure 7A and B. Nonetheless, the extent of lung lesions tended to be higher in non-vaccinated group at day 14 pc (Figure 7A) (P=0.071, after arcsin√ (% macroscopic lung lesions) transformation. Interestingly, 50% of the vaccinated/challenged animals had no or less than 1% of macroscopic lung lesions. Furthermore, this experimental group was not different from non-vaccinated/non-challenged animals ($P>0.05$) (Figure 7A). The proportion of animals having macroscopic lung lesions in non-vaccinated/challenged animals was high, with 67% of animals having a 10% score or more on macroscopic lung lesions (Figure 7A). In addition, this latter group had significantly higher macroscopic

lung lesions scores compared to non-vaccinated/non-challenged and vaccinated/non-challenged animals at 14 days pc (P<0.05) (Figure 7A). At 28 days pc, very few macroscopic lungs lesions were observed at necropsy in all challenged animals, indicating that even non-vaccinated animal lungs were healing from PRRSV FMV12-1425619 strain infection and thus, no statistical difference between experimental groups was observed (Figure 7B). Overall, histopathological findings are in accordance with macroscopic lung lesions evaluation. Histopathological lung lesions were more extensive at 14 days pc compared to 28 days pc. Microscopically, PRRSV-specific lung lesions were characterized by septal thickening and presence of alveolar necrotic debris, macrophages and other mononuclear cells. Similarly to macroscopic lung lesions results, no significant differences were found when vaccinated/challenged animals were compared to non-vaccinated/challenged animals at 14 and 28 days pc (P=0.91 and 0.25, respectively) (Figures 7C and 7D). Noteworthy, at 14 days pc, all challenged animals had significant higher microscopic lung lesions compared to non-vaccinated/non-challenged and vaccinated/non-challenged groups with P values = 0.02 and 0.01, respectively (Figure 7C).

Discussion

The efficacy of PRRSV modified live vaccines depends greatly on the degree of genetic similarity between the vaccine and challenge strains, but the degree of ORF5 homology between infecting and vaccine strain is not always a good predictor of the immune response (20, 21). However, it is well accepted that immunity against genetically related strains is almost completely sterilizing while the immunity against genetically divergent strains will be more variable (22). Here, PRRSV ORF5 genomic analyses revealed that the amino acid homology between the vaccine and the challenge strain was 86.4%. This level of identity clearly illustrates that the two PRRSV strains were heterologous and this divergence may affect the vaccine cross-protection efficacy. In a previous report, the Fostera™ PRRS vaccine showed some efficacy to reduce the level of viremia and severity of PRRSV-induced lesions following challenge with a Korean heterologous strain, that shared, according to our evaluation, 88.4% amino acid (aa) identity, on ORF5 sequence, with the vaccine strain (17). The level of heterogeneity of the present study is similar; however, results from the latter study may not be predictive of the effectiveness of the vaccine since aa homology between the Korean and the Canadian strains is only 91.5 %. To evaluate the efficacy of the vaccine in a Canadian context, piglets were vaccinated with Fostera™PRRS and subsequently infected with a PRRSV heterologous strain that is widely circulating in Eastern Canada swine herds.

PRRSV can cause many clinical manifestations including anorexia, fever, lethargy and severe pneumonia often complicated by concurrent bacterial infections, as well as reduction of weight gain (23, 24). Here, vaccination with attenuated PRRSV strain resulted in a decrease of average daily weight gain over a three week period following

vaccination of approximately 14%. This loss is of the same magnitude as what has been shown previously for other MLV vaccines (21, 25). It should be noted that weight gain reduction decreased from 14 to 8% at day 50, suggesting compensation in weight gain in vaccinated animals. The weight gain reduction caused by the field strain used for experimental infection was much more severe, causing weight gain reduction of about 44%. Vaccination did not have a significant positive impact on weight gain when considering the entire length of the experiment. This could be explained by the short interval between the vaccination and the challenge. Previous reports argued that the maximum heterologous protection could be reached at least 5 weeks after vaccination (21, 26). However, Fostera™ PRRS vaccination increased the growth rate during the post-challenge period by 20%, suggesting a positive impact of the vaccine on growth rate following challenge. When looking at growth rate, vaccinated pigs clearly had an advantage starting at day 24 pc, suggesting a delayed-type immune response. The same conclusions can be drawn when looking at temperature and clinical signs. Indeed, the body temperature declined more rapidly in vaccinated animals starting at day 11 pc. The high body temperature lasted about one week longer in non-vaccinated animals.

In growing pigs, the intensity of clinical signs is usually well correlated with viremia in PRRSV genotype II strain-infected animals (27, 28). Thus, a protective effect can be inferred from the level of viremia (29). Viremia between vaccinated and non-vaccinated animals did not differ significantly except at day 13 pc, when viremia was significantly lower in vaccinated animals. Similar results have been obtained previously for heterologous protection against virulent strains of genotype II (30). This suggests that virus elimination started earlier in vaccinated animals. At day 21 pc, the majority (4/5) of

vaccinated animals had almost completely resolved the infection compared to non-vaccinated group (1/6). The same conclusions can be deduced from the lung viral load, which was significantly lower in the lungs of vaccinated animals at day 14 pc. These results support a previous report concluding that modified live vaccines could be used to reduce viral shedding in the environment (31). No PRRSV vaccine strains were detected in sera, lungs and nasal swabs starting at 44, 50-51 and 36 days post-vaccination, respectively, suggesting that the risk of vaccine virus shedding will be low from day 50-51 post-vaccination in vaccinated animals. Noteworthy, tracheobronchial lymph nodes were still PRRSV positive on the last experimental day for all groups except the non-vaccinated/non-challenged animals, indicating that PRRSV persists for a longer period of time in lymph nodes compared to other samples such as sera and lungs, as previously reported (32, 33). The Fostera™PRRS vaccine strain is not an exception since, at the end of the experiment (50-51 days post-vaccination), PRRSV could still be detected in lymph nodes in all vaccinated/non-challenged animals but not in sera, lungs and nasal swabs. However, the vaccine strain viral load within lymph nodes was significantly lower in vaccinated/non-challenged animals compared to challenged animals, indicating that the vaccine strain virulence was lower compared to the PRRSV FMV12-1425619 strain.

PRRSV is responsible for specific lung lesions that vary from no apparent lesions to severe tan consolidation that are frequently aggravated by lesions resulting from concurrent bacterial infections (24). At day 14 pc, the extent of lung lesions tended to be less important in the lungs of vaccinated animals, evoking again a partial protecting effect.

Fostera™PRRS vaccine did not confer a complete protection against disease induced by heterologous PRRSV Canadian strain tested in this study, but overall, the vaccine has shown some beneficial effects by reducing clinical signs, body temperature, viremia and pulmonary viral load. A significant difference between non-vaccinated and vaccinated animals was detected for some parameters from 11-13 days pc, suggesting that cell-mediated immune response or other delayed responses could play important role than the pre-existing PRRSV antibodies in vaccinated animals in a context of heterologous vaccine protection. However, it has been shown that neutralizing antibody response appears only 28 days after the onset of infection (34). Since the challenge was performed at day 21 post-vaccination, we cannot exclude that neutralizing antibodies could play a role in the positive impact of the vaccine. A previous report has attributed heterologous cross-protection to cell mediated immunity (35).

Acknowledgments

The authors wish to thank Guy Beauchamp for his advice on statistical analyses . This project was financially supported by Zoetis Canada Inc. CS and CP were recipients of Canadian Swine Health Board (CSHB) postdoctoral fellowships. CS was a recipient of a Fonds de recherche du Québec - Nature et technologies (FRQNT) postdoctoral fellowship. FA was a recipient of a graduate scholarship from Fédération des producteurs de porcs du Québec (FPPQ). CAG was financially supported by the Natural Sciences and Engineering Research Council of Canada (NSERC).

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562 cluster) field strain: Clinical protection and cell-mediated immunity. Vaccine2009
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564
565

566 **Figure legend**

567

568 Figure 1. PRRSV ORF5 genomic analyses of amino acid sequences.

569 PRRSV ORF5 amino acid sequence identities between field strain (FMV12-1425619)
570 and vaccine strain (Fostera^{TM} PRRS) was analyzed using SIM alignment tool for protein
571 sequences on bioinformatics resource portal ExPASy.

572 Figure 2. PRRSV-specific antibody response.

573 Blood samples were collected at -3 (A) and 13 days post-challenge (23 and 36 days post
574 vaccination) (B). Sera were tested for presence of specific PRRSV antibodies with a
575 commercial ELISA kit (IDEXX HerdChek-PRRS). Data are expressed in ratio of sample
576 to positive (s:p). Ratios above 0.4 are considered positive. Different superscripts indicate
577 significant difference between groups ($P < 0.05$). The dash bar represents the negative-
578 positive cut-off s/p ratio value.

579 Figure 3. Growth rates and time course of rectal body temperature and clinical signs
580 during infection.

581 (A) Average daily weight gain was calculated for each group by dividing the total weight
582 gain for a period of time by the number of days included in this period of time: from
583 vaccination to challenge, at day 13 post-challenge, at day 27 post-challenge and from
584 vaccination to day 27 post-challenge. (B) A growth rate was obtained by dividing the
585 weight gain by the initial weight before the challenge for each day post-challenge. Rectal
586 temperature (C) and clinical sign scores (D) for each day following challenge. Different

587 superscripts indicate significant difference between groups (P<0.05). * Indicates
588 difference between vaccinated/challenged and non-vaccinated/challenged experimental
589 groups (P<0.05).

590 Figure 4. PRRSV viremia in vaccinated and challenged animals.

591 Blood was collected at day 10 (A), 13 (B), 21(C) and 27 post-challenged (pc) (D) and
592 serum tested for presence of PRRSV RNA by real-time qPCR. Different superscripts
593 indicate significant difference between groups (P< 0.05).

594 Figure 5. Lungs and tracheobronchial lymph nodes viral load.

595 Lung viral load at day 14 post-challenge (pc) (A) and day 28 pc (B), tracheobronchial
596 lymph nodes (L.N.) viral load at day 14 pc (C) and day 28 pc (D). Homogenized samples
597 were tested for presence of PRRSV RNA by real-time qPCR. Different superscripts
598 indicate significant difference between groups (P< 0.05).

599 Figure 6. Nasal viral shedding.

600 Nasal swab were collected at day 7 (A), 13 (B) and day 21 (C) post-challenge. Samples
601 were tested for presence of PRRSV RNA by real-time qPCR. Different superscripts
602 indicate significant difference between groups (P< 0.05).

603 Figure 7. Macroscopic and microscopic lung lesion scores

604 Macroscopic lung lesions were evaluated at days 14 (A) and 27 (B) post-challenge (pc).
605 Microscopic lung lesions were evaluated at days 14 (C) and 27 (D) pc. Different
606 superscripts indicate significant difference between groups (P< 0.05).

Table 1. Clinical signs scoring system.

	Score
Sneezing	1
Non-productive cough	
light	1
moderate	2
severe	3
Productive cough	
light	2
moderate	3
severe	4

	Score
Behavior	
Normal	0
Lethargic	2
Need a stimulus to take a normal position after recumbency	3
Prolonged recumbency	Euthanasia

Total score of 6 = euthanasia

Figure 1

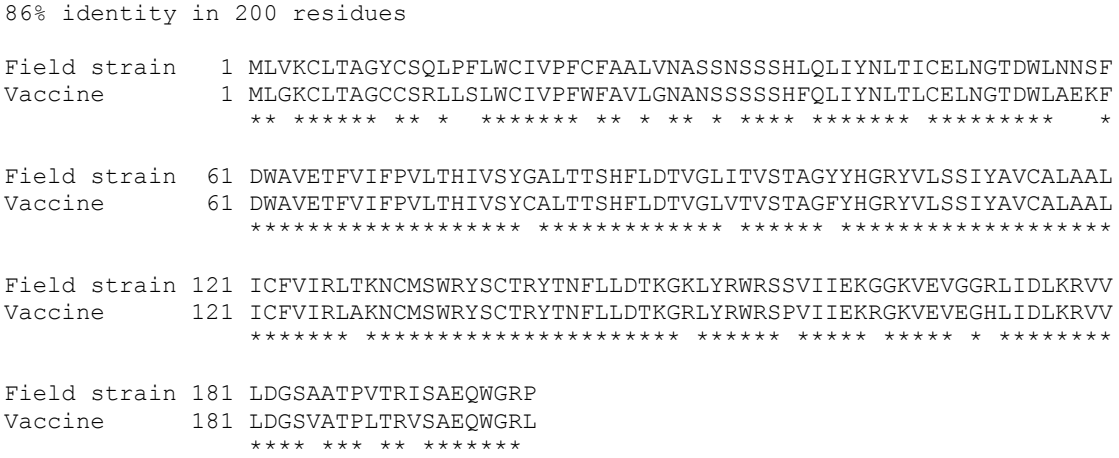


Figure 2

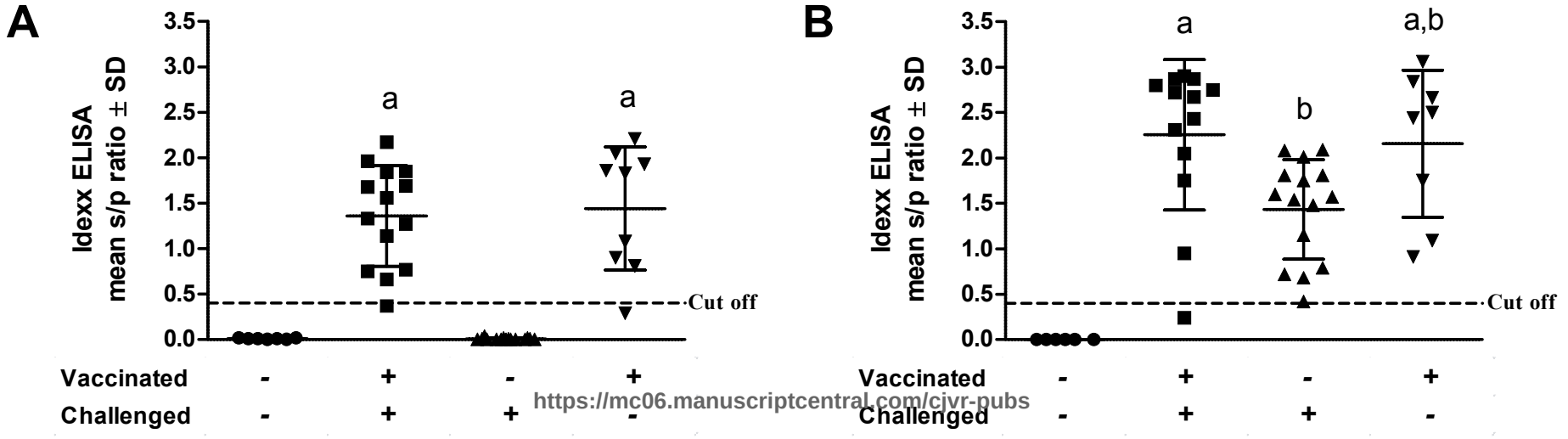


Figure 3

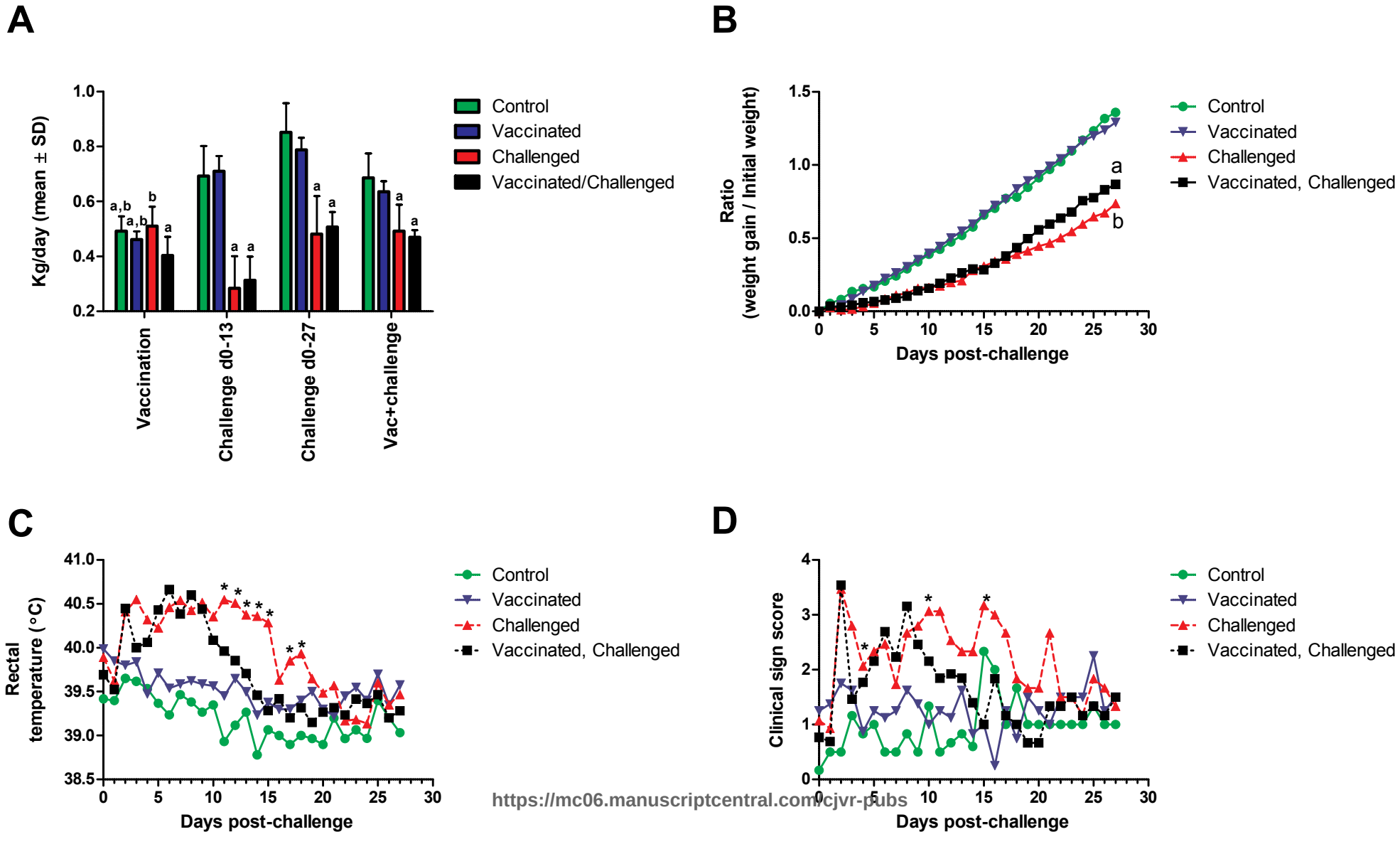


Figure 4

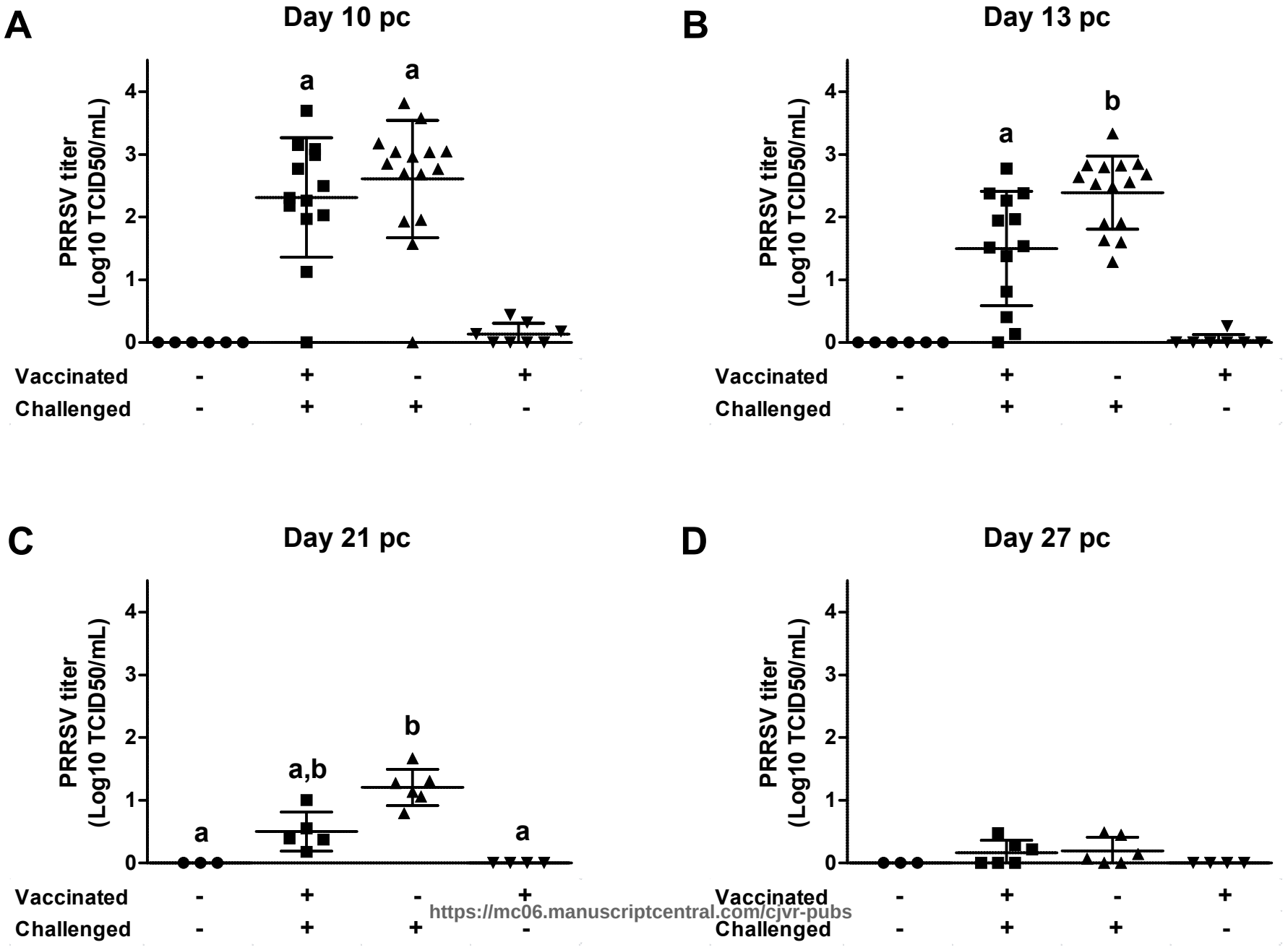


Figure 5

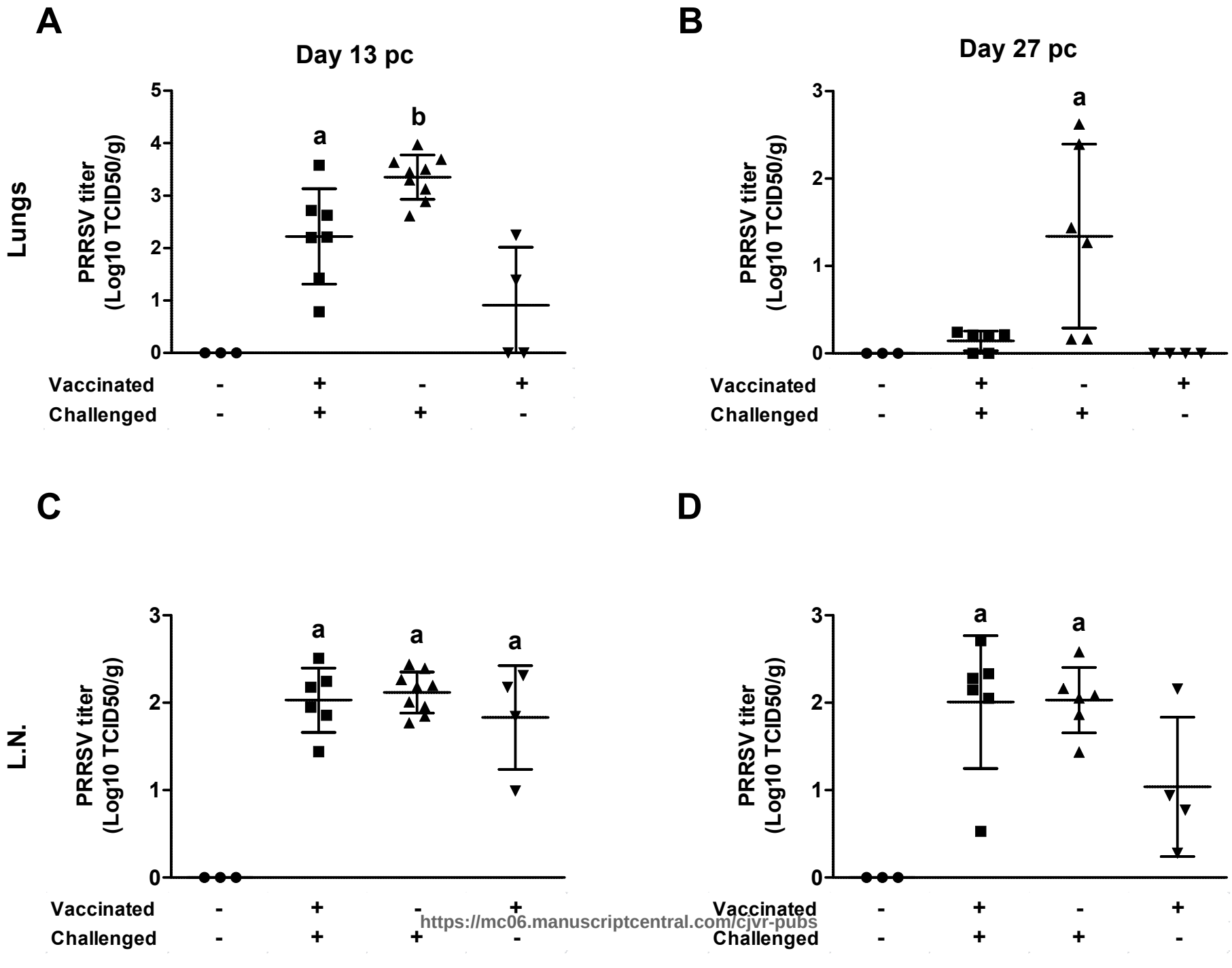


Figure 6

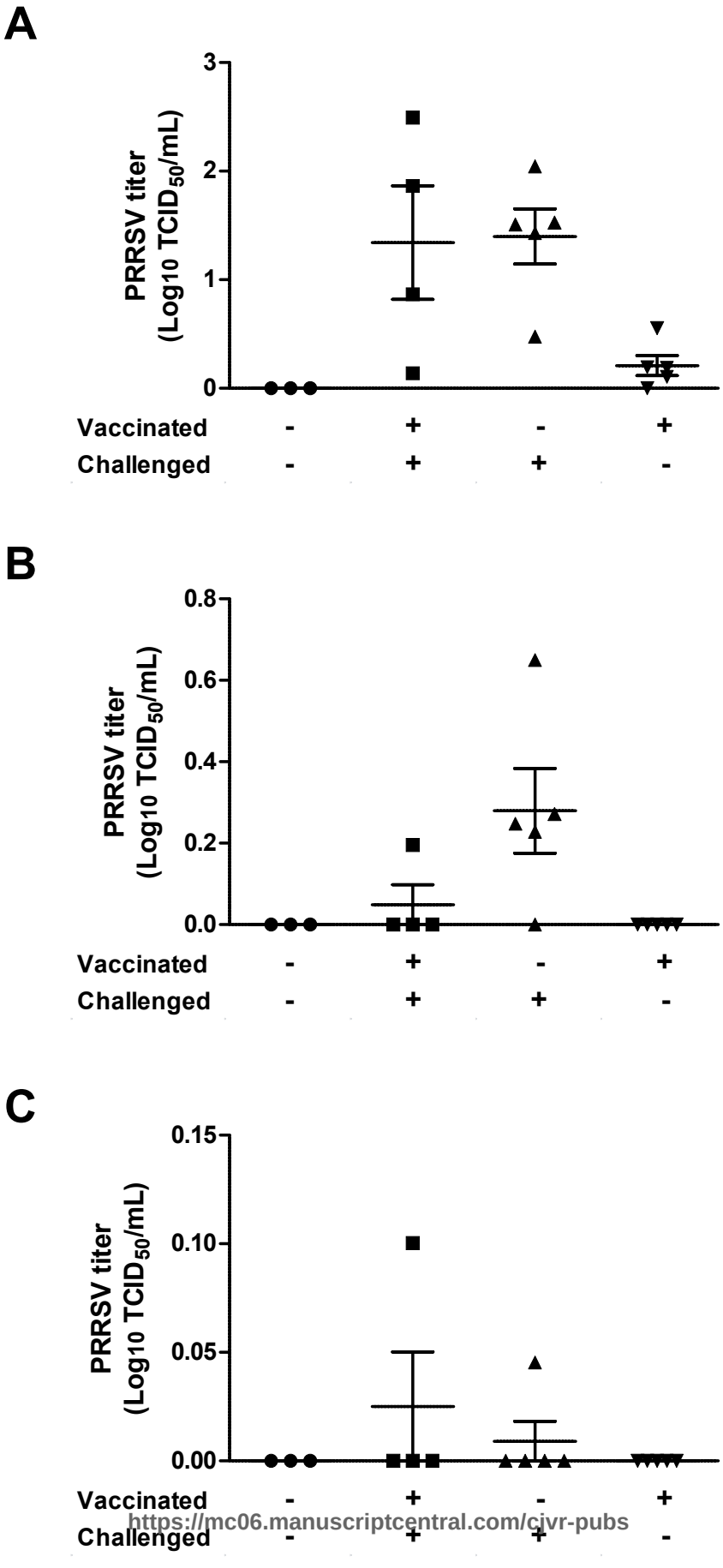
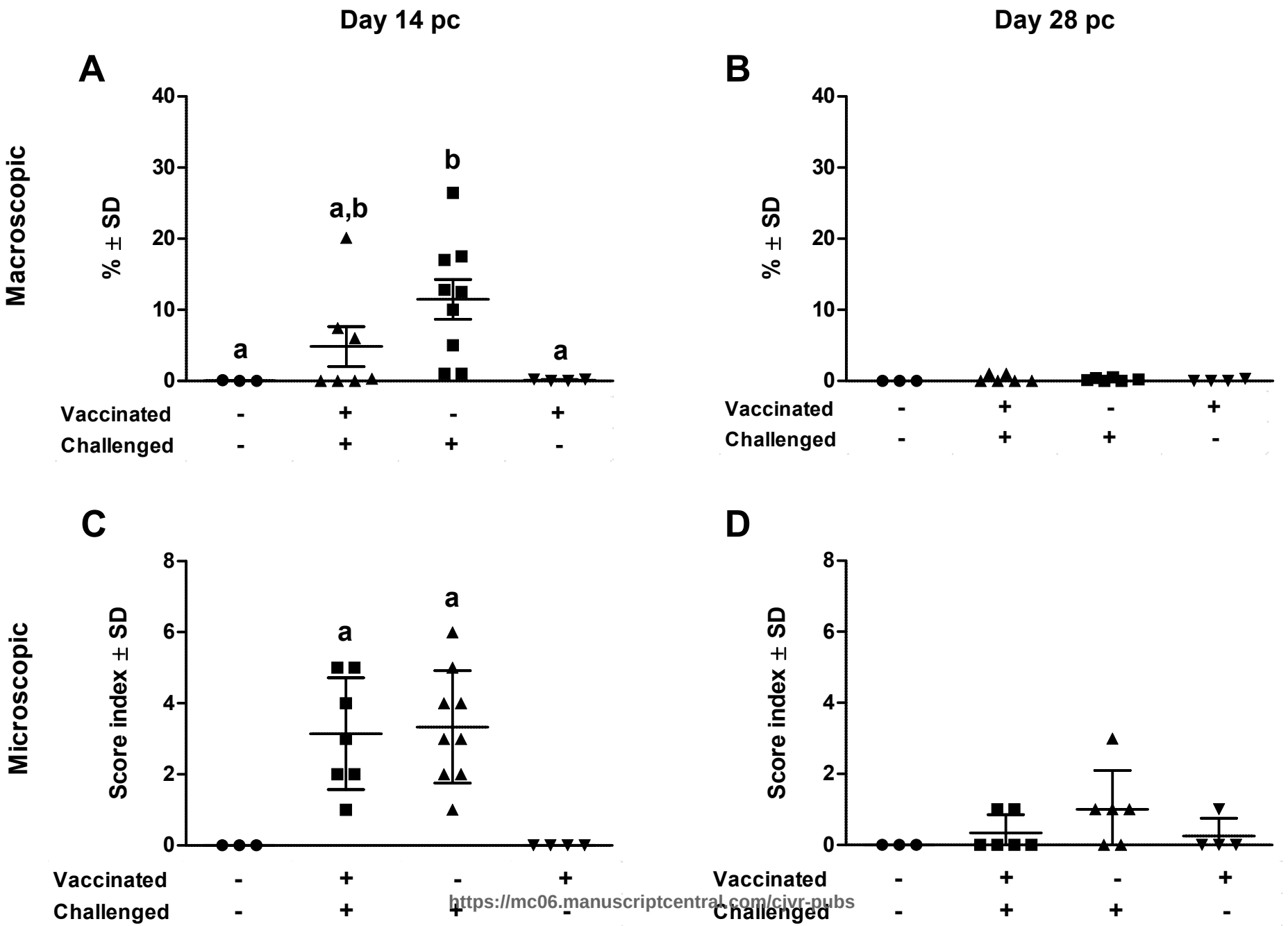


Figure 7


Day 14 pc
Day 28 pc
A
B
C
D
Macroscopic
Microscopic
% ± SD
Score index ± SD
a
a,b
b
Vaccinated
Challenged
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