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Ivermectin treatment inhibits the replication of Porcine circovirus 2 (PCV2) *in vitro* and mitigates the impact of viral infection in piglets

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ABSTRACT

Porcine circovirus 2 (PCV2) capsid protein (Cap) has a nuclear localization signal (NLS) and can enter the nucleus. In this study, ivermectin, a small-molecule nuclear import inhibitor of proteins was used to determine the role of nuclear localization of Cap on PCV2 replication. Observation by fluorescence microscopy of the intracellular localization of Cap and Cap NLS in cells cultured with ivermectin (50 μg/mL) determined that Cap and Cap NLS were located in the cytoplasm; in contrast, for cells cultured without ivermectin, they accumulated in the cell nucleus. Ivermectin treatment also reduced nuclear transport of Cap derived from PCV2 infection as well as PCV2 replication in PK-15 cells. In addition, lower levels of PCV2 in tissues and sera of piglets treated with ivermectin were detected by qPCR. These results established for the first time that ivermectin has potent antiviral activity towards PCV2 both *in vitro* and *vivo*.

1. Introduction

Porcine circovirus type 2 (PCV2) is the primary causative agent of post-weaning multisystemic wasting syndrome (PMWS) and other related diseases in pigs (Allan and Ellis, 2000). The virus is a member of *Circoviridae* with a circular single-strand DNA genome approximately 1.7 kb in length (Lv et al., 2014). At least 11 overlapping open reading frames (ORF) have been computationally predicted in this small genome (Lv et al., 2014). Of these, ORF 1 and 2 are the major ORFs: ORF 1, also known as the *rep* gene, encodes replicase, and ORF 2 encodes the unique structural component of the virion, the capsid protein Cap (Allan and Ellis, 2000). Nuclear localization of Cap was demonstrated in PCV2-infected cells in previous research (Finsterbusch et al., 2005) and enhanced nuclear import of Cap increased the progeny yield of PCV2 (Liu et al., 2016). As circovirus genomic DNA synthesis is performed in the nucleus (Cheung, 2012), nuclear transport of viral DNA after viral invasion is of great importance for the replication of Circovirus.

Ivermectin has been identified as a specific inhibitor of importin α/β-mediated nuclear import, with no effect on a range of other nuclear import pathways, including that it is mediated by importin β1 alone (Wagstaff et al., 2012). Ivermectin inhibited nuclear accumulation of HIV-1 integrase and non-structural protein 5 (NS5) polymerase proteins of dengue virus and, therefore, was observed to decrease HIV-1 and dengue virus replication in cell cultures (Wagstaff et al., 2012; Fraser et al., 2014). Additionally, ivermectin inhibited entrance into the nucleus and viral replication of DNA polymerase accessory subunit UL42 of the pseudorabies virus *in vitro* and *vivo* (Lv et al., 2018).

The protein Cap of PCV2 is mainly located in cell nuclei and the nuclear location of Cap is mediated by typical NLS through the importin α/β-mediated nuclear import pathway (Hou et al., 2018). Therefore, it is quite possible that ivermectin plays a role in controlling the intracellular localization of Cap. Moreover, Cap is a multifunctional protein and plays a very important role in the PCV2 replication cycle. Thus, the prevention of nuclear import of Cap may have a large impact on PCV2 growth.

2. Materials and methods

2.1. Cells, viruses and plasmids

PCV2 isolate SH1 was grown in PK-15 cells, which were cultured in DMEM with FBS (10%), penicillin (100 μg/mL) and streptomycin (100 μg/mL) at 37 °C in a 5% CO_2 atmosphere.

The sequences of Cap (the full length sequence of Cap without stop codon) and NLS (the front 122 bp, coding 1-41 aa of Cap) (Hou et al., 2018; Liu et al., 2001) were amplified from viral genome (GenBank: FJ644919.1) using primers: CapCF, GGAATTCATGTATCCAAGGAGG

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CGT, CapCR, ACGCGTCGACCGCTTAGGGTTAAGTGGGGG and CapNLSCF, GGAATTC ATGTATCCAAGGAGGCGT, CapNLSCR, ACGC GTCGACCGGCCATTTTTCCTTCTCCA. The amplicons were cloned into vector pCMV-C-DsRed (Beyotime) fused with red fluorescent protein by restriction enzyme digestion and DNA ligase ligation. The obtained recombinant vectors, named pRed-Cap and pRed-CapNLS, were sequenced to confirm no mutation or frame shifting.

2.2. CCK-8 assay for evaluation of ivermectin cytotoxicity

Cell viability was assessed using Cell Counting Kit-8 (BestBio, China), as reported (Han et al., 2011). Briefly, cells in 96-well plates with three replicates were treated with ivermectin (0, 50, 100 or 200 μg/mL, Sigma, Shanghai) dissolved in dimethyl sulfoxide (DMSO) for 24 or 48 h. Normal cultured PK-15 cells were used as the control.

Then, CCK-8 reagent (10 μl) was added to the cells after 24 or 48 h treatment with ivermectin and the mixture incubated in the dark for 2 h at 37 °C. The OD_{450} of each well was read using a spectrophotometer (Bio-Rad). The relative viability of the cells treated with ivermectin was compared with that of the control cells treated with DMSO alone.

2.3. Intracellular localization of Cap and NLS of Cap with ivermectin treatment

Transfections were conducted with lipofectamine 2000 (Life Technologies). Briefly, PK-15 cells in 6 well plates with 80% confluence were washed with PBS. The plasmid (4 μg) together with transfection regent (6 μL) in Opti-MEM without fetal bovine serum (240 μL) were added to the cells. After 1 h incubation, cell culture medium (4 ml) with or without ivermectin (50 μg/mL) was added and the cells were incubated at 37 °C in a 5% CO_2 atmosphere.

Intracellular localization of Cap-Red and CapNLS-Red was detected by fluorescence microscopy at 48 h post transfection. The cell nuclei were stained blue with DAPI (4′,6-diamidino-2-phenylindole).

2.4. PCV2 replication in vitro with ivermectin treatment

PK-15 cells with 80% confluence (about 4×10^6 cells per well) in a 6-well plate were infected with PCV2 at a multiplicity of infection (MOI) of 10. After 1 h incubation the cells were washed with PBS, and culture medium (4 ml) with or without ivermectin (0, 50, 100 or 200 μg/ml) was added. There were three replicates for each treatment. Real-time PCR, western blot and indirect immunofluorescence (IFA) were used to determine viral loads in the culture medium and cell lysates, respectively.

2.5. Testing viral loads with real-time quantitative PCR (qPCR)

Viral DNA was extracted according to the manufacturer's instructions (Tiangen, Beijing). Real time PCR was performed in triplicate per sample as described previously (Yu et al., 2007), using the primers: Cap DF, GTTTACATAGGGGTCATAG, Cap DR, TGTGCCCTTTGAATACTAC. The SYBR Green real time PCR detection system (Vazyme Biotech co., Ltd.) and a real-time thermocycler (Four-channel, Tianlong, China) were used for real time PCR analysis as per the manufacturer's instructions (Wang et al., 2017).

The plasmid pRed-Cap was used to generate the standard curve. Briefly, plasmid was extracted from *Escherichia coli* DH5α cells and quantified by measuring the OD_{260} using a NanoDrop™ spectrophotometer. The copies of DNA were calculated using the online NEBio Calculator (https://nebiocalculator.neb.com/#!/ligation). 10^1~10^{12} copies of plasmids per microliter were made by ten-times serial dilutions. Viral DNA copies were calculated according to the obtained standard curves.

2.6. Testing Cap expression by western blot

Cell lysates were separated by 10% SDS-PAGE and transferred to nitrocellulose membrane (Pall Corporation). Membranes were blocked in solution (10% fat-free milk in PBS, PBS-M) at room temperature overnight, then incubated with polyclonal antibody against PCV2 Cap (rabbit anti-PCV2) in PBS-M for 2 h at room temperature, followed by incubation for 1 h with goat anti-rabbit IgG conjugated with horseradish peroxidase (1:2000 in PBS-M; Boshide, Wuhan, China). Detection was performed using chemiluminescence luminol reagents (SuperSignal West PicoTrial Kit, Pierce).

β-actin was used as the internal reference and antibody against porcine β-actin was commercially available (Boshide, Wuhan, China).

2.7. Testing viral loads and subcellular location of viral protein with IFA

IFA was used to detect PCV2 infection and the intracellular localization of Cap as reported (Zhu et al., 2007). Briefly, the cells infected with PCV2 were fixed with absolute ethanol at 4 °C for 15 min, permeabilized with 0.1% Triton X-100 in PBS for 10 min and blocked with 1% BSA in PBS for 1 h. The cells were then incubated with 1:200 rabbit polyclonal antibody against Cap in PBS containing 1% BSA at 37 °C for 2 h. After washing three times with PBS, the cells were stained with Alexa Fluor 647-conjugated goat anti-rabbit IHIgG H&L (1:200 in PBS containing 1% BSA; Sangon Biotech, Shanghai, China). The fluorescent images were observed using an inverted fluorescence microscope (Olympus IX73, Japan).

2.8. In vivo effect of ivermectin on PCV2 infection

Nine healthy piglets (about 15 kg, 30 days old) were purchased from a local farm. PCV2 and Porcine Reproductive and Respiratory Syndrome Virus infection were excluded using PCR or RT-PCR, respectively, and virus-specific antibodies using ELISA.

The piglets were separated into three groups of three. After one week of environmental adaptation, piglets in groups 1 (virus) and 2 (virus + ivermectin) were challenged with $5 \times 10^{3.5}$ $TCID_{50}$ of PCV2 in cell culture medium (5 mL) by intramuscular injection, and piglets in group 3 (negative control) were given cell culture medium (5 mL). The challenged piglets were then given hemocyanin (5 mL) by multiple-site subcutaneous injection at 3, 5, and 7 dpc, as reported (Wang et al., 2007). Ivermectin (0.2 mg/kg) was given to piglets in group 2 at 2, 4 and 6 dpc. Rectal temperatures were recorded every day until the end of the experiment at 21 days. Piglets were weighed at the beginning and the end of the experiment to calculate the daily weight gains, and sera samples were collected at 0, 7, 14 and 21 dpc to test viremia with real-time PCR. Piglets were euthanized at 21 dpc and inguinal lymph nodes (ILNs) were collected and fixed to make slices as described (Seo et al., 2014). Viral loads (copies/g) in heart, liver, spleen, lung, kidney, tonsil and ILNs were tested using real-time PCR.

2.9. Analysis of PCV2-caused tissue lesions by histopathological examination and viral loads with immunohistochemistry (IHC)

The collected ILNs were fixed in 4% buffered formalin solution, embedded in paraffin and sectioned at 4 μm. According to the conventional staining procedure, H&E staining was conducted to observed histopathology in the tissues. Based on lesion characteristics, which include lymphocyte depletion, necrosis and inclusion bodies, a set of evaluation criteria was made in order to evaluate the lesion severity: the presence of lymphocyte depletion was rated from 0 to 3 (0, normal; 1, mild lymphocyte depletion or dispersed single cell necrosis of histiocyte or macrophage linage cells in lymphoid follicle; 2, moderate lymphocyte depletion or aggregated necrotic cells in follicles; 3, severe lymphoid depletion with loss of lymphoid follicle structure) (Opriessnig et al., 2004). A total of three slices and 15 microscope views for each

sample were used to calculate the tissue lesion scores.

For IHC testing, the paraffin wax sections were rehydrated and endogenous peroxidase activity inhibited by treatment with H_2O_2 (3% in methanol) for 30 min. Sections were then washed with PBS for 5 min. After hydration, sections of tissue were incubated in trypsin solution (0.1%) in presence of calcium chloride dihydrate (3 M) for 20 min at 37 °C. Slides were then washed in PBS several times and incubated overnight at 4 °C with rabbit polyclonal antibodies against PCV2 Cap (1:20 diluted in antibody diluent; Sigma-Aldrich, 938B). After being rinsed, the sections were incubated with biotinylated goat anti-rabbit IgG and streptavidin-conjugated HRP (1:500; Bioss Biotech, China). Subsequently, the sections were stained with diaminobenzidine (CW Biotech, China) and then counterstained with haematoxylin. Negative control slides without primary antibody but with rabbit IgG were included for each staining.

The immunohistochemical staining of viral antigen was analyzed using Image-Pro Plus (IPP) 6.0 software (Media Cybernetics, Bethesda, USA) as reported (Fan et al., 2017; Xavier et al., 2005). Briefly, three digital images at 1024 × 768 pixel resolution and 400× magnification were captured by the Motic BA210LED Digital microscope (Motic, Hong Kong). The measurement parameters included integrated optical density (IOD), area sum and mean density. The optical density was calibrated and the area of interest (AOI) was set as follows: hue, 0–30; saturation, 0–255; intensity, 0-255. The image was then converted to a grey-scale image and the values were measured (Fan et al., 2017). The average IOD for each piglet was collected from 3 slices and 15 AOIs.

2.10. Ethical statement

All the animal experimental procedures were reviewed and approved by local ethnical committee and strictly followed the guideline of an independent Animal Care and Use Committee in Shaanxi Province, China.

2.11. Statistical analysis

Multiple group data were analyzed by Bonferroni post-hoc test, while two-group comparisons were performed by unpaired Student's t-test. Data are presented as means ± standard deviation (SD). Significant differences were considered when $p \leq 0.05$.

3. Results

3.1. Ivermectin was not cytotoxic to PK-15 cells at 50 and 100 μg/mL

According to the CCK-8 assay results, when the concentration of ivermectin was 50 or 100 μg/mL, no cytotoxic effects to PK-15 cells were detected at 24- or 48-h post ivermectin treatment (Fig. 1a and 1b). However, when the concentration of ivermectin was increased to 200 μg/mL, PK-15 cell cytotoxicity was observed as the cell viability decreased significantly ($p \leq 0.05$; Fig. 1).

3.2. IVM blocks NLS from working to get cap into the nucleus

Intracellular localization of Cap-Red and CapNLS-Red in cells cultured with or without ivermectin was determined by fluorescence microscopy. Cap without NLS could not enter cell nucleus has been reported previously (Wagstaff et al., 2012). Nuclear accumulation of Cap-Red and CapNLS-Red was observed at 48 h post transfection in cells cultured without ivermectin (Fig. 2): the red fluorescence, representing Cap-Red or CapNLS-Red, overlapped with nuclei shown with DAPI (blue). In contrast, red fluorescence was located in the cytoplasm when ivermectin was added after transfection: the red fluorescence was separate from and located around the blue staining (Fig. 2).

3.3. Ivermectin inhibited PCV2 replication in PK-15 cells

Ivermectin (50 or 100 μg/mL) was added into the cell culture medium after PCV2 infection. The viral DNA copies were determined using PCR and compared with viral growth in cells cultured without ivermectin. As shown in Fig. 3a, addition of 50 and 100 μg/mL ivermectin decreased the viral loads to 41.0% and 28.2%, respectively, at 24 h post infection, and 28.2% and 15.7%, respectively, at 48 h post infection, compared with cell cultures without ivermectin (Fig. 3a). Consistently lower levels of Cap expression in PK-15 cells treated with ivermectin after PCV2 infection were detected by western blot compared with cells cultured without ivermectin, as indicated by the protein bands (Fig. 3b). Experiments were performed in triplicate and repeated three times with similar results.

IFA staining with anti-Cap polyclonal antibody was also conducted to show PCV2 infection in cells and intracellular location of Cap. As predicated, less cells were infected by PCV2 when ivermectin was added to the culture media compared with those cultured without ivermectin after PCV2 infection (Fig.3c). And the mean number of positive cells at 15 views were significantly different ($p \leq 0.05$, Fig. 3d). In addition, Ivermectin also restricted Cap derived from PCV2 infection into cell nuclei as the fluorescence only observed in the cytoplasm, not nuclei (Fig. 3c); in contrast, without ivermectin the fluorescence clustered in cell nuclei (Fig. 3c).

3.4. Ivermectin mitigated PCV2 infection in piglets

PCV2 infection significantly reduced piglet growth. The average daiy weight gains were significantly reduced due to PCV2 infection: piglets in the non-challenge group gained weight by 249 g per day, while piglets challenged with PCV2 without treatment gained 154 g weight per day. Piglets that received ivermectin treatment after PCV2 infection (2, 4 and 6 dpc) gained 198 g weight per day, which was intermediate to the non-challenge control and PCV2 challenge control group weight gains (Fig. 4a).

With the co-stimulation of hemocyanin, PCV2 infection also caused rectal temperature (RT) variation of infected piglets. The days with RT over 40 °C for each piglet were recorded and the average number of

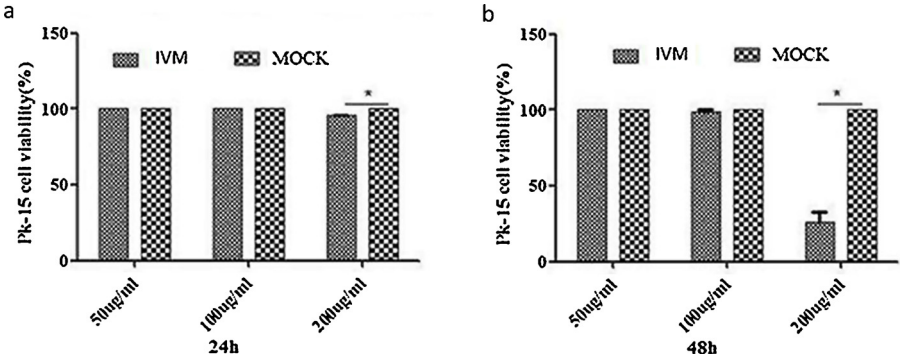

Fig. 1. Relative cell viabilities of the PK-15 cells treated with ivermectin for 24 (a) and 48 h (b). All cell-related experiments were performed in quadruplicate, and the relative cell viability (%) was expressed as a percentage relative to the untreated control cells. Data are presented as mean ± SD, and $p \leq 0.05$ is considered a significant difference.

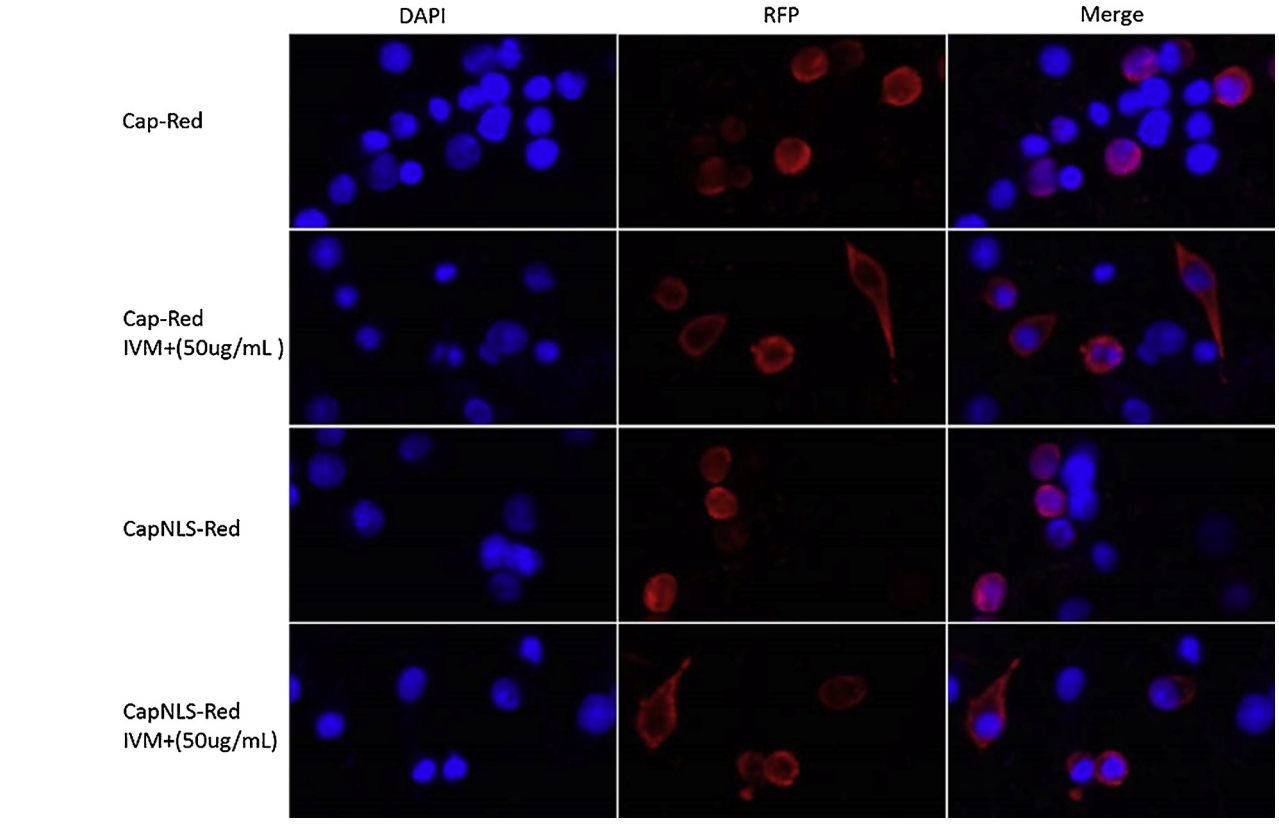

Fig. 2. Ivermectin-inhibited nuclear transport of Cap-Red and CapNLS-Red.
Cell nuclei are stained blue with DAPI. When Cap-Red or CapNLS-Red enters the cell nucleus, red fluorescence (RFP) and blue fluorescence overlap (pink); in contrast, when these proteins are restricted in the cytoplasm, red and blue fluorescence are separate. When ivermectin (50 μg/mL) is added (IVM +), these proteins mainly appear in the cytoplasm; in contrast, when cells are cultured without ivermectin they accumulate in cell nuclei.

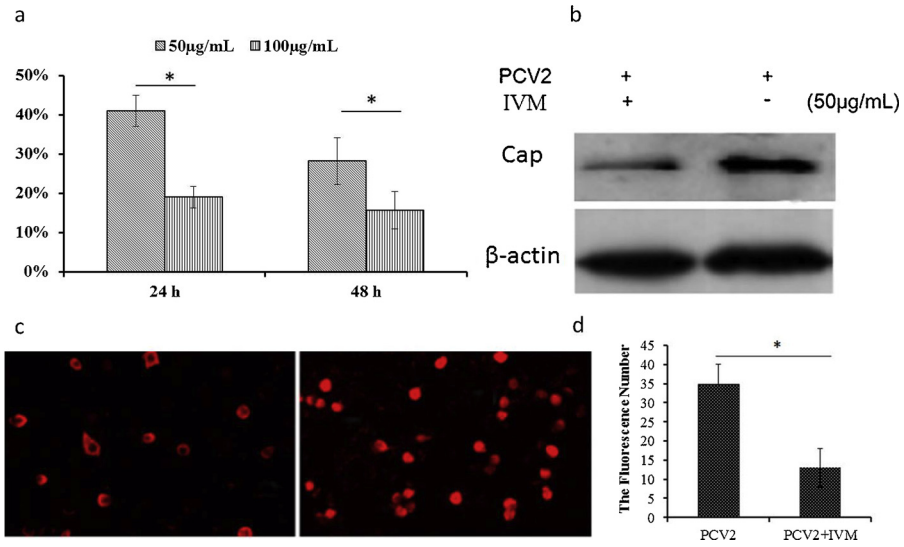

Fig. 3. Ivermectin decreased PCV2 replication *in vitro*.
(a) Adding ivermectin (50 μg/mL) decreased viral DNA copies by 59% and 71.8%, as detected by real-time PCR at 24 and 48 h, respectively, post PCV2 infection relative to cells cultured without ivermectin (control). Adding ivermectin (100 μg/mL) decreased viral DNA copies by 81% and 84.3% at 24 and 48 h, respectively. (b) PCV2 derived Cap expression in cells treated with ivermectin at 48 h after infection decreased, as detected by WB. (c, d) Adding ivermectin decreased the number of PCV2 positive cells and also altered the intracellular localization of Cap at 48 h post infection. Without the influence of Ivermectin, Cap (red) was in nuclei and with ivermectin, Cap (red) was around nuclei. Data are shown as mean ± SD, and $p \leq 0.05$ is considered a significant difference.

days with RT > 40 °C were calculated each week to show the effect of PCV2 infection. The data are shown in Fig. 4b. In the challenge control group, the piglets had 1.3, 3.6 and 3.6 days high temperature fever (> 40 °C) within 1, 2 and 3 weeks; while, the piglets treated with ivermectin after PCV2 infection suffered only 0.3, 1.3 and 1.7 days of fever (> 40 °C), indicating an alleviation of infection by ivermectin treatment. The difference between these two group was significant ($p \leq 0.05$). The piglets in the control group did not experience fever during the experiment.

Ivermectin treatment also significantly decreased viremia ($p \leq 0.05$, at 7, 14 and 21 dpc) and viral loads in tissues ($p \leq 0.05$, collected at 21 dpc), as the viral loads in sera and tissues were lower in piglets from the challenge plus ivermectin treatment group compared with those from the challenge group (Figs. 4c, and d).

During the autopsy, swollen lymph nodes were observed but no other gross lesions. Accordingly, ILNs were collected from pigs in each group and tissue sections were made to analysis the microlesions caused by PCV2 infection. IHC was used to detect PCV2 antigen in lymph nodes. Typically, PCV2 related lesions were observed in PCV2 infected pigs, like lymphoid depletion and histiocytic infiltration, as well as

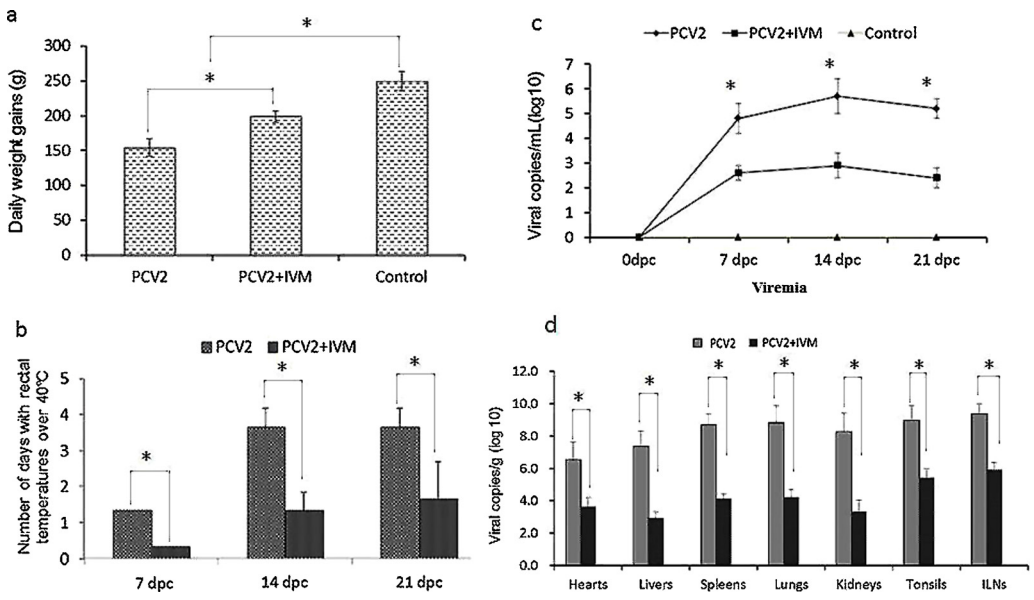

Fig. 4. Ivermectin treatments were beneficial to piglets and reduced viral load in sera and tissues. (a) The daily weight gains of piglets in different groups (PCV2, PCV2 + IVM and Control) after challenge. (b) The average number of days that the piglets in different groups had a fever ($\geq$ 40 °C). (c) Detection by real-time PCR of viral loads in sera collected at 7, 14 and 21 dpc from each group. (d) Detection of viral loads in tissues collected from PCV2-infected pigs with real-time PCR. Data are shown as mean ± SD, and $p \leq 0.05$ is considered a significant difference.

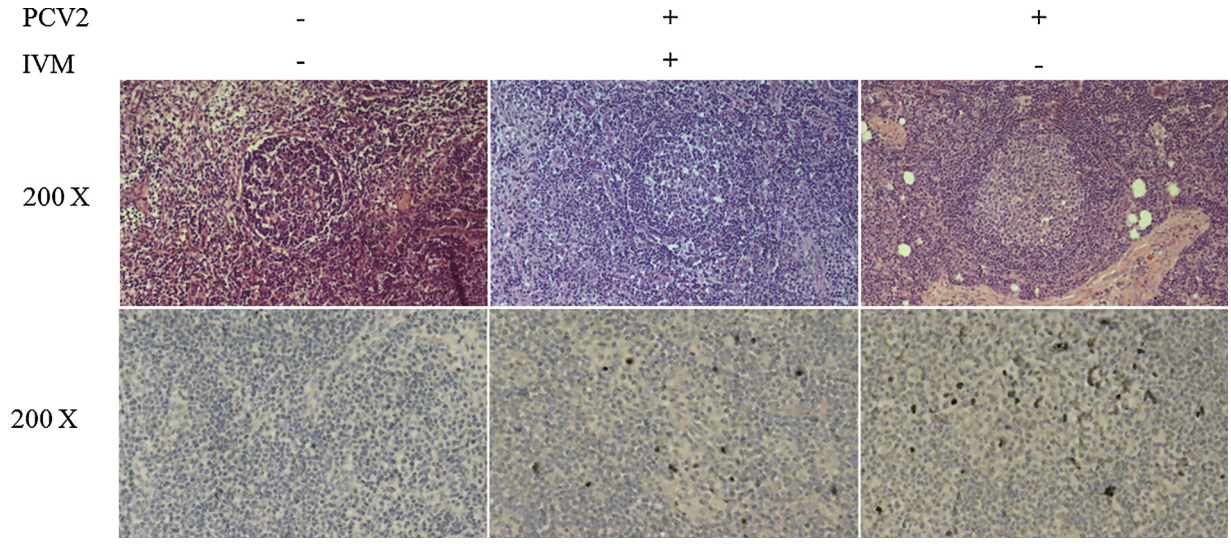

Fig. 5. Histopathological observation and IHC analysis. (a) Microlesions, lymphoid depletion and histiocytic infiltration in different groups. (b) PCV2 antigen detection by IHC; the red-brown are positive.

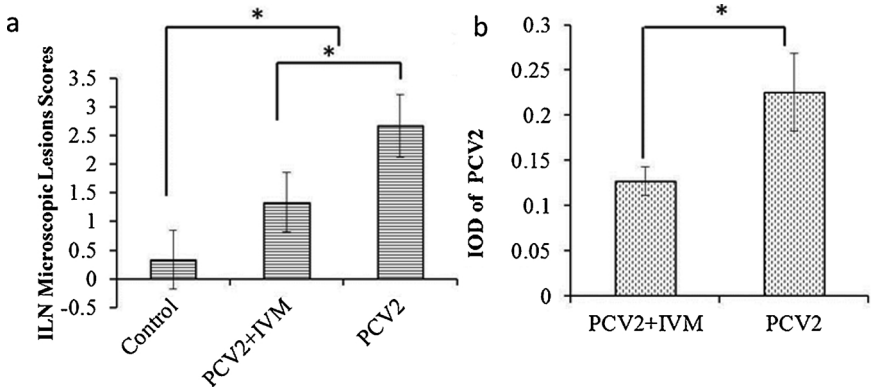

Fig. 6. Analysis of microlesions and viral antigen in ILNs. (a) Microlesion scores in ILNs collected from different groups. (b) Integrated optical density (IOD) of PCV2. Data are shown as mean ± SD, and $p \leq 0.05$ was considered a significant difference.

Table 1
The average IOD values (from 15 views) of each challenged piglets.

IOD	PCV2 + IVM	PCV2
1	0.106 ± 0.021	0.18 ± 0.026
2	0.131 ± 0.032	0.276 ± 0.031
3	0.145 ± 0.017	0.221 ± 0.024

PCV2 antigen (Fig. 5). The lesions in piglets treated with ivermectin were milder compared with those in challenge control piglets. There were also clear differences in the numbers of lymphocytes in lymph follicles and the severity of histiocytic infiltration in the slices collected from these two groups. According to the lesions score system, piglets infected with PCV2 had the most serious microlesions, followed by those treated with ivermectin after PCV2 infection (Fig. 6). The piglets in the control group were quite healthy with no clear histopathological changes in ILNs (Figs. 5 and 6). PCV2-related positive IHC signals were detected in both infected groups but not in the control (Fig. 5). According to IOD (Table 1) analysis, ivermectin treatment decreased the viral signals in ILNs and the difference between these two groups was significant (Fig. 6b).

4. Discussion

Ivermectin is widely used in the treatment of parasitic infestations by invertebrates (nematodes and insects) in veterinary and human medicine. In invertebrates, ivermectin binds selectively to glutamate-gated chloride ion channels in muscle and nerve cells and increases the permeability of the cell membrane to chloride ions (Campbell and Benz, 2010). This leads to parasitic paralysis and death (Cully et al., 1994). In vertebrates, ivermectin can affect the central nervous system (CNS) through reactions at the receptor for the gamma-aminobutyric acid (GABA) neurotransmitter: ivermectin acts as a GABA receptor agonist. GABA type A gated chloride channels (GABA A receptors) are only present in the central nervous system (CNS) (Estrada-Mondragon and Lynch, 2015). As the blood-brain barrier (BBB) can efficiently prevent ivermectin penetration into the CNS, ivermectin is quite safe for mammals except for some dog breeds that have an ABCB1 gene defect resulting in a lack of functional P-glycoprotein (Neff et al., 2004), since P-glycoprotein is a component of the BBB and transports some substances, including ivermectin, back across the cell membrane. Ivermectin has a wide safety margin in feeder swine, with no adverse reactions observed at dosages of up to 50 times the recommended level (Sanford et al., 1988). At 100 times this level, CNS signs similar to those seen in these neonatal piglets were reported (Campbell and Benz, 2010). However, ivermectin toxicosis in neonatal pigs has been reported previously (Sanford et al., 1988).

Currently, vaccination is a useful method for control of PCV2-related disease (Segalés, 2015). However, an early-life vaccine is necessary as PCV2 infection may occur during the early growth stage of piglets (Sno et al., 2016). Only inactivated and subunit vaccines have recently been available (Karuppannan and Opriessnig, 2017); these kinds of vaccines need a relatively longer induction period for effective immune protection. Additionally, as the immune systems of young piglets are underdeveloped, immunization with an oil-adjuvant vaccine would not induce an ideal immune response or could even be harmful to their immune system. This has led to conflicting ideas about the ideal immunization time, which is a key element influencing PCV2 control.

This new finding of the anti-PCV2 effect of ivermectin may provide a solution: as PCV2-related pig diseases always happen at a specific time, that is, after weaning for PMWS (Madec et al., 2000) and before or at the early fattening stage for most porcine dermatitis and nephropathy syndrome (Sipos et al., 2005), pretreatment of piglets with ivermectin prior to the disease onset period may be helpful for disease control. Thus, ivermectin could provide a useful way to prevent or control PCV2 before effective immune responses are available.

Decreased PCV2 replication by inhibiting virus transport to the nucleus has been reported in previous research (Cao et al., 2015). The nuclear transport process of PCV2 is mediated by the cytoplasmic intermediate chain 1(IC1) of dynein, which interacts with viral capsid protein. Knockdown of IC1 or inhibition of microtubule depolymerization with nocodazole decreased virus transport and replication (Cao et al., 2015). Accordingly, inhibiting virus transport to the nucleus could be an approach to control viral infection.

The accumulation of Cap protein into the nucleus is mediated by a special arginine-rich N-terminus (position 1-41) NLS. Cap could not enter the nucleus when the NLS were removed and the NLS of Cap could enable other proteins like green fluorescent protein to enter the nucleus (Liu et al., 2001). This study demonstrated that ivermectin prevents Cap and the NLS of Cap from entering the cell nucleus. This suggests that ivermectin may work on an NLS-mediated nuclear import pathway of Cap, but further research is needed to confirm the mechanism.

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