Strain-dependent differences in the capacity of Peste des Petits Ruminants virus to infect antigen presenting cells

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Running Head: APC Infection Differences Among two PPRV Strains

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ABSTRACT

The ability of morbilliviruses to infect antigen-presenting cells (APCs) plays a major role in the outcome of infection, as these cells transport the virus from the respiratory tract to the lymph nodes before spreading to lymphoid and epithelial tissues. This study used two cell models, — monocyte-derived macrophages (MoMs) and monocyte-derived dendritic cells (MoDCs), — from three host species (goat, sheep, cattle), to evaluate the differential ability of APCs to be infected by two strains of peste des petits ruminants virus (PPRV) with contrasting virulence in goats. Infection was analyzed by flow cytometry, infectious titers ($TCID_{50}$/mL), RT-qPCR and immunofluorescence. Goat and sheep MoDCs proved highly permissive to both virus strains, with viral titers at 48 hpi in the range of 10^4–10^5 $TCID_{50}$/mL and a high percentage of infected cells, unlike bovine MoDCs, which were weakly infected and had no detectable viral titer, as expected for this dead-end host. In comparison, MoMs showed reduced permissiveness to PPRV in goats but not in sheep. Post-infection supernatants in goat MoMs infected with the low-virulent strain showed low or undetectable $TCID_{50}$/mL, despite persistence of detectable viral genome up to 96 hpi. Immunofluorescence analyses revealed the presence of double-stranded RNA but absence of nucleoprotein of the low virulent PPRV strain in goat MoMs suggests a blockage at the level of viral protein translation. These results suggest that this combined in vitro model of MoDC and MoM infections would be useful in anticipating host susceptibility and PPRV strain virulence, providing complementary tools for PPR control efforts.

IMPORTANCE

Peste des petits ruminants (PPR) disease affects goats, sheep and multiple other hosts across the globe, with major impacts on the economy, food security and biodiversity. The sensitivity of the host species to PPR virus infection varies considerably, with clinical manifestations and disease progression being shaped by the viral strain, host species, and infected breed. In vitro models focusing on immune cells, which are the primary target of PPRV could provide a relevant tool for studying the mechanisms of viral adaptation to the host and cellular antiviral responses, while contributing to surveillance and control strategies. The results obtained here suggest that a dual-cell model focusing on macrophages and dendritic cells could be useful for pre-screening studies aimed at assessing the susceptibility of a given host species to a PPRV strain and estimating its relative virulence. Testing this in vitro approach in additional host species and a wider range of strains is required to confirm its relevance.

INTRODUCTION

Peste des Petits Ruminants virus (PPRV) is a member of the Paramyxoviridae family, belonging to the Morbillivirus genus, such as the Measles virus (MeV), Rinderpest virus (RPV) and Canine Distemper virus (CDV). PPRV has a linear, non-segmented, single-stranded RNA genome with a negative polarity (1). First identified in Ivory Coast in 1942, PPRV has since spread extensively, becoming endemic in large regions of Africa, the Middle East, and Asia (1,2), and has recently emerged in several European countries (3,4). The virus primarily affects domestic small ruminants, such as goats and sheep, but it can also infect suids and a variety of wild species within the order Artiodactyla, including antelopes and gazelles (5).

PPRV is transmitted primarily through direct contact with infected animals, via respiratory secretions, feces, and contaminated feed or water sources. The virus spreads rapidly in high-density livestock populations, especially where biosecurity measures are insufficient. Peste des petits ruminants (PPR) is characterized by severe clinical manifestations in sheep and goats, with consequences ranging from morbidity to livestock mortality, leading to significant economic losses for farmers. Given its high transmissibility and the significant economic impact, PPR is classified as a notifiable disease by the World Organisation for Animal Health (WOAH, formerly OIE) (6). Compared with rinderpest, which has been eradicated since 2011, the PPR virus has been the focus of a control program jointly led by the WOAH and the Food and Agriculture Organization of the United Nations (FAO) since 2015, aiming for its eradication by 2030 (7). However, the sensitivity of the host species to PPRV infection varies considerably, with clinical manifestations and disease progression being shaped by the viral strain, the host species and infected breed (8,9). The reasons for this variability are still not clearly understood, although it seems to be linked with differences in early inflammatory responses (10). A better understanding of PPRV virulence and host susceptibility is considered as a research priority for disease control (11).

The main targets of the PPR virus and other Morbilliviruses are immune cells via the SLAMF1 (Signaling Lymphocytic Activation Molecule Family 1) receptor, also known as CD150 (12,13), and epithelial cells via the Nectin-4 receptor (14). At the early stage of infection, Morbilliviruses infect antigen-presenting cells (APCs), such as macrophages and dendritic cells, which transport the viral particles to lymphoid organs, in particular lymph nodes and tonsils, where lymphocytes reside (1). Infected cells enter the bloodstream, causing viremia which facilitates the spread of the virus to various organs and tissues. Infection of epithelial cells occurs once viremia is established, with the Nectin-4 receptor playing an essential role in viral dissemination and excretion (12,14).

For PPRV, direct infection of APCs in vivo has not yet been demonstrated. However, draining lymphoid tissues such as the tonsils and lymph nodes, are the first major sites of replication (15). Recently, studies have shown that monocyte-derived dendritic cells are permissive to PPRV in vitro (16). Permissiveness is defined as the ability of the virus to replicate and produce infectious virions. What is known for other morbilliviruses is that permissiveness varies depending on the cell type and the virus that infects these cells (17,18). Due to the importance of APCs in morbillivirus infection, it is possible that the observed variability in PPRV virulence and host susceptibility can be related to differences in viral replication capacity in APCs.

Among APCs, dendritic cells and macrophages are essential for detecting viral infections and initiating innate immunity. Macrophages are key cells in innate immunity, with a high capacity for phagocytosis (19). These cells secrete pro- or anti-inflammatory cytokines and also act as APCs. Macrophages are able to recruit and activate other immune cells, in particular T cells (19,20). Macrophages play a more prominent role in amplifying ongoing immune responses and modulating the inflammatory microenvironment through cytokines secretion (21–23). Dendritic cells (DCs) have a high level of phagocytic activity in their immature state, actively monitoring their environments by capturing and processing antigens (24,25). Following pathogen recognition by pattern recognition receptors (PRRs), DCs undergo a maturation process characterized by upregulation of MHC class II and co-stimulatory molecules such as CD80 and CD86, which are essential for effective priming and regulation of T cell responses (26,27). Macrophages and DCs are derived from monocyte differentiation and can be distinguished by their morphology (25,28).

Thus, the study of infection of monocyte-derived macrophages (MoMs) and monocyte-derived dendritic cells (MoDCs) could be a relevant and reproducible model for comparing

host species permissiveness to PPRV. Here, we will focus on the infection of MoDCs and MoMs from goats, sheep and cows (identified as dead-end hosts for PPRV (29)) with two strains of PPRV. The two strains used in this study, Morocco 2008 (MA08) and Ivory Coast 1989 (IC89), demonstrated different levels of virulence in certain breeds of goat (30). Strain MA08 belongs to lineage IV of the PPRV and was isolated from an Alpine goat that was severely affected during the 2008 outbreak in Morocco (31). The IC89 strain belongs to lineage I. It was isolated from a goat in Ivory Coast in 1989 and is considered to be a mildly virulent strain, inducing a mild disease in various goat breeds (30,32,33), although it is used as a virulent strain for the study of immune response in sheep (34).

Based on representative known virulence to goats of the two strains of PPRV, low for strain IC89 and high for strain MA08, the aim of this study was to assess the comparative in vitro replication of these PPRV strains in both MoMs and MoDCs of goats, sheep and cows. The research question was to evaluate whether any difference could be observed between these APCs in relation to PPRV strain virulence.

RESULTS

1. Infections of MoMs and MoDCs by PPRV

To determine the infectivity of the two PPRV strains, IC89 and MA08, for macrophages and dendritic cells, circulating monocytes from the blood of the different animal species studied were derived into dendritic cells (MoDCs) and macrophages (MoMs). These two cell types were infected with each of the viral strains studied, IC89 and MA08, for 48 h at a Multiplicity of infection (MOI) of 0.1. Infection was assessed by flow cytometry after intracellular

labelling with an antibody targeting the virus nucleoprotein (anti-NPPRV) coupled to a fluorochrome.

In goat MoMs, an average of 20% of the cells were positive for the N protein of PPRV strain IC89, compared with an average of 32.6% for those infected with strain MA08; MA08 infected MoMs significantly more than IC89 (P=0.0058) (Figure 1A). For goat MoDCs, no significant difference was observed between the two viral strains and in most cases the cells were positive for NPPRV with a range of 43 to 73% (Figure 2B). Intercellular comparison (i.e. MoMs vs MoDCs) revealed a significant difference in the rate of NPPRV-positive cells; MoDCs being twice as infected than MoMs, which was observed for both PPRV strains (Fig. 1C and 1D).

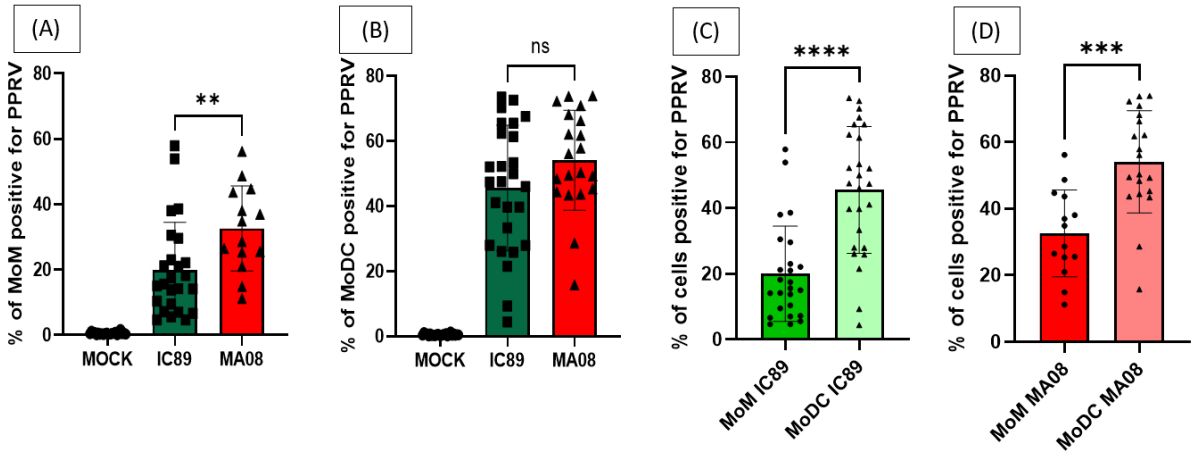


Figure 1: Infection of goat APCs with PPRV. Percentages of goat MoMs (A) and MoDCs (B) positive for PPRV at 48hpi after in vitro infection with IC89 or MA08 PPRV strain. The MOCK group corresponds to uninfected control cells. Data are reported as individual biological replicates from 3 goats and with minimum n=14 replicates, mean ± SD; **P=0.0058; ns=non-significant; Mann-Whitney U test . Comparison of infection by strain IC89 (C) or MA08 (D) between goat MoMs and MoDCs at 48hpi; ****P<0.0001; ***P=0.0002; Mann–Whitney U test.

For sheep MoMs, there was no significant difference in the rate of NPPRV-positive cells between the two PPRV strains, and the variability of responses was high, with an average rate of 14.7% for IC89 and 21% for MA08 (Fig. 2A). Similarly, for sheep MoDCs, there was no significant difference between these two viral strains in terms of percentage of NPPRV-

positive cells: in both cases, the average was 30%, with comparable extreme values (Fig. 2B). A significant difference was observed between sheep MoMs and MoDCs infected with IC89, again MoDCs being about twice as infected than MoMs (P=0.0092) (Fig. 2C). In the case of PPRV strain MA08, although there was a visually higher infection rate of MoDCs, with an average of 30%, there was no significant difference when compared to MoMs (Fig. 2D).

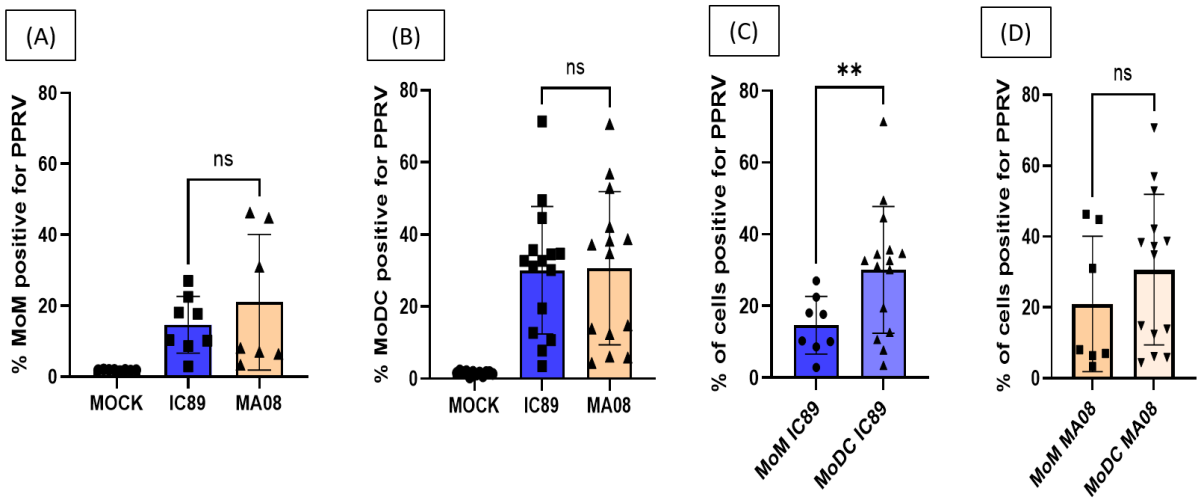


Figure 2: Infection of sheep APCs with PPRV. Percentages of sheep MoMs (A) and MoDCs (B) positive for PPRV at 48hpi after in vitro infection with IC89 or MA08 PPRV strain. The MOCK group corresponds to uninfected control cells. Data are reported as individual biological replicates from 3 sheep and with minimum n=7 replicates, mean ± SD; ns=non-significant; Mann-Whitney U test. Comparison of infection by strain IC89 (C) or MA08 (D) between sheep MoM and MoDC at 48hpi; **P=0.0092; Welch's t test; ns=non-significant; Mann–Whitney U test.

MoMs and MoDCs from cattle were also infected with IC89 and MA08 strains using the same parameters. Flow cytometry analysis after intracellular staining of the NPPRV protein revealed very low levels of NPPRV-positive cells, whatever the cell type and viral strain used (Supplemental fig. S1A, S1B).

2. Replication of PPRV in MoMs and MoDCs

To study viral replication in MoMs and MoDCs of different hosts, post-infection supernatants were added to cultures of SLAM-expressing Vero cells (VDS) (35) at dilutions

from 10^{-1} to 10^{-7} with a minimum of four replicates per dilution. The cytopathic effect (CPE) was observed under the microscope and viral titers were calculated as described in the Materials and Methods.

In goats, post-infection supernatants from MoDCs had, on average for both PPRV strains MA08 and IC89, significantly higher titers than supernatants from MoMs (Fig 3A). For MoMs, the viral titers of the post-infection supernatants for MA08 reached an average $TCID_{50}$/mL of $3x10^3$ whereas, for the IC89 strain, the viral titer was barely detectable with an average $TCID_{50}$/mL of 34 (Fig 3A) including 9 out of 17 replicates where no titer was detected (circled in Fig 3A).

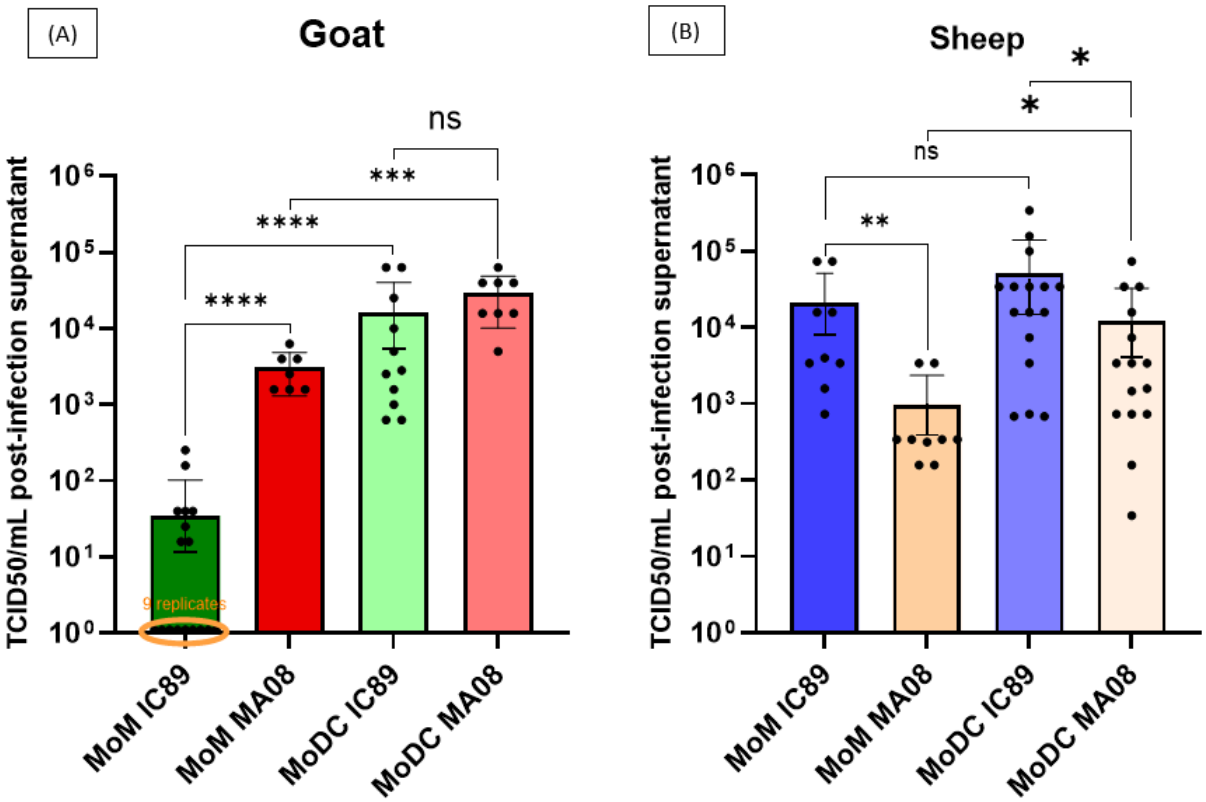


Figure 3: Viral titers ($TCID_{50}$/mL) at 48 hpi in goat (A) and sheep (B) MoMs and MoDCs. Data are shown as individual biological replicates from 3 goats and 3 sheep and with minimum n=7 replicates, mean ± SD. Replicates with no titer observed are highlighted with a circle. Statistical comparisons were performed using the Mann–Whitney U test; ****P<0.0001; ***P=0.0005; ns = not significant (A). MoMs MA08 vs MoDCs MA08 *P=0.0229; MoDC IC89 vs MoDC MA08 *P=0.0417; **P=0.0014; ns=not significant (B).

In sheep, post-infection supernatants from MoDCs infected with strain MA08 had, on average, significantly higher titers than those from MoMs infected with MA08 (Fig 3B) with

a TCID50 of around $1.2x10^4$ for MoDCs infected with MA08 compared with $9.8x10^2$ for MoMs infected with MA08 (P=0.0229). There were no significant differences between MoMs and MoDCs infected with the strain IC89 even though there was a trend towards higher titers for post-infection supernatants from MoDCs. The strain IC89 yielded a significantly higher mean titer than the strain MA08 in both MoDCs (P=0.0417) and MoMs (P=0.0014) (Fig 3B).

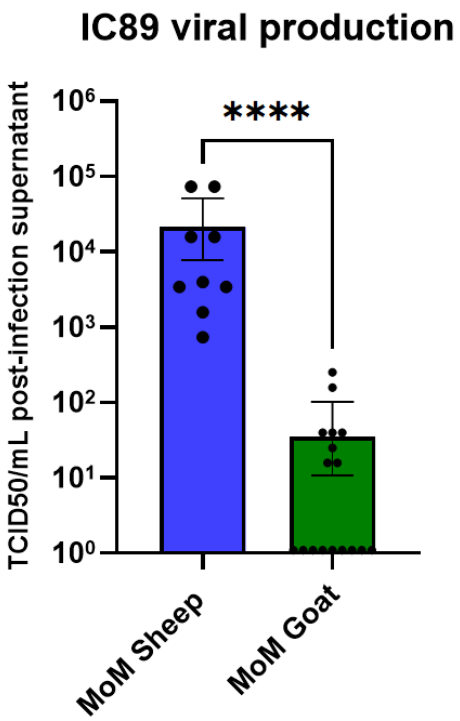


Figure 4: Comparison of viral titer of strain IC89 in MoMs from sheep and goat. Data are shown as individual biological replicates from 3 goats and 3 sheep and with minimum n=9 replicates, mean ± SD. Statistical comparisons were performed using the Mann–Whitney U test, ****P<0.0001.

The titration of 48hpi supernatants of the strain IC89 on sheep MoMs vs goat MoMs, indicated a significant difference (P<0.0001), sheep MoMs exhibiting high titers with an average TCID50 of $2x10^4$ whereas goat MoMs infected with IC89 have titers that are barely or not at all measurable (Fig 4). In cows APCs, no viral titer was detected in the post-infection supernatants (Supplemental fig. S2).

3. Kinetics of infection of goat MoMs by strain IC89 of PPRV

a. Infection and replication

After observing low or non-detectable viral titers in supernatants of goat MoMs infected for 48h with the IC89 strain, infection kinetics were studied to see if replication was somewhat delayed for this low virulent strain. Goat MoMs were infected for 24 h, 48 h, 72 h and 96 h, at an MOI of 0.1. As previously, the rate of NPPRV-positive cells was measured using a flow cytometer and post-infection supernatants were collected and viral titers were determined.

The results show that the rate of infected cells is relatively stable over time, with an average NPPRV positivity of around 30% regardless the infection time. No significant increase in the percentage of NPPRV-positive cells was observed between 24hpi and 96hpi (Fig 5).

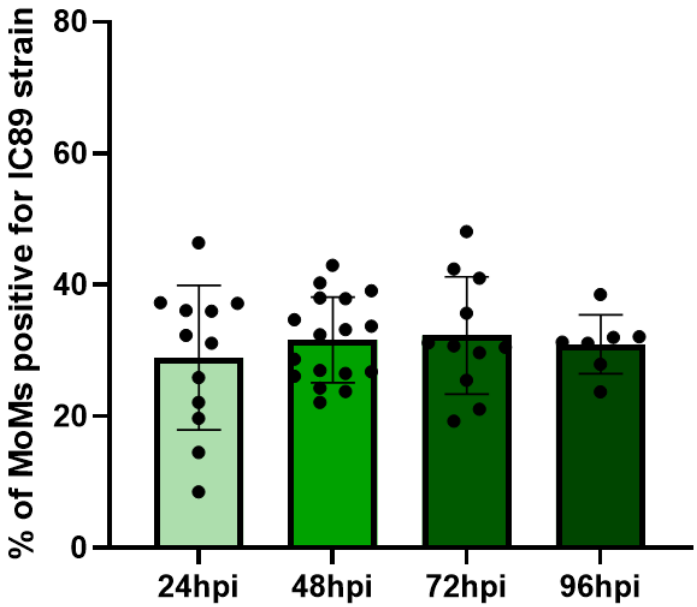


Figure 5: Kinetics of infection of goat MoMs by strain IC89 of PPRV. Percentages of NPPRV+ cells were determined by flow cytometry at 24, 48, 72, and 96hpi. Data are shown as individual biological replicates with 3 goats and with minimum n=6 replicates, mean ± SD. Statistical comparisons were performed using the ordinary one-way Anova.

The post-infection supernatants from IC89-infected goat MoMs were collected in triplicates throughout the kinetics, and titrated on VDS. No $TCID_{50}$/mL viral titers could be detected for any time point during the kinetics, with values remaining below the test detection threshold, and cytopathic effects (CPEs) sometimes observed in a single well of the first dilution (10^{-1}).

The titration plates used for this kinetics analysis were then labelled with the monoclonal antibody 38.4 tragetting the NPPRV coupled with tetramethylrhodamine isothiocyanate (or TRITC) in order to observe the presence or absence of the virus at the cellular level. We observed the presence of the virus in VDS cells in the first dilution, and sometimes up to the second and third dilutions (10^{-2} and 10^{-3}). However, this detection occurred in the absence of cytopathic effect (CPE), or with CPE limited to a single well in the first dilution, which is insufficient to calculate a reliable viral titer (Supplemental fig. S3).

b. Detection of viral RNA

To determine the presence of viral genomic RNA, an RT-qPCR was performed on post-infection supernatants throughout the kinetic. The cycle number (Cq) between 24 hpi and 48hpi remained stable at around 20.5. A decrease in the Cq value was first observed at 72hpi, falling to an average of 19.5, with an even more significant decrease at 96hpi with an average Cq of 17.8 (Fig. 6), representing an increase in genetic material of IC89 strain detected in supernatants.

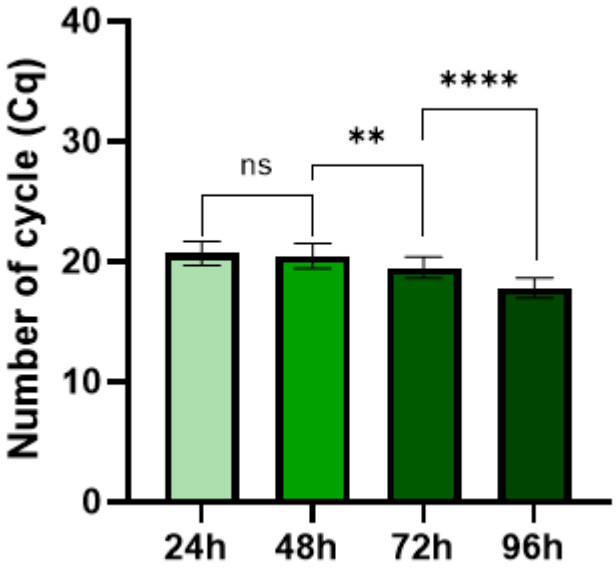
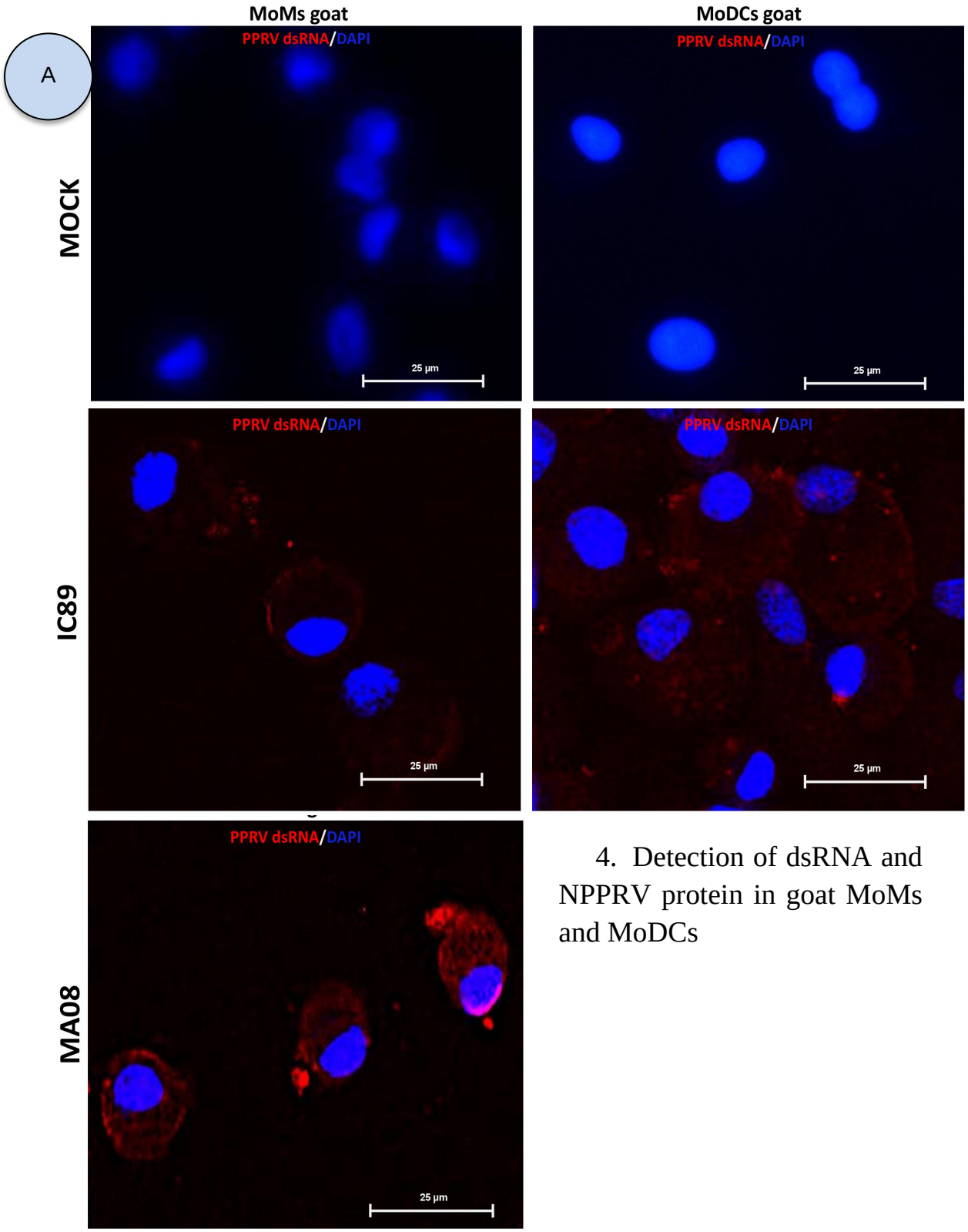
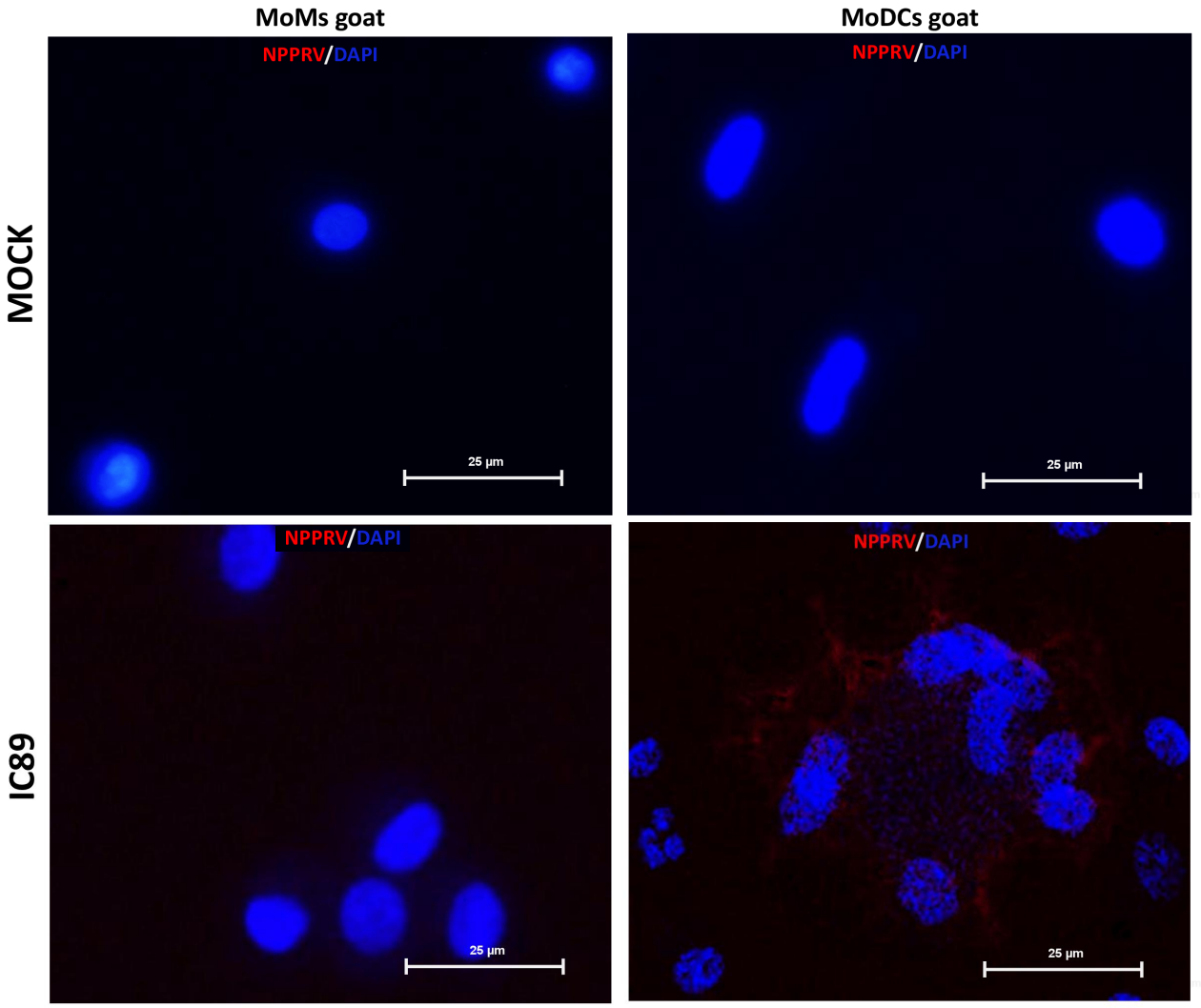


Figure 6: Kinetics of viral shedding in MoMs supernatant infected by IC89 strain at 24h, 48h, 72h and 96h.Viral shedding was monitored by RT-qPCR (expressed in Cq) detecting the presence of the PPRV N gene. Data are shown as individual biological replicates with mean ± SD. Statistical comparisons were performed using the Mann–Whitney U test, **P=0.0028; ****P<0.0001



4. Detection of dsRNA and NPPRV protein in goat MoMs and MoDCs



B

7: Immunofluorescent detection of viral double-stranded RNA (dsRNA) (A) and Nucleoprotein PPRV protein (NPPRV) (B) MoMs and MoDCs infected with IC89 or MA08. Infected (IC89 or MA08) and uninfected (MOCK) MoMs and MoDCs were ed with DAPI to identify cell nuclei, dsRNA (A) and NPPRV (B) are detected here in red, scale bar = 25 µm.

To investigate a possible blockage or slowdown in the replication cycle of the IC89 strain in goat MoMs, the detection of double-stranded RNA (dsRNA) molecules, an intermediate marker characteristic of the replication of single-stranded RNA viruses such as PPRV, can indicate whether viral replication is occurring. In addition, PPRV nucleoprotein (NPPRV) detection was performed to assess the presence of viral proteins in these cells. Immunofluorescence was used to detect dsRNA and NPPRV separately in infected MoMs, and the profiles obtained were compared with those observed in goat MoDCs and with the MA08 strain. A positive dsRNA signal is observed in both cell types in the presence of IC89 and in MoMs only in the presence of MA08 (Fig. 7A). NPPRV is detected in goat MoDCs infected with IC89, but not in goat MoMs (Fig. 7B).

DISCUSSION

PPRV infection can lead to a variety of outcomes ranging from acute symptoms and death of the infected host to subclinical infection. Surveillance and control measures must be adapted for efficient response. However, so far, it is hard to predict disease outcomes in the different hosts and prepare accordingly. A better understanding of differences in sensitivity of the host to viral strains, studied in vitro, would make it possible to anticipate disease impact and develop a predictive model that can demonstrate both a host's sensitivity to different strains but also the differences in virulence expressed by the same strain depending on the host (36,37). To move towards this objective, we selected two strains of the PPRV with distinct virulence profiles in goats, mildly virulent IC89 and highly virulent MA08 (10,33,38). Goat and sheep cells were used because these species are the main natural hosts of PPRV, while bovine cells served as a relevant negative control, as cattle are considered a dead-end host (29,39). This in vitro experimental model focused on a comparative overview of PPRV

infection in MoMs and MoDCs in order to describe possible differences in sensitivity and replication profile between cell types, host species and depending on the viral strain.

Overall, our study shows that MoDCs from goats and sheep are more permissive to PPRV than MoMs. On average, the rate of infected cells (or NPPRV-positive cells) and viral titers are higher for MoDCs than for MoMs. This higher permissiveness of MoDCs could be explained by the higher presence of the C-type lectin DC Specific Intercellular adhesion molecule 3-Grabbing Non integrin DC-SIGN (or CD209) compared to MoMs. This promotes the capture of the virus on the cell surface, improving the interaction between PPRV envelope glycoproteins and the signaling lymphocytic activation molecule family (SLAMF), leading to more efficient viral entry and replication (40–43). In cattle, the absence of infection and of measurable replication in MoDCs and MoMs confirms the dead-end host status of this species. No significant differences in viral titers were observed between the IC89 and MA08 viral strains in goat MoDCs. However, in sheep MoDCs, the mean titer of the IC89 strain was approximately 0.6 $\log_{10}$ higher than those of the MA08 strain. MoDCs results concur with the multiple field and experimental studies showing that cows are not affected and cannot transmit PPRV unlike their main small ruminant hosts (29,44). In small ruminants, MoDCs confirmed their susceptibility to PPRV infection, but did not reveal any significant difference between goats and sheep, which does not allow for more precise information to be provided about differences in susceptibility between viral strains. This cell model could therefore potentially serve as an indicator of the ability of the virus to infect a given species. This would be of high interest notably for wild Artiodactyls of high conservation value for which there is limited information on susceptibility to PPRV (45). Such indicator could inform efforts to limit the impact of PPRV infection and the corresponding variety of disease symptoms on biodiversity, or in improving our

understanding of the potential role of different atypical host species in modulating PPRV virulence during the transmission cycle (46).

In contrast to MoDCs, MoMs are less permissive but more discriminating host cells for PPRV. In goats, strain MA08 infected MoMs more efficiently than strain IC89. By contrast, IC89 produces detectable titers that are significantly higher than MA08 in sheep MoMs. Furthermore, the titers observed with IC89 in sheep MoMs were significantly higher than those obtained in goat MoMs. This suggests that the ability of this strain to replicate will depend on the host species and the type of cell infected. Based on this, and on knowledge of the virulence of these strains in vivo (10,30,33,38,47), we propose that this infection model with MoMs could serve as an indicator of strain-specific virulence in small ruminant breeds and may highlight host-specific signatures of viral adaptation.

For the remainder of the study, infection kinetics were performed on goat MoMs infected with IC89, since the discrepancy was most pronounced in this model (i.e. cellular infection was detected by flow cytometry, but the viral titer was undetectable). The objective was to determine whether the absence of a viral titer was due to a delay in replication or a persistent blockage of the viral cycle. Throughout the kinetics (24h to 96h infection), the rate of infected cells remained stable on average between 28 and 32% with no significant increase. Titrations of supernatants at all time points remained negative, indicating the absence of measurable infectious virions production. Nevertheless, RT-qPCR revealed a significant decrease in Cq between 48, 72 and 96 hpi, reflecting a gradual accumulation of extracellular viral genomic RNA. It should be mentioned that RT-qPCR does not distinguish between infectious and non-infectious viral particles. In addition, immunofluorescence microscopy of the titration plates showed that viral antigens were present in the cells because of the NPPR

protein detection in VDS cells, at different levels but without CPE, which supports the viral titer determined in supernatants and the cytometry results.

Despite the absence of measurable infectious titer, the detection of viral RNA in the supernatants suggests an incomplete or an abortive replication cycle. This could result in release of non-infectious or defective viral particles, which has already been observed with several RNA viruses (48–50), including morbilliviruses (51–54). Presence of dsRNA detected by immunofluorescence in goat MoMs infected with IC89 indicates the initiation of viral genome replication. But no NPPRV signal was detected in these IC89 strain-infected cells. This means that in MoMs, the viral cycle progresses to the synthesis of replication intermediates, but that translation could be strongly inhibited or that structural proteins, including nucleoprotein N, do not accumulate in a proper form. Another hypothesis could be a delayed or ineffective release of complete virions. Altogether, these data indicate that some level of viral genome expression occurs in infected cells, but this process does not appear to result in the production of infectious viral particles, suggesting a restricted or semi-permissive infection of goat MoMs by the IC89 strain.

Further work is needed to precisely understand the observations made within this promising model in order to evaluate its usefulness. It would be interesting to move towards a multiomics approach to understand the different mechanisms involved in PPRV infection of MoMs and MoDCs, in particular those related to innate antiviral immune activity and the link between different immune signaling pathways and the non-structural proteins C and V of morbilliviruses. It would also be relevant to quantify the different cytokines production in the post-infection supernatants of MoMs and MoDCs, and to perform proteomic analysis of these supernatants to detect and characterize viral proteins released during infection. Finally, observing the state of the virion as it leaves the cells under electron microscope would

provide additional structural information on the viral particles produced and their infectious capacity.

Various mechanistic implications depending on cell type and metabolism may explain these differences in viral replication. On the viral side, the expression and the function of essential proteins such as phosphoprotein (P) in combination with polymerase (L) will ensure the replication, transcription, capping and polyadenylation of the viral mRNAs. Similarly, non-structural proteins C and V appear to play roles in viral replication, virulence and immune escape (1). On the host side, the effector cells of innate immunity develop many antiviral mechanisms involving type I and II interferons (IFNs), IFN-stimulated genes (ISGs) and restriction factors that are able of limiting the synthesis or assembly of the viral genome (55,56). MoMs are more restrictive cells, which can be explained by certain antiviral factors. For example, the Zika virus (a flavivirus) replicate much less in human MoMs than MoDCs due to the non-inhibition of STAT1 and STAT2 protein phosphorylation. These proteins mediate an innate antiviral response via type I and III IFNs and ISGs (57,58). If the antiviral response intensifies, the production of complete infectious viral particles will be limited, but abortive replication will still be possible. In other words, the virus can enter the host cell and synthesize viral RNA and structural proteins, but virion assembly will be incomplete or impaired and thus defective viral particles will be excreted. This semi-permissiveness is consistent with what is observed for the IC89 strain in goat MoMs.

Early activation of interferon-dependent antiviral pathways could be responsible for the selective blocking of viral protein translation while maintaining partial genome replication. Protein kinase RNA-activated (PKR) is an IFN-induced protein that influences translation. The accumulation of dsRNA activates PKR, which then phosphorylates eukaryotic initiation factor 2α (IF2α), thus initiating global inhibition of translation (59). In the type I interferon

response, ISGs limit viral infection through various mechanisms, particularly by inhibiting translation and thus restricting the production of viral proteins (60,61).

The viral proteins C and V of morbilliviruses play an important role in regulating signaling pathways and antiviral effects. Protein C interferes with the JAK-STAT signaling pathway, in particular by inhibiting the phosphorylation of STAT1 (62,63). Protein V interacts with Jak1/Tyk2 and interferes with the activation of IFN receptor-associated kinases, thereby blocking the type 1 and type 2 IFN signaling pathways (43,64,65). The MAVS/RIG-I and JAK-STAT pathways are connected to another major signaling pathway, the nuclear factor-kappa B (NF-κB) pathway (66). The NF-κB pathway is a central transcription factor in innate immunity and is an important regulator of the inflammatory response to pathogens (67,68). The non-structural proteins C and V can interact with the p65 subunit of NF-κB and modulate NF-κB-regulated transcriptional activity (69), as is the case with the measles virus. In the case of the IC89 strain in goat MoMs, it is possible that activation of the NF-κB pathway is not completely inhibited. The antiviral response is therefore activated and the productive viral cycle is blocked. Conversely, in other cases, for example MA08 in goat MoMs, the NF-κB pathway is blocked and viral replication is more efficient. All of this suggests that this signaling pathway is an important element in cellular permissiveness and could be a specific signature between strain and host species. These hypotheses could be tested by evaluating NF-κB activation by assessing p65 phosphorylation under different conditions. Finally, it would be interesting to observe the effect on viral titers in the presence vs absence of NF-κB inhibitor (70) or JAK inhibitor (71).

Additionally, it is possible that the varying ability of PPRV strains to regulate autophagy via the phosphoinositide 3 kinase/AKT/mammalian target of rapamycin or PI3K/AKT/mTOR axis has a role in the contrasting permissiveness observed in goat MoMs. The CDV V protein

induces autophagy via the PI3K/AKT/mTOR pathway, again by modulating the phosphorylation of these proteins. Inhibition of the phosphorylation of these proteins promotes viral replication. Conversely, activation of PI3K inhibits the expression of autophagy and, by extension, also inhibits replication (72). Proteomic and transcriptomic studies of our infection model would allow the exploration of these various hypotheses.

In summary, we highlighted here the difference in permissiveness to PPR virus strains between MoDCs and MoMs. As demonstrated by the results we obtained with the two strains IC89 and MA08 in the two cell models obtained from three different hosts (goats, sheep, cattle), in vitro infections of MoDCs may enable us to determine whether a given host is susceptible to the PPR virus, and MoMs could serve as a cell model to determine the virulence of a given strain among susceptible hosts. With IC89 strain, an abortive infection is observed in goat MoMs, while sheep MoMs are completely permissive. MoMs from goat or sheep also remain permissive for the MA08 strain. The use of bovine MoMs and MoDCs in this study constitutes a relevant negative control due to its status as a dead-end host. This combined model of two cell types (MoDCs and MoMs) would be useful for pre-screening studies to assess the sensitivity of species to a given strain of PPRV and thus estimate relative virulence. It would therefore be interesting to test other PPRV strains with goat, sheep and cattle MoMs and MoDCs, and to extend this to other host species.

In 2024 and 2025, Europe has been facing an emergence of PPR, with small ruminants infected in 6 different countries and showing a wide variety of symptoms (73–76). This recent dynamic in PPRV spread shows us that the epidemiological situation remains unstable and that the virus still crosses borders, making it essential to strengthen risk assessment tools and early detection capacities. The risk is also present in areas that interface with wildlife, with cases observed in atypical hosts and large wild ruminants in contact with domestic

animals (46,77–80). Furthermore, the impact of PPRV on wild species remains poorly documented, as does their potential role in the transmission or maintenance of the virus and modulation of virulence. As global eradication efforts led by the Food and Agriculture Organization of the United Nation (FAO) and the World Organization of Animal Health (WOAH) intensify, having tools to anticipate host susceptibility and strain virulence is a major asset in guiding early detection and rapid response measures.

MATERIALS AND METHODS

Cell lines and viral production

All viral strains were produced from Vero expressing canine SLAM receptor (or Vero-Dog-SLAM cells, VDS cells) (35). VDS cell were cultured in complete Dulbecco's Modified Eagle's Medium (DMEM, Gibco) supplemented with 10% fetal bovine serum (FBS, Dominique Dutscher). Virus stocks were harvested from infected cell supernatants, clarified by centrifugation, and stored at –80°C until use. The PPRV strains IC89 (lineage I) and MA08 (lineage IV) are from production used in previous experimental studies (30).

Differentiation of macrophages and dendritic cells from CD14+ monocytes

Peripheral blood samples were obtained from clinically healthy goats (*Capra hircus*, N = 3), sheep (*Ovis aries*, N = 3) and cattle (*Bos taurus*, N = 6) housed at the ASTRE research facility (CIRAD, Baillarguet, France). Animal care and handling followed institutional ethical guidelines and was approved by the Languedoc-Roussillon regional ethics committee

(French CE-LR#36) in the authorized project using animals for scientific purposes APAFIS#2628.

Peripheral blood mononuclear cells (PBMCs) were isolated from the whole blood of the animals studied by Ficoll-Hypaque density gradient centrifugation using Histopaque-1077 (Sigma-Aldrich), following standard protocols (81). After collection, viable PBMCs were counted in a hemocytometer using the Trypan Blue exclusion method.

$CD14^+$ monocytes were positively selected using CD14 MicroBeads UltraPure (Miltenyi Biotec) and LS MACS columns, according to the manufacturer's instructions. After elution, $CD14^+$ monocytes were cultured in Roswell Park Memorial Institute medium and GlutaMAX™ (RPMI 1640 + GlutaMAX™, Gibco™) supplemented with 10% FBS and 1% antibiotics (Penicillin-Streptomycin). A minimum purity of 80% $CD14^+$ monocytes is required to ensure homogeneous differentiation into MoMs or MoDCs (Supplemental fig. S5).

For differentiation into monocyte-derived dendritic cells (MoDCs), cultures were supplemented with in house bovine recombinant IL-4 and GM-CSF (50 ng/ml) From plasmid prepared with EndoFree Plasmid Purification Maxi Kit Qiagen (reference 12362), cytokines were then produced by transfection on HEK 293T cells. For the differentiation into monocyte-derived macrophages (MoMs), no exogenous growth factors were added and monocytes were allowed to spontaneously differentiate into macrophages under standard culture conditions. This approach, although less controlled than cytokine-supplemented protocols, mimics a more physiological environment and results in a heterogeneous macrophage population (82,83). Differentiation was carried out for 6 to 7 days with a medium change on day 2 or 3 (supplemental fig. S4). Viral infection assays were performed at the end of the differentiation period.

Viral infection

MoDCs and MoMs were infected with either MA08 or IC89 strains at a multiplicity of infection (MOI) of 0.1. The cells were incubated with virus for 1 hour at 37°C in serum-free RPMI. After incubation, cells were washed with PBS and cultured for 48 hours in RPMI supplemented with 2% FBS. After 48 h post-infection, the culture supernatants were harvested, aliquoted, and stored at –80°C for downstream analysis.

Flow cytometry

Following infection, adherent cells in culture were detached using TrypLE™ Express Enzyme 1X (Gibco) at 37°C for 10 minutes. Cells were harvested into PBS, centrifuged at 300 x g) for 5 minutes at 4°C, and fixed with 4% paraformaldehyde (or 4% PFA) for 10 minutes at 4°C.

For intracellular PPRV detection, cells were permeabilized with 0.3% saponin and stained with FITC-conjugated monoclonal antibody (clone 38.4) targeting the N nucleoprotein of PPRV developed by G. Libeau (84) and produced by Proteogenix. No secondary antibody was required due to conjugation of Mab 38.4 with FITC. Stained cells were analyzed using a BD FACSCanto II flow-cytometer (BD Biosciences) and FlowJo_V10 software (Supplemental fig. S6).

Titration of PPRV

Viral production and cell culture supernatants were titrated on VDS cells seeded in 96-well plates using the Spearman-Kärber method (85–88). Infectious titers were calculated as

$TCID_{50}$/mL with $TCID_{50}$ calculator developed by Marco Binder. (v2.1—20-01-2017_MB* by Marco Binder; adapted @ TWC, https://www.klinikum.uni-heidelberg.de/fileadmin/inst_hygiene/molekulare_virologie/Downloads/TCID50_calculator_v2_17-01-20_MB.xlsx. The titration plates were marked with antibody 38:4 (anti-NPPRV) coupled with TRITC in order to observe the presence or absence of the virus (Supplemental fig. S3), according to the protocol of Eloiflin *et al.* (89).

RNA extraction from supernatant and real-time qPCR analyses

Viral RNA was extracted from supernatants using the IndiMag Pathogen Kit (384 reactions, Indical Biosciences), following the manufacturer's protocol. RNA isolation was performed on the IDEAL™ 32 automated extraction system (Innovative Diagnostics), based on magnetic bead technology.

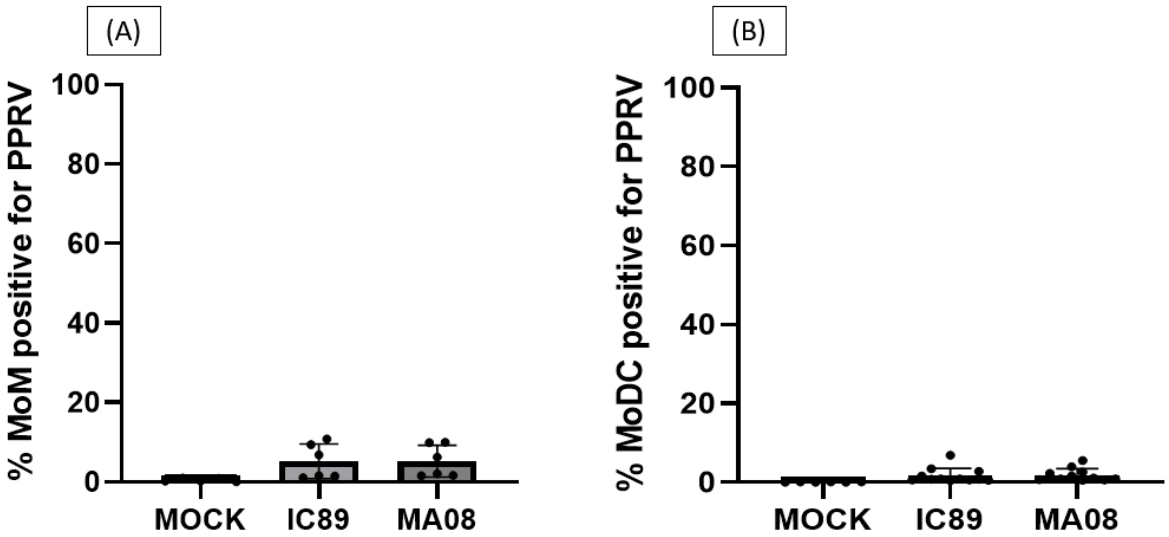
The specific RNA of PPRV present in the supernatants was quantified by real-time PCR by partial amplification of the N protein gene using the ID GeneTM Peste des Petits Ruminants Duplex kit (IDvet), then was performed with LightCycler (Roche).

Immunofluorescence staining and inverted microscopy

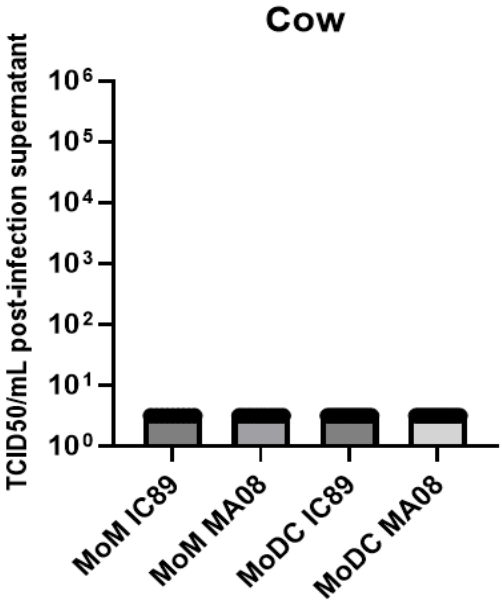
The cells, whether infected with PPRV or not, were fixed at 48 hpi using 4% paraformaldehyde (PFA) for 20 minutes at room temperature. After fixation, permeabilization was performed with 0.1% Triton X-100 in 1X PBS for 5 minutes at room temperature. The cells were then incubated in a buffer composed of 1X PBS, 10% FBS and 1 g BSA per 50 mL for 30 minutes. Primary staining was performed with anti-dsRNA monoclonal antibody J2 (IgG2a, 1 mg/mL, SCICONS™, Jena Bioscience), diluted 1:300, and

incubated for 2 hours at room temperature. For detection, cells were incubated with DAPI diluted 1:1000 (Sigma-Aldrich, MBD0015) and either a Donkey anti-Mouse IgG secondary antibody (H+L) Alexa Fluor™ 555 secondary antibody (2 mg/mL) applied at 4 µg/mL (Thermo Fisher Scientific, Cat# A-31570, RRID:AB_2536180), or with TRITC-coupled anti-NPPRV (Mab 38.4) antibody diluted 1:250. In both cases, incubation took place for 1 hour at room temperature, in the dark. The samples were then imaged using an AXIOVERT A1 inverted microscope (Zeiss, France) with Archimed 6.1.4 software, then analysed with ImageJ (version supplied with Java 1.8.0_172 64-bit).

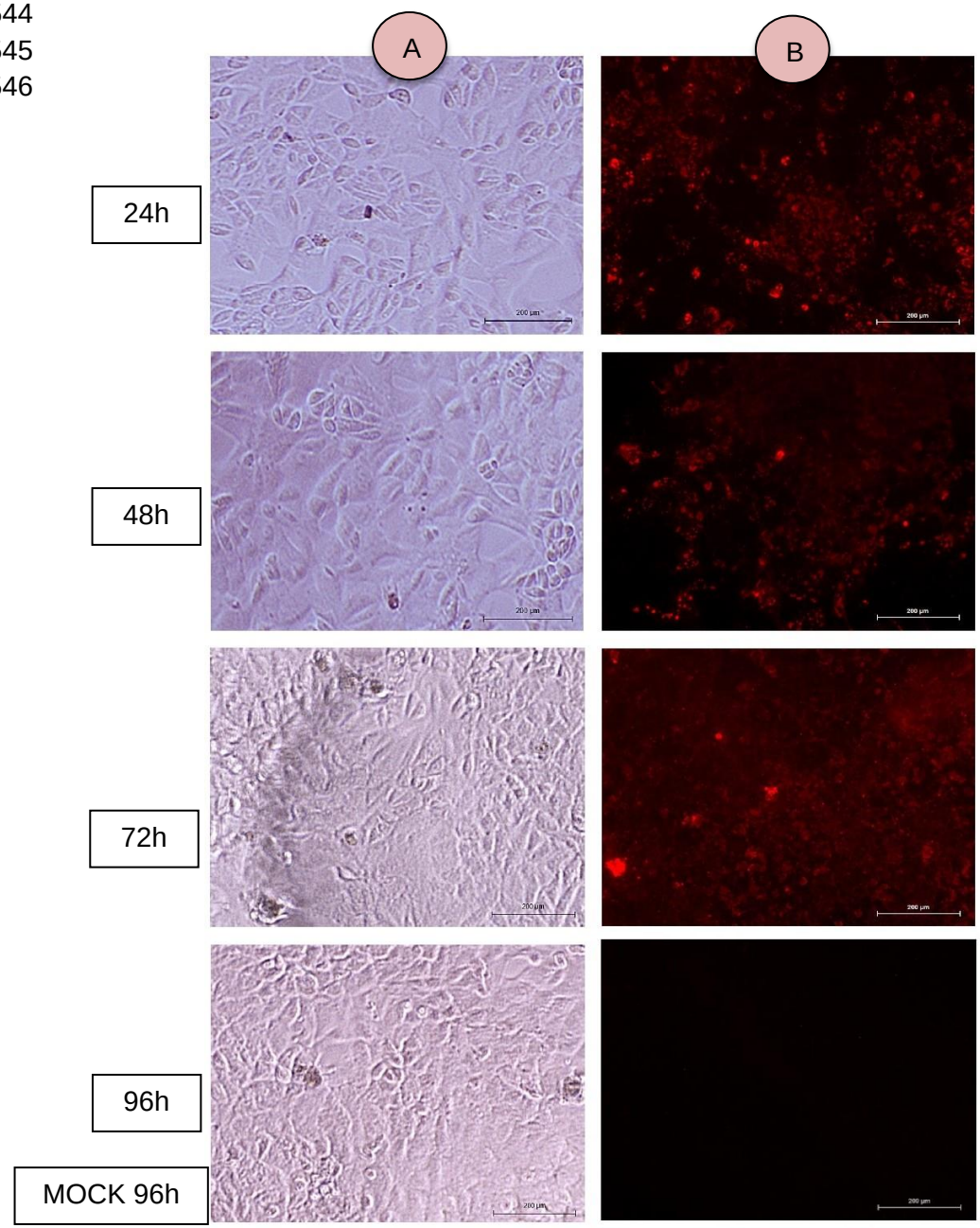
Supplemental Material



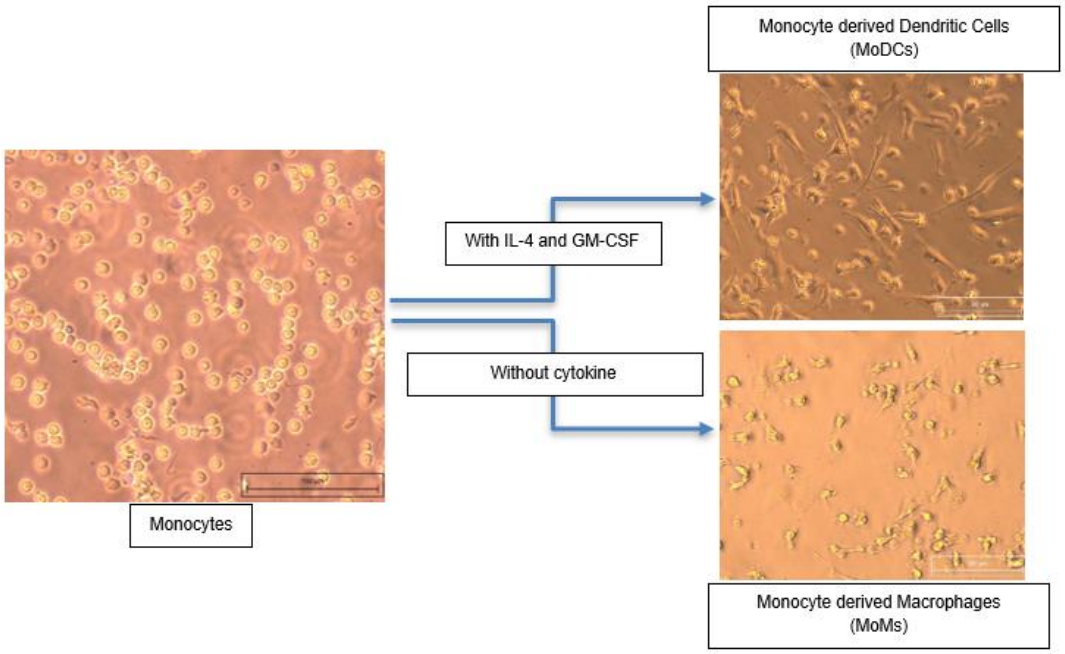
Supplementary Figure S1: Infection of cow APCs with PPRV. Percentages of cow MoMs (A) and MoDCs (B) positive for PPRV at 48hpi after in vitro infection with IC89 or MA08 PPRV strain. The MOCK group corresponds to uninfected control cells. Data are reported as individual biological replicates from 6 cows and with minimum n= 6 replicates, mean ± SD.



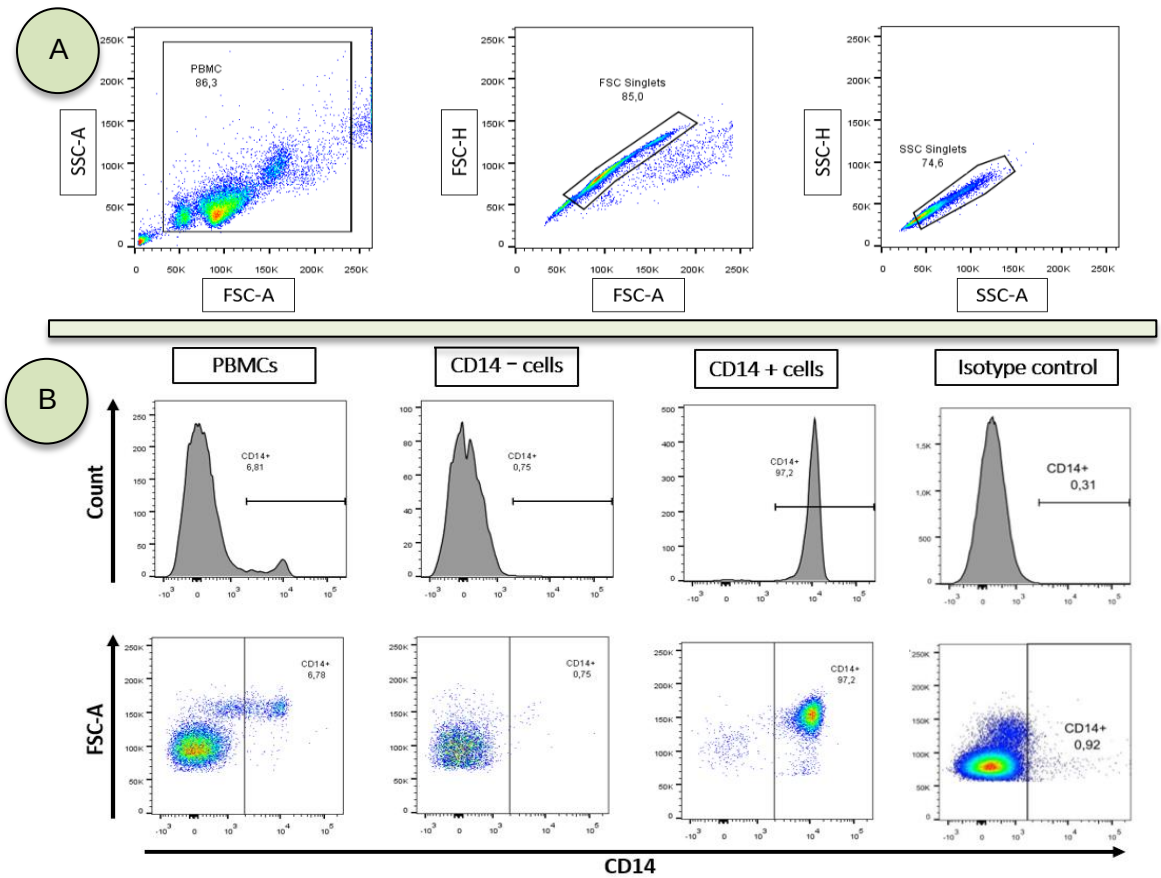
Supplementary Figure S2: Viral titers ($TCID_{50}$/mL) at 48 hpi in cow MoMs and MoDCs. Data are shown as individual biological replicates from 6 cows and with minimum n=9 replicates.



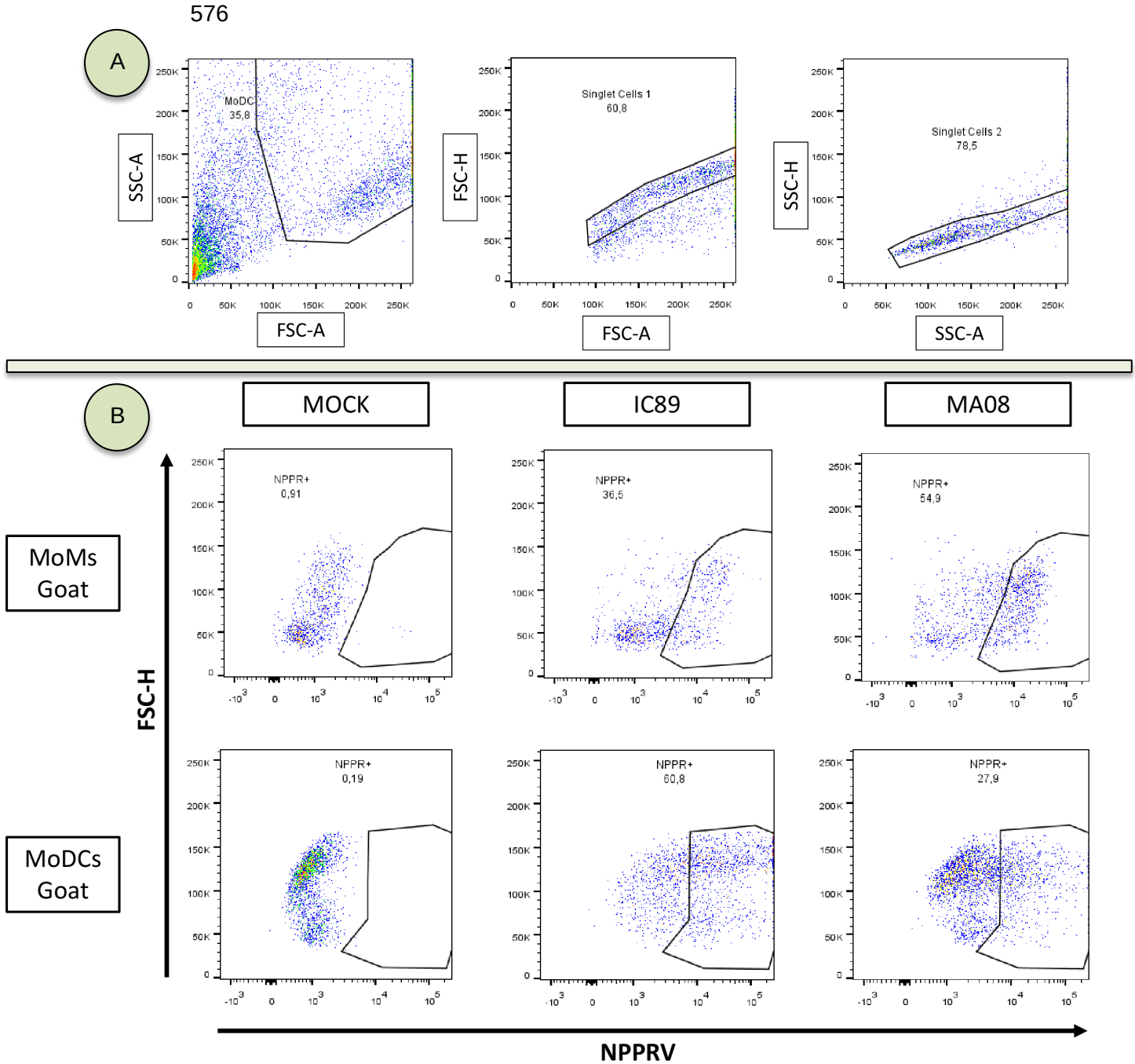
Supplementary Figure S3: Identification of the N protein of PPRV on titration plates of post-infection supernatants from the kinetics of strain IC89 on goat MoMs. VDS cells were labelled with the 38:4 anti-NPPR antibody coupled to TRITC The titration plate was observed with (B) or without (A) Cy3 filter using the Zeiss AXIOVERT A1 inverted microscope (Zeiss, France) with Archimed 6.1.4 software, magnification x20 (scale: 200 µm).



Supplementary Figure S4: Optical microscope image of monocytes and their differentiation into monocyte-derived dendritic cells (MoDCs) and monocyte-derived macrophages (MoMs)



Supplementary Figure S5: (A) Gate selection for PBMCs using FSC/SSC, removal of debris and cell doublets using FSC/SSC dot plots. (B) Isolation of CD14+ target cells discriminated by labelling the cells with an IgG1 isotype anti-CD14 antibody by flow cytometry. Example of PBMCs from a goat. The same types of parameters were set for the other goat, sheep and bovine replicates.



Supplementary Figure S6: (A) Gate selection for MoDCs, removal of debris and cell doublets. The same selection was performed on MoMs. (B) Detection of viral nucleoprotein (NPPR+) by flow cytometry on goat MoMs and MoDCs. The same types of parameters have been implemented for other goat, sheep and cattle replicas.

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Declarations of interest

The authors declare no conflict of interest.

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