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 infection outcome, because these cells transport peste des petits ruminants virus (PPRV) 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 and cattle) to evaluate the differential ability of APCs to be infected by two strains of PPRV with contrasting virulence in goats. Infection was analysed using flow cytometry, infectious titers ($TCID_{50}$/mL), RT-qPCR and immunofluorescence. Goat and sheep MoDCs proved highly permissive to both 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. Cattle MoDCs 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 revealed double-stranded RNA but no nucleoprotein of the low-virulent strain in goat MoMs, suggesting a partial engagement of the viral cycle, resulting in a restricted or semi-permissive infection. Overall, 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 economies, food security and biodiversity. The sensitivity of host species to PPRV infection varies considerably, with clinical manifestations and disease progression being shaped by the viral strain, host species and breed infected. 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, which includes 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 the 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. PPR can cause severe clinical manifestations in sheep and goats**,** with high morbidity and mortality, leading to significant economic losses for farmers. Given its high transmissibility and significant economic impact, PPR is classified as a notifiable disease by the World Organisation for Animal Health (WOAH, formerly OIE) [6], and has been the focus of a control programme jointly led by 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

71 strain, host species and breed infected[8,9]. A better understanding of PPRV virulence and host
72 susceptibility is considered a research priority for disease control [10].

73 The main targets of PPRV and other morbilliviruses are immune cells via the SLAMF1
74 (Signalling Lymphocytic Activation Molecule Family 1) receptor, also known as CD150 [11,12],
75 and epithelial cells via the Nectin-4 receptor [13]. At the early stage of infection, morbilliviruses
76 infect antigen-presenting cells (APCs), such as macrophages and dendritic cells, which
77 transport the viral particles to lymphoid organs, in particular lymph nodes and tonsils, where
78 lymphocytes reside [1]. Infection of epithelial cells occurs once viraemia is established, with the
79 Nectin-4 receptor playing an essential role in viral dissemination and excretion [11,13].

80 For PPRV, direct infection of APCs in vivo has not yet been demonstrated. However, draining
81 lymphoid tissues such as the tonsils and lymph nodes, are the first major sites of replication [14].
82 Recent studies have shown that monocyte-derived dendritic cells are permissive to PPRV in
83 vitro [15]. Permissiveness is defined as the ability of the virus to replicate and produce infectious
84 virions. We know that for other morbilliviruses permissiveness varies depending on the cell
85 type and the virus infecting these cells [16,17]. Due to the importance of APCs in morbillivirus
86 infection, it is possible that the observed variability in PPRV virulence and host susceptibility
87 can be related to differences in the viral replication capacity in APCs. Among APCs, dendritic
88 cells and macrophages are essential for detecting viral infections and initiating innate
89 immunity[18,19].

90 So, the study of infection of monocyte-derived macrophages (MoMs) and monocyte-derived
91 dendritic cells (MoDCs) could be a relevant and reproducible model for comparing the
92 permissiveness of host species to PPRV. Here, we will focus on the infection of MoDCs and
93 MoMs from goats, sheep and cattle (the latter being identified as dead-end hosts for PPRV [20])
94 with two strains of PPRV. The two strains used in this study, Morocco 2008 (MA08) and Ivory

95 Coast 1989 (IC89), demonstrated different levels of virulence in certain goat breeds [21]. Strain
96 MA08 belongs to lineage IV of PPRV and was isolated from an Alpine goat that was severely
97 affected during the 2008 outbreak in Morocco [22]. The IC89 strain belongs to lineage I. It was
98 isolated from a goat in Ivory Coast in 1989 and is considered to be a mildly virulent strain,
99 inducing a mild disease in various goat breeds [21,23,24], although it is used as a virulent strain for
100 the study of immune response in sheep [25].

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

106 RESULTS

107 1. PPRV infections of MoMs and MoDCs

108 To determine the infectivity of the two PPRV strains, IC89 and MA08, for macrophages and
109 dendritic cells, circulating monocytes from the blood of the different animal species studied
110 were differentiated into dendritic cells (MoDCs) and macrophages (MoMs). These two cell
111 types were infected with each of the viral strains studied, IC89 and MA08, for 48 hours at a
112 multiplicity of infection (MOI) of 0.1. Infection was assessed by flow cytometry after
113 intracellular labelling with an antibody targeting the virus nucleoprotein (anti-NPPRV) coupled
114 to a fluorochrome.

115 In goat MoMs, an average of 20% of the cells were positive for the N protein of PPRV strain
116 IC89, compared with an average of 32.6% for those infected with strain MA08. However, no
117 significant difference was observed (Figure 1A). In addition, for goat MoDCs, no significant

118 difference was observed between the two viral strains and in most cases the cells were positive
119 for NPPRV with a range of 40 to 73% (Figure 1B). Intercellular comparison (i.e. MoMs versus
120 MoDCs) revealed a significant difference in the rate of NPPRV-positive cells, with MoDCs
121 being twice as infected as MoMs. This was observed for both PPRV strains (Fig. 2A and 2B).

122 For sheep MoMs, there was no significant difference in the rate of NPPRV-positive cells
123 between the two PPRV strains, and the variability of responses was high, with an average rate
124 of 14.7% for IC89 and 21% for MA08 (Fig. 1A). Similarly, for sheep MoDCs, there was no
125 significant difference between these two viral strains in terms of percentage of NPPRV-positive
126 cells. In both cases, the average was 30%, with comparable extreme values (Fig. 1B). A
127 significant difference was observed between sheep MoMs and MoDCs infected with IC89,
128 again MoDCs being about twice as infected as MoMs ($P<0.0001$) (Fig. 2A). In the case of
129 PPRV strain MA08, the infection rate of MoDCs was significantly higher than in MoMs
130 ($P<0.001$), with an average of 30% compared to 22% for MoMs (Fig. 2B).

131 MoMs and MoDCs from cattle were also infected with IC89 and MA08 strains using the same
132 parameters. Flow cytometry analysis after intracellular staining of the NPPRV protein revealed
133 very low levels of NPPRV-positive cells, no matter which cell type and viral strain was used
134 (Fig. 1A, 1B)

135 2. Replication of PPRV in MoMs and MoDCs

136 To study viral replication in MoMs and MoDCs of different hosts, post-infection supernatants
137 were added to cultures of SLAM-expressing Vero cells (VDS) [26] at dilutions from 10^{-1} to 10^{-7}
138 with a minimum of four replicates per dilution. The cytopathic effect (CPE) was observed under
139 the microscope and viral titers were calculated as described in Materials and Methods.

140 In goats, post-infection supernatants from MoDCs had, on average for both PPRV strains MA08
141 and IC89, significantly higher titers than supernatants from MoMs ($P<0.0001$) (Fig. 3A and
142 3B). For MoMs, the viral titers of the post-infection supernatants for MA08 reached an average
143 $TCID_{50}$/mL of $3x10^3$ (Fig3B) whereas, for the IC89 strain, the viral titer was barely detectable
144 with an average $TCID_{50}$/mL of 34 (Fig 3A) including 9 out of 17 replicates where no titer was
145 detected (Circled in Fig 3A).

146 In sheep, post-infection supernatants from MoDCs infected with strain MA08 had, on average,
147 significantly higher titers than those from MoMs infected with MA08 (Fig. 3B) with a $TCID_{50}$
148 of around $1.2x10^4$ for MoDCs infected with MA08 compared with $9.8x10^2$ for MoMs infected
149 with MA08 ($P<0.0001$). There were no significant differences between MoMs and MoDCs
150 infected with the IC89 strain even though there was a trend towards higher titers for post-
151 infection supernatants from MoDCs. The IC89 strain yielded a significantly higher mean titer
152 than the MA08 strain in both MoDCs ($P<0.001$) and MoMs ($P<0.0001$) (Supplemental fig. S1).

153 The titration of 48hpi supernatants of the IC89 strain on sheep MoMs versus goat MoMs,
154 indicated a significant difference ($P<0.0001$), with sheep MoMs exhibiting high titers with an
155 average $TCID_{50}$ of $2x10^4$. In contrast, whereas goat MoMs infected with IC89 had titers that
156 are barely or not at all measurable (Fig. 3A). In cattle APCs, no viral titer was detected in the
157 post-infection supernatants (Fig. 3A and 3B).

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

159 a. Infection and replication

160 After observing low or non-detectable viral titers in supernatants of goat MoMs infected for 48
161 hours with the IC89 strain, infection kinetics were studied to see if replication was somewhat
162 delayed for this low virulent strain. Goat MoMs were infected for 24 h, 48 h, 72 h and 96 h, at

163 an MOI of 0.1. As previously, the rate of NPPRV-positive cells was measured using a flow
164 cytometer and post-infection supernatants were collected and viral titers were determined.

165 The results show that the rate of infected cells is relatively stable over time, with an average
166 NPPRV positivity of around 30% regardless of the infection time. No significant increase in
167 the percentage of NPPRV-positive cells was observed between 24hpi and 96hpi (Fig. 4A).

168 The post-infection supernatants from IC89-infected goat MoMs were collected in triplicates per
169 animal throughout the kinetics, and titrated on VDS. No $TCID_{50}$/mL viral titers could be
170 detected for any time point during the kinetics, with values remaining below the test detection
171 threshold (Fig. 4B), and cytopathic effects (CPEs) sometimes observed in a single well of the
172 first dilution (10^{-1}). The titration plates used for this kinetic analysis were then labelled with the
173 monoclonal antibody 38.4 targeting the NPPRV coupled with tetramethylrhodamine
174 isothiocyanate (TRITC) in order to observe the presence or absence of the virus at the cellular
175 level. We observed the presence of the virus in VDS cells in the first dilution, and sometimes
176 up to the second and third dilutions (10^{-2} and 10^{-3}). However, this detection occurred in the
177 absence of a cytopathic effect (CPE), or with CPE limited to a single well in the first dilution,
178 which is insufficient to calculate a reliable viral titer (Supplemental fig. S2).

179 ### b. Detection of viral RNA

180 To determine the presence of viral genomic RNA, an RT-qPCR was performed on post-
181 infection supernatants throughout the kinetic. The cycle number (Cq) between 24 hpi and 48hpi
182 remained stable at around 20.5. A decrease in the Cq value was first observed at 72hpi, falling
183 to an average of 19.5, with an even more significant decrease at 96hpi, when the Cq averaged
184 17.8 (Fig. 4C), representing an increase in genetic material of the IC89 strain detected in
185 supernatants.

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

187 To investigate a possible blockage or slowdown in the replication cycle of the IC89 strain in
188 goat MoMs, immunofluorescence was used to detect separately double-stranded RNA
189 (dsRNA), an indicator of the accumulation of aberrant viral RNAs in morbilliviruses, and the
190 PPRV nucleoprotein (NPPRV), a marker of viral protein expression, in infected MoMs. The
191 profiles obtained were compared with those observed in goat MoDCs. A positive dsRNA signal
192 was observed in both cell types in the presence of IC89, although the dsRNA signal appeared
193 slightly less intense in MoMs (Fig. 5A). The dsRNA signal appeared stronger in goat MoMs
194 when infected by MA08 (Supplemental fig. S3). NPPRV is detected in goat MoDCs infected
195 with IC89, but not in goat MoMs (Fig. 5B).

196

197 ## DISCUSSION

198 PPRV infection can lead to a variety of outcomes ranging from acute symptoms and death of
199 the infected host to subclinical infection. Surveillance and control measures must be adapted to
200 provide an efficient response. However, we have limited capacity to predict disease outcomes
201 in different hosts and prepare accordingly. A better understanding of differences in sensitivity
202 of the host to viral strains, studied in vitro, would make it possible to anticipate disease impacts
203 and develop a predictive model that can demonstrate not only the sensitivity of a host to
204 different PPRV strains but also the differences in virulence expressed by the same strain
205 depending on the host [27,28]. To move towards this objective, we selected two strains of PPRV
206 with distinct virulence profiles in goats, mildly virulent IC89 and highly virulent MA08 [24,29,30].
207 Goat and sheep cells were used because these species are the main natural hosts of PPRV, while
208 bovine cells served as a relevant negative control, as cattle are considered a dead-end host [20,31].

209 This in vitro experimental model focused on a comparative overview of PPRV infection in
210 MoMs and MoDCs in order to describe possible differences in sensitivity and replication profile
211 between cell types, host species and depending on the viral strain.

212 Overall, our study shows that MoDCs from goats and sheep are more permissive to PPRV than
213 MoMs. On average, the rate of infected cells (or NPPRV-positive cells) and viral titers are
214 higher for MoDCs than for MoMs. In cattle, the absence of infection and of measurable
215 replication in MoDCs and MoMs confirms the dead-end host status of this species.

216 Despite significant inter-individual variability observed in the number of cells infected during
217 the experiment, the measurement of viral replication via titration provided a robust and
218 biologically meaningful reading for defining permissiveness. The data obtained 48 hours post-
219 infection at MOI of 0.1 likely reflect the combined effect of several infectious cycles,
220 incorporating both the efficiency of viral replication and the virus's capacity for secondary
221 spread in cell cultures. Experiments at lower MOI and at different time post infection may give
222 additional information on the PPRV primary infectious cycle in both cells, however our results
223 show that the conditions used in our in vitro experiments are suitable to detect differences of
224 interest for the overall goal of this study.

225 No significant differences in viral titers were observed between the IC89 and MA08 viral strains
226 in goat MoDCs. However, in sheep MoDCs, the mean titer of the IC89 strain was approximately
227 0.6 $\log_{10}$ higher that of the MA08 strain. MoDCs results concur with the multiple field and
228 experimental studies showing that cattle are not affected and cannot transmit PPRV, unlike the
229 main small ruminant hosts [20,32]. In small ruminants, MoDCs confirmed their susceptibility to
230 PPRV infection, but did not reveal any significant difference between goats and sheep. This
231 cell model could therefore potentially serve as an indicator of the ability of the virus to infect a
232 given species.

233 In contrast to MoDCs, MoMs are less permissive but more discriminating host cells for PPRV.
234 In goats, strain MA08 infected MoMs more efficiently than strain IC89. Oppositely, IC89
235 produced detectable titers that were significantly higher than MA08 in sheep MoMs.
236 Furthermore, the titers observed with IC89 in sheep MoMs were significantly higher than those
237 obtained in goat MoMs. This suggests that the ability of this strain to replicate will depend on
238 the host species and the type of cell infected. Based on this, and on knowledge of the virulence
239 of these strains in vivo [29,21,24,30,33], we propose that this infection model with MoMs could serve
240 as an indicator of strain-specific virulence in small ruminant breeds and may highlight host-
241 specific signatures of viral adaptation.

242 For the remainder of the study, infection kinetics were performed on goat MoMs infected with
243 IC89, since the discrepancy was most pronounced in this model (i.e. cellular infection was
244 detected by flow cytometry, but the viral titer was undetectable). The objective was to determine
245 whether the absence of a viral titer was due to a delay in replication or a persistent blockage of
246 the viral cycle. Throughout the kinetics (24h to 96h infection), the rate of infected cells
247 remained stable, averaging between 28 and 32% with no significant increase, suggesting
248 continuous infection of a sub-population of macrophages present in the culture. Titrations of
249 supernatants at all time points remained negative, indicating the absence of measurable
250 infectious virion production. Nevertheless, RT-qPCR revealed a significant decrease in Cq
251 between 48, 72 and 96 hpi, reflecting a gradual accumulation of extracellular viral genomic
252 RNA. It should be noted that RT-qPCR does not distinguish between infectious and non-
253 infectious viral particles. In addition, immunofluorescence microscopy of the titration plates
254 showed that viral antigens were present in the cells because of the NPPR protein detection in
255 VDS cells, at different levels but without CPE, which supports the viral titer determined in
256 supernatants and the cytometry results.

257 Despite the absence of measurable infectious titer, the detection of viral RNA in the
258 supernatants suggests an incomplete or an abortive replication cycle. This could result in the
259 release of non-infectious or defective viral particles, which has already been observed with
260 several RNA viruses[34–36], including morbilliviruses[37–40].

261 In negative-sense single-stranded RNA viruses, such as PPRV, viral genome replication is
262 closely linked to nucleocapsid assembly, meaning that dsRNA is not considered a reliable
263 indicator of productive replication [41]. Nevertheless, the presence of dsRNA is more likely to be
264 an indicator of the accumulation of aberrant viral RNA, such as defective interfering RNA (DIs)
265 or defective viral genomes (DVG)[39]. Such DIs and DVGs can be produced during virus
266 production, but the contrasting levels of dsRNA observed in MoMs and MoDCs infected with
267 IC89 suggests differences in the accumulation of these particles during infection. Importantly,
268 no NPPRV signal was detected in MoMs infected with the IC89 strain, unlike in MoDCs. This
269 would suggest that the IC89 viral cycle in MoMs appears to be engaged until the accumulation
270 of aberrant viral RNA forms (DIs or DVGs), while nucleoprotein expression or accumulation
271 is severely limited or absent. Therefore, while partial expression of the viral genome is
272 detectable, this does not result in the production of infectious particles, indicating a restricted
273 or semi-permissive infection of goat MoMs by the IC89 strain.

274 Such a restriction may be associated with the activation of interferon-dependent antiviral
275 pathways known to limit viral protein synthesis[42,43].

276 Further work is needed to precisely understand the observations made within this promising
277 model in order to evaluate its usefulness. It would be interesting to move towards a multiomics
278 approach to understand the different mechanisms involved in PPRV infection of MoMs and
279 MoDCs, in particular those related to innate antiviral immune activity and the link between
280 different immune signalling pathways and the non-structural proteins C and V of

281 morbilliviruses. It would also be relevant to quantify the different production of cytokines in
282 the post-infection supernatants of MoMs and MoDCs, and to perform proteomic analysis of
283 these supernatants to detect and characterise viral proteins released during infection. Finally,
284 observing the state of the virion as it leaves the cells under electron microscope would provide
285 additional structural information on the viral particles produced and their infectious capacity.

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

299 Since 2024, Europe has been facing the emergence of PPR, with small ruminants infected in
300 seven different countries and showing a wide variety of symptoms [44–47]. This recent dynamic
301 in the spread of PPRV shows that the epidemiological situation remains unstable and that the
302 virus still crosses borders, making it essential to strengthen risk assessment tools and early
303 detection capacities. The risk is also present in areas that interface with wildlife, with cases
304 observed in atypical hosts and endangered wild ruminants in contact with domestic animals [48–
305 52]. Furthermore, the impact of PPRV on wild species remains poorly understood, as does their

306 potential role in the transmission or maintenance of the virus and modulation of virulence. As
307 global eradication efforts led by FAO and WOAH intensify, having tools to anticipate host
308 susceptibility and strain virulence would be a major asset in guiding early detection and rapid
309 response measures.

310 MATERIALS AND METHODS

311 Cell lines and viral production

312 All viral strains were produced from Vero expressing the canine SLAM receptor (or Vero-Dog-
313 SLAM cells, VDS cells) [26]. VDS cell were cultured in complete Dulbecco's Modified Eagle's
314 Medium (DMEM, Gibco) supplemented with 10% foetal bovine serum (FBS, Dominique
315 Dutscher). Virus stocks were harvested from infected cell supernatants, clarified by
316 centrifugation, and stored at -80°C until use. The PPRV strains IC89 (lineage I) and MA08
317 (lineage IV) are from productions used in previous experimental studies [21]. Any risk of genetic
318 drift of PPRV strains during cell passages was assessed by sequencing the full genome of the
319 virus production used for the infection experiments using a protocol described elsewhere[53].
320 Genomic comparison showed that no mutations were detected following the passages required
321 for production.

322

323 Differentiation of macrophages and dendritic cells from CD14+ monocytes

324 Peripheral blood samples were obtained from clinically healthy goats (*Capra hircus*, N = 3),
325 sheep (*Ovis aries*, N = 3) and cattle (*Bos taurus*, N = 6) housed at the CIRAD animal facility
326 (Montpellier, France). Animal care and handling followed institutional ethical guidelines and
327 was approved by the Languedoc-Roussillon regional ethics committee (French CE-LR#36) in
328 an authorised project using animals for scientific purposes APAFIS#2628.

329 Peripheral blood mononuclear cells (PBMCs) were isolated from the whole blood of the
330 animals studied by Ficoll-Hypaque density gradient centrifugation using Histopaque-1077
331 (Sigma-Aldrich), following standard protocols [54]. After collection, viable PBMCs were counted
332 in a hemocytometer using the Trypan Blue exclusion method.

333 $CD14^+$ monocytes were positively selected using CD14 MicroBeads UltraPure (Miltenyi
334 Biotec) and LS MACS columns, according to the manufacturer's instructions. After elution,
335 $CD14^+$ monocytes were cultured in Roswell Park Memorial Institute medium and GlutaMAX™
336 (RPMI 1640 + GlutaMAX™, Gibco™) supplemented with 10% FBS and 1% antibiotics
337 (Penicillin-Streptomycin). A minimum purity of 80% $CD14^+$ monocytes is required to ensure
338 homogeneous differentiation into MoMs or MoDCs (Supplemental fig. S4).

339 For differentiation into monocyte-derived dendritic cells (MoDCs), cultures were supplemented
340 with in-house bovine recombinant IL-4 and GM-CSF (50 ng/ml) From plasmid prepared with
341 EndoFree Plasmid Purification Maxi Kit Qiagen (reference 12362), cytokines were then
342 produced by transfection on HEK 293T cells. For the differentiation into monocyte-derived
343 macrophages (MoMs), no exogenous growth factors were added and monocytes were allowed
344 to spontaneously differentiate into macrophages under standard culture conditions. This
345 approach, although less controlled than cytokine-supplemented protocols, mimics a more
346 physiological environment and results in a heterogeneous macrophage population[55,56].
347 Differentiation was carried out for six to seven days with a medium change on day two or three
348 (Supplemental fig. S5). In goats, characterization based on CD205 marker expression was
349 performed to distinguish monocytes from cells differentiated into MoMs or MoDCs
350 (Supplemental fig. S6A). Similarly, the CD209 marker was used in cattle[57] (Supplemental fig.
351 S6B). To distinguish MoMs from MoDCs in goats and sheep, the CD172a marker was used
352 (Supplemental fig. S6C). In addition, SLAMF9 receptor (homolog of SLAMF1) labeling was
353 also performed on goat cells (Supplemental fig S6D). Viral infection assays were performed at

354 the end of the differentiation period. In addition to phenotyping, microscopic observation
355 (Supplemental fig. S5) confirmed that the cells had been differentiated in agreement with
356 previous work[15,57,58].

357

358 ## Viral infection

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

364

365 ## Flow cytometry

366 Following infection, adherent cells in culture were detached using TrypLE™ Express Enzyme
367 1X (Gibco) at 37°C for 10 minutes. Cells were harvested into PBS, centrifuged at 300 x g) for
368 5 minutes at 4°C, and fixed with 4% paraformaldehyde (or 4% PFA) for 10 minutes at 4°C.

369 For intracellular PPRV detection, cells were permeabilized with 0.3% saponin and stained with
370 FITC-conjugated monoclonal antibody (clone 38.4) targeting the N nucleoprotein of PPRV
371 developed by G. Libeau [59] and produced by ProteoGenix (Schiltigheim, France). No secondary
372 antibody was required due to conjugation of Mab 38.4 with FITC. Stained cells were analyzed
373 using a BD FACSCanto II flow-cytometer (BD Biosciences) and FlowJo_V10 software
374 (Supplemental fig. S7).

375 For phenotyping, the primary antibodies anti-CD14 (CAM36A), anti-CD205 (ILA114A), anti-
376 CD209 (209MD26A), anti-CD172a (DH59B) and anti-SLAMF9 CACT206A were provided

377 by WSU Monoclonal Antibody Center Veterinary Microbiology and Pathology (Washington
378 State University – Pullman). The secondary antibodies used, rat anti-mouse IgG1,
379 eBioscience™, PE-Cyanine7 Clone M1-14D12 (Reference: 24-4015-82), Invitrogen by
380 Thermo Fisher Scientific and Alexa Fluor™ goat anti-mouse IgG2a (Reference: A21241),
381 Invitrogen by Thermo Fisher Scientific.

382

383 ## Titration of PPRV

384 Viral production and cell culture supernatants were titrated on VDS cells seeded in 96-well
385 plates using the Spearman-Kärber method [60–63]. Infectious titers were calculated as $TCID_{50}$/mL
386 using the $TCID_{50}$ calculator developed by Marco Binder. (v2.1—20-01-2017_MB* by Marco
387 Binder; adapted @ TWC https://www.klinikum.uni-
388 heidelberg.de/fileadmin/inst_hygiene/molekulare_virologie/Downloads/TCID50_calculator_v
389 2_17-01-20_MB.xlsx). The titration plates were marked with antibody 38:4 (anti-NPPRV)
390 coupled with TRITC in order to observe the presence or absence of the virus (Supplemental fig.
391 S2), according to the Eloiflin *et al.* [64] protocol.

392

393 ## RNA extraction from supernatant and real-time qPCR analyses

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

398 RNA of PPRV present in the supernatants was quantified using real-time PCR by partial
399 amplification of the N protein gene using the ID GeneTM peste des petits ruminants Duplex kit
400 (Innovative Diagnostics) and then performed with LightCycler (Roche).

401

402 Immunofluorescence staining and inverted microscopy

403 Cells were fixed at 48 hpi using 4% paraformaldehyde (PFA) for 20 minutes at room
404 temperature. After fixation, permeabilization was performed with 0.1% Triton X-100 in 1X
405 PBS for 5 minutes at room temperature. The cells were then incubated in a buffer composed of
406 1X PBS, 10% FBS and 1 g BSA per 50 mL for 30 minutes. Primary staining was performed
407 with anti-dsRNA monoclonal antibody J2 (IgG2a, 1 mg/mL, SCICONS™, Jena Bioscience),
408 diluted 1:300, and incubated for 2 hours at room temperature. For detection, cells were
409 incubated with DAPI diluted 1:1000 (Sigma-Aldrich, MBD0015) and either a Donkey anti-
410 Mouse IgG secondary antibody (H+L) Alexa Fluor™ 555 secondary antibody (2 mg/mL)
411 applied at 4 µg/mL (Thermo Fisher Scientific, Cat# A-31570, RRID:AB_2536180), or with
412 TRITC-coupled anti-NPPRV (Mab 38.4) antibody diluted 1:250. In both cases, incubation took
413 place for 1 hour at room temperature, in the dark. The samples were then imaged using an
414 AXIOVERT A1 inverted microscope (Zeiss, France) with Archimed 6.1.4 software, and
415 analysed with ImageJ (version supplied with Java 1.8.0_172 64-bit).

416

417 Statistical analysis

418 Statistical analyses and histograms were performed using GraphPad Prism v.10.6.1 and R
419 software v.4.5.2. For the analysis of infection rates based on the number of cells infected by the
420 two strains studied on the different hosts, generalized linear mixed models (GLMM) with a
421 binomial distribution were used. Species, cell type, and strain were included as fixed effects,

422 and animal identify and/or experimental batches was included as a random effect to account for
423 repeated measurements within individuals.
424 Viral titers were analysed using generalized linear mixed-effects models with a negative
425 binomial distribution. Species, cell type and strain were included as fixed effects and animal
426 identify and/or experimental batches as a random effect.
427 For kinetic experiments, the analysis was performed using generalised mixed models with
428 binomial or beta-binomial distribution in cases of overdispersion, with post-infection time (hpi)
429 as a fixed effect and animal identify and/or experimental batches as a random effect.
430 RT-qPCR data from kinetics were analysed using linear mixed models (LMM) with the
431 restricted maximum likelihood (REML) method, with hpi as a fixed effect and animal identify
432 as a random effect to account for repeated measurements in the same animal.
433 Overdispersion and zero-inflation were evaluated where appropriate. Pairwise comparisons
434 between groups were performed using Tukey's multiple comparison test with adjustment for
435 multiple testing.
436
437
438 Figure legends
439
440 **Figure 1: Percentage of MoM (A) and MoDC (B) from cow, sheep and goat, positive for PPRV at 48hpi after in vitro infection with**
441 **IC89 or MA08 strain.** The MOCK group corresponds to uninfected control cells. Data are shown as individual biological replicates from a
442 minimum of three animals per species and per condition with mean ± SD; ns=non-significant; *P<0.05; Statistical analysis was performed
443 using a generalized linear mixed-effects model (GLMM). Post-hoc comparisons were conducted using estimated marginal means with Tukey
444 adjustment for multiple comparisons.
445
446 **Figure 2: Percentage of MoM and MoDC from sheep and goat, positive for PPRV at 48hpi after in vitro infection with IC89 (A) or**
447 **MA08 (B) strain.** The MOCK group corresponds to uninfected control cells. Data are shown as individual biological replicates from a
448 minimum of three animals per species and per condition with mean ± SD; ***P<0.001; ****P<0.0001; Statistical analysis was performed
449 using a generalized linear mixed-effects model (GLMM). Post-hoc comparisons were conducted using estimated marginal means with Tukey
450 adjustment for multiple comparisons.
451
452 **Figure 3: Viral titers ($TCID_{50}$/mL) of IC89 (A) and MA08 (B) at 48 hpi in cow, sheep and goat MoMs and MoDCs.** Nine of seventeen
453 replicates were below the limit of detection in goat MoMs infected with IC89 (circled in A). Data are shown as individual biological replicates
454 from a minimum of three animals per species and per condition with mean ± SD; ns=non-significant; ****P<0.0001. Replicates from goat
455 with no titer observed are highlighted with a circle. Statistical analysis was performed on viral titers using a generalized linear mixed-effects

model (GLMM) fitted with a negative binomial distribution. Post-hoc comparisons were performed using estimated marginal means with Tukey adjustment for multiple comparisons.

Figure 4: (A) 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=7 replicates, mean ± SD. A beta-binomial GLMM revealed no significant effect of time post-infection on infection rates (all $P > 0.76$). Post-hoc pairwise comparisons using Tukey-adjusted estimated marginal means confirmed the absence of significant differences between 24, 48, 72 and 96 hpi (all adjusted $P > 0.99$). **(B) Viral titration kinetics of MoMs supernatants from goats infected with strain IC89 at 24, 48, 72 and 96hpi.** Data are shown as individual biological replicates from 3 goats with n=9 replicates per time point. **(C) 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 from 3 goats with n ranging from 12 to 20 replicates per time point, mean ± SD; *$P<0.05$; $P<0.0001$. Statistical analysis was performed using a linear mixed-effects model (LMM) fitted by restricted maximum likelihood (REML). Pairwise comparisons were conducted using estimated marginal means with Tukey adjustment for multiple comparisons (Kenward–Roger approximation for degrees of freedom).

Figure 5: Immunofluorescent detection of viral double-stranded RNA (dsRNA) (A) and Nucleoprotein PPRV protein (NPPRV) (B) in goat MoMs and MoDCs infected with IC89. Infected and uninfected (MOCK) MoMs and MoDCs were stained with DAPI to identify cell nuclei, dsRNA (A) and NPPRV (B) are detected here in red, scale bar = 25 µm.

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Declarations of interest

The authors declare no conflict of interest.

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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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The order of authors reflects the relative contributions of the individuals listed up to S. Guendouz. The last three authors, P. Holzmuller, P. Totté, and A. Bataille, contributed to the study in supervisory roles.

KEYWORDS: Morbillivirus, monocyte-derived macrophage, monocyte-derived dendritic cell, virulence, host susceptibility

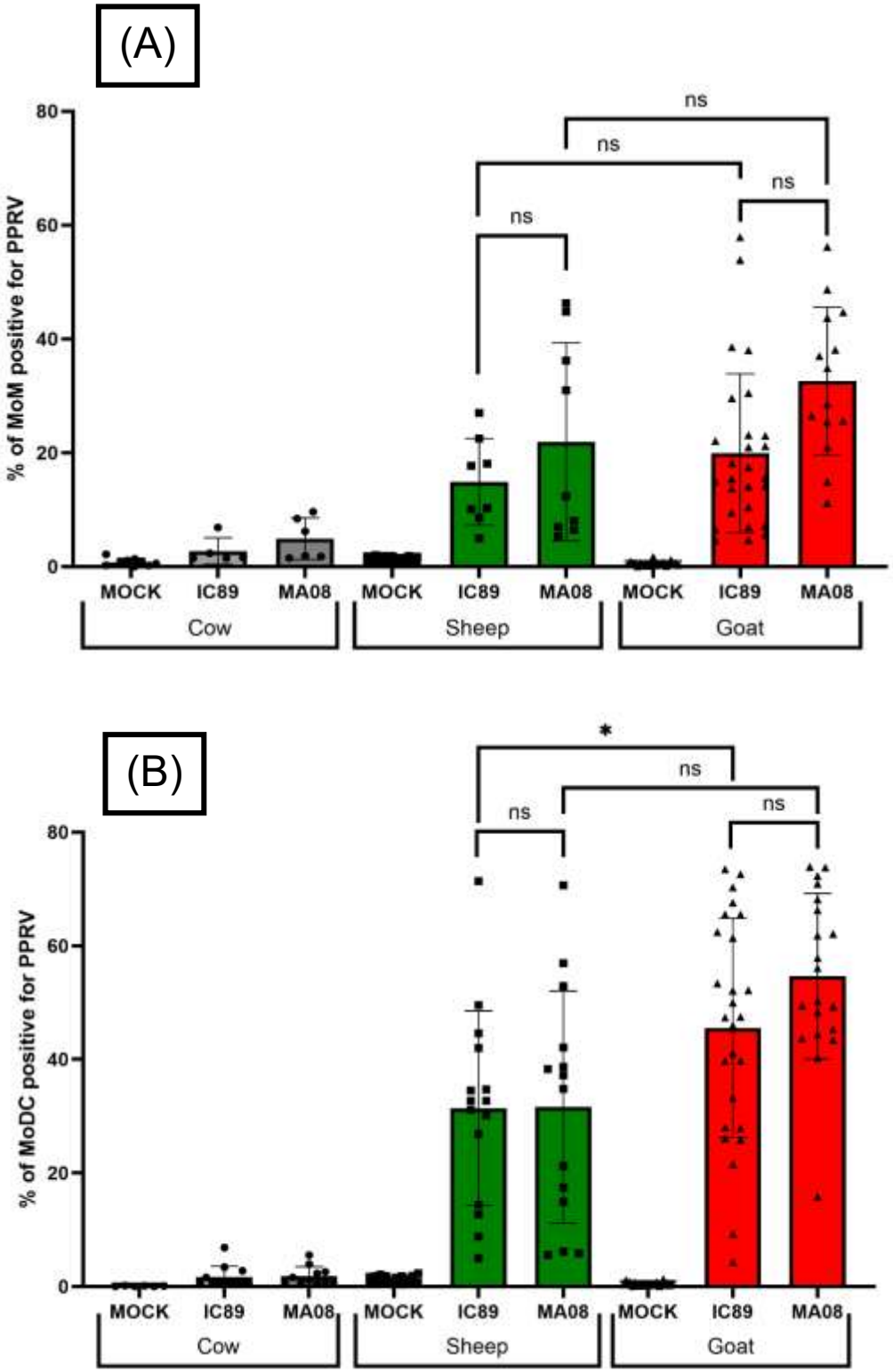

Figure 1: Percentage of MoM (A) and MoDC (B) from cow, sheep and goat, positive for PPRV at 48hpi after in vitro infection with IC89 or MA08 strain. The MOCK group corresponds to uninfected control cells. Data are reported as individual biological replicates from minimum 3 animals per species and per condition with mean ± SD; ns=non-significant; *P<0.05; Statistical analysis was performed using a generalized linear mixed-effects model (GLMM). Post-hoc comparisons were conducted using estimated marginal means with Tukey adjustment for multiple comparisons.

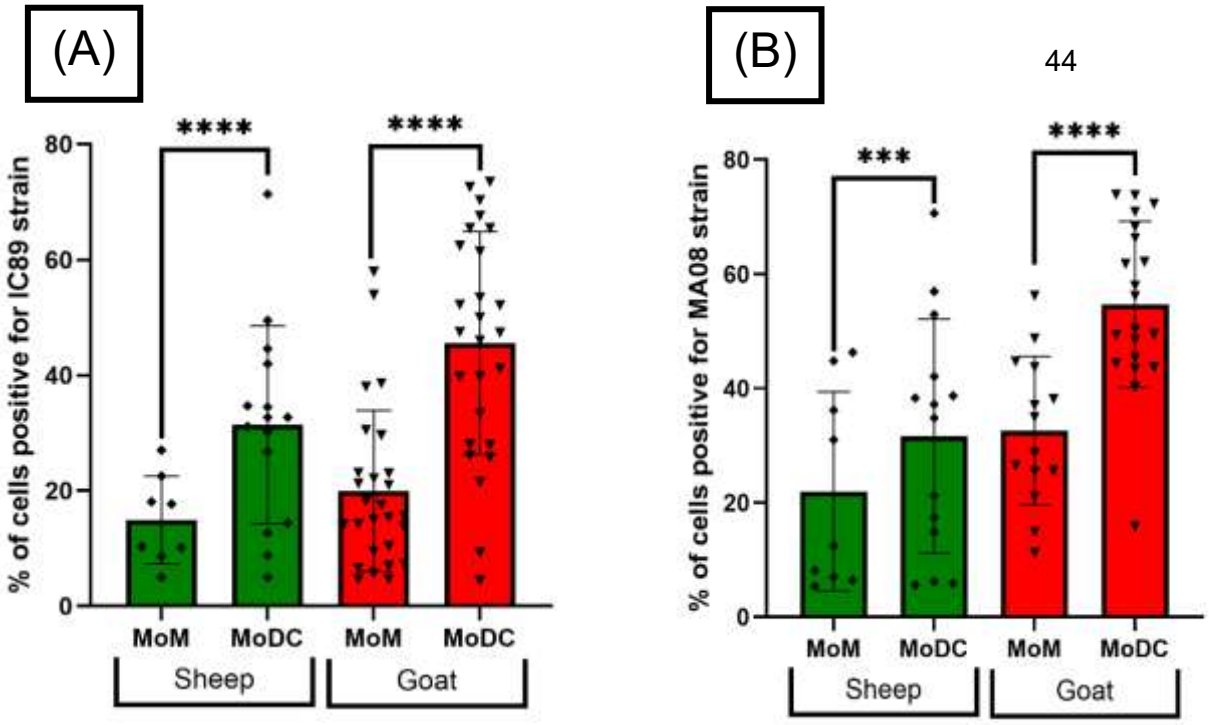

Figure 2: Percentage of MoM and MoDC from sheep and goat, positive for PPRV at 48hpi after in vitro infection with IC89 (A) or MA08 (B) strain. The MOCK group corresponds to uninfected control cells. Data are reported as individual biological replicates from minimum 3 animals per species and per condition with mean ± SD; ***P<0.001; ****P<0.0001; Statistical analysis was performed using a generalized linear mixed-effects model (GLMM). Post-hoc comparisons were conducted using estimated marginal means with Tukey adjustment for multiple comparisons.

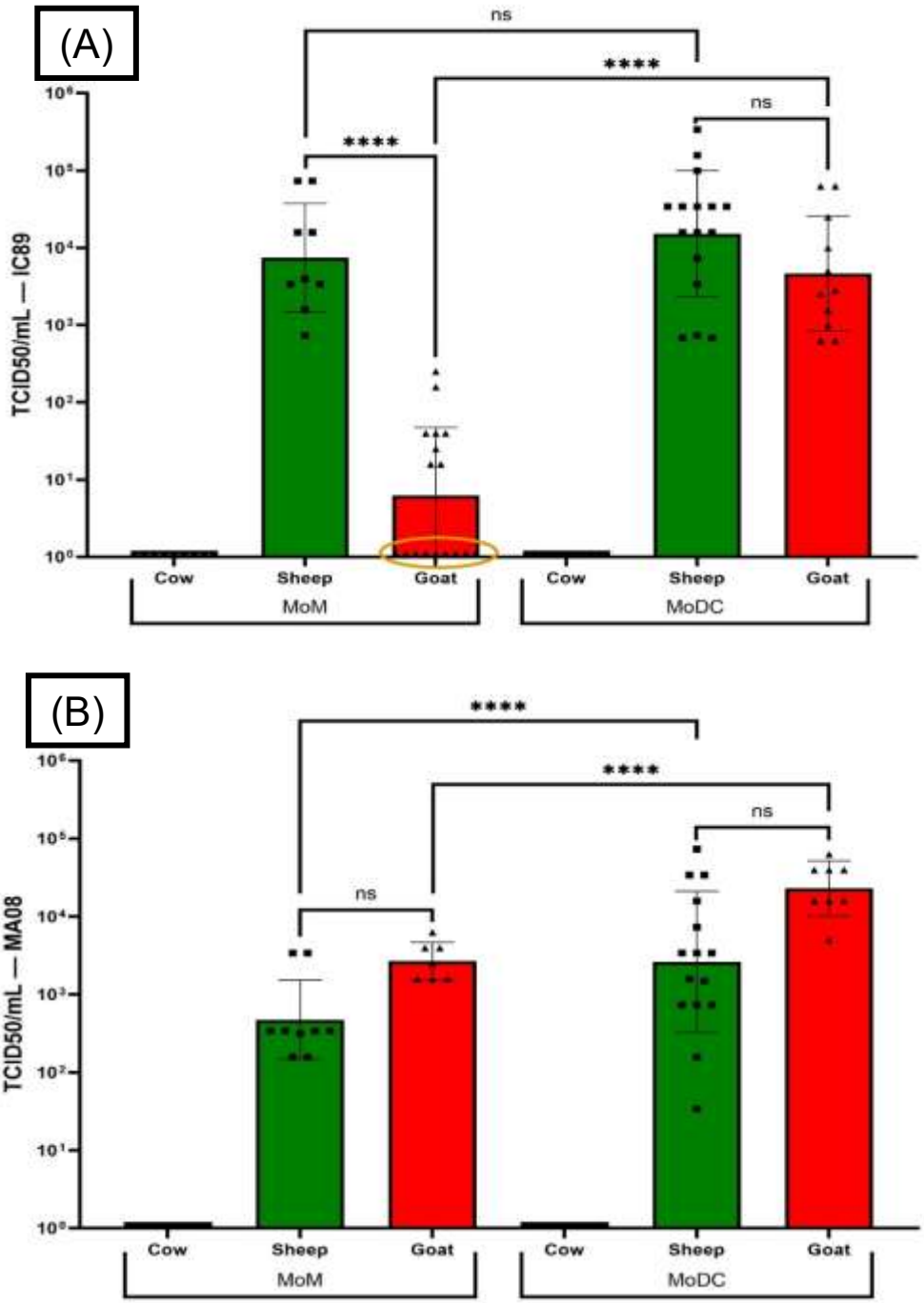

Figure 3: Viral titers ($TCID_{50}$/mL) of IC89 (A) and MA08 (B) at 48 hpi in cow, sheep and goat MoMs and MoDCs. Nine of seventeen replicates were below the limit of detection in goat MoMs infected with IC89 (circled in A). Data are shown as individual biological replicates from a minimum of three animals per species and per condition with mean ± SD; ns=non-significant; ****P<0.0001. Replicates from goat with no titer observed are highlighted with a circle. Statistical analysis was performed on viral titers using a generalized linear mixed-effects model (GLMM) fitted with a negative binomial distribution. Post-hoc comparisons were performed using estimated marginal means with Tukey adjustment for multiple comparisons

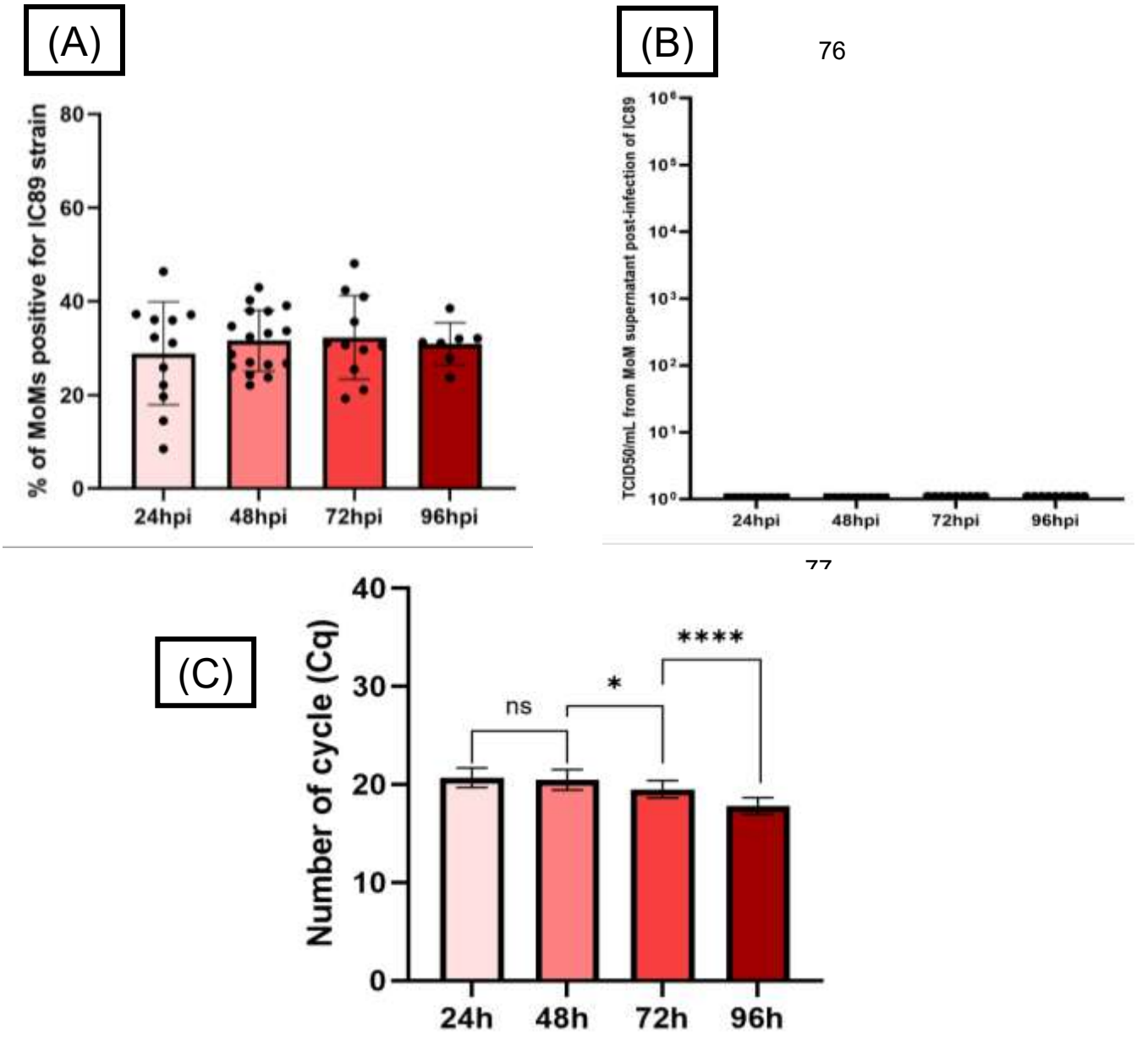

Figure 4: (A) Kinetics of infection of goat MoMs by strain IC89 of PPRV. Percentages of NPPRV+ cells were determined by flow cytometry at 24, 48, 72, and 96hpi. Data are shown as individual biological replicates with 3 goats and with minimum n=7 replicates, mean ± SD. A beta-binomial GLMM revealed no significant effect of time post-infection on infection rates (all P > 0.76). Post-hoc pairwise comparisons using Tukey-adjusted estimated marginal means confirmed the absence of significant differences between 24, 48, 72 and 96 hpi (all adjusted P > 0.99). **(B) Viral titration kinetics of MoMs supernatants from goats infected with strain IC89 at 24, 48, 72 and 96hpi.** Data are shown as individual biological replicates from 3 goats with n=9 replicates per time point. **(C) 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 from 3 goats with n ranging from 12 to 20 replicates per time point, mean ± SD; *P<0.05; P<0.0001. Statistical analysis was performed using a linear mixed-effects model (LMM) fitted by restricted maximum likelihood (REML). Pairwise comparisons were conducted using estimated marginal means with Tukey adjustment for multiple comparisons (Kenward–Roger approximation for degrees of freedom).

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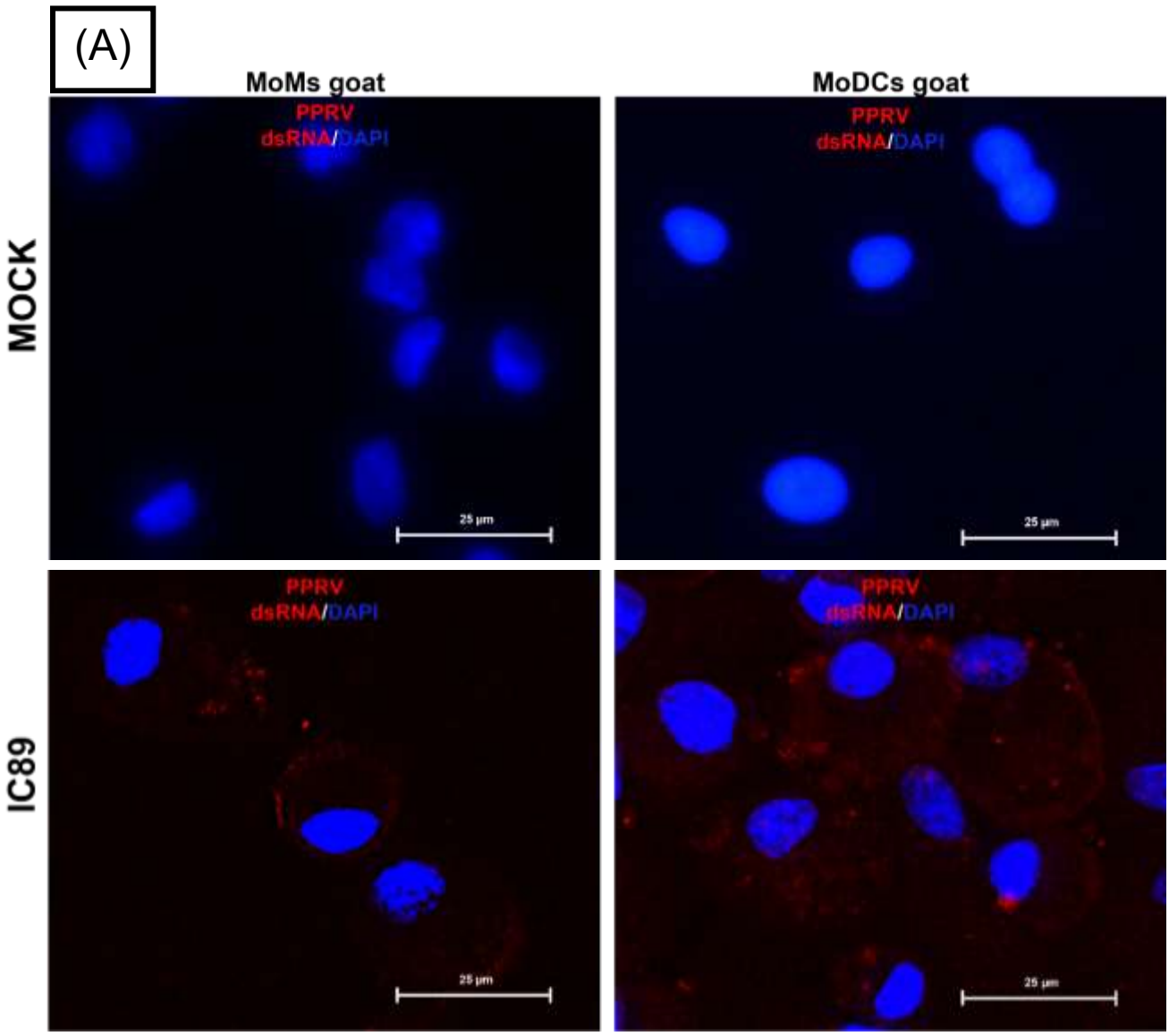

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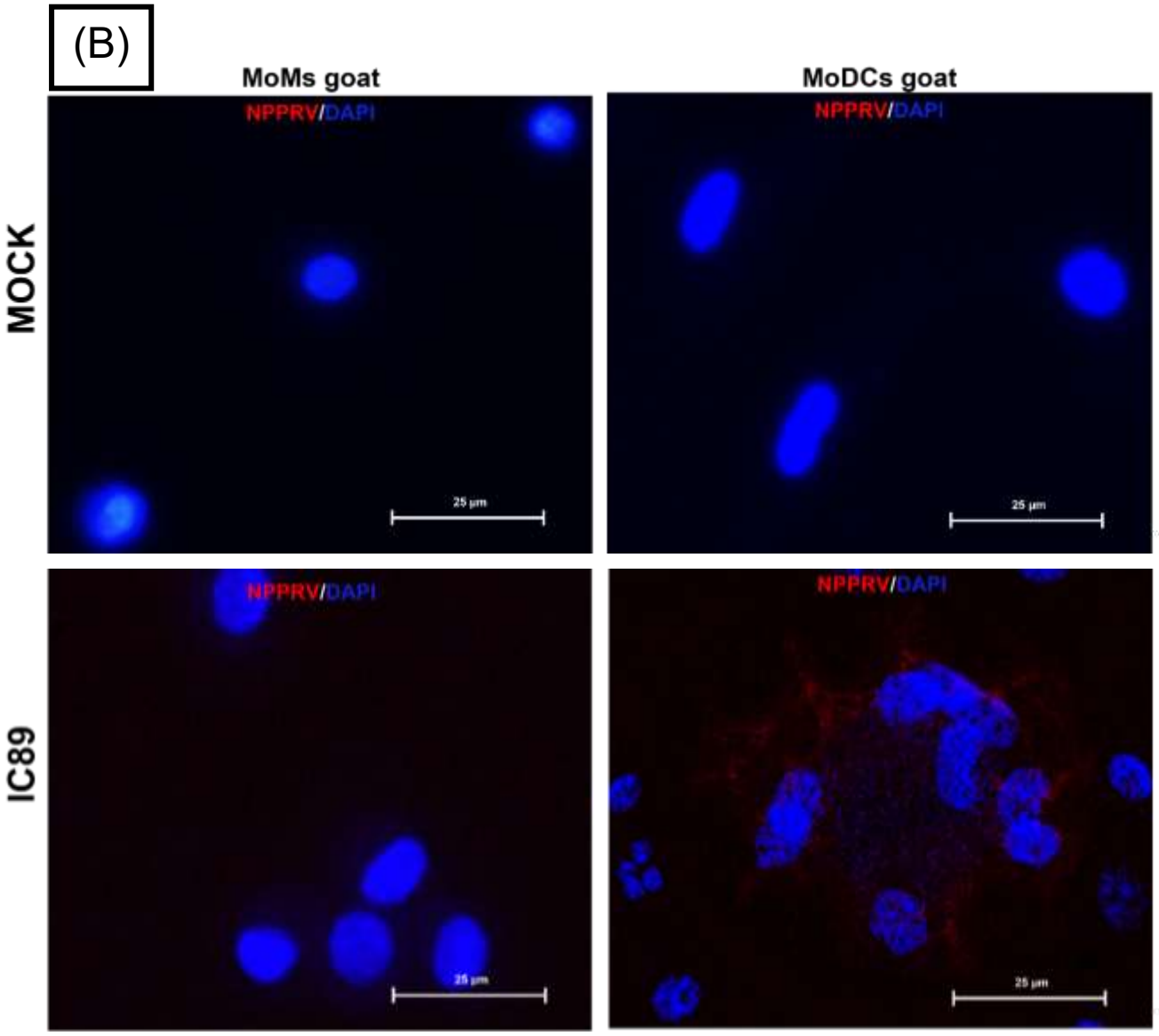

Figure 5: Immunofluorescent detection of viral double-stranded RNA (dsRNA) (A) and Nucleoprotein PPRV protein (NPPRV) (B) in goat MoMs and MoDCs infected with IC89. Infected and uninfected (MOCK) MoMs and MoDCs were stained with DAPI to identify cell nuclei, dsRNA (A) and NPPRV (B) are detected here in red, scale bar = 25 µm.