1 **Ivermectin repurposing for COVID-19 therapy: Safety and pharmacokinetic**

2 **assessment of a novel nasal spray formulation in a pig model**

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22 **Running Head:** *Nasal spray ivermectin for COVID 19 therapy*

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Abstract

High ivermectin (IVM) concentrations suppress *in vitro* SARS-CoV-2 replication. Nasal IVM spray (N-IVM-spray) administration may contribute to attaining high drug concentrations in nasopharyngeal (NP) tissue, a primary site of virus entrance/replication. The safety and pharmacokinetic performance of a new N-IVM spray formulation in a piglet model were assessed. Crossbred piglets (10–12 kg) were treated with either one or two (12 h apart) doses of N-IVM-spray (2 mg, 1 puff/nostril) or orally (0.2 mg/kg). The overall safety of N-IVM-spray was assessed (clinical, haematological, serum biochemical determinations), and histopathology evaluation of the application site tissues performed. The IVM concentration profiles measured in plasma and respiratory tract tissues (nasopharynx and lungs) after the nasal spray treatment (one and two applications) were compared with those achieved after the oral administration. Animals tolerated well the novel N–IVM-spray formulation. No local/systemic adverse events were observed. After nasal administration, the highest IVM concentrations were measured in NP and lung tissues. Significant increases in IVM concentration profiles in both NP-tissue and lungs were observed after the 2-dose nasal administrations. The nasal/oral IVM concentration ratios in NP and lung tissues (at 6 h post-dose) markedly increased by repeating the spray application. The fast attainment of high and persistent IVM concentrations in NP tissue is the main advantage of the nasal over the oral route. These original results are encouraging to support the undertaking of further clinical trials to evaluate the safety/efficacy of the nasal IVM spray application in the treatment and/or prevention of COVID-19.

Introduction

Ivermectin (IVM) [a mixture of 22, 23-dihydro-avermectin B1a (80%) and 22, 23-dihydro-avermectin B1b (20%)], is a macrocyclic lactone, discovered in 1975 by Satoshi Omura as a fermentation product of the actinomycete *Streptomyces avermitilis* and developed in the early eighties to treat parasitic diseases. Its high lipophilicity and efficacy allow its use through different routes, being effective to control endo and ecto-parasites in animals and humans. Shortly after its introduction in the veterinary market, the drug was approved for human use. Nowadays, after decades of intensive, safe and effective use, IVM is indicated to treat several neglected tropical diseases, including onchocerciasis, helminthiases, and scabies. It had also been evaluated for its potential to reduce the rate of malaria transmission by killing mosquitoes (1). Overall, IVM has been widely used, demonstrating an excellent safety profile. Additionally, in the last few years, new knowledge guided the repurposing of the drug towards the treatment of other diseases. IVM antibacterial (2), antiviral[3]and antimitotic activities (4, 5, 6) have been experimentally observed.

IVM antiviral activity against Dengue virus (3), West Nile virus (7), Venezuelan Equine Encephalitis virus (8), and Influenza virus (9), has been reported. Recently, Caly et al. (10) reported that IVM inhibits *in vitro* the replication of SARS-CoV-2 (severe acute respiratory syndrome coronavirus) using high concentrations in the range of 2.5-5 µM. Furthermore, there is now available information from a randomized clinical trial on IVM antiviral activity in SARS-CoV-2 infected patients (11) .The mechanism by which IVM inhibits SARS-COV-2, seems to be the same described for other RNA viruses, i.e. inhibition of transport across the nuclear membrane mediated by importin α/β1 heterodimer, carrier of some viral molecules indispensable for the replication process (12, 13).

SARS-CoV-2 is the etiological agent of Covid-19 (coronavirus disease 2019), a viral disease causing a pandemic since December 2019, inducing from asymptomatic to life-threatening disease. It is highly transmissible with a primary respiratory entrance and airborne transmission, which explains its extensive distribution worldwide. The information available to date indicates that SARS-CoV-2 colonizes the oropharynx and nasopharynx (NP), from where is transmitted even before the appearance of any symptoms. With viral replication in this area (14), the first symptoms (odynophagia, anosmia, dry cough, and fever) and lung parenchyma colonization appear. In the context of the current COVID-19

82   pandemic, it is relevant to determine the best way to administer IVM to optimise its potential

83   in vivo therapeutic usefulness.

84

85   The IVM pharmacokinetic features, based on high lipophilicity and a large volume of

86   distribution, allow its high availability in the respiratory tract (15, 16). Thus, considering the

87   gateway of the virus, the administration of a nasal IVM spray (N-IVM spray) intended to

88   deposit the drug in the upper respiratory tract, could represent a practical tool to expose the

89   of SARS-CoV-2 virus (or the cells where the viral particles are located) to high

90   concentrations of IVM. Hence, a reduction of the viral load at the beginning of the infection,

91   preventing viral replication, transmission and disease aggravation might be achieved.

92

93   Only limited information on inhaled IVM in rats is available (17) and to the best of our

94   knowledge, this is the first time that a nasal IVM spray formulation is developed and its

95   safety and pharmacokinetic performance determined in a pig model, the most appropriate

96   animal model to use in translational research into humans. In an attempt to achieve high

97   IVM concentrations in tissues where entry and transmission of SARS-CoV-2 occurs (where

98   large viral loads are found at the early stages of the infection), the main goal of the work

99   described here was to assess the safety and pharmacokinetic performance of a novel IVM-

100   spray formulation for intranasal administration in piglets. The IVM concentration profiles

101   measured in plasma, NPand lung tissues after the intranasal treatment (one and two

102   applications) were compared with those achieved in the same tissues after the oral (tablets)

103   administration of the antiparasitic dose of 0.2 mg/kg approved for human use. The work

104   reported here is fully supported by recently available scientific evidence on both the

105   potential preventive effect of IVM in SARS-CoV-2 transmission (18), and the concentration-

106   dependent IVM effect on the viral load decay rate observed in a recently completed

107   controlled clinical trial in COVID-19 infected patients (11). This clinical trial was

108   simultaneously performed with the work described here by the same authors as a part of

109   large public-private joint research collaboration in Argentina.

110

111

112 **Material and methods**

113 *Study formulations*

114 N-IVM spray formulation, N-IVM-free methylene blue coloured spray formulation, IVM

115 tablets 2.0 mg and IVM tablets 0.5 mg were developed, manufactured and quality controlled

116 according to Good Manufacturing Practices and supplied byLaboratorio Elea-Phoenix,

117 Argentina. The N-IVM spray was designed to deliver 1 mg IVM, in a 0.1 mL puff. Each

118 container provides 100 puffs and calibrated microdroplets to produce a high NP tissue

119 deposit.

120

121 *Experimental animals*

122 Forty healthy Landrace-Duroc Jersey-Yorkshire crossbred piglets (weighing 10 to 12 kg)

123 were used. The animals were housed in the farm of origin. They were kept with the usual

124 diet during the trial (antibiotic-free diet) and ad libitum access to water. Management and

125 euthanasia of the animals were performed according to approved Good Veterinary

126 Practices (19) and Principles of Animal Welfare (20). The study was fully performed in

127 compliance with ethical, animal procedures *and* management protocols approved by the

128 Ethics Committee on Animal Welfare Policy of Biogenesis Bago, Argentina (Pol-UE 0001).

129

130 *Pre-trial*

131 A pre-trial with the N-IVM-free coloured spray was performed in two animals to assure that

132 the target tissue areas were properly covered after one dose application and to determine

133 the sampling methodology for NP-tissue, based on the observation of colorant presence.

134

135 *Objectives and study design*

136 The main goals of the work described here were: 1) to assess the safety of the N-IVM spray

137 in single and double dose administration to healthy piglets, and 2) to determine the IVM

138 concentration profiles in NP tissue, lung tissue and plasma, at different times after its

139 intranasal spray (one and two applications) and oral administration.

140 The animal phase of the study was conducted at an intensive pig farming establishment ("El

141 Campito", SieteBochos S.R.L., Buenos Aires, Argentina). Clinical laboratory evaluations

142 were performed at Microdiag Laboratory, La Plata, Argentina. The tissue histopathological

143 evaluations, drug analysis and pharmacokinetic evaluation were performed at Centro de
144 InvestigaciónVeterinaria de Tandil (CIVETAN), UNCPBA-CICPBA-CONICET, Tandil,
145 Argentina.

146 The experimental design was based on a three-group animal phase study. Group 1 (22
147 animals) received one dose (2 mg, 1 puff/nostril) of N-IVM-spray, Group 2 (10 animals)
148 received two (2 mg each, 1 puff/nostril) doses N–IVM-spray 12 h apart, and animals in
149 Group 3 (8 animals) were treated with IVM (0.2 mg/kg) oral tablets. Animals with body
150 weight from 10 to 12 kg were selected to allow an equivalent standard-dose treatment for
151 one dose N-IVM administration and one dose oral (0.2 mg/kg) treatment. A summary of
152 dosing and group design is shown in Table I.

153

Safety assessment of the N-IVM spray formulation

155 The overall safety and local tissue tolerability of theN-IVM spray formulation were assessed.
156 The IVM-treated animals were monitored by a careful clinical examination. Vital signs,
157 haematological/serum biochemistry analysis and histopathology of tissues at the drug
158 application area were assessed. All the experimental animals were carefully monitored for
159 adverse effects throughout each dosing period.

160

161 During the first 6 h after administration and then at 12 and 24 h, careful clinical control was
162 performed, looking for any signs of nasal or respiratory discomfort, abnormal behavior, the
163 appearance of the stool, feed and water consumption. Immediately after each drug
164 administration and at 2, 6, 12 and 24 h after treatment, physical/visual examination of the
165 application sites was performed. External and internal mouth inspection was performed to
166 determine any possible adverse effect, which included visual observation of any possible
167 abnormal manifestation in the NP epithelium area as a consequence of the N-IVM spray
168 application.

169

Clinical laboratory evaluation

171 Blood samples were collected at baseline (before treatment) (12 samples) and 24 h after
172 single-dose (6 samples) and double-dose (6 samples) treatments to perform the
173 haematologic and serum biochemical analysis. Haematology included measurements of

174   hematocrit, red blood cell count, hemoglobin, mean corpuscular volume (MCV), mean

175   corpuscular hemoglobin (HCM), mean-corpuscular hemoglobin concentration (MCHC) and

176   counts of leukocytes, band neutrophils, segmented neutrophils, eosinophils, basophils,

177   lymphocytes, monocytes and platelets. Serum concentrations of urea, creatinine, and the

178   activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline

179   phosphatase (ALP) enzymes were determined.

180   ***Blood, nasopharyngeal and lung tissues sampling for drug measurement***

181   Four (4) animals from each of the experimental groups were randomly selected to be

182   euthanized at each sampling time following approved animal guidelines. The head of the

183   animals was opened following a sagittal line, the nasal septum removed and discarded, and

184   a scrape of NP mucosa, submucosa, turbinates and soft palate were integrated to form the

185   NP-tissue sample. After opening the chest, a portion of the upper lobe of the right lung was

186   obtained.

187   Following a single dose of the N-IVM spray administration (Group 1), samples of blood, NP

188   and lung tissues were collected at 2, 6, 12 and 24 h post-dose to measure IVM

189   concentrations. After the double dose N-IVM spray administration (Group 2) and oral

190   administration (Group 3), samples of blood, NP-tissue and lung were collected at 6 and 24

191   h post-dosing. Plasma was separated by centrifugation at 2500 rpm for 15 min. The plasma

192   and collected tissue samples were placed into plastic tubes and frozen at -20°C until

193   analysis byHigh Performance Liquid Chromatography (HPLC).

194

195   ***Histopathological study***

196   NP and oropharynx epithelia were carefully examined post-mortem for assessing possible

197   macroscopic abnormalities induced by the N-IVM spray application. Samples of NP-tissue

198   from the soft palate region were obtained at 24 h post-administration of the N-IVM spray

199   formulation (animals receiving one or two intranasal applications) to perform the

200   histopathological assessment.

201

202   ***Analytical development. Measurement of IVM tissue concentration profiles***

203   Concentrations of IVM in NP-tissue, lung and plasma samples were determined by HPLC

204   with fluorescence detection following the technique previously described (15). An aliquot of

7

205   plasma and tissues were homogenized and combined with moxidectin as an internal

206   standard. Full validation of the analytical procedures used to measure IVM concentrations

207   in the different tissues was performed. After acetonitrile-mediated chemical extraction, IVM

208   was converted into a fluorescent molecule using N-methylimidazole and trifluoroacetic

209   anhydride (Sigma Chemical, St Louis, MO, USA). An aliquot (100 µl) of this solution was

210   injected directly into the HPLC system (Shimadzu Corporation, Kyoto, Japan). The

211   determination coefficients (r^2) of the calibration curves for the different tissues analysed

212   ranged between 0.989 and 0.999. The mean absolute drug recovery percentages were

213   94% (NP-tissue), 86% (lung tissue) and 97% (plasma). The relative error values (accuracy)

214   was in the range between 2.9% and 9.4%. The method exhibited a high degree of inter-day

215   precision with a coefficient of variation below 7%. The limits of drug detection were 0.45

216   ng/g (NP-tissue), 0.19 ng/g (lung) and 0.20 ng/mL (plasma). The limits of quantification

217   (LOQ) were 0.70 ng/g (NP-tissue), 0.30 ng/g (lung) and 0.34 ng/mL (plasma).

218   Concentration values below the quantitation limits were not considered for the

219   pharmacokinetic analysis.

220   ***Pharmacokinetic and statistical analysis of the data***

221   The IVM concentration versus time curves obtained for each tissue/fluid after each

222   experimental treatment were fitted with the PK Solutions 2.0 (Ashland, Ohio, US) computer

223   software. The area under the concentration-time curves (AUC) was calculated by the

224   trapezoidal rule (21) to determine the IVM exposure (tissue availability) at each assayed

225   tissue. The statistical analysis was performed using the Instat 3.0 software (GraphPad

226   Software, CA, US). IVM concentrations after the different treatments were statistically

227   compared using a non-parametric Kruskal-Wallis test. The data from the hematological and

228   biochemical determinations were compared by basic statistical analysis using the Info Stat,

229   2016 software.

230

231   **Results**

232   The N-IVM spray was well tolerated after either one or two applications to the Animal

233   model. The piglets were considered clinically healthy by specialised veterinarians

234   throughout the whole experimental trial. All had normal skin and mucous membranes color,

235   body condition and behaviour activity. No adverse events or intolerance were evident along

236   the whole study period in animals treated either orally or with the spray formulation once or
237   twice.

238   There were no macroscopic changes at the tissue area of spray application at different
239   examination times. Furthermore, no histopathological changes (lesions) were observed in
240   the mucosa or submucosa of the soft palate in spray-treated animals. A mild to moderate
241   inflammation was observed in the tonsils (both before and after spray application), which is
242   normal in pigs because of the immunological role of the area. The serum biochemical and
243   hematological values did not show any alteration that would lead to adverse effects. Range
244   values for hematological and biochemical determinations before and after the
245   administration of the N-IVM spray formulation are summarized in Table II.

246   IVM was recovered in plasma, NP and lung tissues following a single dose application of
247   the N-IVM-spray formulation (Group 1). Although some degree of variability was observed
248   in the patterns of tissue concentration among the animals treated with the spray
249   formulation, the highest IVM concentrations were always measured in NP-tissue.
250   Additionally, high IVM concentrations were measured in lung tissue, with a limited passage
251   into the central compartment (systemic absorption), reflected in the low plasma levels
252   recovered in the animals treated with N-IVM spray. The comparative IVM concentration
253   profiles in NP-tissue, lung and plasma obtained over the first 24 h post-administration (one
254   dose) of the N-IVM spray formulation, are shown in Figure 1.

255   A significant positive correlation between the IVM concentrations in NP and lung tissues (r=
256   0.735) was observed between the 4 h and 24 h post-administration. The IVM exposure
257   (measured as AUC values) was calculated for each of the assayed tissues and plasma. The
258   highest drug exposure after the one dose nasal application was observed at the NP and
259   lung tissues. The drug availability (exposure) expressed as AUC values in each tissue and
260   the relationship (ratio) between IVM exposure in lung and plasma compared to NP tissue
261   are shown in Table III.

262   IVM concentrations were also measured following the two doses of N-IVM-spray
263   administration as well as after the treatment with the oral tablets in pigs. The repeated spray
264   treatment increased significantly the IVM concentrations in NP and lung tissues compared
265   to those measured after the single intranasal administration, without any significant
266   increment on IVM concentrations in the bloodstream, reflecting a limited IVM systemic

267   absorption. These results confirm the hypothesis of a high IVM availability in the NF tissue
268   area following the nasal spray administration. Besides, a high distribution of IVM was
269   observed after its oral administration, with higher lung concentration profiles compared to
270   NP tissue measured at 6 h post-treatment. The comparative mean IVM concentrations in
271   each tissue after the three experimental treatments are shown in Table IV.

272   Using the oral tablet administration as a reference, the relationship between IVM
273   concentrations recovered at 6 h in target tissues (NP and lung) after the spray application
274   was estimated. The spray/oral concentration relationship increased significantly from 0.88
275   (one spray application) to 2.10 (two spray applications) in NP tissue and from 0.24 to 0.63
276   in lung tissue. A less marked increase for the same spray/oral ratio was observed in plasma
277   (from 0.25 to 0.57), as it can be observed in Figure 2.

278   **Discussion**

279   The work reported here illustrates the safety and pharmacokinetic assessment of a novel
280   pharmaceutical formulation aimed to attain high IVM concentrations in NP and lung tissues
281   with low systemic availability. The pig was chosen as the test animal model to assess the
282   safety, application site tolerability and kinetic performance of the new formulation and
283   innovative route of IVM administration. Pigs and humans have anatomical and physiological
284   similarities. The pig is the animal species most used in translational research in studies of
285   pathophysiology, cardiovascular and gastrointestinal surgery, preclinical toxicological
286   testing of pharmaceuticals, and lately for the understanding of the anatomy of the
287   respiratory system and training in lung transplantation (22).

288

289   The assessment of safety and pharmacokinetics for the novel IVM spray formulation in a
290   pig model is described for the first time. The N-IVM-spray was shown to be safe and well
291   tolerated. Neither clinical adverse effects, haematological, serum biochemical nor
292   histopathological changes on the tissue area of drug application, were observed in animals
293   treated with the spray formulation.

294

295   The work reports original data on IVM concentration profiles on NP-tissue after an
296   intranasal and oral administration in a pig animal model. While the repetition of the
297   intranasal dose at a 12 h interval determined a significant increase in IVM concentrations in
298   NP and lung, both identified as target tissues for SARS-CoV-2, only minimal drug systemic

10

299 exposure (low plasma levels) was observed. After 0.2 mg/kg oral tablet administration, IVM

300 plasma concentration levels were in agreement with those previously published for piglets

301 (23, 24). IVM was well absorbed and extensively distributed, resulting in higher

302 concentrations in lung tissue than in plasma (see Figure 2), a pattern already observed in

303 cattle subcutaneously treated with IVM15, and confirmed by a recent pharmacokinetic

304 simulation report looking at IVM lung exposure in humans. Using a minimal physiological

305 pharmacokinetic model, this work establishes that a lung mean concentration as high as

306 193 ng/g may be achieved after a single oral treatment of 30 mg (16).

307

308 The variability observed among treated animals on the patterns of IVM concentration in NP

309 and lung tissues may be related to the fact that it is not possible to control the ventilator

310 state of the animals at the moment of the spray drug application. As a consequence, some

311 animals could have different degrees of inspiration during the spray administration, which

312 may help to understand individual variation on drug concentrations measured both in NP

313 and lung tissues. The situation could be different if this type of N-IVM-spray is used by

314 humans, since the user could be instructed to slightly inspire or remain in apnea, with a

315 predictable increase in drug penetration into the respiratory tree achieving higher lung drug

316 concentrations.

317

318 Several randomized controlled trials are ongoing to investigate the efficacy of IVM against

319 COVID-19, using oral treatments at different doses. Moreover, many uncontrolled oral

320 treatments are using the approved antiparasitic dose of 0.2 mg/kg. Recently reported

321 data18 has shown the potential preventive effect of IVM in SARS-CoV-2 transmission.

322 Additionally, we have recently demonstrated the concentration-dependent IVM effect on the

323 viral clearance in a controlled clinical trial in COVID-19 infected patients (11). The scientific

324 evidence of the in vivo effects of IVM on reducing the SARS-CoV-2 viral load gives

325 prominence to the data on the assessment of the spray formulation in a pig model

326 described here.

327

328 It may be expected that repeated intranasal administration increases IVM concentrations in

329 NP-tissue and lungs. Asa low systemic absorption was observed, and considering the

330 intrinsic safety of the drug, there would be no anticipated significant risks of IVM systemic

331 toxicity after repeated nasal administration. Compared to the oral administration, the

332   intranasal administration in humans may provide fast, high and persistent IVM
333   concentration at the NP tissue area at much lower doses. For instance, in a 60 kg body
334   weight person, higher IVM concentrations in NP tissue may be reached with 4 mg of N-IVM
335   (2 spray doses) than giving 12 mg orally (1 dose 0.2 mg/kg). Thus, the daily administration
336   of one puff in each nostril to health workers would allow the persistence of high IVM
337   concentrations in NP epithelium during an entire working period. The design and execution
338   of clinical trials to confirm safety and to determine the efficacy of N-IVM spray should
339   evaluate these potential benefits. This could include recently diagnosed COVID-19 patients,
340   their close contacts, and/or a preventive usage in health workers. Based on the
341   pharmacokinetic data shown here, the administration of more than one puff per nostril a day
342   would allow IVM accumulation in NP tissue, reaching a local drug exposure not feasible to
343   be achieved by the oral route. In the same direction, further research is also needed to
344   evaluate the potential advantages of a combined nasal plus oral treatment regimen to
345   further contribute to IVM repurposing in COVID 19 therapy.

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Author Contributions

361   J. Errecalde. Protocol design, IVM spray design. Animal phase work (Spray administration
362   and sampling). Data analysis. Overall integration/discussion of the data. Manuscript writing.

363 A. Lifschitz. Protocol design. HPLC analysis. PK data analysis. Overall
364 integration/discussion of the data. Manuscript writing.

365 G. Vecchioli. Protocol design. Data analysis. Overall integration/discussion of the data.
366 Manuscript writing.

367 L. Ceballos. Analytical development. Method validation. HPLC analysis. Data integration

368 F.Errecalde. Animal phase work (treatments/sampling).

369 M. Ballent. Analytical development. Method validation.

370 G. Marín. Dosage calculation. Manuscript´s revision.

371 M. Daniele. Animal phase work (treatments/sampling).

372 E. Spitzer, F. Toneguzzo, S. Gold., Protocol Design. Pharmaceutical Spray development.
373 Regulatory Discussion. Overall Integration/analysis/discussion of the data.

374 A. Krolewiecki. Protocol design. Overall integration/discussion of the data.

375 L. Alvarez. Protocol design. Overall discussion of the data. Manuscript writing.

376 C. Lanusse. Protocol design. Overall integration/discussion of the data. Manuscript writing.

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References

1. Campbell WC. 2020. Ivermectin and Malaria-Putting an Elderly Drug to a New Test. Am J Trop Med Hyg 102:1.

2. Lim LE, Vilchèze C, Ng C, Jacobs Jr W, Ramón-García S, Thompson C. 2013. Anthelmintic avermectins kill Mycobacterium tuberculosis, including multidrug-resistant clinical strains. Antimicrob Agents Chemother 57: 1040–1046.

3. Tay MY, Fraser J E, Chan W K, Moreland N J, Rathore A P, Wang C, Vasudevan S G, Jans D A. 2013. Nuclear localization of dengue virus (DENV) 1-4 non-structural protein 5; protection against all 4 DENV serotypes by the inhibitor IVM. Antiviral Res 99: 301–306.

4. Ashraf S, Prichard R. 2016. IVM exhibits potent antimitotic activity. Vet Parasitol 226: 1–4.

5. Juarez M, Schcolnik-Cabrera A, Dueñas-Gonzalez A. 2018. The multitargeted drug IVM: from an antiparasitic agent to a repositioned cancer drug. Am J Cancer Res 8: 317–331.

6. Intuyod K, Hahnvajanawong C, Pinlaor P, Pinlaor S. 2019. Anti-parasitic drug IVM exhibits potent anticancer activity against gemcitabine-resistant cholangiocarcinoma in vitro. Anticancer Res 39: 4837–4843.

7. Yang S N, Atkinson S C, Wang C, Lee A, Bogoyevitch M, Borg N, Jans D. 2020. The broad spectrum antiviral IVM targets the host nuclear transport importin α/β1 heterodimer. Antiviral Res 177: 104760.

8. Lundberg L, Pinkham C, Baer A, Amaya M, Narayanan A, Wagstaff K M, Jans D, Kehn-Hall K. 2013. Nuclear import and export inhibitors alter capsid protein distribution in mammalian cells and reduce Venezuelan Equine Encephalitis Virus replication. Antiviral Res 100: 662–672.

9. Götz V, Magar L, Dornfeld D, Giese S, Pohlmann A, Höper D, Kong B-W, Jans D A, Beer M, Haller O, Schwemmle M. 2016. Influenza A viruses escape from MxA restriction at the expense of efficient nuclear vRNP import. Sci Rep 6:23138.

10. Caly L, Druce J, Catton M, Jans D A, Wagstaff K M. 2020. The FDA-approved Drug IVM inhibits the replication of SARS-CoV-2 *in vitro.* Antiviral Res 2020, 178:104787.

14

406 **11.** ClinicalTrials.gov[Internet]. Ivermectin Effect on SARS-CoV-2 Replication in Patients with
407 COVID-19, October 2, 2020.
408 https://clinicaltrials.gov/ct2/show/NCT04381884?term=ivermectin+covid&draw=2&rank=4

409 **12.** Wagstaff K M, Rawlinson SM, Hearps A, Jans DA. 2011. An AlphaScreen(R)-based
410 assay for high-throughput screening for specific inhibitors of nuclear import. J Biomol
411 Screen 16:192–200.

412 **13.** Wagstaff KM, Sivakumaran H, Heaton SM, Harrich D, Jans D A. 2012. IVM is a specific
413 inhibitor of importin α/β-mediated nuclear import able to inhibit replication of HIV-1 and
414 dengue virus. Biochem J 443: 851-6.

415 **14.** Wölfel R, Corman VM, Guggemos W, Seilmaier M, Zange S, Müller MA, Niemeyer D,
416 Jones TC, Vollmar P, Rothe C, Hoelscher M, Bleicker T, Brünink S, Schneider J, Ehmann
417 R, Zwirglmaier K, Drosten C, Wendtner C. 2020. Virological assessment of hospilatalized
418 patients vith COVID-2019. Nature 581: 465-469.

419 **15.** Lifschitz A, Virkel G, Sallovitz J, Sutra J F, Galtier P, Alvinerie M, Lanusse C. 2000.
420 Comparative distribution of ivermectin and doramectin to parasite location tissues in cattle.
421 Vet Parasitol 87: 327–338.

422 **16.** Jermain B, Hanafin PO, Cao Y, Lifschitz A, Lanusse C, Rao G. 2020. Development of a
423 minimal physiologically-based pharmacokinetic model to simulate lung exposure in humans
424 following oral administration of Ivermectin for COVID-19 drug repurposing. J Pharm Sci
425 2020; Sep 4: S0022-3549(20)30495-0. doi: 10.1016/j.xphs.2020.08.024.

426 **17.** Ji L, Cen J, Lin S, Hu C, Fang H, Xu J, Chen J. 2016. Study on the subacute inhalation
427 toxicity of ivermectin TC in rats. Comp Med 26: 70-4.

428 **18.** ClinicalTrials.gov [Internet]. Prophylactic Ivermectin in COVID-19 Contacts, August 27,
429 2020. https://clinicaltrials.gov/ct2/show/results/NCT04422561?view=results.

430 **19.** VICH Guideline 9. Veterinary Medicines and Information Technology Unit.
431 CVMP/VICH/595/98-FINAL. The European Agency for the Evaluaion of Medicinal Products
432 (EMEA) 2001.

433 **20.** Chambers PG, Grandin T, Heinz G, Srisuvan, T. 2001. Guidelines for human handling,

434 transport and slaughter of livestock. Food and Agriculture Organisation (FAO) Publishers.

435 Thailand.

436 **21.** Gibaldi M, Perrier D. 1982.Pharmacokinetics. In: Revised and Expanded, 2nd ed.

437 Marcel Dekker, New York, USA, pp 45-109.

438 **22.** Fernández L, Velásquez M, Sua L F, Cujiño I, Giraldo M, Medina D, Burbano M, Torres

439 G, Muñoz-Zuluaga C, Gutierrez-Martinez L. 2019. The porcine biomodel in translational

440 medical research: From biomodel to human lung transplantation. Biomedica 39: 300-313.

441 **23.** Lees P, Cheng Z, Chambers M, Hennessy D, Abbott E M. 2013. Pharmacokinetics and

442 bioequivalence in the pig of two ivermectin feed formulations. J Vet Pharmacol Ther 36:

443 350-7.

444 **24.** Pasay CJ, Yakob L, Meredith HR, Stwart R, Mills P, Dekkers M H, Ong O, Lawellyng S,

445 Hugo R L, McCarthy J S, Devine G J. 2019. Treatment of pigs with endectocides as a

446 complementary tool for combating malaria transmission by Anopheles farauti (**s.s**.) in

447 Papua New Guinea. Parasit Vectors 12: 124.

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1   **Table I**: Summary of the experimental design for the study animal phase.

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	Group 1	**Group 2**	**Group 3**
Formulation	N-IVM spray	N-IVM spray	Oral tablets
Treatment	1 dose (1 puff/nostril)	2 doses (1 puff/nostril) 12h apart	1 oral dose
Dose	2 mg	4 mg	0.2 mg/kg (approx. 2 mg)
Animals (n)	22	10	8
Purpose	Safety Pharmacokinetics	Safety Pharmacokinetics	Pharmacokinetics

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15 **Table II**. Haematological and serum biochemical range values obtained in experimental

16 pigs before and after the treatment with either one or two doses of the N-IVM spray

17 formulation.

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			Post-treatment	
Hematology	**Unit**	**Pre-treatment**	**1 Dose**	**2 Dose**
RBC	m/µL	5.6-7.0	5.3-7.1	5.5-6.4
HGB	g/dL	9-12	8.4-11	9-11.5
WBC	/µL	11700-27000	9300-25900	15500-27300
Neutrophils	/µL	4329-16055	3534-17395	7130-15288
Eosinophils	/µL	0-1080	0-1554	0-1204
Basophils	/µL	0	0	0
Lymphocytes	/µL	5518-11520	3990-9842	4347-10374
Monocytes	/µL	438-2070	490-1881	688-1638
Platelets	/µL	283-500	353-470	303-407
Serum biochemistry				
Urea	g/L	0.13-0.30	0.15-0.24	0.10-0.20
Creatinine	mg/dL	1.00-1.35	0.90-1.17	0.90-1.10
GOT (AST)	U/L	47-74	34-120	32-67
GPT (ALT)	U/L	57-98	58-92	60-103

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20 RBC: red blood cell counts, HGB: hemoglobin, WBC: white blood counts.

21 AST: aspartate aminotransferase ALT: alanine aminotransferase.

The data express range values obtained for each parameter in all the animals in each experimental Group before and after treatment. No statistically significant differences ($P > 0.05$) were observed between pre- and post-treatment values for any of the studied parameters.

31 **Table III**: Ivermectin (IVM) exposure (availability) expressed as AUC values in
32 nasopharyngeal (NP) tissue, lung tissue and plasma following the N-IVM-spray (one dose)
33 administration.

	IVM exposure (mean AUC values)	Relationship with NP exposure*
NP tissue	457ng.h/g	-
Lung Tissue	268ng.h/g	0.59
Plasma	49.5ng.h/mL	0.11

34 *Drug exposure (AUC) ratios in lung/NP tissues and plasma/NP tissue
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37 **Table IV**: Mean (±SD) comparative ivermectin (IVM) concentrations measured in
38 nasopharyngeal tissue, lung tissue and plasma at 6and 24 h after its intranasal (N-IVM
39 spray) (as one and two applications) and oral administration to piglets.

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	IVM concentration (ng/g; ng/mL)		
	N-IVM spray (one 2 mg dose)	N-IVM spray (two 2 mg doses, 12 h apart)	Oral (one 0.2 mg/kg dose)
Nasopharyngeal			
6 h	20.7 ± 2.92^a	49.1 ± 18.7^b	23.6 ± 10.8^a
24 h	6.96 ± 6.01^a	10.9 ± 7.58^a	13.0 ± 5.33^a
Lung tissue			
6 h	13.0 ± 4.38^a	34.4 ± 10.2^b	54.7 ± 14.0^c
24 h	5.42 ± 4.69^a	13.0 ± 9.41^a	25.4 ± 10.3^b
Plasma			
6 h	2.13 ± 0.50^a	4.95 ± 2.92^{ab}	8.60 ± 3.39^b
24 h	1.20 ± 1.04^a	3.37 ± 2.61^b	3.65 ± 0.88^b

41 Different letters indicate statistically significant differences at $P < 0.05$.

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Figure Legends

Figure 1. Mean ivermectin (IVM) concentration profiles in nasopharyngeal (NP) tissue, lung and plasma following the intranasal (N-IVM-spray, one dose) administration to piglets. The insert shows the comparative IVM concentrations obtained between 2 and 6 h post spray administration.

Figure 2. Comparative ivermectin (IVM) concentrations in nasopharyngeal (NP) tissue, lung and plasma at 6 h after oral and N-IVM-spray (one and two doses) treatment. The spray to oral IVM concentration ratios (values in brackets) are shown for each o fthe target tissues/plasma.

Figure 1

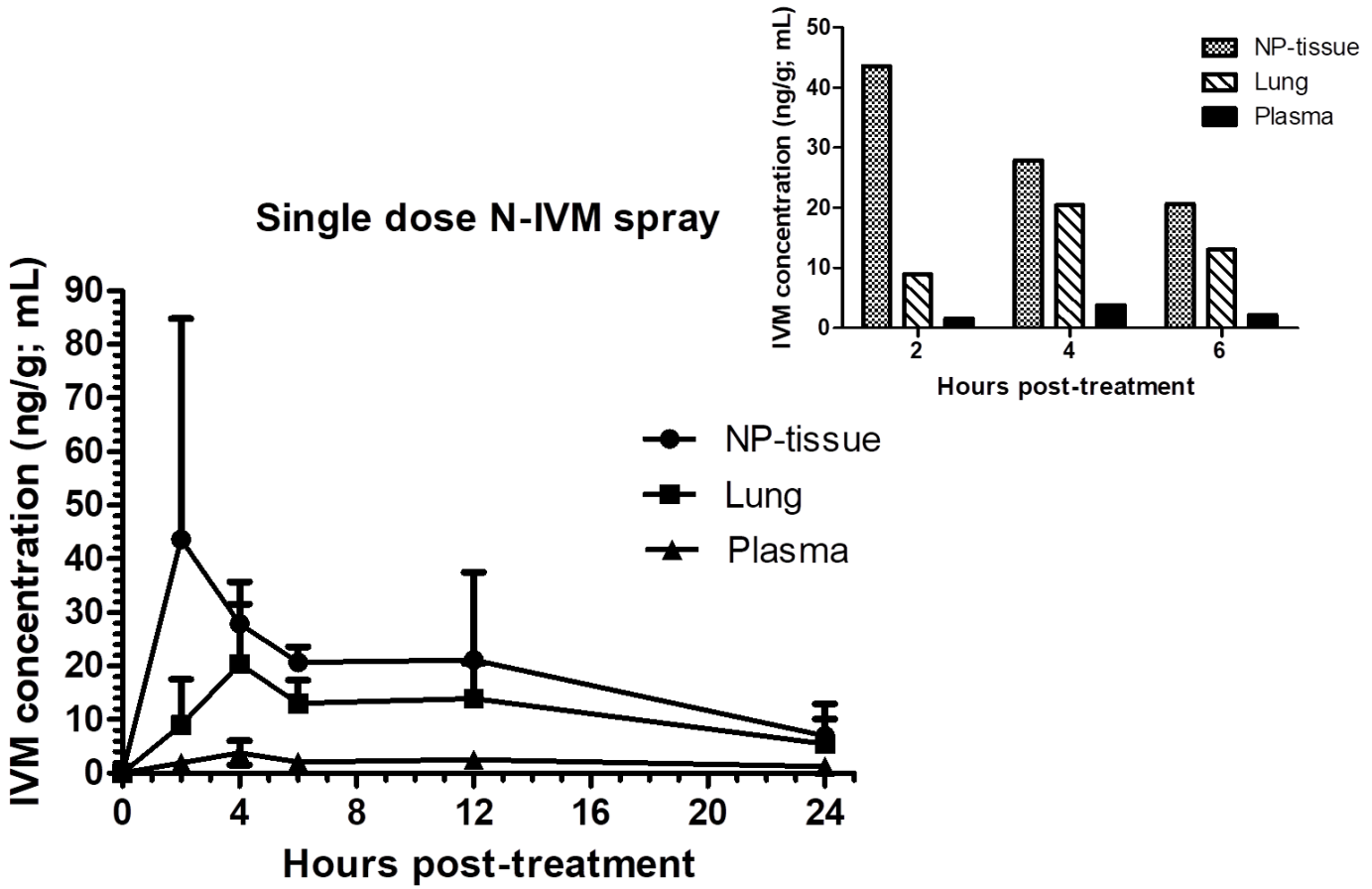**Single dose N-IVM spray**

- NP-tissue
- Lung
- Plasma

IVM concentration (ng/g; mL)

Hours post-treatment

Figure 2

IVM concentrations at 6 h post-treatment

- NP-tissue
- Lung
- Plasma

Concentration (ng/g; mL)

(0.87)
(0.24)
(0.25)
(2.10)
(0.63)
(0.57)

N-IVM spray 1 dose   N-IVM spray 2 doses   Oral 1 dose