Experimental design impacts the vector competence of *Ornithodoros* ticks for African swine fever virus: a meta-analysis of published evaluations

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Abstract

African Swine Fever (ASF) is a highly economically devastating viral disease for swine. Soft ticks of the genus *Ornithodoros* are involved in its epidemiology, as vectors and natural reservoirs of African Swine Fever Virus (ASFV). This constitutes a significant problem as it is currently challenging to eliminate endophilous soft ticks living in inaccessible, sheltered habitats. The vector competence of *Ornithodoros* ticks for ASFV has been mainly studied by mimicking natural tick-to-pig transmission pathways through experimental infections in the laboratory. However, experimental designs are anticipated to exert significant influence on the results, owing to inherent technical limitations of the approaches. By reviewing the original research studies dating back to the 1960s on the vector competence of *Ornithodoros* for ASFV, we estimated the vector competence of 10 tick species in association with 38 viral strains resulting in 51 tick-virus associations. This assessment emphasized the extensive range of protocol designs employed and of vector competence measurements. Those experimental factors have clear impact on the success of tick infection by ASFV. Our results offer valuable insights into how future trials can be refined by curtailing potential experimental biases. They also call for caution when interpreting conclusions drawn from studies on ASFV vector competence.

Keywords: *Ornithodoros* soft ticks, African swine fever virus, vector competence, methodology, meta-analysis

Introduction

African swine fever (ASF) ranks among the most damaging viral diseases affecting swine farming. The etiologic agent is a large DNA virus belonging to the Asfaviridae family (Dixon *et al.*, 2013). African Swine Fever Virus (ASFV) causes haemorrhagic fever in susceptible infected swine, with up to mortality rates of up to 100% in naïve domestic pigs. In Africa, wild suids (warthogs, and possibly bushpigs and giant forest hogs) are natural vertebrate reservoirs that are resistant to the disease (Jori et al. 2013). In Europe, wild boars are susceptible to ASFV and exhibit clinical symptoms and mortality rates comparable to those of domestic pigs. In certain countries, soft ticks of the genus *Ornithodoros* act as vectors or natural reservoirs of ASFV. The virus can persist for several years, potentially leading to epizootic outbreaks in pig farms that have become colonized by ticks, even with the implementation of ASF control measures. (i.e., pig slaughtering, disinfection, and quarantine) (Boinas et al. 2011, Jori *et al.*, 2013). Once ASFV is introduced into a new free area where the presence of *Ornithodoros* ticks is highly suspected, as it occurred in the Caucasus in 2007 or more recently in the Caribbean, the potential for long-term persistence of ASFV in tick reservoirs and ASFV transmission via tick bites raises concern (Beltran Alcrudo *et al.*, 2008, EFSA, 2021.

Vector competence generally refers to the vector's capacity to be infected by a pathogen, to maintain and multiply this pathogen, and finally to transmit it to a new susceptible host (Nuttal, 1994; Weaver et al., 2004). The biological steps taking place within the arthropod vector can be broken down into four stages: (i) the ingestion of an infected blood meal in sufficient amount, (ii) the infection of the vector upon crossing of its intestinal midgut by the pathogen, (iii) the persistence and multiplication of the pathogen within the vector despite the vector's immune response, and (iv) the dissemination of the pathogen inside the vector by crossing organic barriers and ultimately reaching organs necessary for transmission to vertebrate hosts, typically the salivary glands. Sexual transmission of pathogens between males and females of vectors as

54 well as transovarial transmission from female vectors to their progeny can occur through the infection of reproductive
55 organs. Although not strictly included in the formal definition of the vector competence, these pathways of transmission
56 will also be considered in this review.

57 All these biological steps are observed in the interaction between ASFV and *Ornithodoros* tick although results reported
58 varied greatly between studies. In addition to historical demonstration of tick vector competence for ASFV in African *O.*
59 *moubata* sensu lato and Iberian *O. erraticus*, several other *Ornithodoros* tick species succeed to maintain or transmit ASFV
60 under experimental conditions (Endris et al., 1987, Endris et al., 1991, Hess et al., 1987, Mellor & Wilkinson, 1985). This
61 suggests that most *Ornithodoros* ticks might be able to maintain and transmit ASFV (EFSA, 2010b). Based on available
62 results, some researchers have proposed to predict the global role of each tick species as a vector and reservoir for ASFV
63 (Kleiboeker & Scoles, 2001; EFSA, 2010a, 2010b, 2014; Burrage, 2013). However, these comprehensive reports do not
64 account for the evident disparities in vector competence results, which likely rely on intricate tick-virus adaptations as
65 proposed by Pereira de Oliveira (2019, 2020c) and other yet-to-be-identified biological and experimental factors. No paper
66 has considered the substantial diversity of techniques employed to test vector competence except for a study on *O.*
67 *erraticus* infected with two Portuguese ASFV strains (Ribeiro et al., 2015). Nonetheless, the importance of experimental
68 methodology has been demonstrated in other models like mosquitoes with Rift Valley fever, Dengue, Zika or Japanese
69 Encephalitis viruses. (Azar, 2019; Auerswald, 2021; Drouin, 2022). We hypothesize that the experimental design, including
70 the choice of biological materials and the methods used to infect ticks and detect the virus, is an important factor in
71 explaining variation in vector competence, in addition to the influence of tick species, ASFV strains and their combinations.

72 To investigate this hypothesis, a systematic review of studies published up to 2022 was conducted, examining the vector
73 competence of *Ornithodoros* ticks for ASFV. The present paper focuses on the diverse experimental factors that may impact

74 vector competence and determines the overall vector competence of the various associations between *Ornithodoros* tick
75 and ASFV. We provide recommendations on how experimental trials can be refined to minimize biases and obtain more
76 comparable and reliable results on *Ornithodoros* vector competence for ASFV.

77 **Material and Methods**

78 *Collection of bibliographical resources*

79 The first objective of the study was to identify the bibliographic resources related to vector competence of *Ornithodoros*
80 ticks for ASFV, published in English, in peer-reviewed journals or under high publication standards. This was achieved
81 following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Annex 1). Our
82 bibliographic review was conducted until July 2022, using three different search systems: EThOS
83 (http://ethos.bl.uk/Home.do), PubMed (http://www.ncbi.nlm.nih.gov/) and Scopus (http://www.scopus.com/). Queries
84 were built under the keywords "African swine fever" AND "tick". The topics not related with tick competence (e.g.,
85 immunology, genetic, vaccinology, epidemiology) were excluded during the screening phase. Then, during the eligibility
86 phase, studies were excluded if quantitative reliable data could not be extracted from publications. Purely descriptive
87 reviews without any original vector competence results were also excluded. Apart from published scientific papers, some
88 authors presented their results in their PhD manuscript. These data were also considered but all duplicated results were
89 removed to avoid biases in the analyses. Finally, the references cited at the end of the included papers were checked, in
90 order to identify further relevant studies. The final list of included papers that were used to extract all data in soft tick
91 vector competence for ASFV are shown in Table 1.

92 **Table 1 -** Included papers providing original results on the vector competence of *Ornithodoros* ticks for ASFV, with number attributed to each

93 paper by years of publication.

N°	Included papers
1	Parker J, Plowright W, Pierce MA. The epizootiology of African swine fever in Africa. Vet Rec. 1969 Dec 13;85(24):668-74
2	Plowright W, Perry CT, Peirce MA, Parker J. Experimental infection of the argasid tick, *Ornithodoros moubata porcinus*, with African swine fever virus. Arch Gesamte Virusforsch. 1970;31(1):33-50.
3	Plowright W, Perry CT, Peirce MA. Transovarial infection with African swine fever virus in the argasid tick, *Ornithodoros moubata* porcinus, Walton. Res Vet Sci. 1970 Nov;11(6):582-4.
4	Greig A. The localization of African swine fever virus in the tick *Ornithodoros moubata porcinus*. Arch Gesamte Virusforsch. 1972;39(1):240-7.
5	Plowright W, Perry CT, Greig A. Sexual transmission of African swine fever virus in the tick, *Ornithodoros moubata* porcinus, Walton. Res Vet Sci. 1974 Jul;17(1):106-13.
6	Groocock CM, Hess WR, Gladney WJ. Experimental transmission of African swine fever virus by *Ornithodoros coriaceus*, an argasid tick indigenous to the United States. Am J Vet Res. 1980 Apr;41(4):560-4.
7	Thomson GR. The epidemiology of African swine fever: the role of free-living hosts in Africa. Onderstepoort J Vet Res. 1985 Sep;52(3):201-9. Review.
8	Mellor PS, Wilkinson PJ. Experimental transmission of African swine fever virus by *Ornithodoros savignyi* (Audouin). Res Vet Sci. 1985 Nov;39(3):353-6.
9	Hess WR, Endris RG, Haslett TM, Monahan MJ, McCoy JP. Potential arthropod vectors of African swine fever virus in North America and the Caribbean basin. Vet Parasitol. 1987 Dec;26(1-2):145-55.
10	Endris RG, Haslett TM, Geering G, Hess WR, Monahan MJ. A hemolymph test for the detection of African swine fever virus in *Ornithodoros coriaceus* (Acari: Argasidae). J Med Entomol. 1987 Mar;24(2):192-7.
11	Hess WR, Endris RG, Lousa A, Caiado JM. Clearance of African swine fever virus from infected tick (Acari) colonies. J Med Entomol. 1989 Jul;26(4):314-7.
12	Haresnape JM, Wilkinson PJ. A study of African swine fever virus infected ticks (*Ornithodoros moubata*) collected from three villages in the ASF enzootic area of Malawi following an outbreak of the disease in domestic pigs. Epidemiol Infect. 1989 Jun;102(3):507-22.
13	Endris RG, Haslett TM, Hess WR. Experimental transmission of African swine fever virus by the tick *Ornithodoros* (Alectorobius) *puertoricensis* (Acari: Argasidae). J Med Entomol. 1991 Nov;28(6):854-8.
14	Endris RG, Hess WR, Caiado JM. African swine fever virus infection in the Iberian soft tick, *Ornithodoros* (Pavlovskyella) *marocanus* (Acari: Argasidae). J Med Entomol. 1992 Sep;29(5):874-8.
15	Endris RG, Hess WR. Experimental transmission of African swine fever virus by the soft tick *Ornithodoros* (Pavlovskyella) *marocanus* (Acari: Ixodoidea: Argasidae). J Med Entomol. 1992 Jul;29(4):652-6.
16	Endris RG, Haslett TM, Hess WR. African swine fever virus infection in the soft tick, *Ornithodoros* (Alectorobius) *puertoricensis* (Acari: Argasidae). J Med Entomol. 1992 Nov;29(6):990-4.
17	Endris RG, Hess WR. Attempted transovarial and venereal transmission of African swine fever virus by the Iberian soft tick *Ornithodoros* (Pavlovskyella) *marocanus* (Acari: Ixodoidea: Argasidae). J Med Entomol. 1994 May;31(3):373-81.
18	Boinas, FS. The role of *Ornithororos erraticus* in the epidemiolohy af ASFV in Portugal. PhD thesis manuscript. 1994
19	Rennie L. The Persistence of African swine fever virus in the Argasid Tick *Ornithodoros moubata*. PhD thesis manuscript. 1998.

20 Anderson EC, Hutchings GH, Mukarati N, Wilkinson PJ. African swine fever virus infection of the bushpig *(Potamochoerus porcus*) and its significance in the epidemiology of the disease. Vet Microbiol. 1998 Apr 30;62(1):1-15.

21 Kleiboeker SB, Burrage TG, Scoles GA, Fish D, Rock DL. African swine fever virus infection in the argasid host, *Ornithodoros porcinus porcinus*. J Virol. 1998 Mar;72(3):1711-24.

22 Kleiboeker SB, Scoles GA, Burrage TG, Sur J. African swine fever virus replication in the midgut epithelium is required for infection of Ornithodoros ticks. J Virol. 1999 Oct;73(10):8587-98.

23 Rennie L, Wilkinson PJ, Mellor PS. Effects of infection of the tick *Ornithodoros moubata* with African swine fever virus. Med Vet Entomol. 2000 Dec;14(4):355-60.

24 Rennie L, Wilkinson PJ, Mellor PS. Transovarial transmission of African swine fever virus in the argasid tick *Ornithodoros moubata*. Med Vet Entomol. 2001 Jun;15(2):140-6.

25 Burrage TG, Lu Z, Neilan JG, Rock DL, Zsak L. African swine fever virus multigene family 360 genes affect virus replication and generalization of infection in *Ornithodoros porcinus* ticks. J Virol. 2004 Mar;78(5):2445-53.

26 Nix-Rowlands RJ. Interaction of ASFV with its tick vector. PhD thesis manuscript. 2006.

27 Basto AP, Nix RJ, Boinas F, Mendes S, Silva MJ, Cartaxeiro C, Portugal RS, Leitão A, Dixon LK, Martins C. Kinetics of African swine fever virus infection in Ornithodoros erraticus ticks. J Gen Virol. 2006 Jul;87(Pt 7):1863-71.

28 Vial L, Wieland B, Jori F, Etter E, Dixon L, Roger F. African swine fever virus DNA in soft ticks, Senegal. Emerg Infect Dis. 2007 Dec;13(12):1928-31.

29 Rowlands RJ, Duarte MM, Boinas F, Hutchings G, Dixon LK. The CD2v protein enhances African swine fever virus replication in the tick vector, Ornithodoros erraticus. Virology. 2009 Oct 25;393(2):319-28.

30 Ravaomanana J, Michaud V, Jori F, Andriatsimahavandy A, Roger F, Albina E, Vial L. First detection of African Swine Fever Virus in *Ornithodoros porcinus* in Madagascar and new insights into tick distribution and taxonomy. Parasit Vectors. 2010 Nov 30;3:115.

31 Boinas FS, Wilson AJ, Hutchings GH, Martins C, Dixon LJ. The persistence of African swine fever virus in field-infected *Ornithodoros erraticus* during the ASF endemic period in Portugal. PLoS One. 2011;6(5):e20383.

32 Diaz AV, Netherton CL, Dixon LK, Wilson AJ. African swine fever virus strain Georgia 2007/1 in *Ornithodoros erraticus* ticks. Emerg Infect Dis. 2012 Jun;18(6):1026-8.

33 de Carvalho Ferreira HC, Tudela Zúquete S, Wijnveld M, Weesendorp E, Jongejan F, Stegeman A, Loeffen WL. No evidence of African swine fever virus replication in hard ticks. Ticks Tick Borne Dis. 2014 Sep;5(5):582-9.

34 Ribeiro R., Otte J., Madeira S., Hutchings G.H., Boinas F. Experimental Infection of Ornithodoros erraticus sensu stricto with Two Portuguese African Swine Fever Virus Strains. Study of Factors Involved in the Dynamics of Infection in Ticks. PLoS One. 2015 Sep 14;10(9):e0137718.

35 Pereira de Oliveira R, Hutet E, Paboeuf F, Duhayon M, Boinas F, Perez de Leon A, Filatov S, Vial L, Le Potier MF. Comparative vector competence of the Afrotropical soft tick Ornithodoros moubata and Palearctic species, *O. erraticus* and *O. verrucosus*, for African swine fever virus strains circulating in Eurasia. PLoS One. 2019 Nov 27;14(11):e0225657.

36 Pereira De Oliveira, R., Hutet, E., Duhayon, M., Paboeuf, F., Le Potier, M. F., & Vial, L. No experimental evidence of co-feeding transmission of african swine fever virus between Ornithodoros soft ticks. Pathogens 2020; 9(3), 168.

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38 Pereira De Oliveira, R., Hutet, E., Lancelot, R., Paboeuf, F., Duhayon, M., Boinas, F., ... & Vial, L. (2020). Differential vector competence of Ornithodoros soft ticks for African swine fever virus: What if it involves more than just crossing organic barriers in ticks?. Parasites & vectors 2020 13(1), 1-15.

39 Forth, J. H., Forth, L. F., Lycett, S., Bell-Sakyi, L., Keil, G. M., Blome, S., ... & Beer, M. Identification of African swine fever virus-like elements in the soft tick genome provides insights into the virus' evolution. BMC biology, 2020 18(1), 1-18.

94

95 *Extraction of data about tick competence from bibliographical resources*

96 Bibliometric general information on authors and years of publication were collected to describe the general interest in
97 tick vector competence for ASFV and to identify scientific experts. We considered the experts as the persons in first and
98 last position of the included papers. Information on tested *Ornithodoros* tick-ASFV associations was systematically reported.
99 A challenge was to deal with missing or incomplete data for ASFV strains, as well as the ambiguities in tick species
100 identification.

101 Regarding virus, we recorded the strain, the genotype (on the basis of p72 gene sequences, Qu *et al*. 2022), the
102 geographical origin, and the host (vertebrate animals or ticks) from which the ASF virus was isolated. The original names of
103 some ASFV strains were retrieved by crossing information from several papers, according to known authors' collaborations,
104 or by directly contacting the authors. When the determination of strains remained impossible, the strain was identified by
105 indicating the country where the ASFV strain was isolated, the year of the study, and a letter if duplicates existed. One
106 exception was a strain used to infect *O. turicata (*Hess *et al.* 1987*)* without any indication of the country of origin which was
107 named Unknown. Six recombinant ASFV strains, genetically modified and obtained from two natural strains (from papers
108 n°25 and n°26), were excluded from this review since they cannot infect ticks in nature. Genotypes were not available for
109 some strains of ASFV, but they could be inferred based on their geographical origin and ASFV genotype distribution
110 published by Lubisi (Lubisi *et al.*, 2005).

111 Concerning ticks, the species, their geographical origin, their colony origin and status were reported. When ticks were
112 reared for less than five years in the laboratory, they were considered comparable to ticks sampled from the field due to
113 the limited number of generations occurring during this period. Some *Ornithodoros* ticks belong to species complexes and
114 soft tick systematics has greatly changed during the last decades. Tick species identification was updated in accordance

115 with the revision of valid tick names by Guglielmone (Guglielmone *et al.*, 2010) and the mapping of their known distribution
116 (Vial, 2009). The more recent taxonomy study of southern African *Ornithodoros* ticks (Bakkes, 2018) could not be used, due
117 insufficiently precise location to attribute a species name among the *O. moubata* complex of species. Finally, when
118 determining tick geographical origin, 6 studies involving 4 different tick species (*O. coriaceaus, O. parkeri, O. moubata*, and
119 *O. porcinus*) did not report this information but since *O. parkeri* and *O. coriaceus* are known to be limited to North America,
120 especially the United-States (de la Fuente et al. 2008), they were assigned to this country.
121
122 Authors used various measurements to assess the tick vector competence for ASFV. Some authors reported numbers
123 of ticks positively detected with ASFV, while others scrutinized the virus titres in ticks or monitored ASFV kinetics in ticks
124 for several weeks or months, providing more comprehensive information on the virus persistence within its tick reservoir.
125 Some authors preferred to detect ASFV in tick transmission organs (salivary or coxal glands) or secretions (saliva or coxal
126 fluid). Such measurements suggest possible successful transmission to pigs although only transmission experiments can
127 confirm it. In these transmission experiments, clinical signs observed in pigs following tick bites confirm the ability of ticks
128 to transmit the disease. Detection of ASFV in pig blood is usually associated to confirm infection. Transmission between
129 ticks is either tested directly by measuring the proportion of transmitting ticks or the proportion of infected tick or indirectly
130 through the detection of ASFV in male reproductive organs (for sexual transmission) or female ovaries (for transovarial
131 transmission). However, this was not considered sufficient evidence to confirm such transmission pathways. Conversely,
132 the detection of ASFV in transmission secretions (e.g., saliva or coxal fluids) was considered sufficient evidence, as the virus
133 is already excreted in this case.
134

135 Based on these data, it was possible to evaluate three major steps of the vector competence of the ticks: 1) the tick
136 infection (revealed by the crossing of the tick midgut by ASFV), 2) the ability of ticks to transmit ASFV to pigs (named
137 horizontal transmission) and 3) their ability to transmit ASFV to other ticks through sexual or transovarial vertical
138 transmission (named tick-to-tick transmission). Although these steps are not necessarily gradual, transmission to vertebrate
139 hosts through tick bite or from ticks to ticks cannot occur without the previous infection of the tick, which was thus
140 considered as the main step to be assessed. In addition, tick ability to transmit ASFV to pigs or other ticks are less frequently
141 reported (N= 160 and N=54 over 900 experiments, respectively) likely because they are somewhat more difficult to assess
142 than tick infection. Therefore, for each experiment, we computed a binary variable, the infection status, according to any
143 measurements providing direct information on tick infection as well as indirect transmission measurements that imply
144 previous tick infection (Figure 1). More precisely, if ASFV transmission to pigs or ASFV transmission from ticks to other ticks
145 were reported in papers, the ticks were considered to be infected as the virus has necessarily crossed the tick organic
146 barriers to reach transmission organs and be transmitted.

147 If transmission failed or was not tested, the detection of ASFV in ticks was used as an indication of infection status.
148 Obviously when ASFV was not detected, ticks were considered as not infected. The only exception was when the detection
149 occurred on the same day as virus exposure, in which case no virus detection indicates a failure of experimental infection
150 or a too low detection threshold.

151 Different methods have been used by authors to detect ASFV in ticks, namely ASFV isolation/titration, observation of
152 ASFV particles, and viral DNA or RNA detection using multiple designs of Polymerase Chain Reaction (PCR). In the included
153 papers, ASFV isolation/titration was based on haemadsorption (HAD), which is the ability of many ASFV strains to aggregate
154 on red blood cells when they infect macrophages and create typical “rosettes” (Malmquist & Hay 1960); this implies that

155 the virus is able to replicate but does not necessarily confirm its entry in ticks. Similarly, detection of viral RNA also indicates
156 active viral replication. Conversely the detection of viral DNA can be due to residual virus from blood meal and does not
157 mean that the virus is still active. However, we considered that those methods brought equivalent results to assess infection
158 status once the duration after exposure was taken into account.

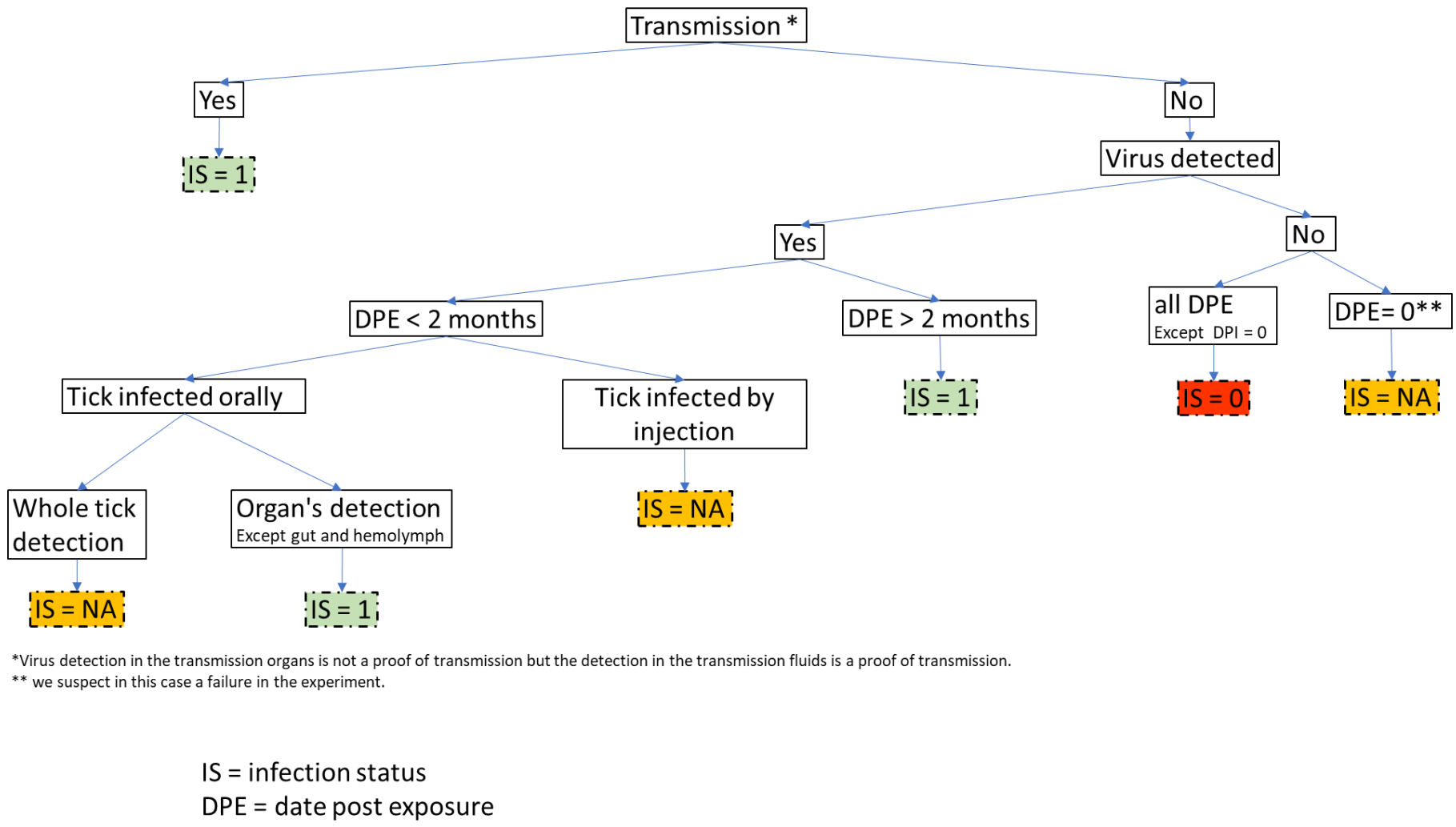
159 Indeed, when ASFV was detected in ticks, a minimum duration of 61 days between tick exposure to ASFV and detection
160 of ASFV in tick (hereafter DPE for duration post exposure) was chosen to distinguish successful viral replication inside the
161 tick from detection of residual virus after the infectious blood meal or virus inoculation. Indeed, the minimum duration for
162 blood meal digestion and clearance of ingested blood in soft ticks is approximately 4 weeks (Rowlands *et al.*, 2009).
163 Furthermore, if tick infection is unsuccessful, ASFV can be detected during 4 weeks by isolation (Ribeiro et al., 2015) and
164 after at least 49 days by qPCR (de Carvalho et al., 2014, see also Bernard, 2015, for a review on ASFV kinetics inside soft
165 ticks). If tick infection is successful, virus generalization in ticks takes about 30 to 56 days (Basto *et al.*, 2006, Burrage, 2013,
166 Kleiboeker *et al.*, 1998, Plowright *et al.* 1970). Therefore, and as a precaution, if ASFV was tested and detected before 61
167 DPE, the infection status was considered unknown unless virus was detected in organs other than the midgut. In this case,
168 this indicates that the virus has crossed the midgut barrier. When inoculation is based on injection into the haemocoel,
169 virus is spread throughout the tick. In this case, as the detection of virus in the organs was doubtful, the infection status
170 was recorded as unknown.

171 The experimental design used in the different studies was also reported, including: i) the exposure route for the tick
172 infection, ii) the ASFV titres in the inoculum used to infect the ticks (infectious dose), iii) the development stage of the ticks
173 tested in the study, iv) the DPE, v) the number of ticks tested in the study. Except for the DPE and the number of ticks
174 monitored, all parameters were categorized in different classes.

175 In field studies, viral titres in the hosts were not necessarily reported but they were considered to be high (>10^6

176 HAD_{50}/mL of blood) considering the viraemia usually observed in ASF diseased pigs (Plowright et al., 1968). Tick stage was

177 classified as early from larva to nymph 3 and as late from nymph 4 to adult.



178

179 **Figure 1 -** Criteria used to determine the infection status of ticks after their virus exposure, with three classes (IS: Infection Status; DPE: Date

180 post exposure; NA: Not Applicable, 1: Infected, and 0: Not Infected).

181

182 *Statistical analysis of the tick infection status*

183 A statistical analysis was carried out to identify factors potentially affecting the tick infection status. More precisely, we
184 investigated if factors related to the nature of tick-virus association or the experimental design influenced the infectious
185 status (see above for details) of the ticks in published experimental trials. The explanatory variables were: the ASFV strain
186 and genotype, the tick species, the potential geographical co-occurrence of tick and virus as an indication of possible tick-
187 virus adaptation (ASFV and ticks from the same country versus from different countries), the viral titre in infection blood
188 meal and the exposure route for tick infection, the tick stage and the tick colony status, the host from which the ASFV strain
189 was sampled. Using a dataset where all ticks were adults, we also tested the influence of tick sex on infectious status (along
190 with all other factors except tick stage). All statistical analyses were performed on R 4.2.2 (R Core Team, 2018). To assess
191 which factors influenced the infection status, we applied a generalized mixed model with a binomial family. The tick species,
192 virus genotype and virus strain were set as random effects to account for the fact that only a random fraction of soft tick
193 species and virus genotypes and strains were sampled. All other factors were set as fixed effects. The significance of random
194 effects was tested using a likelihood ratio test (Zuur et al., 2009). We then selected the best parsimonious models (with the
195 remaining 6 fixed effects) based on the AIC (Akaïke Information Criterion). In this best model, the significance of fixed effects
196 was tested using Chi^2 tests between models and post-hoc tests, with Tukey HSD correction, were performed.
197

198 *Assessment of the overall tick vector competence for ASFV*

199 Although our main assumption is that vector competence results are influenced by the experimental design, we
200 attempted to assess the overall vector competence of the various *Ornithodoros* tick-ASFV associations. Based on the three
201 main steps described above, we scored each tick-ASFV association, according to the following ranking: “score 0” for ticks
202 that were unable to become infected or for which it was impossible to conclude (see flow chart figure 2) ; “score 1” when

203 ASFV was able to cross tick midgut and replicate in ticks; “score 2” when transmission from tick to pig was successful ; and,
204 “score 3” for ticks validating all steps of vector competence, including ASFV transmission from ticks to other ticks. The
205 attribution of a score was based on all data available from the different included papers related to the considered tick-ASFV
206 association, and thus the longest period of monitoring and the best vector competence performance for this association.
207
208 Attributing graduate steps of vector competence sounds as if that associated transmission events are also graduate.
209 This is not the case: even if horizontal transmission scored 2 and sexual or transovarial transmissions scored 3, the success
210 of tick-to-tick transmission does not necessarily imply the efficiency of horizontal transmission. For example, *O. erraticus*
211 showed vertical transmission with the viral strain OURT88/1 but no transmission to pig (Pereira de Oliveira *et al*. 2019,
212 Pereira de Oliveira *et al.* 2020)
213

214 **Results**

215 *Bibliometric description*

216 The initial bibliographic search generated 236 references using Scopus and 138 by Pubmed, with 2 articles detected only
217 by Pubmed. The ETHOS database of thesis manuscripts and our manual bibliography search allowed identifying 8 more
218 resources. Among the final 246 references selected, 75 were reviews, 163 scientific papers and 8 thesis manuscripts. In this
219 pool of bibliographic references, 39 resources presenting vector competence original results were included in the final
220 dataset. They consisted of 5 field studies monitoring natural tick infections, 34 articles of laboratory experiments on ticks
221 and 2 mixed papers (Table 1). As shown in figure 1, the number of publications pertaining to the vector competence of

Ornithodoros ticks for ASFV was consistently low over the years, while the total number of papers related to ticks and ASFV progressively increased from the 1980s to 2022.

Thirty-nine authors were reported as first and/or last authors of the selected publications and were considered as "experts". Their period of expertise, including all their publications on ASFV & ticks, varied from 1 to 34 years. After 2015, 9 out of 39 experts continued to publish in this domain and can be considered the current core of experts on the vector competence of ticks for ASFV.

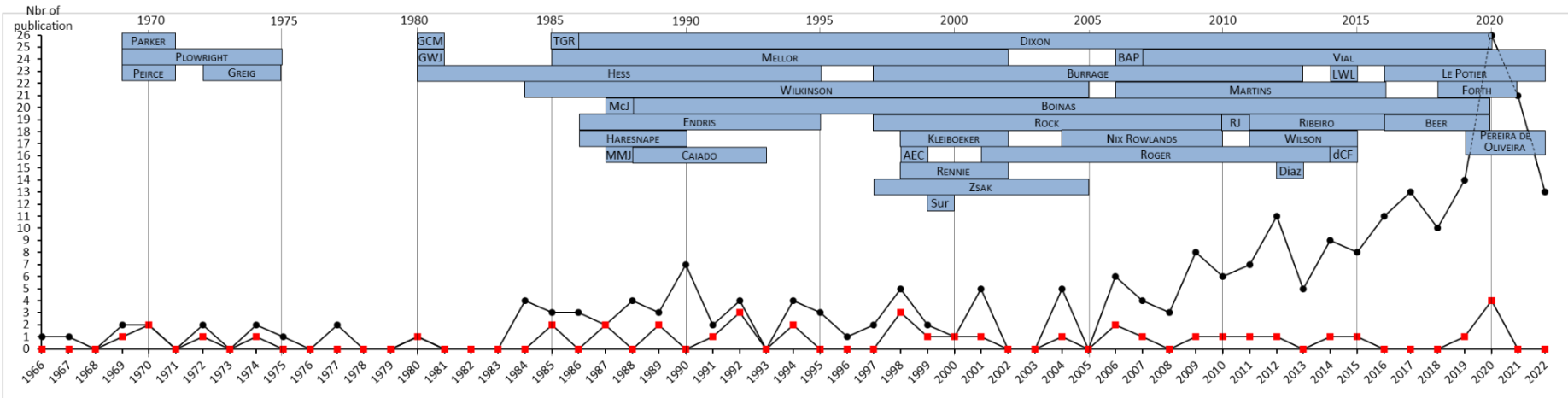


Figure 1 - Number of bibliographic resources published each year, from 1966 to 2022, on ticks and ASFV (black dots = 246 screening papers) and on the vector competence of *Ornithodoros* ticks for ASFV (red dots = 39 included papers). The names of first and last authors of the 39 included papers were reported in blue boxes at the top of the graphic. The length of boxes indicating their respective period of expertise. It is the period where they appear in the list of authors from the 246 screening papers. List of the abbreviation author name: AEC: Anderson E.C.; BAP: Basto A.P.; dCF: de Carvalho Ferreira H.C.; GWJ: Gladney W.J.; GCM: Groocock C.M.; LWL: Loeffen W.L.; McJ: McCoy J.P.; MMJ: Monahan M.J.; RJ: Ravaomanana J.; TGR: Thomson G.R.

Data related to the biological material: ticks and ASFV

237 A database was created to record all the extracted data. Each record comprises a precise combination of any modalities
238 of informed indicators concerning the publication, the ticks, the ASFV strain and the experimental design. From the 39
239 included papers, 2036 records corresponding to 900 unique experiments were extracted. For some papers, authors detailed
240 the results of their experiment for each tick or with multiple records over time for the same ticks. Each experiment was
241 characterized by a combination of tick species, virus strain and experimental conditions (see Annexes 2 and 3). In total, the
242 tick species and ASFV strains represented 51 tick-virus associations and more than half (34/51) appeared in only one
243 publication.

244 For the ticks, 10 species were tested for their vector competence for ASFV. The Iberian tick *O. erraticus* was the most
245 studied (representing 44% of the reported experiments), followed by the *O. moubata* complex of species, including *O.*
246 *porcinus* and *O. moubata* sensu stricto (43%). The North American *O. coriaceus* (6.4%) were third, then they were far
247 followed by the other species *O. puertoricensis, O. verrucosus, O. savignyi, O. parkeri, O. sonrai, O. turicata* (3.7% - 1.0% -
248 0.8% - 0.4% - 0.4% - 0.2 % respectively). The geographical origins of ticks specified in the included papers are presented in
249 figure 2. All *O. erraticus* ticks tested came from Portugal, and most of them from the same region of Alentejo, while *O.*
250 *moubata* and *O. porcinus* ticks came from diverse countries, which is representative of their large distribution range in East
251 and Southern Africa.

252 A total of 38 different ASFV strains were tested. Most ASFV strains used in the studies were isolated from ticks and pigs
253 (43.1% and 40.4% of the reported experiments, respectively). Only 4 strains studied in 16.2% of the experiments were
254 isolated from warthogs and 0.2% of virus strains are from undetermined hosts. ASFV strains were geographically distributed
255 over 17 different countries, mostly in Africa, and belonged to 8 genotypes over the 23 genotypes described so far, with a

large representation of the genotype I (64.9% Figure 2). The genotype of the 114a and Transvaal CV strains from South Africa remains unknown.

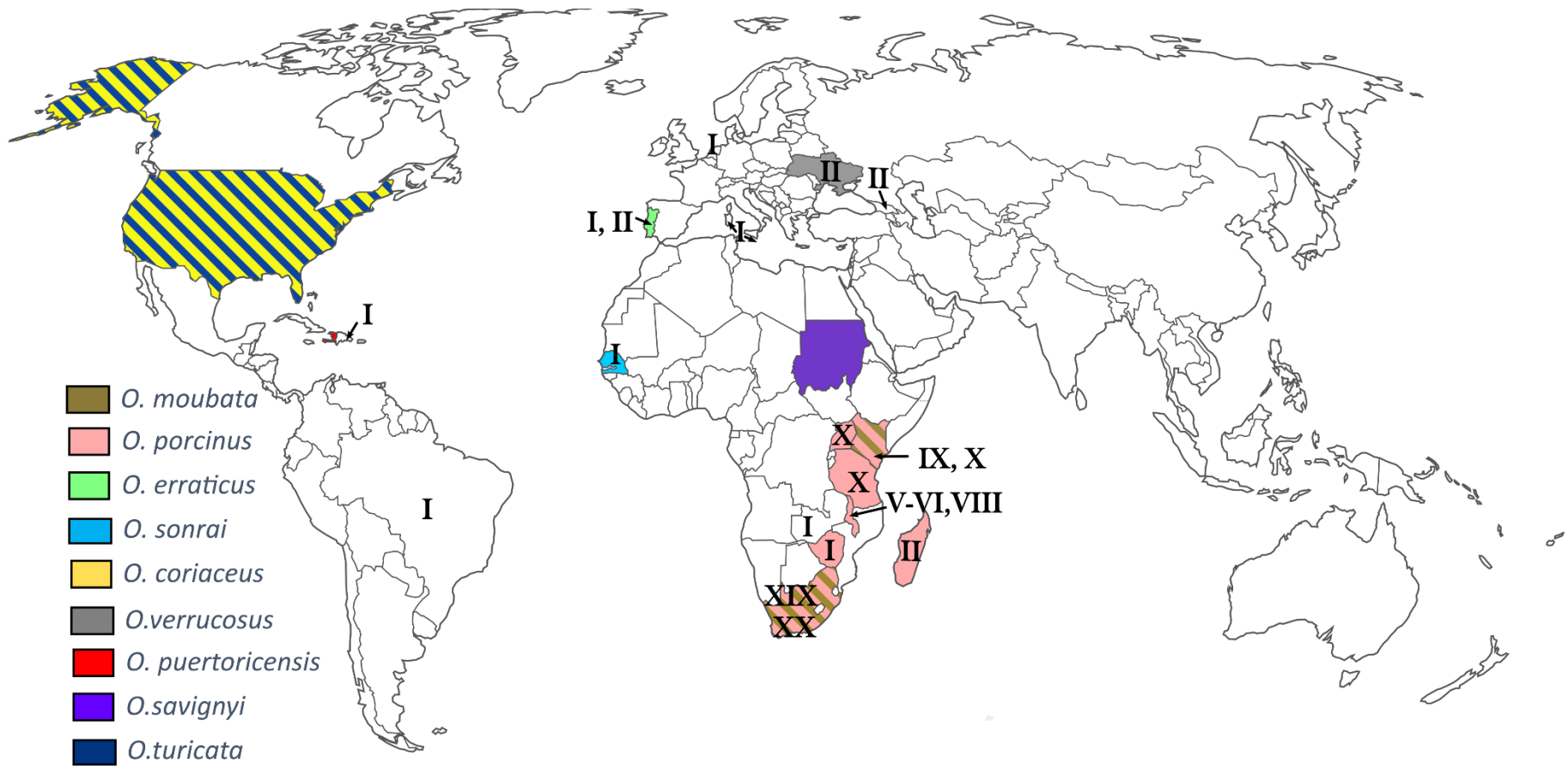


Figure 2 - Countries of origin of *Ornithodoros* tick species (colored according to the species) and ASFV genotypes (indicated by genotype number associated with each country), involved in natural tick-ASFV associations studied in included papers. The origin of the tick *O. parkeri* was not indicated but should be the United-States, as described in the literature.

Data related to the experimental design

266 On the 900 experiments recorded, half used ticks directly sampled from the field (especially *O. erraticus* and *O. porcinus*)
267 or reared in insectary for less than 5 years (48.7%). The other experiments used ticks reared in the laboratory for more than
268 5 years with some reared until 25 years for one study on *O. turicata* (47.2%). It was not possible to find information for the
269 remaining experiments. The majority of experiments (57.9%) involved ticks in their later developmental stages, ranging
270 from nymph 4 to adults. Early stages, from larva to nymph 3, were employed in 23.3% of the experiments. In the remaining
271 experiments, authors either utilized a combination of early and late stages (14.7%) or did not provide information about
272 the specific developmental stage used (4.1%). The number of ticks tested for each experiment was fairly low especially in
273 laboratory experiments (median=7 ticks tested, mean=30.03, range=[1;1141]).

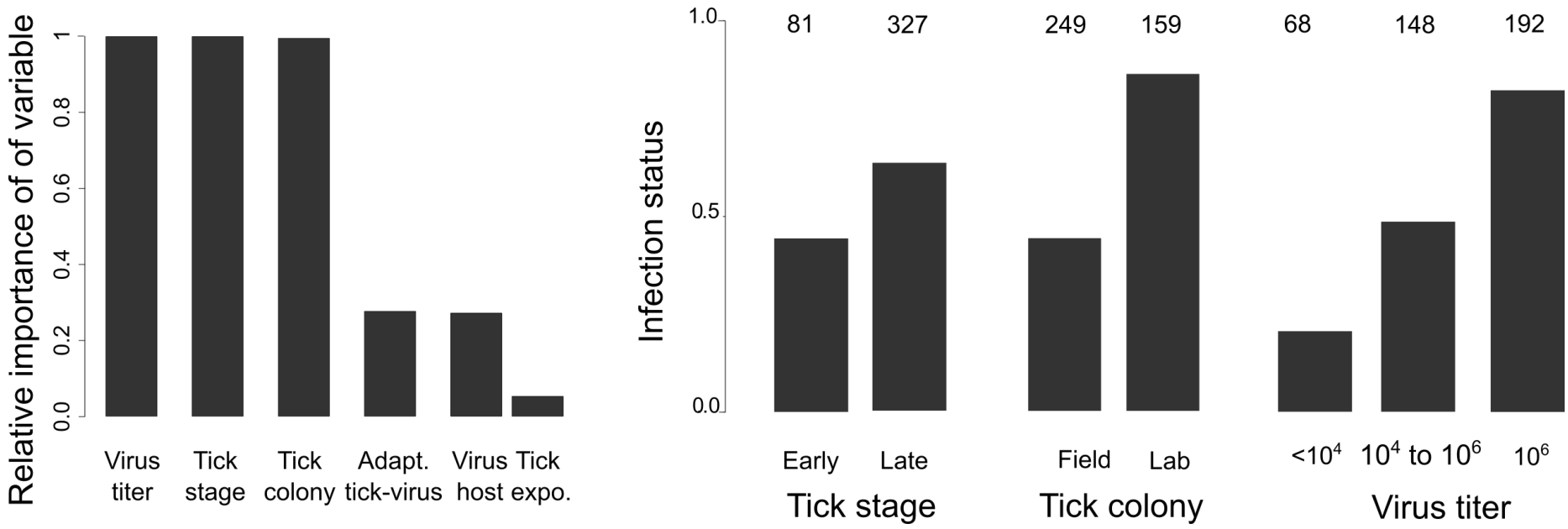
274 Four methods were used to perform viral ASF inoculation to the ticks. Natural blood feeding on vertebrate hosts was
275 reported either in field studies, or in some laboratory experiments using experimentally infected pigs. This method
276 represented almost half of the total experiments conducted (47.4%). Two artificial feeding methods are reported: the
277 longest established method uses capillary tubes inserted on tick mouthparts (8.8%) while a more recent approach uses
278 animal skin, parafilm, or silicone membranes (35.7%). Another artificial infection route was the syringe inoculation of ASFV
279 directly in the tick heamoceal (8.1%). ASFV infectious dose used to infect ticks was very heterogeneous and thus classified
280 in three categories. Most experiments (78,4%) used medium to high viral doses (between 10^4 and 10^6 or over10^6 HAD_{50}/mL
281 of blood). The DPE was very variable, ranging from 1 month to up to 8 years (median value=58.0 days and mean value =
282 115,5 days), meaning that half experiments reported a DPE lower than the duration required to differentiate a successful
283 viral replication inside the tick from a detection of residual virus after the infection.

284

285 *Factors influencing the likelihood of tick infection*

On the initially 900 recorded experiments, we obtained a dataset of 408 observations with full information regarding tick infection status and experimental conditions (Annex 4 and 7). Among the random effects tested, the tick species and virus genotype had no effect on the infection status but the viral strain had (χ_1^2=4.787 p_value= 0.027). Model selection based on AIC ended up with three variables displaying large and comparable importance across all models: virus titre, tick stage and tick colony status (Figure 3 left). All those three variables had significant effects on infectious status (Figure 3 right).

In more details, a higher viral titre corresponded to a higher likelihood of tick infection (χ_2^2X²2= 38.497, p-value=4.369×10-9). Bigger nymphs and adults were more prone to get infected than small nymphs (χ_1^2=17.888, p_value=2.343×10-5). And ticks from laboratory colonies had higher probability of infection than ticks recently sampled in the field (χ_1^2= 14.046, p_value=1.784×10-4). The potential geographical co-occurrence of tick and virus, the exposure route for tick infection and the virus host were not significantly linked with infection status even if they were present in some of the best models (Annex6. Using the dataset containing only experiments with adult ticks showed that sex had no influence on infection status.

300 

301 **Figure 3** - *Left*: Relative importance of model terms across all models explaining infection status. The importance value for a particular predictor
302 is equal to the sum of the weights for the models in which the variable appears. *Right*: Effect of tick stage, tick colony and virus titre on the
303 infection status (all three effects are significant). Mean of infection status (i.e., proportion of experiments where ticks were infected). Values
304 above the bars are the number of experiments in each level. Virus titre in HAD_{50}/mL of blood

305

306 *Vector competence scores for ASFV*

307 Figure 4 summarizes the vector competence score for each of the 51 associations between soft ticks and ASFV virus
308 tested so far. In more details, 23.5% of the tick-ASFV associations presented a vector competence score null (score 0) i.e.,
309 ticks were unable to become infected or it was impossible to conclude on their infection status (Figure 2). For 27.5% of tick-
310 ASFV associations, only tick infection (score 1) was achieved. Transmission from ticks to pigs was tested in 64.7% of tick-
311 ASFV associations and was successful in 29.4% (score 2). Finally, 33.3% of tick-ASFV associations were assessed for ASFV
312 transmission from ticks to ticks and 19.6% succeeded to transmit (score 3).

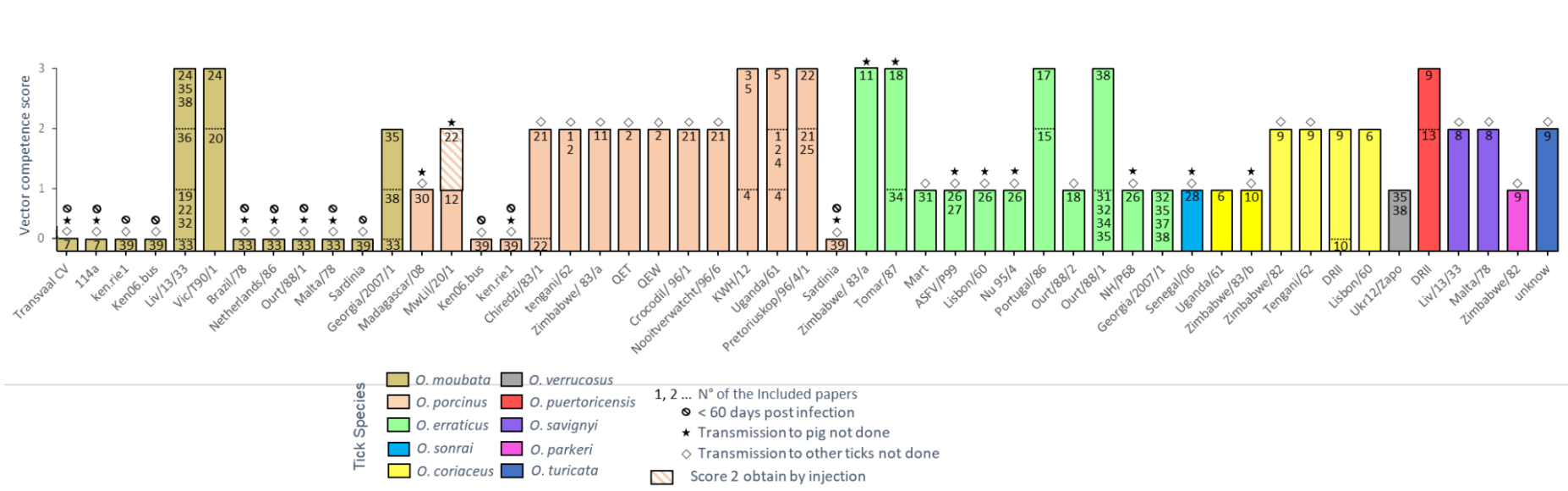


Figure 4 - ASFV vector competence scores for the different tick-ASFV associations studied in the included papers (score 0: no tick infection or no information to conclude, score 1: successful tick infection, score 2: successful ASFV transmission to pigs, score 3: successful ASFV transmission to other ticks). In each histogram bar, the number(s) are related to the included papers recorded in the table n°1. For each included paper, an individual score is given according to available results in this paper. The overall vector competence score for a given tick-virus association is the best vector competence result reported from all included papers related to this tick-ASFV association.

Only 27.5 % of tick-ASFV associations were fully tested for vector competence (including tick infection, transmission to pig, and transmission to other ticks) and 15.7% were fully competent, meaning that they reached scores 1, 2 and 3. Conversely, 3 associations reached score 3, without being tested for horizontal transmission (O. *erraticus*-Zimbabwe/83/a and O. *erraticus*-Tomar/87) or by failing to transmit to pigs when tested (O. *erraticus*-Ourt/88/1). In one tick-ASFV association (*O. porcinus*- MwL1/20/1) transmission capacity was suggested by virus secretion in saliva and in coxal fluid only via virus direct injection into tick hemolymph.

326 At the species level, when considering only scores 1 and 2, most tick species tested were confirmed to be competent
327 vectors for at least one ASFV strain. This competence remains unclear for 3 species that were either tested with only one
328 ASFV strain and failed to transmit the virus to pig and/or to ticks (*O. parkeri* and *O. verrucosus*) or never tested for
329 transmission *(O. sonrai)*. Regarding ASFV transmission to other ticks, 6 tick species were tested and 4 (*O. moubata*, *O.*
330 *porcinus, O. puertoricensis*. and *O. erraticus)* succeeded for at least one ASFV strain whereas 2 (*O. coriaceus* and *O.*
331 *verrucosus)* that were tested with respectively three and one ASFV strains, failed. Finally, high heterogeneity of vector
332 competence scores was observed within a same tick species depending on ASFV strains used (e.g. score 3 for *O. moubata*-
333 LIV13/33 and score 0 for *O. moubata*-Brazil/78), and sometimes for a same tick-ASFV association (e.g. scores 0/1 and 2 for
334 two *O. turicata* colonies of different ages with the same ASFV strain).

335

336 **Discussion**

337 *Bibliographic resources*

338 This paper is the first exhaustive review of all the original studies published on the vector competence of soft ticks for
339 ASFV until 2023. Despite an exhaustive screening of bibliographic resources, only 39 papers were considered and the expert
340 panel had always been limited as exemplified by only 9 publishing experts since 2015. This relative paucity of bibliographic
341 resources regarding soft tick competence for ASFV may be attributed to the difficulty of investigating their vector
342 competence, both in laboratory and in field conditions. Indeed, sampling endophilous *Ornithodoros* ticks and developing
343 relevant methods to detect ASFV in natural tick populations (Jori *et al.*, 2013, Oura 2013) or rearing soft ticks and amplifying
344 ASF virus in the laboratory conditions are long and laborious tasks (Vial 2009, Carrascosa 2011). In addition, although ASF

345 is of high veterinary and economic concern, vectorial transmission is seen as occasional with a relatively minor role in
346 disease spread at least in some regions (Guinat et al., 2016). Nevertheless, soft ticks play a key role in the maintenance of
347 the virus and this is why characterizing competence for tick-virus associations is required to assess the risk of ASFV
348 introduction and spill over as Golnar (Golnar et al.2019) did it for the US.

349

350 *Vector competence and tick-virus co-adaptation*

351 Although all papers included in this study aimed to evaluate the vector competence of soft ticks for ASFV, we noticed a
352 considerable diversity of vector competence measurements that yield distinct information. As a consequence, vector
353 competence was only partially assessed for most tick-ASFV associations, with a noticeable decrease of studies along the
354 successive biological steps of vector competence, from the tick infection to ASFV transmission by tick to pigs, and then by
355 tick to other ticks. In addition, more than half of tick-ASFV associations were characterized by only one single article.
356 Heterogeneous vector competence scores were observed within species between viral strains. For instance, *O. moubata*
357 showed efficient vector competence for only 3 viral strains out of the 12 tested thus far. This could be interpreted either as
358 a lack of sufficient reliable studies or high tick-virus specificity. This latest hypothesis is in agreement with our results
359 showing a significant effect of viral strain on tick infection status.

360 Adaptation between arthropod vectors and viruses is based on genetic features defining susceptibility in both organisms
361 and the selection of susceptibility may be mediated partly by detrimental effects of arboviruses on vector physiology
362 (Weaver et al., 2004). In soft tick-ASFV model, the presence of ASFV MGF360 genes, the lectin-like EP153R gene, or the
363 CD2-like EP402R gene enhance the entry and the replication of ASFV into ticks (Burrage et al., 2004; Rowlands et al., 2009).
364 Leaving their original sylvatic cycle involving warthogs and ticks, some ASFV strains naturally lost these genes through long-

365 term circulation among Iberian domestic pigs, or by multiple passages on pig cell cultures for vaccine production (Portugal
366 et al., 2015). Concerning these vector susceptibility genes, some of them, such as MGF 360, are involved in the suppression
367 of the immune response of vertebrate and arthropod hosts against ASFV (Portugal et al., 2015). In addition, among the
368 large panel of immune responses described in arthropods against transmitted pathogens [44], arthropods are known to
369 display RNA interference as an important immune response against virus infection. The involvement of such molecular
370 pathway has recently been suggested in soft ticks with ASFV- like integrated elements coding for small interfering RNA in
371 *O. moubata* (Forth et al. 2020). More specifically, it appears that a greater number of these small matched with ASFV
372 genomes from genotype II than with genotype I, contributing to the variation in vector competence between viral strains.
373 We can suppose that field ticks, coevolving or not with ASFV, may develop a much more heterogeneous and adaptive
374 response against ASFV infection and show a lower success of infection, as it was already shown in some mosquito-virus
375 models (Weaver et al., 2004). Indeed, some authors previously reported very low ASFV infection rates in field ticks
376 (Plowright 1977, Haresnape & Wilkinson 1989, Boinas et al 2011), as well as over-mortality in laboratory ASFV highly
377 infected ticks (Endris & Hess, 1994, Kleiboeker & Scoles, 2001). In our study, laboratory tick colonies reared for more than
378 five years were shown to be significantly much more infected by ASFV than field ticks or recently established laboratory
379 ticks. In addition, tick from old laboratory colonies may be more prone to efficient artificial blood feeding, increasing the
380 blood volume and ASF virus intake.
381 Despite heterogeneous vector competence according to studies and viral strain tested, experimental evidence shows
382 that 7 *Ornithodoros* species from Africa (*O. moubata, O. porcinus and O. savignyi*), Europe (*O. erraticus*) and North America
383 (*O. coriaceus, O. turicata and O. puertoricensis*) can be infected by at least one strain of ASFV and then transmit it to pigs.
384

385 *Effect of experimental factors on tick competence*

386 Our analysis of vector competence papers demonstrated the importance of the experimental design on vector
387 competence results. Several factors related to the choices of biological material and methodologies were tested. The ASFV
388 titre in the inoculum for infecting ticks appears as a key parameter in the vectorial competence experiments, with likelihood
389 of tick infection above 50% when ASFV load was higher than 10^6 HAD_{50}/mL. Accordingly, a threshold of $10^{5.75}$ HAD_{50}/mL was
390 previously determined by Ribeiro (Ribeiro *et al.* 2015) for the specific ability of *O. erraticus* to maintain and multiply two
391 Portuguese ASFV strains. Similar assumption was also proposed for the likelihood of ASFV transmission by ticks, with a
392 threshold of 10^4 HAD_{50}/mL as a sufficient virus load per tick to insure the success of ASFV dissemination in ticks and the
393 colonization of tick transmission organs (Kleiboeker & Scoles, 2001, Plowright, 1977, Ribeiro *et al.*, 2015). Forth *et al.* (2020)
394 also demonstrated an impact of ASFV titre on tick infection probability and load. Soon after ingestion, ASFV has to cross
395 the tick midgut barrier to be amplified in replication units localized in tick endothelial cells and then enter the tick
396 hemolymph (Burrage et al., 2013). These results may suggest that the tick midgut and other tick membranes act as porous
397 surfaces; the probability of ASFV entry would thus depend on the number of available pores (Franz *et al.*, 2015, Gutiérrez
398 *et al.*, 2012). Tick immune system such as RNAi could also block low level of ASFV infection (Forth *et al.*, 2020).

399 The development stage of ticks had a significant impact on tick infection status, with better results for late stages (from
400 nymph 4 to adults). This pattern confirms previous results on *O. erraticus*-Tomar and *O. erraticus*-OURT88/1 from Ribeiro
401 (Ribeiro *et al.* 2015). This was explained by higher blood volume intake for late and large stages, and therefore highest ASFV
402 titre inside the tick after bloodmeal digestion. In addition, we found no effect of observed sexual dimorphism in adults on
403 the likelihood of tick infection which is in agreement with Ribeiro et al (2015). In this study, the authors suggest that, in
404 adults, the number of ASFV particles already reached a certain threshold necessary to cause tick infection.

405 The route for tick infection was expected to play a role on tick competence. Natural feeding on vireamic pigs may provide
406 higher viral titre (usually >10^6 HAD_{50}/mL) than artificial feeding on membrane or with capillary (from 10^4-10^6 HAD_{50}/mL)
407 due to the difficulty for amplifying ASFV on pig cell cultures. Injection of virus into the haemocoel spread the virus
408 throughout tick body and remains traumatic for the tick (Bonnet & Liu, 2012). Despite differences between inoculation
409 methods, this does not seem to translate into any significant differences on the success rate of infection in our dataset. This
410 could be attributed to our dataset being incomplete and imbalanced, with the virus titre being a confounding factor. The
411 impact of inoculation methods could be specifically addressed by conducting dedicated studies comparing the various
412 methods with a similar virus titre. Similarly, we did not find any effect of the initial host in which viral strain was isolated on
413 tick competence.

414 Our review highlighted the huge diversity of measurements used in the included papers. They differ in their value for
415 informing the various steps of tick competence. Apart from PCR based viral detection, all the other methods reported from
416 included papers show some weakness to assess tick infection. For instance, ASFV isolation and titration on pig alveolar
417 macrophages was largely used by authors but this technique may suffer from inhibition issues due to cytotoxic effect of tick
418 components on pig macrophages and its achievement in at least 6 days is very time-consuming (Oura et al., 2013). In our
419 analysis, the main issue was to differentiate “residual” infection that consisted of remaining virus or viral DNA in the tick
420 midgut before complete digestion, from “true” infection where active virus crossed the tick midgut barrier and began its
421 replication in tick cells. Accordingly, we advise to use detection of viral DNA only 2 months after inoculation. Alternatively,
422 Reverse Transcription quantitative PCR could be used in a shorter delay, as it detects ASFV gene duplication and expression,
423 indicating ASFV crossing of tick midgut barrier where replication units are located. Finally, favoring oral inoculation of the
424 virus, instead of virus injection to the heamolymph, would avoid doubtful detection of ASFV and bypassed the immune

425 system avoiding a counterfeit infection (*O. porcinus*-MwLil/20/1). Since viral titre has the largest effect on tick infection
426 success, preferring blood from vireamic pigs would insure sufficient virus load and probably an ideal virus hemadsorption
427 to erythrocytes. Similarly, when feasible, late nymphs or adult ticks should be used since they will allow large blood intake.
428
429

430 **Conclusion and perspectives**

431 The examination of 39 papers on the vector competence of *Ornithodoros* soft ticks for ASFV highlighted factors
432 influencing vector competence results. For the same tick-ASF association, vector competence results may vary according
433 to the methodology used for infecting ticks (ASFV titre in the inoculum), the choice of biological material (viral strain, tick
434 development stage of tick, tick colony), and the techniques used to monitor and assess the likelihood of tick infection.
435 Although comparisons were difficult due to the scarcity of complete vector competence assessment for many tick-ASFV
436 associations, our analysis highlighted some standards that could be used for further investigations on the vector
437 competence of *Ornithodoros* ticks for ASFV.
438

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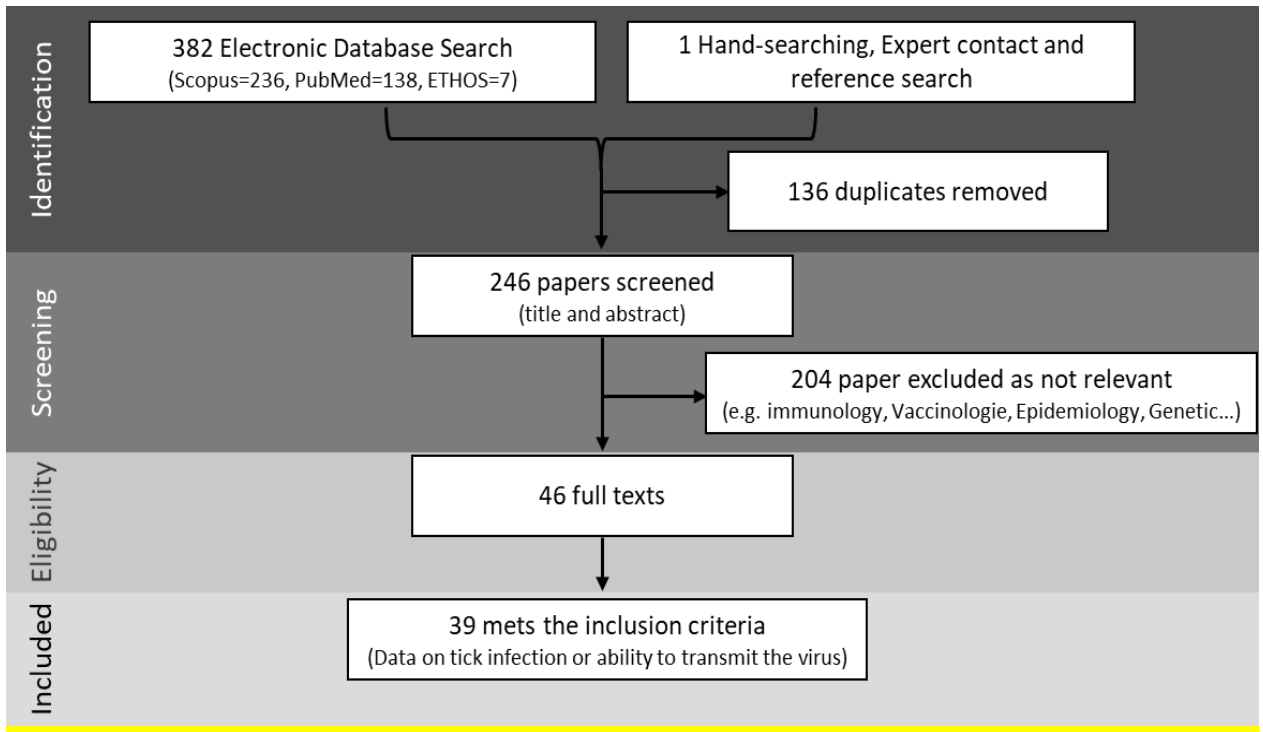
Appendix



Annex 1: Article selection process according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines

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549 Annex 2: Number of experiments reported for each virus strain, virus genotype and tick species in the whole dataset (900 experiments)

Virus strain		Virus genotype		Tick species	
asfvp99	30			coriaceus	58
brazil78	4			erraticus	396
chiredzi831	38			moubata	107
dr2	56			parkeri	4
lisbon60	10			porcinus	279
liv1333	46			puertorice	33
malta78	9			savignyi	8
mart	5			sonrai	4
netherlands86	4			turicata	2
nhp68	30			verrucosus	9
nu954	5	I	584		
ourt881	110				
ourt882	40				
portugal86	51				
sardinia	10				
senegal06	4				
tomar87	93				
vict901	4				
zimbabwe82	7				
zimbabwe83a	17				
zimbabwe83b	11				
georgia20071	42	II	52		
madagascar08	1				
ukr12zapo	9				
tengani62	19	V	19		
mwLil201	22	VIII	22		
kenrie1	20	IX	10		
ken06bus	10	X	176		
kwh12	30				
qet	12				
qew	17				
uganda61	97				
crocodil961	4	XIX	8		
nooitverwatcht966	4				
pretoriuskop9641	19	XX	19		
114a2	1	unknown	10		
Otu87	2				
transvaalcv	7				

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553 Annex 3: Number of experiments reported in each category of the studied experimental factors in the whole dataset (900 experiments). NA:

554 not available.

Exposure route		Tick stage		Tick colony		Virus host		Viral titer		Geographic co-occurrence	
artificial	319	early	210	field	438	pig	364	<10^4	109	yes	747
capillary	79	late	522	lab	425	tick	389	10^4 to 10^	296	no	153
natural	427	mix	131	NA's	37	warthog	145	>10^6	495		
inoculation	75	NA's	37			NA's	2				

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556 Annex 4: Number of experiments reported for each virus strain, virus genotype and tick species in the dataset analysed.

Virus strain		Virus genotype		Tick species	
asfvp99	20			coriaceus	2
chiredzi831	16			erraticus	244
dr2	3			moubata	44
lisbon60	2			parkeri	1
liv1333	29			porcinus	104
malta78	1			puertorice	3
nhp68	26			savignyi	4
nu954	5			verrucosus	6
ourt881	61	I	291		
ourt882	21				
portugal86	25				
sardinia	4				
tomar87	69				
vict901	4				
zimbabwe82	1				
zimbabwe83a	2				
zimbabwe83b	2				
georgia20071	20	II	26		
ukr12zapo	6				
tengani62	6	V	6		
mwLil201	6	VIII	6		
kenrie1	4	IX	5		
ken06bus	5	X	73		
kwh12	21				
qet	7				
qew	8				
uganda61	33				
pretoriuskop9641	1	XX	1		

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559 Annex 5: Number experiments reported in each category of the studied experimental factors in the dataset analysed.

Exposure route		Tick stage		Tick colony		Virus host		Viral titer		Geographic co-occurrence	
artificial	169	early	81	field	249	pig	172	$<10^4$	68	yes	370
capillary	47	late	327	lab	159	tick	174	10^4 to 10^6	148	no	38
natural	154					warthog	62	$>10^6$	192		
inoculation	38										

Annex 6: 5 best models with the included variables influencing infection status (note that all models have « virus strain » as random effect), corrected Akaike Information Criteria and weight of the model (probability that the model is the best out of all models considered).

Adaptation tick/virus	Exposure	Colony type	Tick stage	Viral titer	Viral host	AICc	delta
		+	+	+		393.6	0.00
+		+	+	+		395.6	2.01
		+	+	+	+	395.7	2.07
+		+	+	+	+	397.4	3.84
	+	+	+	+		399.6	5.97