Quantifying and modelling the acquisition and retention of lumpy skin disease virus by haematophagus insects reveals clinically but not subclinically-affected cattle are promoters of viral transmission and key targets for control of disease outbreaks.

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Running Head: Vector-borne transmission of lumpy skin disease virus

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Abstract

Lumpy skin disease virus (LSDV), is a vector- transmitted poxvirus that causes disease in cattle. The vector species involved in LSDV transmission and their ability to acquire and transmit the virus are poorly characterised. Using a highly representative bovine experimental model of lumpy skin disease we fed four model vector species (*Aedes aegypti*, *Culex quinquefasciatus*, *Stomoxys calcitrans* and *Culicoides nubeculosus*) on LSDV-inoculated cattle in order to examine the acquisition and retention of LSDV by these species. We found the probability of LSDV transmission from clinical cattle to vector correlated with disease severity. Subclinical disease was more common than clinical disease in the inoculated cattle, however the probability of vectors acquiring LSDV from subclinical animals was very low.

All four potential vector species studied had a similar rate of acquisition of LSDV after feeding on the host, but *Aedes aegypti* and *Stomoxys calcitrans* retained the virus for a longer time, up to 8 days. There was no evidence of virus replication in the vector, consistent with mechanical rather than biological transmission. The parameters obtained in this study were combined with data from previously published studies of LSDV transmission and vector life history parameters to determine the basic reproduction number of LSDV in cattle mediated by each of the model species. This was highest for *Stomoxys calcitrans* (19.1), *C. nubeculosus* (7.4), and *Ae. aegypti* (2.4), indicating these three species are potentially efficient transmitters of LSDV, which can be used to inform LSD control programmes.

Importance

Lumpy skin disease virus (LSDV) causes a severe systemic disease characterised by cutaneous nodules in cattle. LSDV is a rapidly emerging pathogen, having spread since 2012

from Africa and the Middle East into Europe, Russia, and across Asia. The vector-borne nature of LSDV transmission is believed to have promoted the rapid geographic spread of the virus, however a lack of quantitative evidence about LSDV transmission has hampered effective control of the disease during the current epidemic. Our research has combined experimental and modelling approaches in order to calculate the reproductive number of different insect species, therefore identifying efficient transmitters of LSDV. It has also characterised a subclinical form of LSDV in cattle and shown that these animals play little part in virus transmission. This information can be used to devise evidence-based, proportionate, and effective control programmes for LSD.

Key words: poxvirus, lumpy skin disease, transmission, mosquitoes, flies, midges, basic reproduction number, vector, control, *Aedes aegypti*, *Culex quinquefasciatus*, *Stomoxys calcitrans, Culicoides nubeculosus*

Introduction

Lumpy skin disease virus (LSDV) is a large DNA virus of the family *Poxviridae* and the etiological agent for lumpy skin disease (LSD) in cattle. LSDV is a rapidly emerging pathogen that is mechanically transmitted by vectors. First described in Zambia in cattle in 1929, LSDV subsequently spread throughout Africa and into the Middle East (1). In the past decade the virus has increased its geographical coverage substantially, entering and spreading within Europe and Asia including Russia, India, Bangladesh, Taiwan and China (2-8). This rapid expansion of LSDV distribution has focused attention on improving our knowledge of LSDV transmission, in order to counter the spread of the virus.

LSD is characterised by fever, weight loss, and prominent multifocal necrotising cutaneous lesions (9), and affects cattle of all ages (10). Morbidity in disease outbreaks ranges from 5-26%, and mortality 0.03-2% (2-4, 11-13). Control measures include vaccination, quarantine and partial or complete culling of infected herds. LSD outbreaks and the subsequent control measures cause significant negative economic and welfare impacts in endemic (14-16) and epidemic (17) situations, resulting in food insecurity for affected communities.

Haematophagus dipterans (referred to in this work as “blood-feeding insects”), particularly *Stomoxys calcitrans*, have been associated with outbreaks of LSDV (7, 18-20). In addition, experimental transmission of LSDV from affected to naïve animals (defined by the presence of clinical disease and/or detection of systemic LSDV antigen and/or capripoxvirus-specific antibodies) has being demonstrated via the mosquito *Aedes aegypti* (21), the ticks *Rhipicephalus appendiculatus (22-24)*, *Rhipicephalus decoloratus* (25), *Amblyomma hebraeum* (26), the stable fly *Stomoxys calcitrans,* horseflies *Haematopota* spp. and other *Stomoxys* species (27, 28). LSDV DNA has also been detected in other species after feeding

62 on infected cattle or an infectious blood meal (*Culex quinquefasciatus*, *Anopheles stephensi*,
63 *Culicoides nubeculosus*) (29), or in field-caught pools (*Culicoides punctatus*) (4). However
64 transmission of LSDV to susceptible animals has not been confirmed for these species. To
65 date the mode of LSDV arthropod transmission has been assumed to be mechanical as no
66 evidence of active virus replication in insects or ticks has been found (30).

67 Despite growing evidence of the potential participation of different arthropods in the
68 transmission of the LSDV, there is an important gap in understanding how efficient each
69 vector is in contributing to the transmission of LSDV.

70 LSDV can be detected in skin lesions, blood (primarily in peripheral blood mononuclear
71 cells), and in nasal, oral and ocular excretions of infected cattle (27, 31, 32). Viraemia is
72 considered of short duration and relatively low level, though the virus can survive for longer
73 periods of time in skin lesions (31). LSDV has also been detected in seminal fluid of diseased
74 bulls (33), making venereal transmission a possibility (34-36). Subclinical infections
75 (detection of LSDV in animals without cutaneous lesions) (3, 27, 32) and resistance to LSDV
76 (absence of LSDV and cutaneous lesions following experimental challenge) have been
77 reported, but both are poorly documented. The contribution of subclinical LSD to the
78 transmission of the virus is unclear and a topic of controversy when implementing control
79 measures such as whole-herd culling, particularly when morbidity is low (37, 38).

80 Previous research has assessed if LSDV transmission can occur when mediated by different
81 insects. These studies cannot be solely used to assess the risk of transmission as they were
82 designed with a variety of weaknesses (reduced number of donor cattle and times post-
83 infection, use of virus-spiked blood meals and/or reduced number of insects assayed)
84 resulting in large uncertainty in the parameter estimates. In this study we used new

85 approach to address these weaknesses, incorporating published and new experimental data
86 on LSDV transmission and vector biology. Using our experimental LSD model in cattle we
87 calculated biologically relevant parameters for the mechanical transmission of LSDV using
88 four blood-feeding insect species previously reported to acquire LSDV (*S. calcitrans*, *Ae.*
89 *aegypti*, *Cx. quinquefasciatus* and *C. nubeculosus*). These transmission parameters were
90 used to understand the risk of transmission of the virus from experimentally infected cattle
91 to each model species, and then were combined with data from previous studies to
92 determine the basic reproduction number for each species.

93 **Results**

94 **Experimental infection of calves with LSDV**

95 ***Experimental inoculation of calves with LSDV results in clinical and subclinical disease.***
96 Eight calves were challenged by intravenous and intradermal inoculation of LSDV in order to
97 act as donors on which blood-feeding insects could feed. The clinical and pathological
98 findings have been described previously (9), and resemble those of naturally infected cattle
99 (2, 4, 8, 11, 12, 37). Three calves (calves 3, 5, and 9) developed lumpy skin disease,
100 characterised by severe multifocal dermatitis with necrotising fibrinoid vasculitis consistent
101 with field reports of LSD (Figure S1A). The cutaneous lesions initially appeared in close
102 proximity to the inoculation site at 5 days post challenge (dpc) for calves 5 and 9, and at
103 distant sites in all three clinical calves at 7 dpc. The five remaining calves (calves 2, 4, 7, 8
104 and 10) did not develop lesions other than at the inoculation sites (Figure S1B). All eight
105 inoculated calves developed a fever which was more prolonged in calves with clinical signs
106 (Figure S1C). Superficial lymph nodes, predominantly the superficial cervical lymph node,
107 were enlarged in both groups starting between 2-5 dpc, with larger lymph nodes present in

clinical compared to subclinical calves (Figure S1D). Two non-inoculated in-contact calves (calves 1 and 6) were included in the study and did not develop any clinical signs or lesions consistent with LSD.

LSDV DNA can be detected in blood and skin of clinical and subclinical calves. In the three clinically-affected calves viral DNA was first detected in the blood by qPCR at 5 dpc and remained detectable in all subsequent blood samples (up to 19 dpc). Peak viral DNA levels in the blood (6.9, 5.3 and 5.3 $\log_{10}$ copies/ml in calves 3, 5 and 9, respectively) were reached at 11 dpc (Figure 1). By contrast, viral DNA was detected only intermittently in the blood of four (out of five) subclinically infected calves between 5 dpc and 19 dpc. In addition, genome copy numbers were lower (median: 2.1 $\log_{10}$ copies/ml; range: 1.2 to 2.4 $\log_{10}$ copies/ml) than those in clinically-affected calves (Figure 1). Although negative for LSDV in whole blood, the peripheral blood mononuclear cell (PBMC) fraction of calf 7 was positive for viral DNA on days 7, 9 and 19 post challenge (Data S1). These results indicate that clinical calves had had more viral DNA present in the blood, and for longer compared to subclinical calves. However, LSDV DNA could be detected at least once in all eight challenged animals between 5 and 19 dpc.

Skin biopsies of cutaneous lesions taken at 7 dpc (calf 9) or 9 dpc (calves 3 and 5) contained abundant viral genomes as measured by qPCR (Figure 1). Viral DNA was detected in all subsequent biopsy samples, with the quantities detected remaining at an approximately constant level for the duration of the experiment (Figure 1). The amount of viral DNA present in the skin lesions varied between the three clinical calves in an analogous fashion to the viral DNA in blood, with the highest concentration of viral DNA detected in skin lesions of calf 3 and least in calf 9 (Figure 1). The peak level of viral DNA in skin was reached

after the peak level of viral DNA in blood in all three calves (Figure 1). Viral DNA was detected at three time points in biopsies of normal skin from one subclinical calf (calf 4) at a lower copy number than in the clinically-affected animals; skin biopsies from the other subclinical animals (calves 2, 7, 8 and 10) were all negative for LSDV DNA (Figure 1).

Infectious LSDV is present in larger quantities in the skin compared to blood. Both skin homogenate and PBMC suspension collected between 5 and 19 dpc from clinical calves were titrated to determine the quantity of live virus in these tissues. Although units of measurement are not directly comparable between sample types (i.e. skin vs PBMC), they are representative of the magnitude of exposure that haematophagus insects may encounter during feeding (i.e. mg of skin tissue and µl of blood). In all calves the viral titre from skin homogenate was higher and more constant than from PBMC suspension (Figure 2). Live virus was detected for six consecutive days from 5 dpc in the PBMC fraction of calf 3, whereas in calves 5 and 9 the virus was isolated only in three and two days (respectively) starting at day 7 post challenge. In contrast, all skin samples except one taken from dermal lesions contained live LSDV with a maximum titre of $10^{4.3}$ PFU/mg skin, which is over 10^{3}-fold greater than the maximum level of virus detected in PBMCs, emphasising the strong cutaneous tropism of LSDV. Biopsies collected from normal skin of clinical calves were negative for live virus (i.e. below 10^{-2} PFU/mg, Data S1) suggesting the virus is highly concentrated in the skin lesions of clinical animals. Live virus was not detected in blood or skin from subclinical animals (including samples which were qPCR positive).

Humoral response to LSDV inoculation. Serum from the three clinically-affected calves contained antibodies to LSDV at 15-17 dpc as determined by a commercial ELISA test. By the end of the study period all subclinical animals had also developed detectable LSDV

antibodies at levels lower than those observed in the clinical animals, but above those of the non-challenged controls (Figure S1E). The presence of detectable levels of antibodies confirmed exposure to the virus in all eight challenged animals, although the clinical outcome of challenge varied widely between the eight calves.

Acquisition and retention of LSDV by blood-feeding insects after feeding on donor cattle

We next studied the influence of this disease spectrum on the acquisition and retention of LSDV in blood-feeding insects. To assess the acquisition and retention of LSDV by blood-feeding insects, all eight challenged animals were exposed to two mosquito species, *Ae. aegypti* and *Cx. quinquefasciatus*, one species of biting midge, *C. nubeculosus*, and the stable fly, *S. calcitrans* on days 5, 7, 9, 11, 15, 17 and 19 post challenge. The selected species are potential mechanical vectors with different feeding mechanisms (39), covering those which will feed readily on cattle (*i.e. S. calcitrans*), as well as species models for Culex and Aedes mosquitoes (40, 41) and also biting midges (42, 43) which would feed on cattle. At each time point, a pot of insects of each species (i.e. four pots in total) was placed on a separate cutaneous nodule on a clinical animal and, a corresponding area of normal skin on a subclinical animal. Blood engorgement, as a measure for detection of insect biting activity, was assessed visually. A subset of the insects from each pot was tested for the presence of LSDV DNA by qPCR at 0, 1, 2, 4 and 8 days post feeding (dpf) (Figure S2). The smaller numbers of insects tested at the later time points reflect the lower numbers surviving for long enough to be tested.

Different models for the proportion of positive insects were compared to assess differences in: (i) the probability of transmission from bovine to insect (i.e. of acquiring LSDV) amongst insect species and between clinical and subclinical donors; and (ii) the duration of viral

177 retention amongst insect species (Table S1). Models were compared using the deviance
178 information criterion (DIC), with a model having a lower DIC preferred to one with a higher
179 DIC. Positive insects were those with LSDV DNA amplification by qPCR.

180 ***Probability of transmission from bovine to insect.*** A total of 3178 insects were fed on the
181 eight donor calves (over 7 feeding sessions), of which 180 were positive for viral DNA when
182 tested. A higher proportion of insects were positive after feeding on a clinical donor (173
183 out of 1159) compared to feeding on a subclinical donor (7 out of 2019) (Figure S2).
184 Comparing the proportion of positive insects for each species after feeding in clinical and
185 subclinical calves (Figure 3) revealed that the probability of transmission from bovine to
186 insect (i.e. of acquiring LSDV) does not differ amongst the four insect species, but that this
187 probability does differ between clinical and subclinical donors (Table S1). For a clinical
188 donor, the probability of transmission from bovine to insect was estimated (posterior
189 median) to be 0.22, while for a subclinical donor it was estimated to be 0.006 (Table 1). This
190 means that an insect feeding on a subclinical animal is 97% less likely to acquire LSDV than
191 an insect feeding on a clinical one (Table 1; Figure 3).

192 ***Infectiousness correlates with the level of viral DNA in blood and skin.*** The relationship
193 between the level of viral DNA in the skin or blood of a calf and the proportion of virus-
194 positive insects resulting from a feeding session was examined. For each feeding session
195 that took place on the three clinical calves, the proportion of insects containing viral DNA
196 post-feeding was calculated and compared to the viral DNA copy number present in both
197 the blood sample and the skin biopsy taken from the calf on that day (Figure 4). This
198 revealed a dose-response relationship between the levels of viral DNA in skin and blood and
199 the probability of transmission from bovine to insect (or "donor infectiousness").

Furthermore, this relationship was the same for all four insect species (Table S1), irrespective of their different feeding mechanisms. The relationship differed between levels of viral DNA in blood and skin (Table 2; Figure 4), with the probability of transmission being higher when the level of viral DNA in blood was used compared to skin (Figure 4). The fits of the models using levels of viral DNA in blood or skin are similar, suggesting that both are acceptable proxy measures for infectiousness of the donor.

Combining the dose-response relationship (Figure 4) with the time course for levels of viral DNA in blood or skin for each calf (Figure 1) shows how the infectiousness of an animal changes over time and how it varies amongst animals (Figure 1, right-hand column). This highlights the very low probability of transmission from bovine to insect (<0.01 at all time points; cf. estimate in Table 1) for calves which were only subclinically infected. In addition, for those calves which did develop clinical signs, there were differences in both the timing and level of infectiousness amongst the calves, which is a consequence of the underlying differences in viral dynamics in each animal. This is reflected in both the changes over time in the proportion of insects acquiring virus after feeding and differences in this proportion amongst clinical calves (Figure S3).

Duration of LSDV retention. Viral DNA was detected in *Ae. aegypti* and *S. calcitrans* up to 8 dpf, in *C. nubeculosus* up to 4 dpf and in *Cx. quinquefasciatus* up to 2 dpf (Figure 3). However, few *Cx. quinquefasciatus* mosquitoes survived to 4 or 8 dpf (Figure S2), resulting in uncertainty about the duration of retention in this species (Figures 3 & 4). The mean duration of viral retention differed amongst the four insect species in the present study (Figure 3; Table S1), being the longest for *Ae. aegypti* (5.9 days) and *S. calcitrans* (5.5 days), followed by *Cx. quinquefasciatus* (4.5 days), and *C. nubeculosus* (2.4 days) (Figure 3; Table

223 1). The corresponding virus inactivation rate (i.e. the reciprocal of the mean duration of
224 retention) was 0.17/day for *Ae. aegypti* and 0.18/day for *S. calcitrans*, 0.22/day for *Cx.*
225 *quinquefasciatus* and 0.42/day for *C. nubeculosus* (Table 1).

226 ***Levels of retained LSDV.*** The median amount of viral DNA in homogenized whole insects
227 was the same when tested at different days post feeding for three (out of the four) species:
228 *Ae. aegypti* (Kruskal-Wallis test: χ^2=0.98, df=4, *P*=0.91), *Cx. quinquefasciatus* (Kruskal-Wallis
229 test: χ^2=3.62, df=2, *P*=0.16) or *S. calcitrans* (Kruskal-Wallis test: χ^2=2.74, df=4, *P*=0.60) (Figure
230 S3). However, the median level of viral DNA was lower for individual *C. nubeculosus* tested
231 at later times post feeding (Kruskal-Wallis test: χ^2=10.8, df=3, *P*=0.01) (Figure S4). These
232 results are consistent with a mechanical rather than a biological form of vector-
233 transmission.

234 **Probability of transmission from insect to bovine**

235 Three previous studies have investigated the transmission of LSDV from insects to cattle,
236 where insects of species included in the present study were allowed to feed on an infected
237 donor and were subsequently allowed to refeed on a naïve recipient (21, 27, 29). The
238 number of positive insects refeeding was not determined in these studies. By combining
239 LSDV acquisition and retention results of the present study with challenge outcomes of the
240 aforementioned studies (i.e. whether or not transmission occurred), it is possible to
241 estimate the probability of transmission from insect to bovine. This probability was highest
242 for *Ae. aegypti* (0.56), intermediate for *C. nubeculosus* (0.19) and *Cx. quinquefasciatus* (0.11)
243 and lowest for *S. calcitrans* (0.05) (Table 1). However, there is considerable uncertainty in
244 the estimates for all species, but especially for *Ae. aegypti*, *C. nubeculosus* and *Cx.*
245 *quinquefasciatus* (Table 1), which makes it difficult to compare estimates across species.

Basic reproduction number for LSDV

The basic reproduction number (R_0) is defined as "the average number of secondary cases caused by an average primary case in an entirely susceptible population" (44). For LSDV, R_0 combines the parameters related to transmission (Table 1) with those related to vector life history (i.e. biting rate, vector to host ratio and vector mortality rate; see Table 1 in ref. 45) to provide an overall picture of the risk of transmission by the four insect species (45). The basic reproduction number was estimated to be highest for *S. calcitrans* (median R_0=19.1) (Table 1; Figure 5), indicating that this species is likely to be the most efficient vector of LSDV and would be able to cause substantial outbreaks if it were the sole vector in a region. Both *C. nubeculosus* (median R_0=7.1) and *Ae. aegypti* (median R_0=2.4) are also potentially efficient vectors of LSDV (i.e. R_0>1 for these species) and would be able to sustain transmission if either were the sole vector in a region. Finally, *Cx. quinquefasciatus* (median R_0=0.6) is likely to be inefficient at transmitting LSDV (Table 1; Figure 5). It would not be able to sustain transmission on its own, but it could contribute to transmission if other vector species were also present.

Exploring the contribution of clinical and subclinical animals to the basic reproduction number for each species further emphasises the more limited role played by subclinical animals in the transmission of LSDV (Figure 5). For all species, the R_0 for clinical animals alone is very close to that for both clinical and subclinical animals combined (Figure 5). Moreover, the median R_0 for subclinical animals alone is below one for all species, except *S. calcitrans* (Figure 5).

The R_0 values calculated from our data and previous studies provide a summary of the risk of LSDV transmission. A range of blood-feeding insects are likely to support a disease

outbreak by transmitting LSDV from a clinical to a naïve animal, particularly biting flies such as *S. calcitrans*. The R_0 calculations also highlight that, although there may be a significant subset of subclinical animals in an affected herd, they are likely to play at most a minor role in the transmission of the virus.

Discussion

This study describes a controlled experimental model of LSD that mimics disease features described in field outbreaks (2, 4, 8, 11, 12, 37) and other experimental models (27, 32). Inoculated calves (both clinical and subclinical) were used to measure the acquisition (transmission from bovine to insect) and retention of LSDV by four potential vector species. These data were then used to estimate the risk of transmission by these species with the aim of providing evidence with which to inform decisions during the implementation of measures to control LSDV.

In our experimental model we observed that 37.5% of calves developed generalised LSD with the remaining 62.5% of calves classified as subclinical (no cutaneous nodules, positive qPCR in blood (27)). This attack rate of 0.37 is comparable to other experimental models with field strains of LSDV (0.57 (27) and 0.50 (32)). Reports of animals with subclinical LSD in the field is sparse, with an incidence of up to 31.3% reported (3). The high detection of subclinical infection in our study may be a result of an intense sampling protocol (compared to the limited sampling of individuals during an outbreak investigation). Further investigation of the true incidence of subclinical LSD in field studies is warranted.

Cattle experimentally infected with LSDV, including in our study, have higher concentrations of LSDV in skin lesions than blood (Figures 1 & 2). In clinically infected animals we identified a relationship between the viral load in skin and blood and the proportion of insects positive

292 for the virus, indicating both skin and blood are good predictors of the transmissibility of
293 LSDV from donors to vector. Our study did not extend beyond 21 days post challenge
294 however, and this observation may only be true during the initial stage of the disease when
295 the viraemia is detectable. Donors with different disease severity and therefore different
296 levels of infectiousness would strongly influence the proportion of vectors which acquired
297 virus. This finding may explain the discrepancies between experimental studies which have
298 assessed the transmission of LSDV by vectors (21, 29) when the infectiousness of the donors
299 may have been different.

300 As reported in this study and others (27, 31) LSDV can be detected in the blood of cattle
301 prior to the appearance of skin lesions, 5-8 dpc. However, during this time, viraemia is
302 relatively low and in our study few insects were positive for LSDV after feeding (Figure S3).
303 Viraemia rises and peaks after the multifocal skin lesions appear (at around 7 dpc), and this
304 is when the probability of transmission from bovine to insect starts to increase (Figure 1).
305 The probability remains high while viraemia is high and when skin lesions are present. The
306 appearance of skin lesions therefore marks the start of the risk period for virus transmission,
307 and this means that rapid diagnosis and consequent implementation of control measures
308 should be possible and effective at limiting onwards transmission (46, 47). In this study we
309 were only able to follow the animals for 21 days post-challenge with the last exposure of
310 blood-feeding insects to infected calves on day 19, and thus the period for transmission risk
311 could not be established beyond this time point. Nevertheless, under controlled conditions
312 (31), LSDV has been isolated up to 28 (blood) and 39 (skin) days post-challenge, and
313 detected by PCR up to 91 days post-challenge (in skin biopsies). Therefore, LSDV uptake by
314 vectors may occur beyond the reported period in our study.

315 We found that subclinical donors were much less likely than clinical animals to transmit
316 virus to vectors (Table 1; Figure 3), indicating a substantially reduced role of subclinically
317 infected animals in the transmission of LSDV. For some vector-borne diseases such as
318 dengue fever, malaria, asymptomatic and preclinical individuals may be an important source
319 of the pathogen for vectors and help maintain the transmission cycle (48, 49). The situation
320 with LSDV appears to be different. The viraemia in subclinical animals is low and skin lesions
321 (representing the major viral load) are absent in these animals. Few vectors therefore
322 acquire LSDV from subclinical cattle, and this reduces the chances of onward transmission to
323 a susceptible host. This is the first time the relative contribution of subclinically infected
324 cattle to onward transmission of LSDV has been quantified.

325 Lumpy skin disease virus can be mechanically transmitted by stable and horse flies (27, 28)
326 and mosquitoes (21). Mechanical transmission of viruses by blood-feeding vectors can be
327 influenced by their feeding mechanism, ecology and biting behaviour. Stable flies are
328 aggressive feeders with a painful bite which leads to interrupted feeding and to more than
329 one feeding event per day (50, 51). They are also known to regurgitate previous blood
330 intakes while feeding. To penetrate the skin, stable flies rotate sharp teeth on their
331 proboscis (5-8mm long) and form a pool of blood from which they feed (39). *Culicoides*
332 midges also disrupt the skin barrier using their proboscis (0.1-0.2 mm long). Midges serrate
333 the skin using saw-like blades on their proboscis that cross over each other to produce a
334 pool of blood (39). Biting midges feed generally less frequently than stable flies as feeding is
335 associating to their gonotrophic cycle (7-10 days, but as a temperature-dependent event it
336 can be as short as 2-3 days) (52). Mosquitoes do not produce pools of blood, instead they
337 penetrate the skin “surgically” searching for a capillary with their proboscis of (1.5-2.0 mm
338 long), accompanied by a pushing and withdrawing movement until it hits a capillary from

339 which to withdraw blood [53]. Mosquitoes feeding on blood is also associated to their
340 gonotrophic cycle, but multiple feedings have been reported in some species (54, 55)
341 Despite these variations in feeding behaviour, all four insect species acquire LSDV at the
342 same rate, indicating that virus acquisition is not influenced by feeding behaviour.

343 All four insect species in the present study were able to acquire LSDV through feeding on
344 clinical animals and to retain it for several days (Figure 3). In a small proportion of *Ae.*
345 *aegypti* and *S. calcitrans* LSDV DNA was still present at 8 days post feeding, which was the
346 longest we investigated, thus longer retention cannot be ruled out. Similar to our study,
347 Chihota and co-authors (21), identified that *Ae. aegypti* mosquitoes feeding on animals with
348 clinical LSD were able to acquire and retained the virus for up to six days, and that the
349 proportion of virus-positive insects also decreased with days post feeding. They observed
350 similar dynamics in *Cx. quinquefasciatus* and *Anopheles stephensi* mosquitoes when using a
351 membrane-feeding system with a LSDV-infected bloodmeal, but when they fed *C.*
352 *nubeculosus* and *S. calcitrans* on LSDV-infected calves they did not detect the virus beyond
353 the day of feeding (*C. nubeculosus*) or the following day (*S. calcitrans*) (29). However, we
354 now know that disease severity of the donor can influence the acquisition of LSDV by an
355 insect. This could result in the lower acquisition and retention observed by Chihota et al. In
356 our work we identified that LSDV can be retained longer than previously reported in *S.*
357 *calcitrans* and *C. nubeculosus*, with a decline in virus DNA post-feeding only detectable for *C.*
358 *nubeculosus*.

359 For LSDV, as for other chordopoxviruses including capripox virus, fowlpox virus and myxoma
360 virus, the mode of vector-mediated transmission is assumed to be mechanical (21, 56-59).
361 Our data and that of Chihota and co-authors support the theory that LSDV does not

362 replicate in the insect (at least at detectable levels), but the retention of viral DNA in *Ae.*
363 *aegypti* and *S. calcitrans* at levels similar to those acquired during feeding deserves further
364 investigation (60).

365 Assessment of acquisition and retention of LSDV genome was performed in whole insect
366 homogenates in our study, and further investigations into the location of virus within the
367 insects were not possible. However an earlier study with *Ae. aegypti* (61) indicated that
368 LSDV DNA persist longer in the head than in the thorax/abdomen. This is consistent with
369 research that found myxoma virus was retained on the mouthparts of *Ae. aegypti*
370 mosquitoes up to 28 days post feeding (62). The mechanism by which poxviruses persist for
371 days on the mouthparts of vectors warrants further study.

372 The detection of LSDV in insect vectors in our study was based on the presence of viral DNA
373 rather than infectious virus particles. Virus titration from homogenates of individual insects
374 was attempted however we were able to detect live virus only from pooled homogenates of
375 *S. calcitrans* and of *Ae. aegypti* (data not shown), suggesting low numbers of infectious
376 virions are present on each insect. In previous work live LSDV was detected in individual *Ae.*
377 *aegypti* for up to 6 days following exposure to an infectious calf (21), and live goatpox virus
378 up to 4 days in *S. calcitrans* (59).

379 The aim of the present study was to use the results of feeding four model vector species on
380 LSDV-infected cattle to estimate parameters related to transmission that were not possible
381 with the data from previous studies. Given the large number of insects fed and tested
382 (>3000), the resulting estimates for the probability of transmission from bovine to insect
383 (including the relative risk of transmission from a subclinical animal and the dose-response)
384 are robust, as indicated by the narrow credible intervals for these parameters (Tables 1 and

385 2). The estimates for the duration of virus retention (or, equivalently, the virus inactivation
386 rate) are more uncertain (Table 1), which reflects difficulties in keeping insects alive to later
387 days post feeding, especially *Cx. quinquefasciatus* (Figure S2).

388 Although not assessed in the present study, we used data from previous transmission
389 experiments (21, 27) to estimate the probability of transmission of LSDV from insect to
390 bovine. The small number of studies (and animals in each study) mean that the estimates
391 for this parameter are uncertain, extremely so for *Ae. aegypti*, *Cx. quinquefasciatus* and *C.*
392 *nubeculosus* (Table 1). This uncertainty is less important for *Cx. quinquefasciatus*, which is
393 unlikely to be an important vector even if were able to transmit LSDV efficiently, but it
394 makes it difficult to determine whether or not *C. nubeculosus* is likely be an important
395 vector.

396 One previous study assessed the importance of different vector species by calculating R_0 for
397 LSDV from its component parameters (45), based on data from two studies by Chihota and
398 co-authors (21, 29). Despite different values for the underlying parameters, both this and
399 the present study obtained similar estimates of R_0 for *S. calcitrans* (median: 15.5 vs 19.1)
400 and for *Cx. quinquefasciatus* (median: 0.8 vs 0.6), suggesting the former is likely to be an
401 important vector and the latter is likely to be an inefficient vector of LSDV. The estimates of
402 R_0 for *Ae. aegypti* differed between the studies (median: 7.4 vs 2.4), due to differences in
403 estimates for the mean duration of virus retention (11.2 vs 5.9) and probability of
404 transmission from bovine to insect (0.90 vs 0.22). This suggests that *Ae. aegypti* (or other
405 *Aedes* spp. for which it could be considered a model) may be less efficient vector than
406 previously assumed. Finally, there was a major difference between the studies in their
407 estimates of R_0 for *C. nubeculosus*. The earlier study suggested this species is likely to be a

less efficient vector (median R_0=1.8), but the present one suggests it could be an efficient one (median R_0=7.4). This discrepancy is principally related to markedly different estimates for the mean duration of virus retention (0.01 days vs 2.4 days). Moreover, the estimate of R_0 for this species is highly uncertain, largely as a consequence of uncertainty in the estimate of the probability of transmission from insect to bovine (Table 1). *Culicoides* spp. are ubiquitous on cattle farms (63, 64) and, consequently, would represent a major transmission risk if they proved to be efficient vectors of LSDV. Hence, it is important that their ability to transmit virus to cattle is assessed.

Linking transmission experiments with mathematical modelling is an uncommon and powerful approach to create robust evidence which can inform policy makers involved in controlling the spread of infectious diseases. Here we have used this approach to investigate the transmission of LSDV, which has recently emerged as a significant threat to cattle in Africa, Asia and Europe. Our evidence indicates that *S. calcitrans* is likely to be an important vector species. It also suggests that *Culicoides* biting midges may be a more efficient vector species, than previously considered. Furthermore, we have demonstrated for the first time that subclinical infected cattle pose only very limited risk of onward transmission of LSDV to potential vectors. This evidence supports LSD control programmes which target clinically-affected cattle for rapid removal, rather than complete stamping-out of all cattle in an affected herd.

Methods

Experimental design

Ethical statement, housing and husbandry. The experimental study was conducted under the project license P2137C5BC from the UK Home Office according to the Animals (Scientific Procedures) Act 1986. The study was approved by the Pirbright Institute Animal Welfare and Ethical Review Board. Cattle were housed in the primary high containment animal facilities (Biosafety Level 3 Agriculture) at The Pirbright Institute. The husbandry of the animals during the study was described previously (9).

Challenge study and experimental procedures. Ten Holstein-Friesian male cattle (referred to as calves) were used for the study, which was done in two experimental replicates of five animals each. The median age and weight of the calves was 104 days old, 145 kg in replicate one and 124 days old, 176 kg in the second replicate. Eight calves were challenged by intravenous and intradermal inoculation with a suspension of LSDV containing 10^6 PFU/ml (9). More specifically, 2 ml were inoculated intravenously (jugular vein) and 1 ml was inoculated intradermally in four sites (0.25 ml in each site), two on each side of the neck. The remaining two calves were not challenged and were kept as non-inoculated in-contact controls. Calves were randomly assigned to either the control or challenge groups using a random number generator (excluding control calf 1, which was assigned as a control on welfare grounds following diagnosis with shipping fever pneumonia). The calves were kept for 21 days following the challenge; clinical scores were taken daily and serum, whole blood and skin biopsies (9) collected over the study period. The non-steroidal anti-inflammatory drug meloxicam (0.5 mg/kg body weight) (Metacam 20 mg/ml solution, Boehringer Ingelheim) was used when required on welfare grounds.

450 ***Insect exposure.*** Blood-feeding insects used in the study were: *Aedes aegypti* 'Liverpool'
451 strain, *Culex quinquefasciatus* TPRI line (Tropical Pesticides Research Institute, obtained
452 from the London School of Hygiene and Tropical Medicine, London, UK), *Stomoxys calcitrans*
453 (colony established in 2011 from individuals kindly provided by the Mosquito and Fly
454 Research Unit, USDA Florida) and *Culicoides nubeculosus* (65). All insects were reared at The
455 Pirbright Institute under the following insectary conditions. *Ae. aegypti* were reared in pans
456 of 300 larvae per pan, containing approximately 1 litre of water supplemented with fish
457 food and housed at 28 °C, 70% relative humidity (RH) and 12:12 light/dark cycle. *Cx.*
458 *quinquefasciatus* were reared in pans of 500-800 larvae per larval bowl, containing
459 approximately 1.5 litre of water supplemented with ground guinea pig food and maintained
460 at 26 °C, 50% RH and 16:8 light/dark cycle. *S. calcitrans* were reared in approximately 200
461 eggs per pot, incubated for 12-13 days in larval pots containing a ratio of 3:2:1 (powdered
462 grass meal, water and corn flour) and a table spoon of yeast. *C. nubeculosus* were reared in
463 approximately 10,000 larvae per pan containing 2 litres of dechlorinated water
464 supplemented with oxoid broth and dried grass/wheat germ mix. Pots of 800 *Culicoides*
465 pupae were made with males and females and allowed to emerge. Both *S. calcitrans* and *C.*
466 *nubeculosus* were maintained in insectaries at 27 ± 2 °C, 50% RH, with a 16:8 light/dark
467 cycle.

468 The age and sex composition of the insects at exposure was: female and male *C.*
469 *nubeculosus* between 0-2 days post-eclosion, female *Cx. quinquefasciatus* and *Ae. aegypti* at
470 5-7 days post-eclosion and male and female *S. calcitrans* at an average of 4 days post-
471 eclosion (range: 2-7 days). All adult insects were maintained on 10% sucrose and starved 18-
472 24 hours before exposure to the calves.

473 All eight challenged calves, independent of clinical status, were exposed (for between 5 and
474 20 minutes) to each of the four insect species on days 5, 7, 9, 11, 15, 17 and 19 post
475 challenge. At each time point each pot of insects was placed on a cutaneous nodule on a
476 clinical animal and a corresponding area of normal skin on a subclinical animal. The hair of
477 the calf at each feeding site was clipped and/or shaved, and the insects were held in close
478 contact with the skin of the calves in a container covered by a mesh. Around two hours after
479 exposure, insects were anaesthetised under CO_2 and unfed individuals discarded and blood-
480 engorged individuals collected.

481 For *Ae. aegypti*, *Cx. quinquefasciatus* and *C. nubeculosus* blood engorgement was assessed
482 visually by the presence of blood in the abdominal cavity. However, *S. calcitrans* were all
483 collected "blind" and blood engorgement was confirmed by the detection of the bovine
484 cytochrome *b* gene using qPCR. Those individuals negative for cytochrome *b* at collection
485 were removed from the analysis.

486 Samples from each insect group taken immediately following blood-feeding assessment (dpf
487 0) were stored at -80C, and the rest of the insects were maintained for 1, 2, 4 or 8 dpf. After
488 this incubation period, surviving individuals were collected and stored at -80 ºC after the
489 incubation period. Throughout incubation all insects were maintained on 10% sucrose
490 solution, except *S. calcitrans* which were maintained with defibrinated horse blood (TCS
491 Biosciences Ltd) after 2 dpf. All insects were kept in a temperature-controlled room at
492 biocontainment level 3, with a 10:14 light/dark cycle. For the incubation, cardboard/waxed
493 pots containing the insects were placed inside plastic boxes covered by a mesh which were
494 kept under a plastic shelter to minimise temperature and humidity fluctuations.
495 Temperature (mean: 24.8˚C; range: 22.4˚C – 26.4˚C) and RH (mean: 35.9%; range: 18.5% –

48.9%) of the room and of the incubation area were recorded approximately every 15 minutes (RF513, Comark Instruments and HOBO UX100-003, Onset).

Samples. Skin biopsies were weighed on a calibrated scale (EP613C Explorer Pro, OHAUS®) and homogenised in 500 µl high-glucose Dulbecco's modified Eagle's medium (41965, Life Technologies) supplemented with 5% foetal bovine serum (Antibody Production Services Ltd, Bedford, UK), 100 U/ml penicillin and 100 µg/ml streptomycin (15140122, Life Technologies) and 2.5 µg/ml amphotericin B (15290026, Life Technologies) in a Lysing Matrix A tube (SKU 116910050-CF, MP Biomedicals) using a portable homogeniser (BeadBug Microtube Homogenizer, D1030, Benchmark Scientific Inc.). Whole insects were homogenised using a TissueLyser® (Qiagen, UK) with one or two steel beads of 3 mm (Dejay Distribution, UK) (66) in 200 µl Dulbecco's phosphate buffered saline (PBS, 14190094, Life Technologies), supplemented with penicillin-streptomycin and amphotericin B, as above. Bovine peripheral blood mononuclear cells (PBMC) were isolated from 7 ml of whole blood in EDTA diluted in PBS 1:1. The diluted blood was added to a SepMate™-50 centrifugation tube (Stemcell Technologies) under-layered with Histopaque®-1083 (Sigma-Aldrich). Tubes were centrifuged at 1500×g for 30 minutes, 20 °C with no brake. PBMCs were aspirated from the interface into PBS, washed three times with PBS at 1000×g for 10 minutes at 20 °C. After the final wash, cells were resuspended in 2 ml of RPMI medium (21875091, Life Technologies) supplemented with 10% foetal bovine serum, and penicillin-streptomycin as above. Blood collected without anticoagulants was allowed to clot, spun at 1000×g to 2000×g for 10 minutes in a refrigerated centrifuge and the serum collected. All samples were stored at -80 °C until analysed.

518 ***Laboratory assays.*** Nucleic acid from 200 µl of whole blood, PBMC suspension, skin
519 homogenate or 100 µl of insect homogenate was extracted in a 96-well plate with the
520 MagMAX™ CORE Nucleic Acid Purification Kit (Applied Biosystems, A32700) using protocol A
521 in a KingFisher™ Flex Magnetic Particle Processor (Applied Biosystems) and eluted in 50 µl of
522 buffer. qPCR for LSDV ORF074 detection was performed using a modification of the TaqMan
523 assay described by Bowden *et al.* (67) with the Path-ID™ qPCR Master Mix (Life Technologies
524 #4388644). Briefly, a 20 µl reaction was prepared using 5 µl of template, 400 nM of each
525 primer, 250 nM of the probe and nuclease-free water to the final volume. Samples were
526 prepared in a 96-well plate and assayed using the Applied Biosystems™ 7500 Fast Real-Time
527 PCR System with the program: 95 °C for 10 min, and 45 cycles of 95 °C for 15 s and 60 °C for
528 60 s. Tissue culture derived LSDV positive controls were included in the extraction plates,
529 and the copy number of LSDV genome were quantified using gBlocks® Gene Fragments
530 (Integrated DNA Technologies) to generate the standard curve. The gBlocks® Gene
531 Fragment included the target sequence of the Bowden assay for detection of LSDV ORF074
532 (AAATGAAACCAATGGATGGGATACATAGTAAGAAAAATCAGGAAATCTATGAGCCATCCATTTTCC
533 AACTCTATTCCATATACCGTTTT). Bovine blood intake by insects was determined using a SYBR
534 green assay (PowerUp™ SYBR™ Green Master Mix, A25779, Life Technologies) for the
535 detection of bovine mitochondrial *cytochrome b* as described by Van Der Saag *et al.* (68)
536 with some modifications (forward primer: 5' GTAGACAAAGCAACCCTTAC at 300nM; reverse
537 primer: 5' GGAGGAATAGTAGGTGGAC at 500nM) using the manufacturer cycling conditions
538 for primers with Tm >60 °C. The assay was performed in 10 µl reaction using 2 µl of
539 template. This assay was specific for bovine *cytochrome b* and melt curve analysis was
540 performed to confirm that only specific amplification occurred. For all qPCR assays a
541 constant fluorescence threshold was set which produced a reproducible Cq values for the

positive control samples between runs. A double antigen ELISA (ID Screen® Capripox, IDvet) was used to detect circulating antibodies for LSDV in serum samples following the manufacturer's protocol and analysed with the Multiskan FC Microplate photometer (Thermo Scientific™). Infectious virus titrations of PBMC suspension, insect and skin homogenate was performed by viral plaque quantification in MDBK cells.

Parameter estimation

Full details of parameter estimation are provided in Text S1. Briefly:

Probability of transmission from bovine to insect and virus inactivation rate. The numbers of insects positive for viral DNA after feeding on cattle infected with LSDV were used to estimate the probability of transmission from bovine to insect and the virus inactivation rate. The probability that an insect would be positive when tested is

$$p = \beta \exp(-\gamma t). \quad (1)$$

where β is the probability of transmission from bovine to insect, γ is the virus inactivation rate (i.e. the reciprocal of the mean duration of virus retention) and t is the time post feeding at which the insect was tested. Equation (1) combines the probability that an insect acquired virus (β; i.e. the probability of transmission from bovine to insect) and the probability that the insect retained the virus until it was tested at t days post feeding ($\exp(-\gamma t)$).

Differences amongst insect species in the virus inactivation rate and probability of transmission from bovine to insect and in the probability of transmission between subclinical and clinical animals were explored by comparing the fit of models in which these

563 parameters did or did not vary with species or clinical status of the donor cattle. In addition,
564 the dose-response relationship was investigated by allowing the probability of transmission
565 from bovine to insect to depend on the level of viral DNA (in either blood or skin) in the
566 donor animal, so that,

567 $$\log\left(\frac{\beta}{1-\beta}\right) = d_0 + d_1 V, \quad (2)$$

568 where d_0 and d_1 are the dose-response parameters and V is the level of viral DNA ($\log_{10}$
569 copies/ml in blood or $\log_{10}$ copies/mg in skin) in the donor when the insect fed. The
570 different models were compared using the deviance information criterion (69). The two
571 proxy measures for infectiousness (i.e. level of viral DNA in blood or skin) were compared by
572 computing posterior predictive *P*-values for each insect.

573 ***Probability of transmission from insect to bovine.*** Data on transmission of LSDV from insect
574 to bovine were extracted from the published literature (21, 27, 29). In these experiments,
575 batches of insects (of the same species as used in the present study) were allowed to feed
576 on an infected bovine and then to refeed at later time points on a naïve recipient. The
577 probability of the recipient becoming infected is

578 $$q = 1-(1-b\beta\exp(-\gamma T))^n, \quad (3)$$

579 where b is the probability of transmission from insect to bovine, β is the probability of
580 transmission from bovine to insect, γ is the virus inactivation rate, T is the time interval
581 between feeding on the donor and refeeding on the recipient and n is the number of insects
582 which refed. The probability, (3), is the probability that at least one insect (out of the n
583 refeeding) transmitted LSDV, where the probability that an individual insect will transmit is

584 the product of the probabilities that it acquired the virus during the initial feed (β), retained
585 it until refeeding ($\exp(-\gamma T)$) and that it subsequently transmitted LSDV at refeeding (b).

586 ***Latent and infectious periods in cattle.*** Previous estimates for the latent and infectious
587 periods of LSDV (45) were updated using the data on detection of LSDV in blood and skin
588 collected during the present and other recently published studies (27, 32). In addition, the
589 proportion of cattle that develop clinical disease following challenge was estimated using
590 data extracted from the published literature (27, 31, 32, 70, 71) and the present study.

591 ***Bayesian methods.*** Parameters were estimated using Bayesian methods. For all analyses,
592 samples from the joint posterior distribution were generated using an adaptive Metropolis
593 scheme (72), modified so that the scaling factor was tuned during burn-in to ensure an
594 acceptance rate of between 20% and 40% for more efficient sampling of the target
595 distribution (73). The adaptive Metropolis schemes were implemented in Matlab (version
596 2019b; The Mathworks Inc.) and the code is available online at
597 https://github.com/SimonGubbins/LSDVAcquisitionAndRetentionByInsects. Two chains
598 were allowed to burn-in and then run to generate an effective sample size of around 5,000
599 samples (assessed using the mcmcse package (74) in R (version 3.6.1 (75)). Convergence of
600 the chains was assessed visually and using the Gelman-Rubin statistic provided in the coda
601 package (76) in R (75). Different models for the variation amongst species in virus
602 inactivation and probability of transmission from bovine to insect (Table S1) were compared
603 using the deviance information criterion (69).

604 **Basic reproduction number for LSDV**

The basic reproduction number, denoted by R_0, is the "average number of secondary cases arising from the introduction of a single infected individual into an otherwise susceptible population" (44). The basic reproduction number for LSDV is,

$$R_0 = \sqrt{\frac{b\beta m a^2}{(\mu+\gamma)}\left(p_C \frac{1}{r_C} + (1-p_C)\rho \frac{1}{r_S} \right)}, \quad (4)$$

where b is the probability of transmission from insect to bovine, β is the probability of transmission from bovine to insect, ρ is the relative risk of transmission from a subclinical compared to a clinical bovine, γ is the virus inactivation rate, p_C is the proportion of cattle that develop clinical disease and $1/r_C$ and $1/r_S$ are the mean durations of infectiousness for clinical and subclinical animals, respectively, all of which were estimated in the present study, and a, m and μ are the biting rate, vector to host ratio and vector mortality rate, respectively. The formal derivation of this expression, (4), is given in Text S1.

Replicated Latin hypercube sampling was used to compute the median and 95% prediction interval for R_0 for each insect species (45). Parameters were sampled either from their marginal posterior distributions derived in the present study (b, β, ρ, γ, p_C, $1/r_C$ and $1/r_S$; see Tables 1 and S2) or uniformly from plausible ranges (a, m and μ; see Gubbins (45), their Table 1). The mean duration of infection for clinical animals ($1/r_C$) is based on detection of virus or viral DNA in skin, while that for subclinical animals ($1/r_S$) is based on detection of viral DNA in blood (Table S2).

Data availability

The authors declare that the main data supporting the findings of this study are available within the article and its Supplementary Information files.

Code availability

The code and the data used are available online for readers to access with no restriction at https://github.com/SimonGubbins/LSDVAcquisitionAndRetentionByInsects.

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Conflict of interest

None to report.

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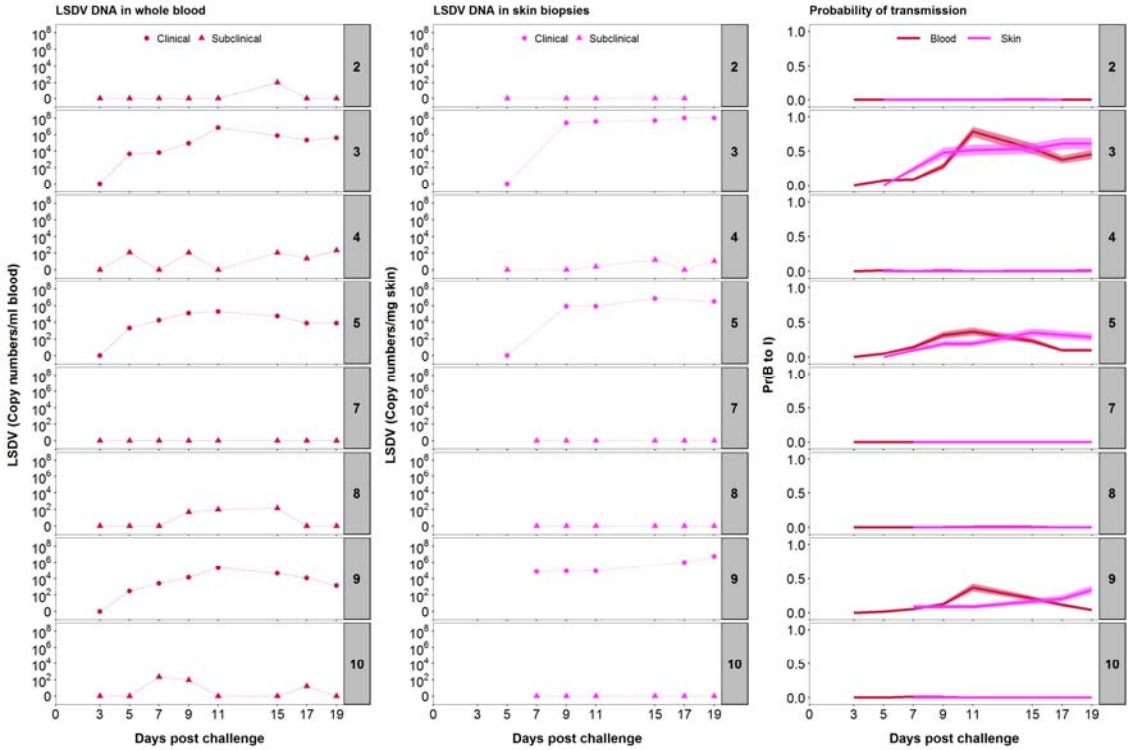
Author contribution

P.M.B., S.G., K.E.D., P.C.H., J.A. and A.J.W. conceptualised the study; B.S.B., L.A., S.B., C.S. and C.B. contributed to the design of the experiments. S.B., W.L., A.V.D., Z.L., E.D., J.S. and M.W. prepared the insects; B.S.B., P.M.B., I.R.H., N.W. and K.E.D. carried out the cattle experiments including collection and preparation of samples. B.S.B. performed the

646 laboratory assays and data acquisition. S.G. performed the statistical analysis and
647 mathematical modelling. B.S.B., S.G. and P.M.B. drafted the paper. All authors discussed the
648 results and commented on the manuscript.

649 **Figures**



650

651 **Figure 1. LSDV inoculation of eight calves results in a spectrum of infectiousness.** Levels of
652 viral DNA in blood (log_{10} copies/ml; first column) and skin (log_{10} copies/mg; second column)
653 of the inoculated calves at different days post-challenge were quantified by qPCR. Based on
654 the viral DNA levels in blood (red) or skin (magenta) the corresponding probability of
655 transmission from bovine to insect ("infectiousness") was calculated using a dose-response
656 relationship (third column). Lines and shading show the posterior median and 95% credible
657 intervals for the probability, respectively.

658

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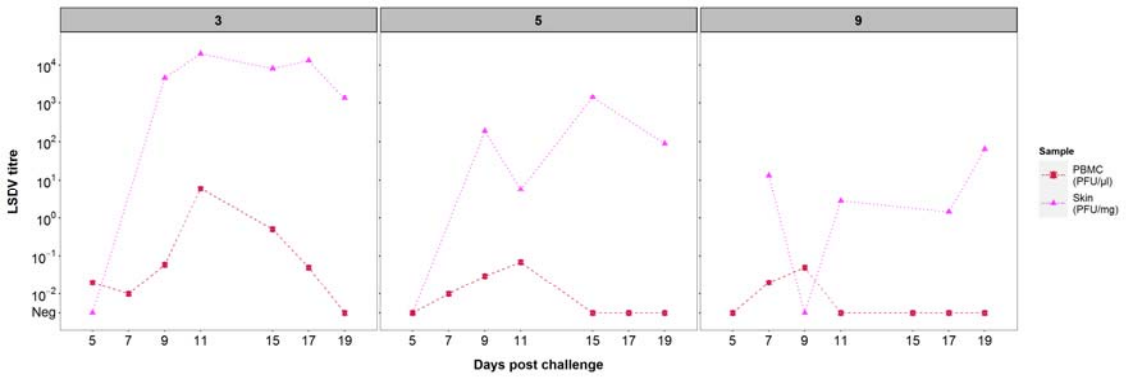


660

661 **Figure 2. LSDV titres vary between three clinical animals but are consistently higher in the**

662 **skin compared to blood.** Levels of infectious lumpy skin disease virus (LSDV) in skin biopsies

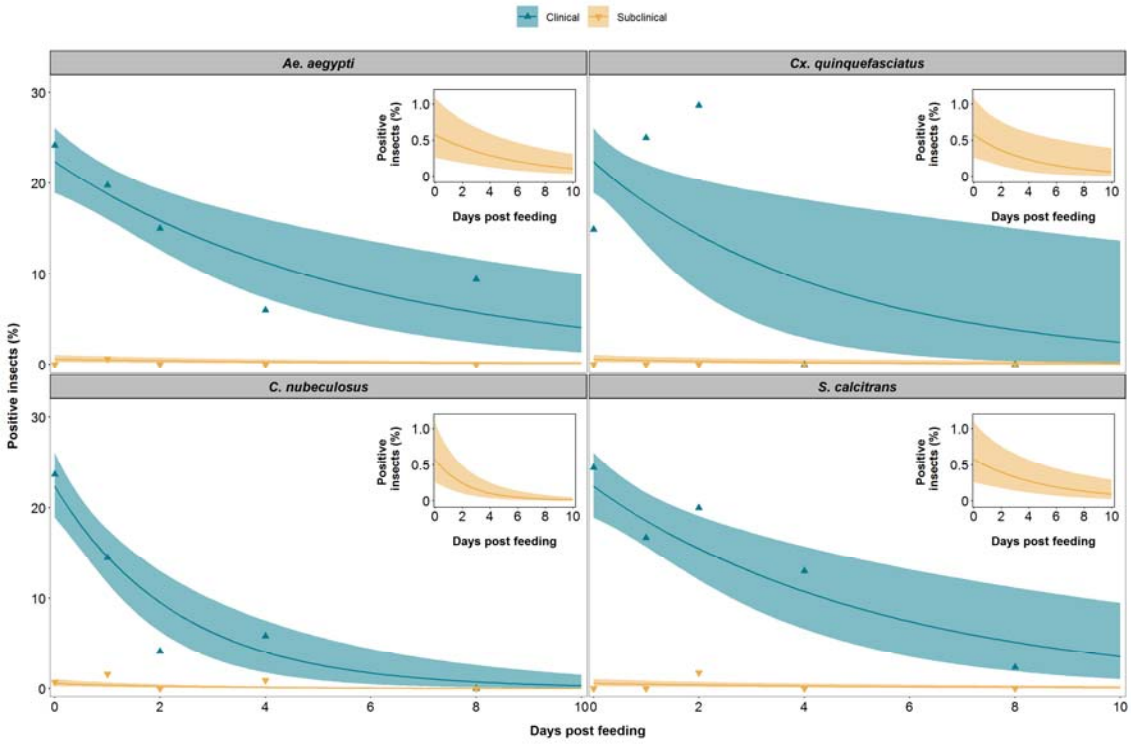
663 (PFU/mg of skin) (magenta triangles) and peripheral blood mononuclear cell (PBMC)

664 fractions (PFU/µl suspension) (red stars) were quantified by titration on MDBK cells.

665 Generalised skin lesions were first noted in all three animals at 7 days post challenge.

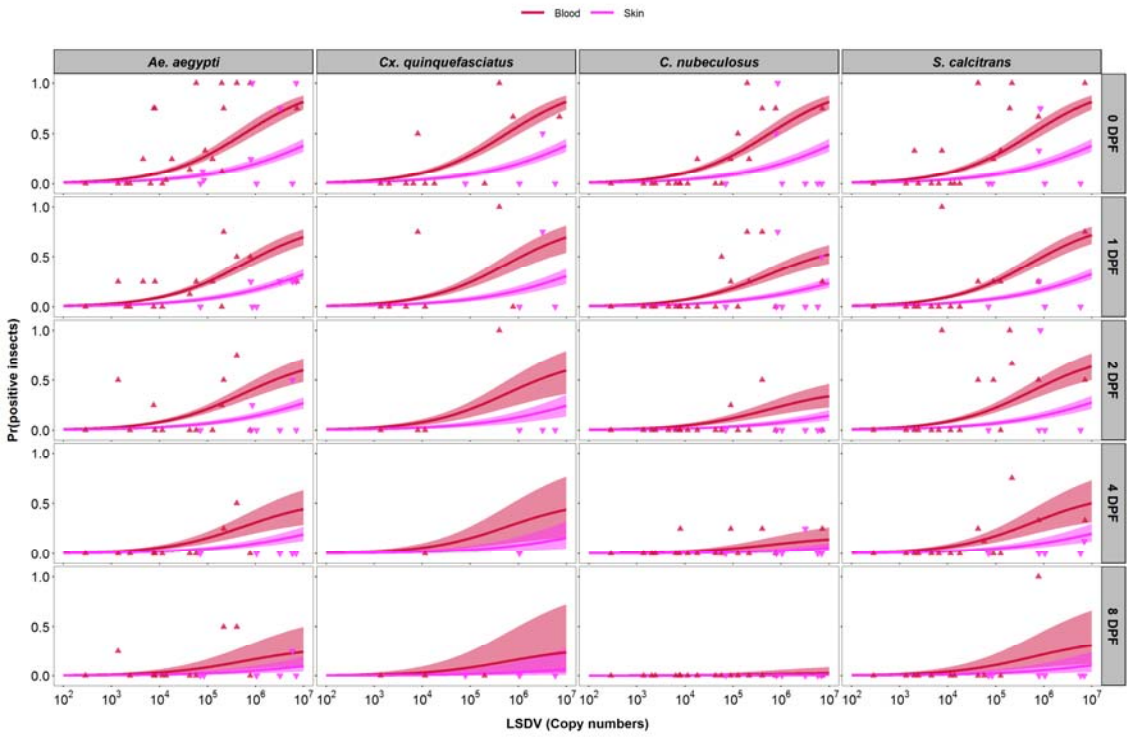
666

667



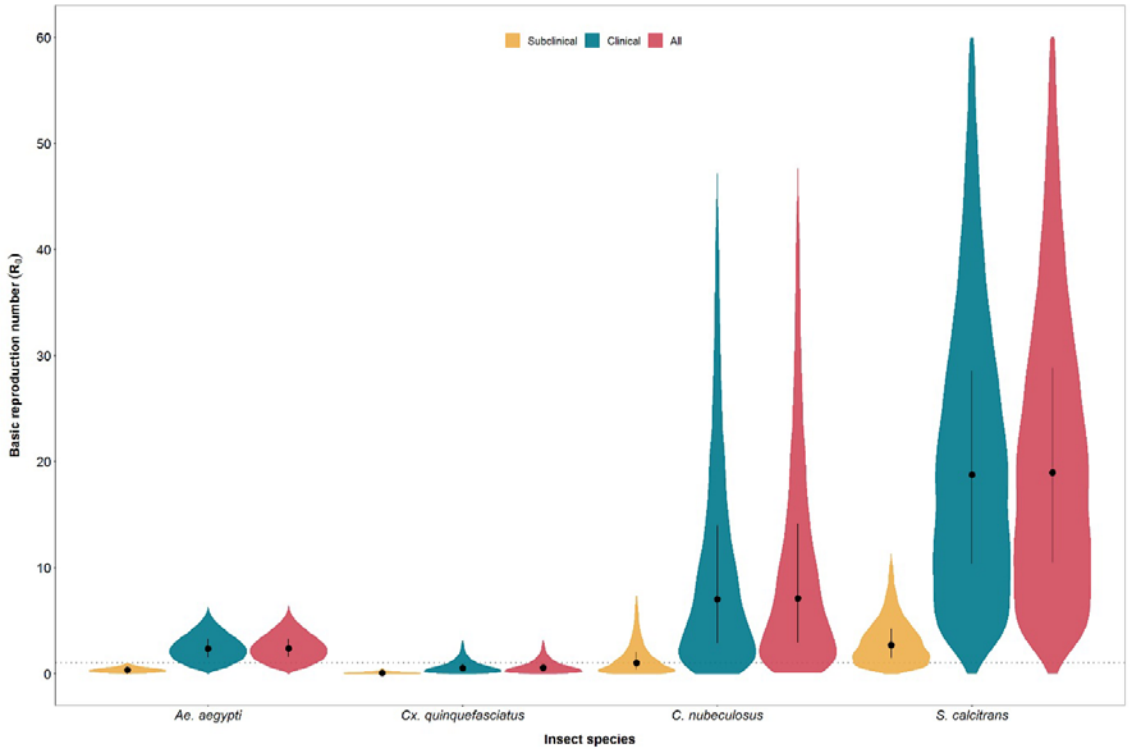
668

669 **Figure 3. LSDV is retained in blood-feeding insects for up to 8 days post feeding.** The
670 proportion of blood-feeding insects positive for lumpy skin disease viral DNA after feeding
671 on a clinical (green) or subclinically (yellow) animal is shown for the four species of insect:
672 *Aedes aegypti*; *Culex quinquefasciatus*; *Culicoides nubeculosus*; and *Stomoxys calcitrans*.
673 Each plot shows the observed proportion of positive insects (triangles) and the expected
674 proportion of positive insects (posterior median (line), and 2.5th and 97.5th percentiles of
675 the posterior distribution (shading)). The inset shows the expected proportion of positive
676 insects after feeding on a subclinical animal using a graph with an expanded y-axis..



677

678 **Figure 4. Levels of lumpy skin disease viral DNA in blood or skin are proxy measures of**
679 **infectiousness.** Each plot shows the dose-response relationship between the probability of
680 an insect being positive for lumpy skin disease virus (LSDV) DNA and the level of viral DNA in
681 the blood (log_{10} copies/ml; red) or skin (log_{10} copies/mg; magenta) of the calf on which they
682 fed. Four species of insect, *Aedes aegypti* (first column), *Culex quinquefasciatus* (second
683 column), *Culicoides nubeculosus* (third column) or *Stomoxys calcitrans* (fourth column) were
684 tested at 0, 1, 2, 4 and 8 days post feeding (rows). Plots show the observed proportion of
685 positive insects (blood: red up triangles; skin: magenta down triangles) and the estimated
686 probability of an insect being positive (posterior median (line) and 2.5th and 97.5th
687 percentiles of the posterior distribution (shading: blood, red; skin: magenta)).



688

689 **Figure 5. Basic reproduction number (R_0) for lumpy skin disease virus (LSDV) in calves**
690 **when transmitted by *Aedes aegypti*, *Culex quinquefasciatus*, *Culicoides nubeculosus* or**
691 ***Stomoxys calcitrans***. For each species, R_0 was calculated for subclinical calves only (yellow),
692 clinical calves only (green) and both combined (red). Violin plots show the posterior median
693 (black circle), interquartile range (black vertical line) and density (shape) for R_0 based on
694 replicated Latin hypercube sampling (100 replicates with the range for each parameter
695 subdivided into 100 steps).

696

Tables

Table 1. Parameters for the transmission of lumpy skin disease virus by four species of biting insect.

parameter		symbol	estimate*
probability of transmission from bovine to insect†			
	clinical donor	β	0.22 (0.19, 0.26)
	subclinical donor	$\rho\beta$	0.006 (0.003, 0.011)
relative risk of transmission from a subclinical compared to clinical bovine†		ρ	0.03 (0.01, 0.05)
virus inactivation rate (/day)		γ	
	Ae. aegypti		0.17 (0.07, 0.29)
	Cx. quinquefasciatus		0.22 (0.05, 0.51)
	C. nubeculosus		0.42 (0.26, 0.64)
	S. calcitrans		0.18 (0.08, 0.31)
mean duration of virus retention (days)		$1/\gamma$	
	Ae. aegypti		5.9 (3.5, 13.4)
	Cx. quinquefasciatus		4.5 (2.0, 22.0)
	C. nubeculosus		2.4 (1.6, 3.9)
	S. calcitrans		5.5 (3.2, 12.3)
probability of transmission from insect to bovine		b	
	Ae. aegypti		0.56 (0.11, 0.98)
	Cx. quinquefasciatus		0.11 (0.004, 0.73)
	C. nubeculosus		0.19 (0.007, 0.91)
	S. calcitrans		0.05 (0.02, 0.15)
basic reproduction number		R_0	
	Ae. aegypti		2.41 (0.50, 5.22)
	Cx. quinquefasciatus		0.55 (0.06, 2.37)
	C. nubeculosus		7.09 (0.24, 37.10)
	S. calcitrans		19.09 (2.73, 57.03)

* posterior median (95% credible interval)

† parameter does not differ amongst species

Table 2. Parameters for the dose-response relationship between levels of viral DNA in blood or skin and the probability of transmission of lumpy skin disease virus from bovine to insect.

parameter	estimate*	
	level of viral DNA in blood	level of viral DNA in skin
dose-response parameters		
intercept (d_0)	-6.89 (-7.74, -6.11)	-6.70 (-7.81, -5.76)
slope (d_1)	1.20 (1.03, 1.38)	0.89 (0.75, 1.06)

* posterior median (95% credible interval)

Supporting information

Data S1. Clinical observations and levels of viral DNA and infectious virus in samples taken from calves infected with lumpy skin disease virus and the outcome when blood-feeding insects were allowed to feed on them.

Figure S1. Clinical characterisation of experimental challenge of cattle with lumpy skin disease virus.

Figure S2. A higher proportion of insects are positive for lumpy skin disease viral DNA after feeding on a clinical compared to a subclinical animal.

Figure S3. The proportion of blood-feeding insects positive for lumpy skin disease viral DNA differs over time post challenge.

Figure S4. The median amount of lumpy skin disease viral DNA in homogenised whole insects was the same over time post feeding in three out of four species tested.

Table S1. Deviance information criterion (DIC) for models assessing variation amongst insect species in the virus inactivation rate, the probability of transmission of lumpy skin disease virus from bovine to insect and the relative risk of transmission from a subclinical bovine.

Table S2. Parameters for the duration of latent and infectious periods for lumpy skin disease virus in cattle.

Text S1. Modelling the transmission of lumpy skin disease virus by haematophagus insects.

723 **References**

724

725 1. Tuppurainen ES, Oura CA. 2012. Review: lumpy skin disease: an emerging threat to
726 Europe, the Middle East and Asia. Transbound Emerg Dis 59:40-8.

727 2. Tasioudi KE, Antoniou SE, Iliadou P, Sachpatzidis A, Plevraki E, Agianniotaki EI, Fouki
728 C, Mangana-Vougiouka O, Chondrokouki E, Dile C. 2016. Emergence of Lumpy Skin
729 Disease in Greece, 2015. Transbound Emerg Dis 63:260-5.

730 3. Sameea Yousefi P, Mardani K, Dalir-Naghadeh B, Jalilzadeh-Amin G. 2017.
731 Epidemiological Study of Lumpy Skin Disease Outbreaks in North-western Iran.
732 Transbound Emerg Dis 64:1782-1789.

733 4. Sevik M, Dogan M. 2017. Epidemiological and Molecular Studies on Lumpy Skin
734 Disease Outbreaks in Turkey during 2014-2015. Transbound Emerg Dis 64:1268-
735 1279.

736 5. OIE. World Animal Health Information Database (WAHIS Interface).
737 https://www.oie.int/wahis_2/public/wahid.php/Diseaseinformation/Immsummary.
738 Accessed

739 6. Beard PM. 2016. Lumpy skin disease: a direct threat to Europe. Vet Rec 178:557-8.

740 7. Mercier A, Arsevska E, Bournez L, Bronner A, Calavas D, Cauchard J, Falala S, Caufour
741 P, Tisseuil C, Lefrancois T, Lancelot R. 2018. Spread rate of lumpy skin disease in the
742 Balkans, 2015-2016. Transbound Emerg Dis 65:240-243.

743 8. Sprygin A, Artyuchova E, Babin Y, Prutnikov P, Kostrova E, Byadovskaya O, Kononov
744 A. 2018. Epidemiological characterization of lumpy skin disease outbreaks in Russia
745 in 2016. Transbound Emerg Dis 65:1514-1521.

746 9. Sanz-Bernardo B, Haga IR, Wijesiriwardana N, Hawes PC, Simpson J, Morrison LR,
747 MacIntyre N, Brocchi E, Atkinson J, Haegeman A, De Clercq K, Darpel KE, Beard PM.
748 2020. Lumpy Skin Disease Is Characterized by Severe Multifocal Dermatitis With
749 Necrotizing Fibrinoid Vasculitis Following Experimental Infection. Vet Pathol 57:388-
750 396.

751 10. Elhaig MM, Selim A, Mahmoud M. 2017. Lumpy skin disease in cattle: Frequency of
752 occurrence in a dairy farm and a preliminary assessment of its possible impact on
753 Egyptian buffaloes. Onderstepoort J Vet Res 84:e1-e6.

754 11. Abutarbush SM, Ababneh MM, Al Zoubi IG, Al Sheyab OM, Al Zoubi MG, Alekish MO,
755 Al Gharabat RJ. 2015. Lumpy Skin Disease in Jordan: Disease Emergence, Clinical
756 Signs, Complications and Preliminary-associated Economic Losses. Transbound
757 Emerg Dis 62:549-54.

758 12. Al-Salihi KA, Hassan IQ. 2015. Lumpy Skin Disease in Iraq: Study of the Disease
759 Emergence. Transbound Emerg Dis 62:457-62.

760 13. Ochwo S, VanderWaal K, Munsey A, Ndekezi C, Mwebe R, Okurut ARA, Nantima N,
761 Mwiine FN. 2018. Spatial and temporal distribution of lumpy skin disease outbreaks
762 in Uganda (2002-2016). BMC Vet Res 14:174.

763 14. Limon G, Gamawa AA, Ahmed AI, Lyons NA, Beard PM. 2020. Epidemiological
764 Characteristics and Economic Impact of Lumpy Skin Disease, Sheeppox and Goatpox
765 Among Subsistence Farmers in Northeast Nigeria. Front Vet Sci 7:8.

766 15. Gari G, Bonnet P, Roger F, Waret-Szkuta A. 2011. Epidemiological aspects and
767 financial impact of lumpy skin disease in Ethiopia. Prev Vet Med 102:274-83.

768 16. Molla W, de Jong MCM, Gari G, Frankena K. 2017. Economic impact of lumpy skin
769 disease and cost effectiveness of vaccination for the control of outbreaks in Ethiopia.
770 Prev Vet Med 147:100-107.

771 17. Casal J, Allepuz A, Miteva A, Pite L, Tabakovsky B, Terzievski D, Alexandrov T, Beltran-
772 Alcrudo D. 2018. Economic cost of lumpy skin disease outbreaks in three Balkan
773 countries: Albania, Bulgaria and the Former Yugoslav Republic of Macedonia (2016-
774 2017). Transbound Emerg Dis 65:1680-1688.

775 18. Kahana-Sutin E, Klement E, Lensky I, Gottlieb Y. 2017. High relative abundance of the
776 stable fly Stomoxys calcitrans is associated with lumpy skin disease outbreaks in
777 Israeli dairy farms. Med Vet Entomol 31:150-160.

778 19. Yeruham I, Nir O, Braverman Y, Davidson M, Grinstein H, Haymovitch M, Zamir O.
779 1995. Spread of lumpy skin disease in Israeli dairy herds. Vet Rec 137:91-3.

780 20. Magori-Cohen R, Louzoun Y, Herziger Y, Oron E, Arazi A, Tuppurainen E, Shpigel NY,
781 Klement E. 2012. Mathematical modelling and evaluation of the different routes of
782 transmission of lumpy skin disease virus. Vet Res 43:1.

783 21. Chihota CM, Rennie LF, Kitching RP, Mellor PS. 2001. Mechanical transmission of
784 lumpy skin disease virus by Aedes aegypti (Diptera: Culicidae). Epidemiol Infect
785 126:317-21.

786 22. Tuppurainen ES, Lubinga JC, Stoltsz WH, Troskie M, Carpenter ST, Coetzer JA, Venter
787 EH, Oura CA. 2013. Mechanical transmission of lumpy skin disease virus by
788 Rhipicephalus appendiculatus male ticks. Epidemiol Infect 141:425-30.

789 23. Lubinga JC, Tuppurainen ES, Coetzer JA, Stoltsz WH, Venter EH. 2014. Transovarial
790 passage and transmission of LSDV by Amblyomma hebraeum, Rhipicephalus
791 appendiculatus and Rhipicephalus decoloratus. Exp Appl Acarol 62:67-75.

792 24. Lubinga JC, Clift SJ, Tuppurainen ES, Stoltsz WH, Babiuk S, Coetzer JA, Venter EH.
793 2014. Demonstration of lumpy skin disease virus infection in Amblyomma hebraeum
794 and Rhipicephalus appendiculatus ticks using immunohistochemistry. Ticks Tick
795 Borne Dis 5:113-20.

796 25. Tuppurainen ES, Lubinga JC, Stoltsz WH, Troskie M, Carpenter ST, Coetzer JA, Venter
797 EH, Oura CA. 2013. Evidence of vertical transmission of lumpy skin disease virus in
798 Rhipicephalus decoloratus ticks. Ticks Tick Borne Dis 4:329-33.

799 26. Lubinga JC, Tuppurainen ES, Mahlare R, Coetzer JA, Stoltsz WH, Venter EH. 2015.
800 Evidence of transstadial and mechanical transmission of lumpy skin disease virus by
801 Amblyomma hebraeum ticks. Transbound Emerg Dis 62:174-82.

802 27. Sohier C, Haegeman A, Mostin L, De Leeuw I, Campe WV, De Vleeschauwer A,
803 Tuppurainen ESM, van den Berg T, De Regge N, De Clercq K. 2019. Experimental

804 evidence of mechanical lumpy skin disease virus transmission by Stomoxys calcitrans
805 biting flies and Haematopota spp. horseflies. Sci Rep 9:20076.

806 28. Issimov A, Kutumbetov L, Orynbayev MB, Khairullin B, Myrzakhmetova B,
807 Sultankulova K, White PJ. 2020. Mechanical Transmission of Lumpy Skin Disease
808 Virus by Stomoxys Spp (Stomoxys Calsitrans, Stomoxys Sitiens, Stomoxys Indica),
809 Diptera: Muscidae. Animals (Basel) 10.

810 29. Chihota CM, Rennie LF, Kitching RP, Mellor PS. 2003. Attempted mechanical
811 transmission of lumpy skin disease virus by biting insects. Med Vet Entomol 17:294-
812 300.

813 30. Sprygin A, Pestova Y, Wallace DB, Tuppurainen E, Kononov AV. 2019. Transmission of
814 lumpy skin disease virus: A short review. Virus Res 269:197637.

815 31. Tuppurainen ES, Venter EH, Coetzer JA. 2005. The detection of lumpy skin disease
816 virus in samples of experimentally infected cattle using different diagnostic
817 techniques. Onderstepoort J Vet Res 72:153-64.

818 32. Moller J, Moritz T, Schlottau K, Krstevski K, Hoffmann D, Beer M, Hoffmann B. 2019.
819 Experimental lumpy skin disease virus infection of cattle: comparison of a field strain
820 and a vaccine strain. Arch Virol 164:2931-2941.

821 33. Irons PC, Tuppurainen ES, Venter EH. 2005. Excretion of lumpy skin disease virus in
822 bull semen. Theriogenology 63:1290-7.

823 34. Annandale CH, Smuts MP, Ebersohn K, du Plessis L, Thompson PN, Venter EH, Stout
824 TAE. 2019. Effect of using frozen-thawed bovine semen contaminated with lumpy

825 skin disease virus on in vitro embryo production. Transbound Emerg Dis 66:1539-
826 1547.

827 35. Rouby S, Aboulsoud E. 2016. Evidence of intrauterine transmission of lumpy skin
828 disease virus. Vet J 209:193-5.

829 36. Annandale CH, Holm DE, Ebersohn K, Venter EH. 2014. Seminal transmission of
830 lumpy skin disease virus in heifers. Transbound Emerg Dis 61:443-8.

831 37. Manic M, Stojiljkovic M, Petrovic M, Nisavic J, Bacic D, Petrovic T, Vidanovic D,
832 Obrenovic S. 2019. Epizootic features and control measures for lumpy skin disease in
833 south-east Serbia in 2016. Transbound Emerg Dis 66:2087-2099.

834 38. EFSA Panel on Animal Health Welfare. 2016. Urgent advice on lumpy skin disease.
835 EFSA Journal 14:e04573.

836 39. Krenn HW, Aspock H. 2012. Form, function and evolution of the mouthparts of
837 blood-feeding Arthropoda. Arthropod Struct Dev 41:101-18.

838 40. Tchouassi DP, Okiro RO, Sang R, Cohnstaedt LW, McVey DS, Torto B. 2016. Mosquito
839 host choices on livestock amplifiers of Rift Valley fever virus in Kenya. Parasit Vectors
840 9:184.

841 41. Hasegawa M, Tuno N, Yen NT, Nam VS, Takagi M. 2008. Influence of the distribution
842 of host species on adult abundance of Japanese encephalitis vectors Culex vishnui
843 subgroup and Culex gelidus in a rice-cultivating village in northern Vietnam. Am J
844 Trop Med Hyg 78:159-68.

845 42. Tomazatos A, Jost H, Schulze J, Spinu M, Schmidt-Chanasit J, Cadar D, Luhken R.
846 2020. Blood-meal analysis of Culicoides (Diptera: Ceratopogonidae) reveals a broad
847 host range and new species records for Romania. Parasit Vectors 13:79.

848 43. Augot D, Hadj-Henni L, Strutz SE, Slama D, Millot C, Depaquit J, Millot JM. 2017.
849 Association between host species choice and morphological characters of main
850 sensory structures of Culicoides in the Palaeartic region. PeerJ 5:e3478.

851 44. Keeling MJ, Rohani P. 2008. Modeling Infectious Diseases in Humans and Animals
852 doi:10.2307/j.ctvcm4gk0. Princeton University Press.

853 45. Gubbins S. 2019. Using the basic reproduction number to assess the risk of
854 transmission of lumpy skin disease virus by biting insects. Transboundary and
855 Emerging Diseases 66:1873-1883.

856 46. Fraser C, Riley S, Anderson RM, Ferguson NM. 2004. Factors that make an infectious
857 disease outbreak controllable. Proc Natl Acad Sci U S A 101:6146-51.

858 47. Charleston B, Bankowski BM, Gubbins S, Chase-Topping ME, Schley D, Howey R,
859 Barnett PV, Gibson D, Juleff ND, Woolhouse ME. 2011. Relationship between clinical
860 signs and transmission of an infectious disease and the implications for control.
861 Science 332:726-9.

862 48. Duong V, Lambrechts L, Paul RE, Ly S, Lay RS, Long KC, Huy R, Tarantola A, Scott TW,
863 Sakuntabhai A, Buchy P. 2015. Asymptomatic humans transmit dengue virus to
864 mosquitoes. Proc Natl Acad Sci U S A 112:14688-93.

865 49. Tadesse FG, Slater HC, Chali W, Teelen K, Lanke K, Belachew M, Menberu T, Shumie
866 G, Shitaye G, Okell LC, Graumans W, van Gemert GJ, Kedir S, Tesfaye A, Belachew F,
867 Abebe W, Mamo H, Sauerwein R, Balcha T, Aseffa A, Yewhalaw D, Gadisa E, Drakeley
868 C, Bousema T. 2018. The Relative Contribution of Symptomatic and Asymptomatic
869 Plasmodium vivax and Plasmodium falciparum Infections to the Infectious Reservoir
870 in a Low-Endemic Setting in Ethiopia. Clin Infect Dis 66:1883-1891.

871 50. Harris RL, Miller JA, Frazar ED. 1974. Horn Flies and Stable Flies - Feeding Activity.
872 Annals of the Entomological Society of America 67:891-894.

873 51. Baldacchino F, Muenworn V, Desquesnes M, Desoli F, Charoenviriyaphap T, Duvallet
874 G. 2013. Transmission of pathogens by Stomoxys flies (Diptera, Muscidae): a review.
875 Parasite 20:26.

876 52. Mullen GR, Murphree CS. 2019. Chapter 13 - Biting Midges (Ceratopogonidae), p
877 213-236. *In* Mullen GR, Durden LA (ed), Medical and Veterinary Entomology (Third
878 Edition) doi:https://doi.org/10.1016/B978-0-12-814043-7.00013-3. Academic Press.

879 53. Ramasubramanian MK, Barham OM, Swaminathan V. 2008. Mechanics of a mosquito
880 bite with applications to microneedle design. Bioinspiration & Biomimetics 3.

881 54. Scott TW, Takken W. 2012. Feeding strategies of anthropophilic mosquitoes result in
882 increased risk of pathogen transmission. Trends Parasitol 28:114-21.

883 55. Santana SR, Andrade PDS, Urbinatti PR, Almeida R, Lima-Camara TN. 2019.
884 Gonotrophic discordance in Culex quinquefasciatus (Diptera: Culicidae) in the city of
885 Sao Paulo, Brazil. Rev Soc Bras Med Trop 53:e20190277.

886 56. Kitching RP, Mellor PS. 1986. Insect transmission of capripoxvirus. Res Vet Sci
887 40:255-8.

888 57. Fenner F, Day MF, Woodroofe GM. 1952. The mechanism of the transmission of
889 myxomatosis in the European rabbit (Oryctolagus cuniculus) by the mosquito Aedes
890 aegypti. Aust J Exp Biol Med Sci 30:139-52.

891 58. Kligler IJ, Muckenfuss RS, Rivers TM. 1929. Transmission of Fowl-Pox by Mosquitoes.
892 J Exp Med 49:649-60.

893 59. Mellor PS, Kitching RP, Wilkinson PJ. 1987. Mechanical transmission of capripox virus
894 and African swine fever virus by Stomoxys calcitrans. Res Vet Sci 43:109-12.

895 60. Gray SM, Banerjee N. 1999. Mechanisms of arthropod transmission of plant and
896 animal viruses. Microbiol Mol Biol Rev 63:128-48.

897 61. Chihota CM. 2000. The role of arthropod vectors in the epidemiology of lumpy skin
898 disease. Degree of Doctor of Philosophy. University of Reading.

899 62. Day MF, Fenner F, Woodroofe GM, McIntyre GA. 1956. Further studies on the
900 mechanism of mosquito transmission of myxomatosis in the European rabbit. J Hyg
901 (Lond) 54:258-83.

902 63. Lassen SB, Nielsen SA, Kristensen M. 2012. Identity and diversity of blood meal hosts
903 of biting midges (Diptera: Ceratopogonidae: Culicoides Latreille) in Denmark. Parasit
904 Vectors 5:143.

905 64. Purse BV, Carpenter S, Venter GJ, Bellis G, Mullens BA. 2015. Bionomics of temperate
906 and tropical Culicoides midges: knowledge gaps and consequences for transmission
907 of Culicoides-borne viruses. Annu Rev Entomol 60:373-92.

908 65. Boorman J. 1974. The maintenance of laboratory colonies of Culicoides variipennis
909 (Coq.), C. nubeculosus (Mg.) and C. riethi Kieff. (Diptera, Ceratopogonidae). Bulletin
910 of Entomological Research 64:371-377.

911 66. Veronesi E, Mertens PP, Shaw AE, Brownlie J, Mellor PS, Carpenter ST. 2008.
912 Quantifying bluetongue virus in adult Culicoides biting midges (Diptera:
913 Ceratopogonidae). J Med Entomol 45:129-32.

914 67. Bowden TR, Babiuk SL, Parkyn GR, Copps JS, Boyle DB. 2008. Capripoxvirus tissue
915 tropism and shedding: A quantitative study in experimentally infected sheep and
916 goats. Virology 371:380-93.

917 68. MR VDS, Gu X, Ward MP, Kirkland PD. 2016. Development and evaluation of real-
918 time PCR assays for bloodmeal identification in Culicoides midges. Med Vet Entomol
919 30:155-65.

920 69. Spiegelhalter DJ, Best NG, Carlin BP, Van Der Linde A. 2002. Bayesian measures of
921 model complexity and fit. Journal of the Royal Statistical Society: Series B (Statistical
922 Methodology) 64:583-639.

923 70. Carn VM, Kitching RP. 1995. An investigation of possible routes of transmission of
924 lumpy skin disease virus (Neethling). Epidemiol Infect 114:219-26.

925 71. Babiuk S, Bowden TR, Parkyn G, Dalman B, Manning L, Neufeld J, Embury-Hyatt C,
926 Copps J, Boyle DB. 2008. Quantification of lumpy skin disease virus following
927 experimental infection in cattle. Transbound Emerg Dis 55:299-307.

928 72. Haario H, Saksman E, Tamminen J. 2001. An adaptive Metropolis algorithm. Bernoulli
929 7:223-242.

930 73. Andrieu C, Thoms J. 2008. A tutorial on adaptive MCMC. Statistics and Computing
931 18:343-373.

932 74. Flegal JM, Hughes J, Vats D, Dai N. 2017. mcmcse: Monte Carlo Standard Errors for
933 MCMC. R Package version 1.3-2.,

934 75. Wallace DB, Weyer J, Nel LH, Viljoen GJ. 2007. Improved method for the generation
935 and selection of homogeneous lumpy skin disease virus (SA-Neethling)
936 recombinants. J Virol Methods 146:52-60.

937 76. Plummer M, Best N, Cowles K, Vines K. 2006. CODA: convergence diagnosis and
938 output analysis for MCMC. R News 6:7-11.