Spatial and temporal patterns of human Puumala virus (PUUV) infections in Germany

Sarah Cunze Corresp., 1, 2 , **Judith Kochmann** 1, 2 , **Thomas Kuhn** 1, 2 , **Raphael Frank** 3 , **Dorian D Dörge** 1, 2 , **Sven Klimpel** 1, 2

1 Goethe University Frankfurt, Institute of Ecology, Diversity and Evolution, Frankfurt am Main, Germany

2 Senckenberg Biodiversity and Climate Research Centre, Senckenberg Nature Research Society, Frankfurt am Main, Germany

3 Institute of Medical Microbiology and Hospital Hygiene, Heinrich-Heine Universität Düsseldorf, Düsseldorf, Germany

Corresponding Author: Sarah Cunze
Email address: cunze@bio.uni-frankfurt.de

Background. Worldwide, the number of recorded human hantavirus infections as well as the number of affected countries is on the rise. In Europe, most human hantavirus infections are caused by the Puumala virus (PUUV), with bank voles (*Myodes glareolus*) as reservoir host. Generally, infection outbreaks have been related to environmental conditions, particularly climatic conditions, food supply for the reservoir species and land use. However, although attempts have been made, the insufficient availability of environmental data is often hampering accurate temporal and spatially explicit models of human hantavirus infections.

Methods. In the present study, dynamics of human PUUV infections between 2001 and 2015 were explored using ArcGIS in order to identify spatio-temporal patterns.

Results. Percentage cover of forest area was identified as an important factor for the spatial pattern, whereas beech mast was found explaining temporal patterns of human PUUV infections in Germany. High numbers of infections were recorded in 2007, 2010 and 2012 and areas with highest records were located in Baden-Wuerttemberg (southwest Germany) and North Rhine-Westphalia (western Germany).

Conclusion. More reliable data on reservoir host distribution, pathogen verification as well as an increased awareness of physicians are some of the factors that should improve future human infection risk assessments in Germany.

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4 Sarah Cunze[*,1,2], Judith Kochmann[1,2], Thomas Kuhn[1,2], Raphael Frank[3], Dorian D Dörge[1,2],

5 Sven Klimpel[1,2]

7 [1]Goethe University Frankfurt, Institute of Ecology, Diversity and Evolution, Frankfurt am Main,

8 Germany

9 [2]Senckenberg Biodiversity and Climate Research Centre, Senckenberg Gesellschaft für

10 Naturforschung, Frankfurt am Main, Germany

11 [3]Institute of Medical Microbiology and Hospital Hygiene, Heinrich-Heine Universität

12 Düsseldorf, Düsseldorf, Germany

17 [*]Corresponding author: Sarah Cunze, sarahcunze@gmail.com

Abstract

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27 climatic conditions, food supply for the reservoir species and land use. However, although

28 attempts have been made, the insufficient availability of environmental data is often hampering

29 accurate temporal and spatially explicit models of human hantavirus infections.

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31 were explored using ArcGIS in order to identify spatio-temporal patterns.

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33 pattern, whereas beech mast was found explaining temporal patterns of human PUUV infections

34 in Germany. High numbers of infections were recorded in 2007, 2010 and 2012 and areas with

35 highest records were located in Baden-Wuerttemberg (southwest Germany) and North Rhine-

36 Westphalia (western Germany).

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38 increased awareness of physicians are some of the factors that should improve future human

39 infection risk assessments in Germany.

40

Introduction

Hantaviruses (family *Hantaviridae*) are tri-segmented, negative-stranded enveloped RNA viruses consisting of at least 23 recognized species (*Vaheri* et al., 2013; *Plyusnin & Sironen*, 2014). According to the ninth report of the International Committee on Taxonomy of Viruses (ICTV) another 30 species potentially belong to this family. Hantaviruses can be found in different regions worldwide with distributions clearly linked to the distribution of their hosts (*Vapalahti* et al., 2003). Since the first isolation in 1978 (*Lee, Lee & Johnson*, 1978), Hantaviruses (named after the river Hantaan in Korea where the first detection of human infections was made) turned into an important subject for researchers and the public, since they constitute an increasing threat to humans with the number of infections globally rising and new viruses being described continuously (*Reusken & Heyman*, 2013; *Lee, Vaheri & Schmaljohn*, 2014; *Carver* et al., 2015). Clinical symptoms and severity of human infections generally depend on the Hantavirus species (*Krautkrämer & Zeier*, 2014). Two syndromes are known so far: the haemorrhagic fever with renal syndrome (HFRS) and Hantavirus cardiopulmonary syndrome (HCPS) (*Krüger, Ulrich & Lundkvist*, 2001). Whereas the latter is only found in North and South America, HFRS occurs in Europe, Asia and Africa (*Hooper* et al., 2001; *Witkowski* et al., 2014). The most severe clinical course of HFRS is primarily characterized by acute symptoms such as fever, circulatory collapse with hypotension, hemorrhage, and acute kidney injury (AKI) (*Jiang* et al., 2016). In Europe, the most frequent infections in humans seem to be caused by the Dobrava-Belgrade orthohantavirus (DOBV) as well as the Puumala orthohantavirus (PUUV), with the latter causing a usually milder or moderate form of HFRS, called nephropathia epidemica (NE) (*Heyman* et al., 2011; *Hofmann* et al., 2014; *Watson* et al., 2014). Although rarely fatal (< 0.25%), NE patient might exhibit a broad range of flu-like symptoms such as an acute onset of fever, headache, backpain as

64   well as signs of renal involvement that might necessitate more intensive medical attention

65   including ghaemodialysis (*Settergren*, 2000). PUUV infections, with bank voles (*Myodes*

66   *glareolus*) as reservoir host species (*Brummer-Korvenkontio* et al., 1980; *Klingström* et al.,

67   2002), are the most prevalent type of human hantavirus infections in Germany (*Schwarz* et al.,

68   2009), although the DOBV has also been identified in the eastern part of Germany, where the

69   host species of its genotype Kurkino, the striped field mouse (*Apodemus agrarius*), occurs

70   (*Schlegel* et al., 2009; *Klempa* et al., 2013).

71   Spatial patterns of PUUV infections have been linked to the geographical distribution of the

72   reservoir host (*Settergren*, 2000; *Schwarz* et al., 2009; *Watson* et al., 2014). Thus, it has been

73   suggested that the number of recorded human PUUV infections is directly related to the

74   population density of bank voles, i.e. the higher the abundance of bank voles, the higher the

75   number of infected bank vole individuals (due to the higher chance of transmission between

76   individuals) (*Vaheri* et al., 2013; *Reil* et al., 2015), consequently increasing the chances of

77   contact between infected bank voles and humans. In Germany, the heterogeneous spatial

78   distribution of human PUUV infections has been explained by an association of PUUV with the

79   Western evolutionary lineage of the bank vole (*Drewes* et al., 2017a). Higher abundance of the

80   reservoir host can be largely explained by temporal dynamics of environmental conditions

81   affecting the presence of food resources as well as suitable habitats. Bank voles can adapt to a

82   large range of climatic conditions as can be derived from their vast distributional range covering

83   a broad spectrum of different climatic conditions (cf. *Vaheri* et al., 2013). Climatic conditions

84   are, however, only indirectly linked to bank vole abundances, i.e. mostly through weather effects

85   on food supply (*Imholt* et al., 2015). Bank voles are mainly granivorous and profit from high

86   seed production during autumn in mast years (i.e. years with intensive fruiting of beech and oak

87 trees (*Fagus sylvatica*, *Quercus robur* and *Q. petraea*). The high food supply of seeds, mainly

88 beechnuts, favours winter survival of voles and leads to early breeding in spring e(*Eccard &*

89 *Herde*, 2013), which in turn results in high number of offspring in early summer ultimately

90 giving rise to PUUV-associated NE outbreaks in humans (*Klempa*, 2009; *Imholt* et al., 2015).

91 The driver of human PUUV infection risk is the likelihood of contact with the virus. The

92 transmission of the PUUV is assumed to be affected by climatic conditions; milder winters and

93 wetter summers are supposed to favour the survival of the virus outside the host species and thus,

94 favour the risk of infection (*Heyman* et al., 2012). The main pathway of PUUV transmission

95 from bank voles to humans is by inhalation of aerosols contaminated with excreta (urine, feces,

96 and saliva) from infected animals (*Ulrich* et al., 2008; *Schwarz* et al., 2009). According to

97 *Watson* et al., 2014, various outdoor activities can be considered a risk factor for infections, e.g.

98 entering or cleaning long-abandoned places such as cabins or attics, and special risk groups

99 comprise: forest and farm workers, muskrat hunters, military staff, camper, dog owners, cat

100 owners and people living near the forest (*Zöller* et al., 1995; *Sane* et al., 2014; *Watson* et al.,

101 2014).

102 Figure 1 summarizes the most important factors that are assumed to influence the spatial and

103 temporal patterns of human PUUV infections.

104 Although human infections with PUUV in Europe are on the rise, data from comprehensive

105 long-term monitoring of virus distribution in hosts and patients is often not extensive or

106 incomplete (but see *Reusken & Heyman*, 2013; *Drewes* et al., 2017a; *Drewes* et al., 2017b).

107 Better insights into the role of abiotic and biotic factors and the increasing incidence of PUUV

108 infections in Europe are urgently needed. In Germany, PUUV infections are known since the

109 1980s (*Ulrich* et al., 2008). Since the notification requirement in Germany in 2001, data on the

110 number and location of human PUUV infections reported are available at a spatial level of

111 German   administrative   districts   (SurvStat@RKI   2.0,

112 https://survstat.rki.de/Content/Query/Create.aspx/).

113 The aim of the present study was to visualize spatial and temporal patterns of human infections

114 for the period 2001 – 2015. We focused on the following questions:

115   1. Where are hot spots of PUUV infections in Germany? (spatial patterns)

116   2. Which years have been affected by especially high numbers of PUUV infections?

117   (temporal patterns)

118   3. Are there any seasonal patterns?

119   4. What are the driving factors for the spatial and temporal patterns in Germany?

120   5. Has there been an increase of PUUV infections over the last 15 years? Overall or in

121   certain regions?

122   6. Does the spatial extent of areas strongly affected by PUUV infections expand? What

123   might be reasons for this?

124 More specifically, we considered the percentage of strong beech fructification (food supply), the

125 percentage of forest and urban area (land cover) as well as precipitation and temperature

126 (climatic conditions) with regard to their influence on temporal and spatial patterns of the

127 recorded number of PUUV infections in Germany.

128

Materials and Methods

130

131 **PUUV infections in Germany**

132 Data on PUUV infections , i.e. the number of reported individual cases of human infections was

133 derived from SurvStat@RKI, provided by the Robert Koch Institute, (www.rki.de) at a spatial

134 level of German administrative districts (German: "Landkreise"). For PUUV, the spatial,

135 temporal and seasonal patterns were investigated. We considered the number of recorded

136 infections for each year from 2001-2015 (status as of May 2017) and for each of the 402 German

137 administrative districts with a temporal resolution of one calendar week. The geometry of the

138 German administrative districts and information about the number of inhabitants were obtained

139 from the "Geodatenzentrum", GeoBasis-DE / BKG (http://www.geodatenzentrum.de).

140 **Climatic conditions**

141 Data on climatic conditions were provided by the German Meteorological Office (Deutscher

142 Wetterdienst (DWD), www.dwd.de, ftp://ftp-cdc.dwd.de/pub/CDC/). Temporal patterns of

143 human PUUV infections were contrasted with mean temperature in July (averaged mean of daily

144 air temperature in 2 m height above ground in July), mean precipitation in July and mean

145 temperature and precipitation in summer (i.e. June, July, August). In addition, we considered

146 annual mean temperature and annual precipitation. Based on the raster data of these variables

147 with a spatial resolution of 1 km² we first calculated averages of the respective climatic variable

148 (2001-2015) for each grid cell and then calculated averages for each of the 402 German

149 administrative districts. The latter was defined as the final spatial resolution of the study, which

150 was set by the spatial scale available for the data on human PUUV infections.

151 **Habitat structure and beech fructification**

152 The presence of suitable habitats as well as food resources for the reservoir host are considered

153 important factors affecting the temporal and spatial pattern of recorded PUUV infections. We

154 thus took data on habitat structure as well as on beech fructification (affecting the availability of

155   food for the bank voles) into account. For habitat structure we considered the percentage cover of

156   forest and urban area per district. These data were derived from the CORINE Land Cover (CLC)

157   data (provided by the European Environment Agency, https://www.eea.europa.eu/data-and-

158   maps/data/clc-2006-raster-4). The CLC is a seamless European land cover vector database which

159   is based on satellite remote sensing images on a scale of 1:100,000 as the primary information

160   source. Land cover information is separated into 44 classes. For each of the 402 German districts

161   we calculated the proportion of forest considering the classes broad-leaved forest (CLC 3.1.1) or

162   mixed forest (CLC 3.1.3), as well as the proportion of urban area of the classes: CLC 1.1.1,

163   1.1.2, 1.2.1, 1.2.2, 1.2.3, 1.2.4, 1.3.1, 1.3.2, 1.3.3 and 1.4.2 (i.e. urban, fabric, industrial,

164   commercial and transport units, mines, dumps and construction sites, sport and leisure facilities).

165   For beech mast we exemplarily considered data from the German federal state of Hesse (located

166   in Central Germany). Data on percentage of old beeches with medium or strong fructification in

167   Hesse were taken from a report on the state of the forest in Hesse in 2016 ("Waldzustandsbericht

168   2016, Hessen", *Hessisches Ministerium für Umwelt, Klimaschutz, Landwirtschaft und*

169   *Verbraucherschutz*, 2016).

170   **Analysis**

171   To analyse the factors impacting the spatial patterns we correlated the number of recorded

172   human PUUV infections with land cover (i.e. the percentage of forest and urban area) and

173   climatic conditions (i.e. annual mean temperature and annual precipitation) for the 402 districts.

174   In addition, we used a generalized linear model (GLM) to identify variables that could be used to

175   predict incidence rates. Four different variables were included in the model: forest area, urban

176   area, annual mean temperature average over 2001 to 2015 (AMT), annual precipitation average

177   over 2001 to 2015 (AP) and their two-way interactions. Data of percentage cover of forest and

178   urban area was arcsine-transformed and the model used a Gaussian error distribution and an

179   identical link function.

180   To estimate whether a tendency of spatial expansion of the area strongly affected by PUUV

181   infections exists, we carried out a linear regression over the number of recorded PUUV

182   infections for the years 2001 to 2015 in each district.

183   Mast years are linked to an increased bank vole population density, which in turn likely increases

184   the numbers of PUUV infections. Drought in early summer seems to be a strong predictor for

185   intensive fruiting of beech in the subsequent year (*Piovesan & Adams*, 2001). According to

186   *Overgaard, Gemmel & Karlsson*, (2007), temperature in July can also be used as a predictor for

187   beech mast in the next year. The likelihood of a strong fructification after a dry and warm

188   summer was found to be even higher when a moist and cool summer preceded the year before

189   the drought (*Piovesan & Adams*, 2001). Allowing for these time lags, we correlated the number

190   of recorded PUUV infections of the respective year with beech fructification of the previous

191   year, which in terms was related to the climatic conditions in the years before; mean temperature

192   in July and mean precipitation in July two years before), and mean temperature and precipitation

193   in summer (June, July, August) three years before).

194   Analysis (e.g. correlation analysis, GLM) was carried out using R (version 3.2.1). ESRI ArcGIS

195   (version 10.3) was used for maps as well as for calculating the percentages of forest and urban

196   area. A map outlining the affected regions in Germany is given in Figure 2.

197   ## Results
198   Spatial patterns of infections were similar between the years 2001 and 2015 with high numbers

199   of recorded PUUV infection in the states of Baden-Wuerttemberg in southwestern Germany

200 (especially in the state capital Stuttgart and the surrounding districts) as well as parts of North

201 Rhine-Westphalia (western Germany) and eastern Bavaria (southern Germany, Fig. 2, Fig. 3).

202 North-eastern Germany was less affected. Within the considered time period of 15 years, there

203 were no human PUUV infection cases in 101 of the 402 districts of Germany (i.e. about one

204 quarter). The maximum number within the considered time period was found in the district

205 "Reutlingen" in the state of Baden-Wuerttemberg (see Fig. 2 for location) with a total number of

206 573 recorded PUUV infections, of which 188 PUUV infections were recorded in 2007.

207 Based on the existing data, there is currently no indication for a clear expansion of the area

208 strongly affected by PUUV infections at the country level. However, in eight out of the 402

209 districts a significant positive trend, i.e. significant increase of recorded infections from 2001-

210 2015, could be demonstrated (Fig S1 in the Supplementary). Five of these eight districts show

211 very low numbers of recorded PUUV infections (up to three per year). The other three districts

212 that show a significant positive trend are situated near the regional hotspot in Baden-

213 Wuerttemberg (Ravensburg), and in North Rhine-Westphalia (Oberhausen and Wesel, Fig. 2.).

214 Although the pattern of recorded infections over the 15 considered years is similar, a strong

215 temporal variation between single years can be observed (Fig. 4). The years 2007, 2010 and 2012

216 stand out due to their high numbers of recorded PUUV infections, especially within the three

217 hotspot regions: Baden-Wuerttemberg, North Rhine-Westphalia and eastern Bavaria (Fig. 4).

218 There is no significant trend ($p = 0.27$) in the annual number of recorded PUUV infections in

219 Germany between 2001 and 2015 (Fig. 4, bottom).

220

221   With regard to the temporal pattern we displayed the relation between the number of recorded

222   PUUV infections (from 2001 to 2015) and the percentage of old beeches with medium or strong

223   fructification in the German federal state Hesse previous to that year, but also the climatic

224   conditions during previous summers two and three years before (Fig. 4). Significant correlations

225   occurred between the number of PUUV infections and beech mast of the previous year in Hesse

226   (r(s) = 0.69, p < 0.01) and the number of PUUV infections and precipitation in July two years

227   earlier in Germany (r(s) = -0.003, p < 0.01. Each year with a high number of recorded PUUV

228   infections was preceded by a mast year but not vice versa (Fig. 4).

229   There was a clear seasonal pattern especially in years with a high number of recorded PUUV

230   infections, with low values during winter and a clear increase in spring (from the end of April)

231   which reached a maximum in early summer (~27th calendar week) (Fig. 5). The number of

232   recorded PUUV infections decreased in late summer and autumn. This seasonal pattern was

233   observed when considering the mean of all years between 2001 and 2015 (black line in Fig. 5)

234   and was more pronounced when considering only the years with a high number of PUUV

235   infections (red line in Fig. 5).

236   Based on current knowledge on factors likely affecting the spatial pattern of the number of

237   recorded PUUV infections in Germany and with respect to data availability, we focused on four

238   environmental factors: the percentage of forest area and urban area regarding habitat structure

239   and annual mean temperature and annual precipitation as climatic variables. The percentage of

240   forest area and the number of recorded PUUV infections was positively correlated (Spearman

241   correlation coefficient r(s) = 0.36, p<0.001, Fig. S2 in the Supplementary), which was also

242   confirmed by the GLM with significant interactions of percentage of forest area and annual mean

243   temperature or annual precipitation (Table 1).

244 **Discussion**

245 *Spatial patterns*

246 Our analyses revealed similar spatial patterns over the considered 15 years and confirmed several

247 hotspots of PUUV infections in Germany: in the federal states of Baden-Wuerttemberg, adjacent

248 areas in Bavaria, the north of North Rhine-Westphalia and the neighbouring part of Lower

249 Saxony (see *Weber de Melo* et al., 2015). Except for few locations where a significant rise in the

250 number of recorded PUUV infections occurred, no significant positive trend or a clear expansion

251 of the area strongly affected by PUUV infections was demonstrated at the country level, which

252 corroborates results of previous studies (see e.g. *Faber* et al., 2013; *Drewes* et al., 2017a). Some

253 of the locations where a rise in infection numbers was noted (i.e. Ravensburg in Baden-

254 Wuerttemberg and Oberhausen and Wesel in North Rhine-Westphalia, cf. Fig. 2 for location of

255 the mentioned districts) are adjacent to already known hot spots of human PUUV infections and

256 may indicate an expansion of the area affected.

257 Identification of the drivers of PUUV outbreaks is of major importance as it provides the

258 opportunity to predict future outbreaks. For the spatial patterns, forest cover is supposed to be the

259 most important factor, because bank voles as host reservoir species are known to be forest-

260 dwelling. Indeed, we showed that forest cover overlaps with hot spot regions of infections in

261 Germany and detected a highly significant correlation between the percentage of area covered

262 with forest and the number of recorded PUUV infections. Higher risk for human PUUV

263 infections is thus associated with forest rich regions. This association confirms results of

264 previous studies from other European countries; the most plausible assumption and widely

265 suggested risk factor for the transmission of the virus to humans has been their exposure within

266 forested areas through, e.g. working in the forest, living nearby, cleaning utility rooms, visiting

267   forest shelters or using wood for heating and building (see *Heyman* et al., 2012; *Reil* et al., 2015).

268   This is also supported by results from the Netherlands where PUUV infections are known since

269   2008 (*Sane* et al., 2014). In the Netherlands, numbers of recorded PUUV infections have been

270   generally low (*Sane* et al., 2014), potentially explained by the relatively low percentage of forest

271   cover in the country. Despite this, peaks have also been recorded for 2010 and 2012 in the

272   Netherlands (*Sane* et al., 2014), which coincided with two important outbreaks in Germany.

273   Generally, the spatial pattern of the occurrence of infected bank voles is expected to be strongly

274   driven by spatial autocorrelation due to bank voles infecting each other. Data on population

275   dynamics of bank voles and their PUUV seroprevalence would therefore improve the capacity of

276   further testing this relationship (but see discussion on data quality further below). Another

277   strategy would be the establishment of a long-term monitoring of rodents as recently suggested

278   by *Jacob* et al., 2014.

279

280   *Temporal patterns*

281   Mast years are commonly considered as main drivers of bank vole abundance in Central Europe

282   (for Germany see *Reil* et al., 2015). Our results are generally in accordance with this assumption.

283   Strong fluctuations among years occur with PUUV outbreaks in 2007, 2010 and 2012 preceded

284   by a year with a   high percentage of fructification of older beech trees (in Hesse). This

285   mechanism seems plausible and has been suggested before: The food supply due to the high seed

286   production in the previous autumn is assumed to favour winter survival of rodents and is thus

287   associated with early breeding in spring, resulting in high densities in early summer, which then

288   gives rise to PUUV outbreaks (*Klempa*, 2009).

289   As a consequence of climate change, PUUV outbreaks might become more frequent in the future

290   (*Imholt* et al., 2015). The three outbreaks of 2007, 2010 and 2012 occurring in rather quick

291   succession in Germany have been considered a possible sign for this climate change induced

292   shortening of outbreak intervals (see also pattern in *Reil* et al., 2015 and *Imholt* et al., 2016). The

293   expected shortening of intervals between years with high numbers of human PUUV infections is

294   due to the shortening of the time intervals between mast years of oak and beech trees in Europe

295   (*Overgaard, Gemmel & Karlsson*, 2007), which provides optimal food conditions for bank voles

296   in Europe more frequently. Despite the possible positive influence of climate change on the

297   frequency of mast years, the occurrence of mast years remains tied to the presence of nutrient

298   reserves and can thus hardly occur for several consecutive years.

299   However, our results show that not every mast year is followed by a year with a high number of

300   recorded PUUV infections. The years 2006, 2009 and 2011 have been mast years in Germany,

301   followed by years with a high number (> 1,000 recorded cases) of recorded human PUUV

302   infections. In contrast, the years 2002, 2004 and 2014 have also been mast years at least for

303   beech in Hesse, butthe number of recorded PUUV infections in Germany was only slightly

304   higher than average in the following years (2003, 2005 and 2015) andnot comparable to those in

305   the years 2007, 2010 and 2012. In the first few years after 2001, the year of the introducing the

306   reporting obligation, not every case of PUUV might have become reported, which may partially

307   explain lower numbers than expected. In 2005, the fifth highest number of PUUV infections

308   (within years of 2001 to 2016) was observed, with a slightly different spatial pattern. A disease

309   hotspot was present in North Rhine-Westphalia but not in Baden-Wuerttemberg. The higher

310   number of PUUV infections in North Rhine-Westphalia corresponds to the observed higher

311   number of PUUV infections in the neighbouring regions in Belgium (*Mailles* et al., 2005). In

312   addition, there is a strong spatial variation of the strength of beech mast. Beech mast is supposed

313   to be largely regulated by climate with greater temporal than spatial dynamics. *Reil* et al., 2015

314   displayed spatial variation of beech fructification among federal States in Germany for the years

315   2001 to 2012, which were, however, disregarded in this study as we chose to only exemplarily

316   consider data from Hesse. The year 2014 was considered a Germany-wide mast year, which led

317   to the expectation of a PUUV outbreak in 2015 (*Reil* et al., 2016). In fact, 542 PUUV infections

318   were reported in Germany in 2015. Although this is the fourth highest number of recorded

319   PUUV infections since the introduction of the reporting obligation in 2001 in Germany, the

320   number was clearly lower in comparison to the PUUV outbreaks in 2007, 2010 and 2012 with

321   1654, 1893 and 2476 reported cases.

322   *Seasonal patterns*

323   We found a clear seasonal pattern of PUUV infections in years of disease outbreak with its

324   maximum in early summer as previously shown by *Krautkramer, Kruger & Zeier*, (2012). This

325   seasonal pattern slightly differs from the seasonal pattern in Northern Europe with two seasonal

326   peaks per annum (*Vaheri* et al., 2013). In Northern Europe a minor peak usually occurs in

327   summer (perhaps related to summer vacation with many people staying outdoors) and a second

328   stronger peak in winter (November – February) (*Vaheri* et al., 2013). Bank voles undergo 3-4

329   litters per year, with a peak in abundance in late autumn and winter (*Vaheri* et al., 2013). The

330   second seasonal peak might thus be ascribed to the high abundance of bank voles during this

331   period. In addition, bank voles tend to frequent the proximity of human dwellings when

332   conditions are adverse, leading to increasing contact with humans and hence infection risk

333   (*Porkert*, 1975; *Olsson* et al., 2009; *Muyangwa* et al., 2015). It was hypothesized that bank voles

334   might move closer to human housings in very cold winters also in Germany, where winter peaks

335   of PUUV infection cases have been observed in some previous years (*Faber* et al., 2010;

336   *Heyman* et al., 2012).

337   *Data quality*

338   Apart from the factors used in this study, bank vole abundance and certainly seroprevalence are

339   crucial factors to be taken into account for predictions of rodent-associated human infections.

340   Both factors were recently used in a study by *Drewes* et al., (2017b), focusing on south-western

341   Germany. Another problem to consider is the current methodology and practice of virus

342   identification. Commonly used methodologies can fail to correctly distinguish antibodies of

343   PUUV and DOBV. In addition, also Tula orthohantavirus can trigger human infections, but

344   cannot be easily identified with standard serological tests but only by neutralization and/or

345   nucleic acid detection and subsequent sequencing (personal comment Rainer Ulrich). Thus,

346   when using data on the respective virus infections, potential identification errors should be taken

347   into account and addressed. More recently, virus shedding (*Voutilainen* et al., 2015) has been

348   proposed as an alternative method. Another data bias might be introduced through the reporting

349   system; reported infections with rodent-associated pathogens usually refer to the patient's place

350   of residence, which might be different from the place of infection. As shown here but also

351   recently by *Drewes* et al., 2017a, north-eastern Germany is less affected. In fact, cases detected

352   in Dessau-Roßlau and Northeim might likely be an example of such mismatch between the

353   location of virus transmission and infection reporting. Furthermore, in the case of a PUUV

354   infection, only 5-10% of the patients display clinical symptoms (*Heyman* et al., 2009). If mild

355   and strong progressions are recognized as such, a correct analysis of PUUV infection trends

356   might still possible. However, there may be a lack of awareness of some doctors for the disease.

357   Thus, the true number of infections is expected to be much higher than recorded even if

358 awareness of physicians, access to diagnostic tests and reporting mechanisms have been

359 improved in recent years (*Watson* et al., 2014).

360 **Conclusion**

361 Human PUUV infection hotspots are located in the federal states of Baden-Wuerttemberg and

362 adjacent areas in Bavaria, and the north of North Rhine-Westphalia and the adjacent part of

363 Lower Saxony. There have been strong fluctuations in the total number of recorded PUUV

364 infections among years with high numbers in 2007, 2010 and 2012. Different environmental

365 factors have been discussed and used to better understand and predict the number of human

366 PUUV infections. Interactions between these factors add to the complexity of the situation and

367 may provide the opportunity to further improve models to predict epidemics (*Heyman* et al.,

368 2012). Reliable long-term, replicated, high-resolution data on population densities of bank voles

369 and other rodents as well as on seroprevalences in host populations could improve the

370 understanding of the ecology of rodent-associated diseases and resulting predictions (e.g. *Jacob*

371 et al., 2014; *Reil* et al., 2015; *Khalil* et al., 2017). Generally, the spatial pattern of infected bank

372 voles is expected to be strongly driven by spatial autocorrelation due to the presence of PUUV in

373 certain regions in Germany and bank voles infecting each other. A higher risk of human PUUV

374 infections can be assumed (i) in areas with a high percentage of forest area (spatial pattern), (ii)

375 in years following a mast year (temporal pattern) and (iii) in early summer (seasonal pattern).

376 Due to ongoing climate change human PUUV infections may gain increased significance in

377 Germany and better risk assessments to predict disease outbreaks are needed.

378

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382

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559

560 **Figure legends**

561

562 Figure 1: Factors that potentially affect the number of recorded PUUV infections.

563

564 Figure 2: Sum of the recorded PUUV infections between 2001 and 2015. The eight districts that

565 show a significant positive trend between 2001 and 2015 are labelled and hatched. Figure based

566 on data provided by the Robert Koch Institute and the GeoBasis-DE / BKI. Figure was built

567 using Esri ArcGIS 10.3.

568

569 Figure 3: Incidence of PUUV infections: number of recorded PUUV infections per 100,000

570 inhabitants in Germany. Note that most recent data for the year 2016 (P) is shown in this figure

571 for completeness only, but was not included in any other analysis. Figure based on data provided

572 by the Robert Koch Institute and the GeoBasis-DE / BKI. Figure was built using Esri ArcGIS

573 10.3.

574

575 Figure 4: Temporal patterns of the number of recorded PUUV infections and related factors.

576 A) Precipitation and temperature in summer (i.e. June, July and August); B) Precipitation and

577 temperature in July; C) Percentage of old beech trees with medium or strong fructification in

578 Hesse, Germany; D) Number of recorded PUUV infections between 2001 to 2016 (2017, data as

579 from 14.11.2017) in Germany. Note the different time lags (also described in Material and

580 Methods). Figure based on data provided by the Robert Koch Institute, the GeoBasis-DE / BKI,

581 the Deutscher Wetterdienst, the Hessisches Ministerium für Umwelt, Klimaschutz,

582 Landwirtschaft und Verbraucherschutz. Figure was built using R 3.4.2.

583

584 Figure 5: Seasonal pattern of recorded human PUUV infections in Germany. In black: mean

585 average over the years 2001 to 2015 and (in grey: mean average +/- standard error), in red: mean

586 average over the three years with a high number of recorded PUUV infections (2007, 2010 and

587   2012) (in light red: mean average +/- standard error), in blue: mean average over the 11 years

588   with a low number of recorded PUUV infections (2001 to 2006, 2008, 2009, 2011, 2013 and

589   2014) (in light blue: mean average +/- standard error). Figure based on data provided by the

590   Robert Koch Institute. Figure was built using R 3.4.2.

591

592   Figure 6: Spatial pattern of four environmental factors that are supposed to affect the spatial

593   pattern of recorded PUUV infections (cf. Fig. 2). A) Annual mean temperature[°C]; B) Annual

594   precipitation [mm]; C) Percentage of forest area [%]; D) Percentage of urban area [%]. Figure

595   based on data provided by the Deutscher Wetterdienst, the European Environment Agency.

596   Figure was built using Esri ArcGIS 10.3.

597

598

599   **Tables**

600

601   Table 1: GLM for the incidences with four variables (forest – arcsine transformed percentage of

602   forest area, urban – arcsine transformed percentage of urban area, AMT – annual mean

603   temperature average over 2001 to 2015, AP – annual precipitation average over 2001 to 2015)

604   and their two-way interactions (Gaussian error distribution). Significance codes: '***' 0.001

605   'and '*' 0.05 and '.' 0.1. GLM based on data provided by the Robert Koch Institute, the

606   Deutscher Wetterdienst, the European Environment Agency. Analysis was preformed using R

607   3.4.2.

608

Figure 1

Factors that potentially affect the number of recorded PUUV infections.

Figure 2

Sum of the recorded PUUV infections between 2001 and 2015.

The eight districts that show a significant positive trend between 2001 and 2015 are labelled and hatched. Figure based on data provided by the Robert Koch Institute and the GeoBasis-DE / BKI. Figure was built using Esri ArcGIS 10.3.

Number of recorded PUUV infections
Sum 2001 - 2015

- 0
- 1
- 2 - 5
- 6 - 20
- 21 - 40
- 41 - 80
- 81 - 160
- 161 - 320
- >321

Kleve

Wesel

Northeim

Dessau-Roßlau

Obernhausen

North Rhine-Westphalia

Bonn

Hesse

Bavaria

Stuttgart

Freudenstadt

Reutlingen

Baden-Wuerttemberg

Ravensburg

N

0   50   100
Kilometers

Federal states of Germany (selection)

districts mentioned in the text
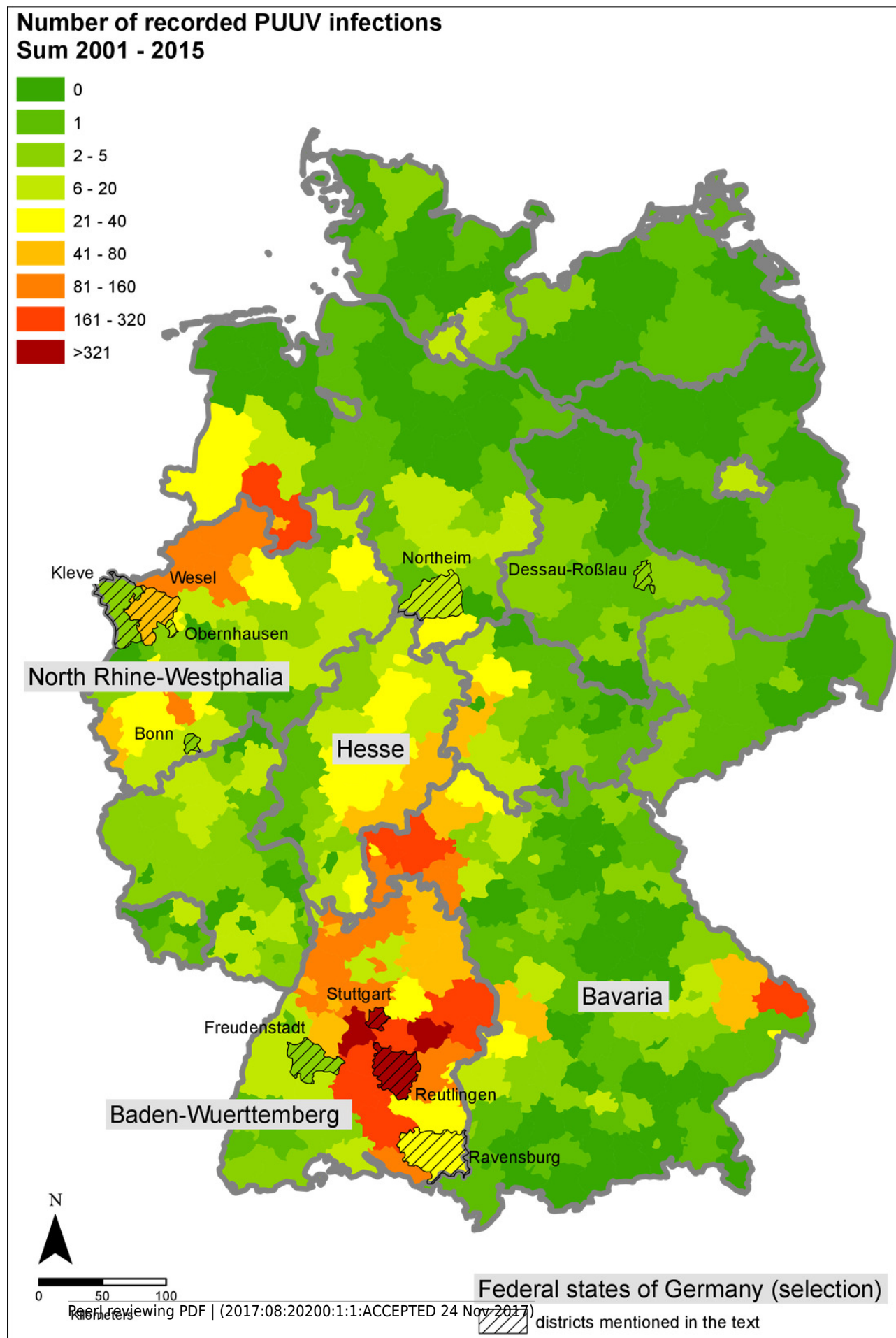
Figure 3

Incidence of PUUV infections: number of recorded PUUV infections per 100,000 inhabitants in Germany.

Note that most recent data for the year 2016 (P) is shown in this figure for completeness only, but was not included in any other analysis. Figure based on data provided by the Robert Koch Institute and the GeoBasis-DE / BKI. Figure was built using Esri ArcGIS 10.3.
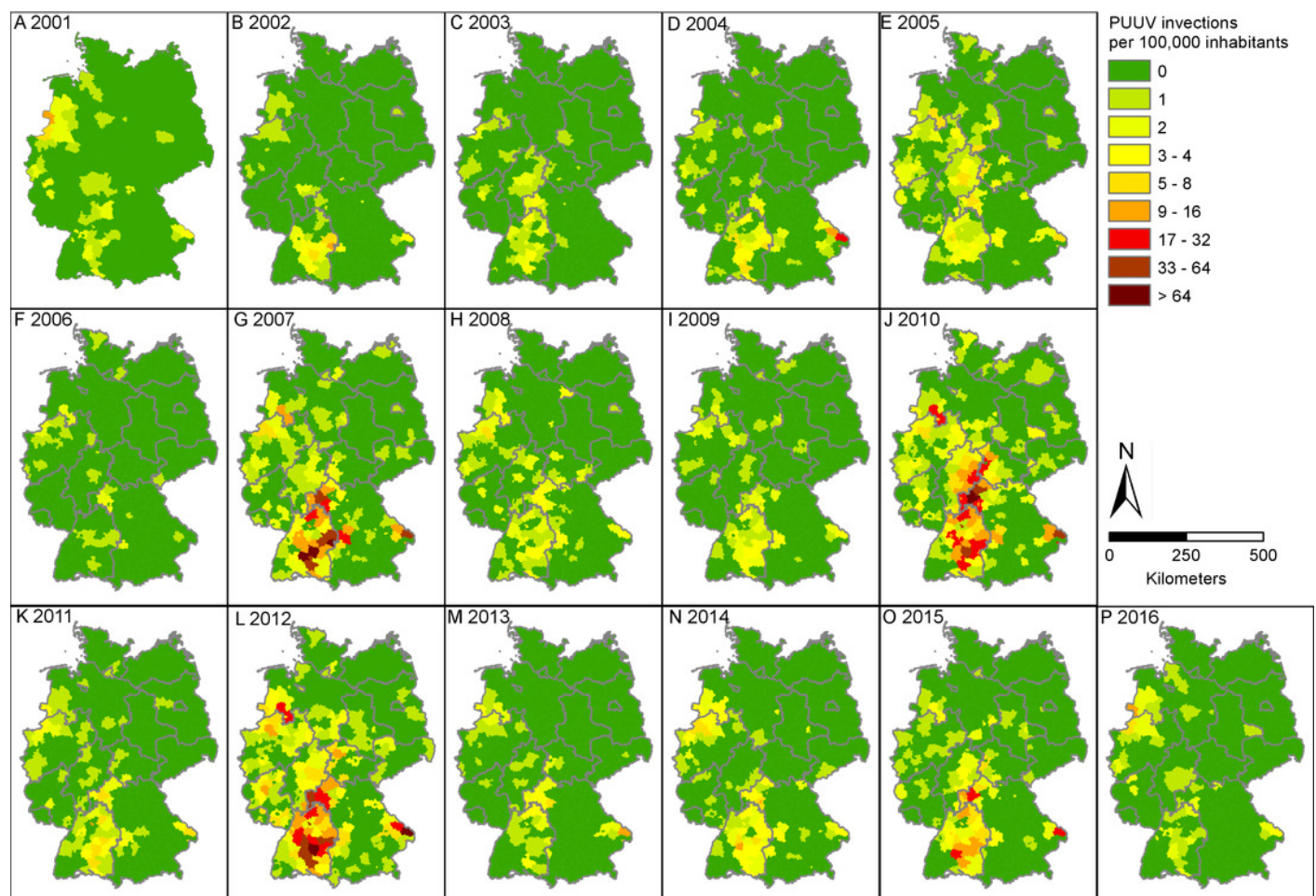
Figure 4

Temporal patterns of the number of recorded PUUV infections and related factors.

A) Precipitation and temperature in summer (i.e. June, July and August); B) Precipitation and temperature in July; C) Percentage of old beech trees with medium or strong fructification in Hesse, Germany; D) Number of recorded PUUV infections between 2001 to 2016 (2017, data as from 14.11.2017) in Germany. Note the different time lags (also described in Material and Methods). Figure based on data provided by the Robert Koch Institute, the GeoBasis-DE / BKI, the Deutscher Wetterdienst, the Hessisches Ministerium für Umwelt, Klimaschutz, Landwirtschaft und Verbraucherschutz. Figure was built using R 3.4.2.
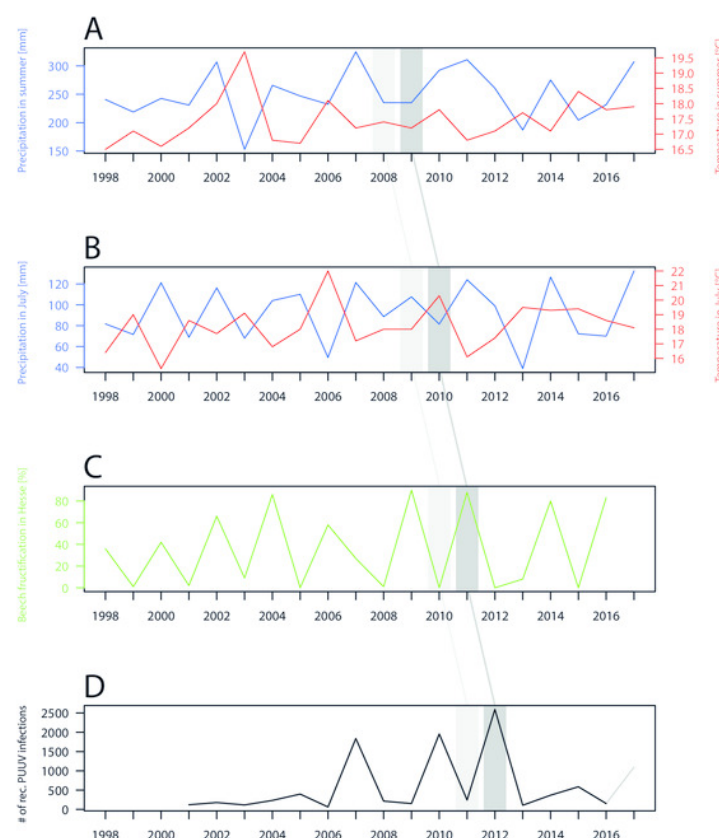
Figure 5

Seasonal pattern of recorded human PUUV infections in Germany.

In black: mean average over the years 2001 to 2015 and (in grey: mean average +/-
standard error), in red: mean average over the three years with a high number of recorded
PUUV infections (2007, 2010 and 2012) (in light red: mean average +/- standard error), in
blue: mean average over the 11 years with a low number of recorded PUUV infections (2001
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Figure based on data provided by the Robert Koch Institute. Figure was built using R 3.4.2.

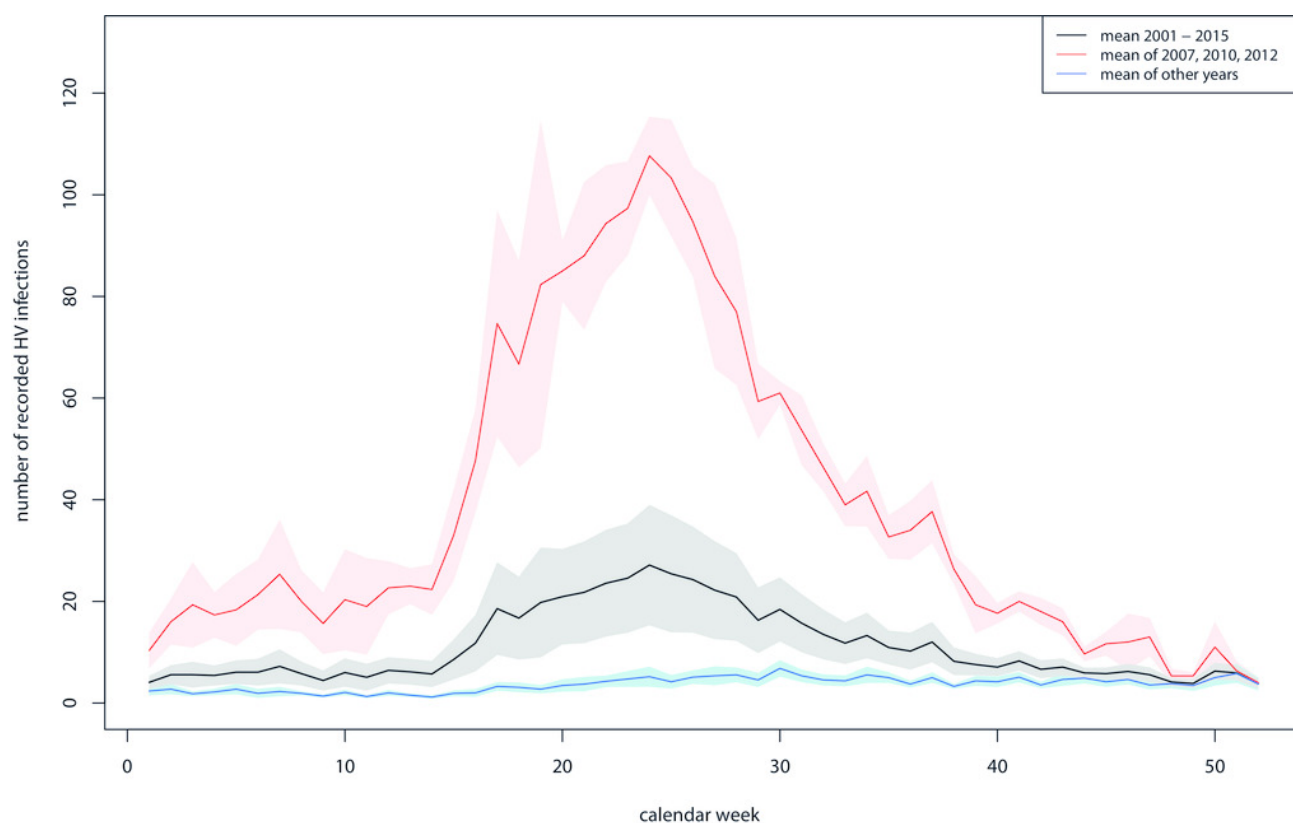



Figure 6

Spatial pattern of four environmental factors that are supposed to affect the spatial pattern of recorded PUUV infections (cf. Fig. 2).

A) Annual mean temperature[°C]; B) Annual precipitation [mm]; C) Percentage of forest area [%]; D) Percentage of urban area [%]. Figure based on data provided by the Deutscher Wetterdienst, the European Environment Agency. Figure was built using Esri ArcGIS 10.3.

A Annual mean temperature [°C]
   Mean over 2001 - 2015

■	< 7
■	7 - 8
■	8 - 9
■	9 - 10
■	10 - 11
■	> 11

B Annual precipitation [mm]
   Mean over 2001 - 2015

■	< 693
■	694 - 816
■	817 - 1000
■	1001 - 1344
■	> 1345

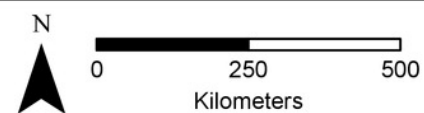
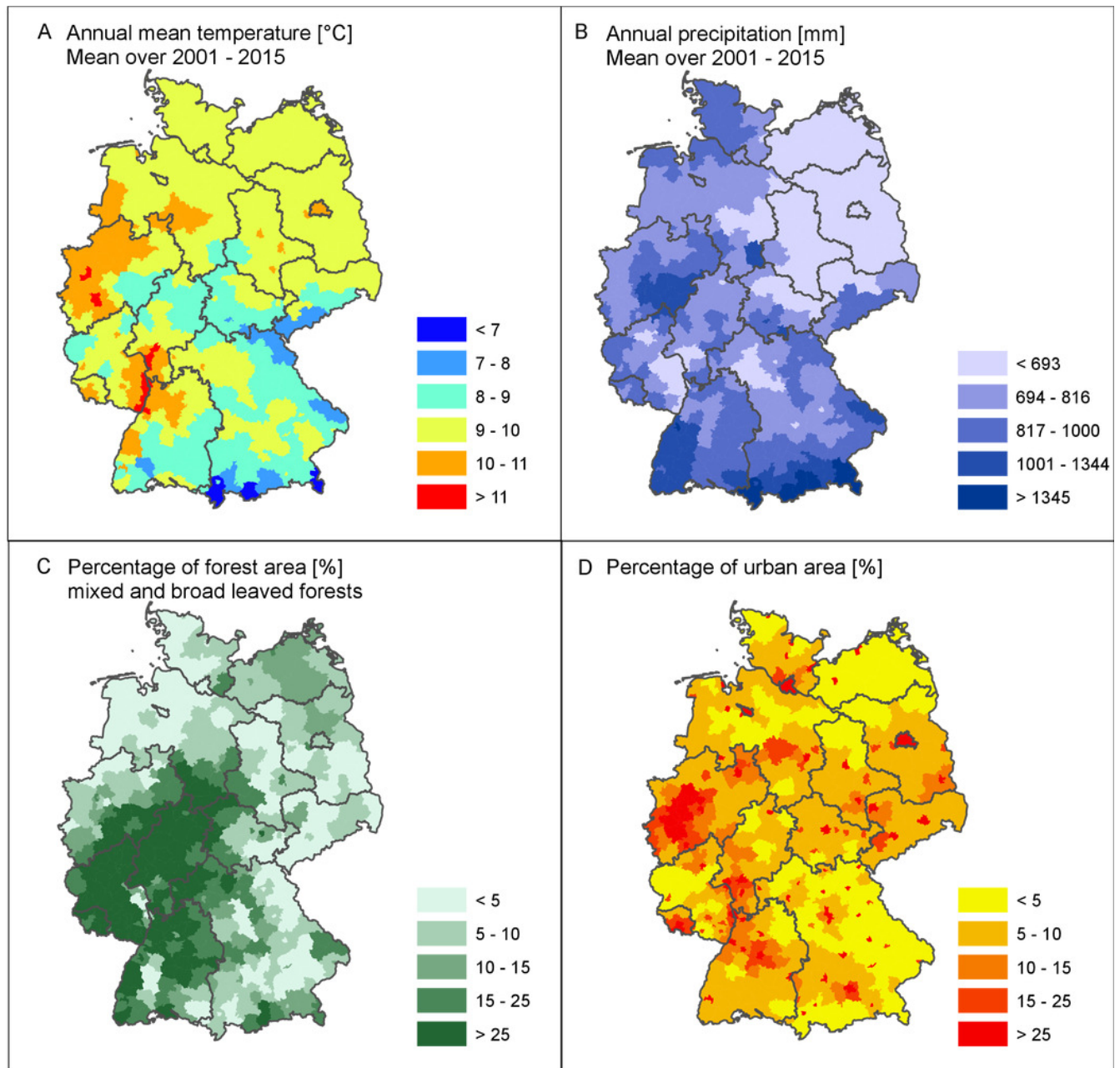
C Percentage of forest area [%]
   mixed and broad leaved forests

■	< 5
■	5 - 10
■	10 - 15
■	15 - 25
■	> 25

D Percentage of urban area [%]

■	< 5
■	5 - 10
■	10 - 15
■	15 - 25
■	> 25

N

0   250   500
Kilometers

GLM for the PUUV incidences with four variable.

GLM for the incidences with four variables (forest – arcsine transformed percentage of forest area, urban – arcsine transformed percentage of urban area, AMT – annual mean temperature average over 2001 to 2015, AP – annual precipitation average over 2001 to 2015) and their two-way interactions (Gaussian error distribution). Significance codes: '***' 0.001 'and '*' 0.05 and '.' 0.1. GLM based on data provided by the Robert Koch Institute, the Deutscher Wetterdienst, the European Environment Agency. Analysis was preformed using R 3.4.2.

1 Table 1: GLM for the incidences with four variables (forest – arcsine transformed
2 percentage of forest area, urban – arcsine transformed percentage of urban area,
3 AMT – annual mean temperature average over 2001 to 2015, AP – annual
4 precipitation average over 2001 to 2015) and their two-way interactions (Gaussian
5 error distribution). Significance codes: '***' 0.001 'and '*' 0.05 and '.' 0.1

Coefficients:	Estimate	Std. Error	t value	Pr(>|t|)	
(Intercept)	4.12E-04	6.95E-04	0.592	0.554	
forest	9.59E-03	2.21E-03	4.341	1.81E-05	***
urban	-2.95E-04	1.43E-03	-0.207	0.8359	
AMT	-7.10E-06	7.38E-06	-0.961	0.337	
AP	-9.15E-07	5.96E-07	-1.535	0.1257	
forest:urban	1.38E-03	1.11E-03	1.242	0.2149	
forest:AMT	-8.21E-05	2.01E-05	-4.074	5.61E-05	***
forest:AP	-1.59E-06	7.92E-07	-2.012	0.0449	*
urban:AMT	8.69E-06	1.24E-05	0.704	0.4819	
urban:AP	-8.75E-07	7.00E-07	-1.25	0.2119	
AMT:AP	1.37E-08	7.25E-09	1.884	0.0602	.