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Retrospective Analysis of the Oral Immunisation of Wild Boar Populations against Classical Swine Fever Virus (CSFV) in region Eifel of Rhineland-Palatinate

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1 **Retrospective Analysis of the Oral Immunisation of Wild Boar Populations**
2 **against Classical Swine Fever Virus (CSFV) in region Eifel of Rhineland-**
3 **Palatinate**

4
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12

13

14 **Abstract**

15 In the present study the effect of control measures implemented during the Classical
16 Swine Fever (CSF) epidemic in wild boar in the Eifel region of the German federal
17 state of Rhineland-Palatinate from 1999 to 2005 was assessed. During the first three
18 years after official confirmation of virus detection these measures comprised
19 intensive hunting, especially of young animals and hygiene measures. Subsequently
20 oral immunisation (o.i.) using a modified live virus vaccine was introduced as an
21 additional control tool.

22 All shot wild boar from the restricted area were tested virologically and serologically
23 for CSF. The laboratory results from over 110000 animals accompanied by
24 information about age, gender and geographical origin of the animals were collected
25 in a relational database. In total about 82 % of all virologically positive wild boars
26 were piglets, thus confirming the importance of this age group in the perpetuation of
27 the epidemic. An analysis of the hunting bag showed that piglets were under
28 represented compared to older animals throughout the eradication programme. This
29 finding indicated that hunters did not comply with the control strategy of intense

30 targeting of young animals. Before as well as after the implementation of o.i. a
31 significantly higher virological prevalence and a significantly lower serological
32 prevalence were observed in piglets compared to yearlings and adults. Shortly after
33 the begin of the vaccination campaign in February 2002 CSFV prevalence decreased
34 significantly whereas the serological prevalence increased markedly in all age
35 classes. In order to test the influence of age and vaccination on the serological
36 prevalence a logistic regression model was used. Our results strongly suggest that
37 under the field conditions in the Eifel region vaccination against CSFV had a crucial
38 influence on the increase of seroprevalence rate and the elimination of CSFV. The
39 last virus-positive pig was found 13 months after start of o.i.

40

41

42 **Introduction**

43

44 Classical swine fever (CSF) is a dreaded infectious disease of domestic pigs as well
45 as wild boar (*Sus scrofa scrofa*) with highly economic impact. CSF virus (CSFV) is a
46 positive single-stranded RNA virus that belongs to the family *Flaviviridae*, genus
47 *Pestivirus*. (Moennig, 2000). During the last two decades, CSF outbreaks occurred in
48 wild boar populations of several EU member states and neighbouring countries. CSF
49 in wild boar is a serious threat to domestic pig farming (Laddomada, 2000; Artois et
50 al., 2002; Kaden et al., 2002). Approximately 60% of all CSF outbreaks in domestic
51 pigs during 1993 to 1998 in Germany could be attributed to direct or indirect contact
52 with infected wild boar (Fritzemeier et al., 2000). During this time in Germany, wild
53 boar populations of the federal states Lower Saxony, Mecklenburg-Western
54 Pomerania and Brandenburg were affected by CSF. The disease was also transiently
55 present in Baden-Wuerttemberg, North Rhine-Westphalia and Saxony-Anhalt (Kaden

56 et al., 2003; Kaden et al., 2005). First control measures consisted of increased
57 hunting, targeting especially the young age classes that are held responsible for the
58 perpetuation of the epidemic. Hygienic precautions were additional means. However,
59 it soon became clear that the spread of CSF could not be stopped. In 1994 Kaden
60 and coworkers began to revisit the option of oral immunisation (o.i.) as an additional
61 control tool in order to reduce the number of susceptible animals and thereby
62 transmission rate of CSFV in wild boar populations (Kaden and Lange, 1998; Kaden
63 et al., 2000). In several federal states field trials were performed (Kaden et al., 2002;
64 Kaden et al., 2004). In 2001 emergency vaccination of wild boar against CSF was
65 implemented in EU legislation (Anonymus, 2001).

66 In the Eifel region (northern part of Rhineland-Palatinate) CSFV was first diagnosed
67 in January 1999 in the district Bitburg-Prüm. The genotype 2.3 Rostock of CSFV was
68 confirmed both in wild boar and domestic pigs and control measures based on the
69 first EU CSF Directive (Anonymus, 1980) were applied. The infection showed a
70 tendency to spread massively and could not be stopped by intensive hunting and
71 hygiene measures. As a consequence o.i. was introduced in February 2002.

72 The control of CSF in the Eifel region was unique because for the first three years of
73 the epidemic attempts were made to control the epidemic using conventional
74 measures only, i.e. increased hunting and hygiene measures. Vaccination was
75 banned. The subsequent implementation of o.i. allowed the comparative analysis of
76 the course of the epidemic without and with o.i., using a large set of georeferenced
77 data collected three years before and three years after the beginning of vaccination.

78

79

80 **Materials and methods:**

81

82 Study area and vaccination procedure

83 The Eifel region is located in Rhineland-Palatinate in the western part of Germany
84 bordering Belgium and Luxembourg. After the initial diagnosis of CSFV in wild boar in
85 January 1999 in the Eifel region, increased hunting of young wild boar and hygiene
86 measures (e.g. drawing all shot wild boar at official collecting points; Anonymus,
87 2001) were used to control CSF. Whenever CSFV was newly detected in wild boar
88 zones were enlarged, so that the size of the restricted area changed 38 times from
89 about 20 km² to 8550 km² in total. A serological monitoring was adopted within not
90 infected region of Rhineland Palatinate in a so called “monitoring area” (data not
91 shown), which girdled the CSF-infected area. The Oral vaccination using a modified
92 live vaccine based on the C-strain (Chinese) of CSFV administered in maize baits
93 (Kaden et al., 2000; Kaden and Lange, 2001) was started in February 2002. One
94 vaccination campaign consisted of two vaccinations with a 4 weeks interval (“double
95 vaccination”). These campaigns were repeated three times per year with intervals of
96 three to four months. Double vaccination campaigns were carried out in spring
97 (February, March), summer (June, July) and autumn (September, October) in 2002,
98 2003, and 2004 in an area covering approximately 8600 km² (Fig. 1). The vaccination
99 area was treated as restricted area with all trade barriers for pig farmers, according to
100 the “Emergency vaccination plan of Rhineland-Palatinate” (Anonymus, 2002). Within
101 the vaccination areas two feeding places per km² of hunting area were used on
102 average. Depending on the estimated density of the regional wild boar population 30
103 to 40 vaccine baits per feeding place and vaccination campaign were laid out
104 manually by hunters. The baits were placed on the feeding places and were covered
105 with a thin layer of soil (Anonymus, 2002). In total, approximately 4.4 million baits
106 were laid out from February 2002 to October 2004.

107

108 Sampling design, diagnostic program, and database

109 All wild boar shot or found dead in the restricted area were serologically and
110 virologically investigated by the State Veterinary Laboratory (*Landesuntersuchungs-*
111 *amt*, LUA) of Rhineland-Palatinate in Koblenz, Germany. For detection of CSFV
112 antigen, samples were examined using commercial Enzyme-linked Immunosorbent
113 Assays (ELISA) “CHEKIT CSF virus I, II, and III” (IDEXX Laboratories, Ludwigsburg,
114 Germany). Positive and doubtful results were confirmed by virus isolation in cell
115 culture using PK-15 cells.

116 Sera were tested for antibodies using the commercial ELISAs “CSF-Sero” or
117 “HerdCheck CSFV Antibody” (IDEXX Laboratories, Ludwigsburg, Germany). Positive
118 and doubtful results were further investigated by virus neutralisation test, which was
119 performed as a Neutralisation-Peroxidase-Linked Assay (Hergarten et al., 2001).

120 Individual data sets including e.g. sex, estimated age and origin (municipality) of the
121 wild boar were recorded by hunters in questionnaires especially designed for
122 epidemiological analysis. The age classification was carried out according to the
123 colour of the coat, body weight and/or dental estimation. All wild boar were stratified
124 into three age classes comprising animals < 12-months (piglets), 12- to < 24-months
125 (yearlings) and > 24-months of age (adults).

126 Data from the questionnaires combined with the diagnostic results were linked to the
127 geographical origin within administrative municipal boundaries (software ArcGIS
128 ArcView 9.0, ESRI Redlands, CA, USA) and entered into a relational database
129 (Staubach et al., 2003).

130

131 Investigated area and time period

132 A descriptive analysis was carried out using data from the temporarily changing
133 restricted area (01/01/1999 to 31/03/2005). In order to be able to compare data from

134 the pre-vaccination period with the years after implementation of o.i. campaigns, a
135 harmonised template scheme matching a part of the immunisation area from
136 February 2002 with the same area in 1999 and in March 2005 was used. Thereby a
137 standardisation across space-time distribution was achieved and the efficacy of o.i.
138 could be assessed utilising all collected data of wild boar out of this defined area for a
139 period of six years (three years before and three years after the beginning of o.i.). In
140 addition, the hunting bag (with focus on age structure) was evaluated utilising wild
141 boar data from three CSF-infected areas during different time periods (size: 70 km² =
142 six years; 1425 km² = four years and 2000 km² = two years).

143

144 Statistical Analysis

145 All statistical analyses were performed using the open-source software-package *R*
146 (www.r-project.org) to assess statistical differences of serological and virological
147 prevalence rates. Depending on the sample size we used χ^2 -test or Fisher's exact
148 test (Cavalli-Sforza, 1964). The significance level was set to p-value ≤ 0.05 . Critical
149 significance levels between the three age classes were Bonferroni corrected
150 according to Rice (1989) taking into account multiple tests on the same data. We
151 calculated exact upper and lower 95% confidence limits assuming binomial
152 distribution for prevalence data within the wild boar population according to the
153 method of Willer (1982). A descriptive, retrospective data analysis was performed to
154 explore potential factors which influenced serological prevalence.

155 We modelled a stepwise forward linear regression with logit transformed
156 seroprevalence data (i. e. a logistic regression) in accordance with Hosmer and
157 Lemeshow (2000):

158

$$159 \quad \text{logit (SP)} = \ln\left(\frac{SP}{1-SP}\right) = a + b_1 \bullet \text{Age}_1 + b_2 \bullet \text{Age}_2 + b_3 \bullet \text{Vacc}$$

160

161 As binary dependent (outcome) variable the seroprevalence (SP) (serologically
162 diagnosed positive = [1] and negative = [0]) was used.

163 We decomposed the logit of SP into an intercept 'a' and the two independent
164 (predictor) variables 'Age' of wild boar and the status of the vaccination area 'Vacc':
165 wild boar from vaccination area = [1], wild boar from non-vaccination area = [0].
166 Variable 'Age' was converted into dummy-variables 'Age₁' (yearlings) and 'Age₂'
167 (adults). In compliance with Kreienbrock and Schach (2005) the largest group was
168 used as reference group, in this case the age class of piglets. Analysis of variance
169 (ANOVA) was applied to test the influence of age and vaccination on the
170 seroprevalence. Within the ANOVA we made use of Wald-Test.

171

172 **Results**

173

174 Description of the sample and analysis of hunting bag

175 During the CSF control measures in the Eifel region 114163 georeferenced samples
176 of wild boar were collected in a relational database. According to this database
177 111451 serological and 112895 virological investigations were performed from
178 January 1999 to March 2005. Within each year the number of samples collected
179 during different months varied corresponding to the main hunting season for wild
180 boar with a peak in autumn and winter (months October to January).

181 To analyse the hunting bag we reviewed data from three CSF-infected areas as
182 described above. According to the age structure of the hunting bag in the infected
183 areas analysed approximately 54% to 59% of animals were piglets, 30% to 35%

184 yearlings and 11% to 12% were adults. The overall hunting bag and its age structure
185 did not change over the time period of the investigation.

186

187 Virological investigations

188 Over the three years before the start of o.i. 271 piglets (3.24%) , 49 yearlings (1.15%)
189 and 19 adults (1.12%) were positive for the CSFV out of 8359 investigated piglets,
190 4257 yearlings, and 1693 adults, respectively (Fig 2). Within the first three years after
191 beginning of the immunisation 135 piglets (out of 43087; 0.31%), 12 yearlings (out of
192 28185; 0.04%) and 8 (out of 10270; 0.08%) adults were virologically positive. Hence,
193 the CSFV prevalence decreased significantly after the launch of the vaccination
194 measures (piglets: $p < 0.001$; $\chi^2 = 766.10$; FG = 1; yearlings: $p < 0.001$; $\chi^2 = 236.37$;
195 FG = 1; adults: Fisher-Exakt, $p < 0.001$), and the last CSFV positive case was
196 diagnosed thirteen months after implementation of vaccination.

197 The virological prevalence was significantly different in piglets, yearlings and adults.
198 The difference was observed before ($p < 0.001$; $\chi^2 = 66.22$; FG = 2) as well as after
199 start of o.i. ($p < 0.001$; $\chi^2 = 73.34$; FG = 2). In total about 82% of all virologically
200 positive wild boar were piglets (Fig. 2).

201

202 Serological investigations

203 An analysis of the serological prevalence (SP) over a period of six years revealed a
204 significant increase within all age classes after the start of o.i. (piglets: $p < 0.001$;
205 $\chi^2 = 1849.52$; FG = 1), (yearlings: $p < 0.001$; $\chi^2 = 2243.85$; FG = 1), (adults:
206 $p < 0.001$; $\chi^2 = 986.33$; FG = 1) (Fig. 3). Prior to the vaccination campaigns
207 serologically positive animals were found in piglets on average 18.37% (1649 out of
208 8977 investigated animals), in yearlings 33.35% (1589 out of 4764), and in adults
209 40.61% (811 out of 1997), The serological prevalence was significantly different in

210 the age groups piglets, yearlings and adults ($p < 0.001$; $\chi^2 = 630.89$; $FG = 2$). After the
211 launch of the o.i. 42.72% piglets (17776 serologically positive piglets out of 41614),
212 69.08% yearlings (19076 out of 25616), and 75.87% adults (7660 out of 10096) were
213 detected positive for CSFV antibodies. The highest seroprevalence (SP) rates were
214 observed after o.i. in adults, followed by yearlings and finally piglets. Furthermore,
215 the seroprevalence rates were also significantly different between the groups ($p <$
216 0.001 ; $\chi^2 = 6517.59$; $FG = 2$).

217 Furthermore we evaluated temporal differences in SP on a monthly basis during the
218 immunisation period within each age class. A wavelike development of the SP was
219 seen in the age class of piglets (Fig. 4). Following the o.i. campaign in spring SP
220 increased slightly from March to June to values between 45% and 60%. During late
221 summer SP decreased continuously to values of about 30% within each year.
222 Between October and February constant SP-rates in the range of 38% to 45% could
223 be detected.

224 The monthly SP-rates of yearlings and adults revealed a continuous increase after
225 the first vaccination campaigns (yearlings: Fig. 5; adults: Fig. 6). During the whole
226 immunisation period SP-rates of the yearlings ranged constantly at a level of about
227 70%. After completion of the last o.i. in winter 2004 SP declined slightly to a level of
228 approximately 64% (Fig. 5). The SP within the age class of adults increased
229 constantly after the first four immunisation campaigns and reached a stable rate of
230 77% - 87% in the following two years (Fig. 6).

231

232

233 Influence of oral immunisation on serology

234 ANOVA was used to investigate the influence of the factors age and vaccination on
235 the dependent variable SP of a logistical regression model. All independent variables

236 revealed a significant influence on the SP ($p < 0.001$). The ANOVA of the logistical
237 regression model showed a 14 times higher influence of vaccination on serology
238 compared to the influence of age (Wald test-statistic: 4442.2 vs. 316.0; Tab. 1).

239

240

241 **Discussion**

242 Historic observations and experiences have shown that outbreaks of CSF in wild
243 boar populations were self-limiting (Terpstra, 1988). This can no longer be
244 generalised, since several crucial parameters have changed: Over the last decades
245 wild boar populations were growing, and this phenomenon is still observed all over
246 Europe (Artois et al., 2002). CSFV strains currently circulating are of medium
247 virulence compared to strains of supposedly higher virulence in circulation about 30
248 to 50 years ago. Thus the impact of CSFV introductions into wild boar populations
249 today is likely to be less severe compared to outbreaks that were caused by highly
250 virulent strains decimating animal populations heavily. Whereas at present in
251 naturally confined regions, e.g. mountainous terrain, CSF outbreaks are still likely to
252 be self-limiting (Schnyder et al., 2002), the infection may become endemic in wide
253 open areas with high densities of wild boar and no effective natural barriers. This was
254 the case in CSF outbreaks in the early 90ties of the last century in two regions of
255 Northern Germany. First control measures applied, e.g. increased hunting and
256 hygiene, were not suitable to decrease the number of susceptible animals sufficiently
257 to prevent further spread of infection. Therefore, in 1994 Kaden and coworkers
258 revisited the option of vaccination. They started first experimental o.i campaigns in
259 Lower Saxony in order to decrease the number of susceptible animals below a
260 critical threshold, where the rate of reproduction of infection would fall <1 ($R_0 < 1$), thus
261 leading to elimination of CSFV from the affected wild boar population. Over the

262 following years more o.i. campaigns followed in several Federal States and the
263 vaccination strategy was refined: First vaccination campaigns consisted of double
264 vaccination at an interval of 14 or 28 days twice yearly (Kaden et al., 2000, Kaden et
265 al., 2002). In 1999, an improved protocol based on three double vaccinations per
266 year, in spring, summer and autumn, was first implemented in Baden-Wuerttemberg
267 (Kaden et al., 2005). Since that time, vaccination of wild boar was carried out
268 accordingly.

269 However, since the vaccination strategy was still under development and the early
270 o.i. campaigns were immediate responses to outbreaks it was difficult to quantify the
271 influence of vaccination on the outcome of the epidemic. There was no “control
272 situation”. This changed when in 1999 CSFV was introduced into the wild boar
273 population of the Eifel region of Rhineland Palatinate. The epidemic spread rapidly
274 and the local government attempted to eradicate CSF using hunting measures and
275 hygienic precautions. Emergency vaccination was not allowed. After three years
276 (2002) when no improvement of the epidemiological situation was in sight the
277 strategy was changed and o.i. was introduced. Accumulated data sets collected over
278 6 years allowed for the first time the direct comparison of control strategies with and
279 without oral vaccination in identical areas.

280 After the initial CSF outbreak and prior to the launch of the vaccination campaign, the
281 size of restricted areas permanently increased from about 70 km² to 8850 km². This
282 correlated with a continuous increase in sample numbers. Data from more than
283 110000 shot wild boar were collected and could be analysed. These data were
284 representative for the affected area, because all shot wild boar had to be tested for
285 CSF during the whole investigation period. Using large template schemes and long
286 time periods pre and post vaccination spatial and temporal dependencies and
287 seasonal effects could be minimised (Staubach et al., 2002).

288 An important criterion for assessing the effect of hunting measures was the
289 stratification of the shot animals in three age classes: 0-1 year old (piglets), 1-2 years
290 old (yearlings) and more than 2 years old (adults).

291

292 Young animals are major reservoirs for CSFV and they contribute to the persistence
293 of the infection in wild boar populations. As described in former studies in Lower-
294 Saxony, Brandenburg, Mecklenburg-Western Pomerania, Saxony-Anhalt and Baden-
295 Wuerttemberg (Kaden et al., 2002; Kaden et al., 2003), in the Eifel region the age
296 class of piglets constituted with 82% the major group of CSFV infected wild boar
297 during the whole investigation period. Therefore one persistent element of CSF
298 control strategy in the Eifel region was the intent to increase hunting of piglets, in
299 order to reduce the number of susceptible animals. However, the analysis of the
300 hunting bag of the affected area over the whole time period revealed that this goal
301 was not reached because the age class of piglets with only 54% to 59% was
302 underrepresented, even though hunters had been asked to specifically target piglets,
303 apparently they did not comply for a number of reasons ranging from financial to
304 ethical considerations. The main reason for decreasing attractiveness of young wild
305 boar piglet hunting seems to be the low carcass weight and lacking trophies.

306 During the three years before starting oral vaccination more than 3% of the
307 investigated piglets were virus-positive in contrast to about 1% in the classes of
308 yearlings and adults. Analysis of the number of virus-positive animals on a monthly
309 basis showed that these values did not decline despite all control efforts with
310 emphasis on intensive hunting and hygiene (data not shown). The crucial role of
311 piglets for the persistence of the infection was confirmed when SP data were
312 reviewed. The age class of piglets showed the lowest SP ratio with about 18% before
313 oral vaccination. In contrast yearlings and adults reached 33% and 40%,

314 respectively. In conclusion, the data supported the notion that conventional control
315 measures could not prevent CSF from becoming endemic in the wild boar population
316 of the Eifel region.

317 In the Eifel region the latest protocol using three double vaccinations was used.
318 Vaccination had a fast effect on seroprevalence within all age classes. In yearlings
319 and adults seroprevalence rose to 69% and 76%, respectively. In contrast to
320 vaccination campaigns in Brandenburg, Lower Saxony and Mecklenburg Western-
321 Pomerania where wavelike courses of seroprevalences of yearlings and adults were
322 observed (Kaden et al., 2002), SP rates in the Eifel region remained stable
323 throughout the duration of the campaign. In piglets only 43% seroprevalence was
324 reached seasonal fluctuations were observed. These findings correspond well with
325 observations made in Baden-Wuerttemberg (Kaden et al., 2005). Depending on the
326 earlier immunisation schemes first field trials in other parts of Germany showed even
327 lower seroprevalences in piglets with average values of 20% - 25% (Kaden et al.,
328 2000; Kern and Lahrmann, 2000; Kaden et al., 2002). A number of reasons are held
329 responsible for the relatively poor seroconversion rates in piglets, e.g., age-
330 dependent poor bait acceptance, suboptimal bait formulation for this age group, and
331 the discrimination of young animals during food (bait) uptake due to the hierarchical
332 structure of wild boar families. Poor seroconversion rates in the age class of piglets
333 are still considered a problem and targeting of these animals in o.i. campaigns should
334 be improved.

335 After the start of o.i. the number of virus-positive animals decreased significantly in all
336 age classes to about 0.3% in piglets and lower than 0.1% in yearlings and adults. In
337 later phases of the programme the rate dropped below the detection threshold, and
338 in March 2003 the last virus-positive piglet case was found in the Eifel region.
339 However, for the assessment of o.i. and freedom from CSFV the rate of virus-positive

340 animals should not be the only parameter (Rossi et al., 2005). According to the
341 present practice, o.i. was continued in the Eifel region for more than one year after
342 the last virus-positive case.

343 This comparative study clearly demonstrated the pivotal role of oral vaccination in the
344 control of CSF in wild boar. The vaccination scheme using three double
345 immunisations over the year quickly led to a marked decrease of virus positive cases
346 and a significant increase of seroprevalence resulting in a reduction of target animals
347 for CSFV. This led to the elimination CSF from the Eifel region in 2003.

348 In October 2005 CSF reemerged in a small restricted area in North-Rhine Westphalia
349 north of the Eifel region. This incident can be explained by a very high local density of
350 wild boar and the apparent non-compliance of hunters with control measures
351 imposed by state veterinary authorities.

352 A final epidemiological assessment of the interrelation of vaccine induced immune
353 response and disease eradication will only be possible after use of a live attenuated
354 marker vaccine. At the present no such vaccine is available. The development of live
355 marker vaccines against CSF for the use in wild boar should therefore be promoted.

356

357

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Table 1: Results of the stepwise forward logistic regression and ANOVA for **region Eifel** with the 'SP' as dependent variable and 'Age₁' (b₁), 'Age₂' (b₂) and 'Vacc' (b₃) as independent variable. Df = degrees of freedom.

variable	Estimate	Std. error	WALD-χ^2	df	p-value
a (intercept)	-1.09567	0.01813		1	< 0.001
b₁ (yearlings)	0.1849	0.0104	316	1	< 0.001
b₂ (adults)	-0.1736	0.01026	286.4	1	< 0.001
b₃ (vaccination)	1.28277	0.01925	4442.2	1	< 0.001

Figure 1: An outline map of the restriction areas within region Eifel of Rhineland Palatinate is shown with state of 14th of February 2002 according to “emergency vaccination plan” (Anonymus, 2002). The first vaccination area in region Eifel is illustrated, furthermore the surveillance area and monitoring area.

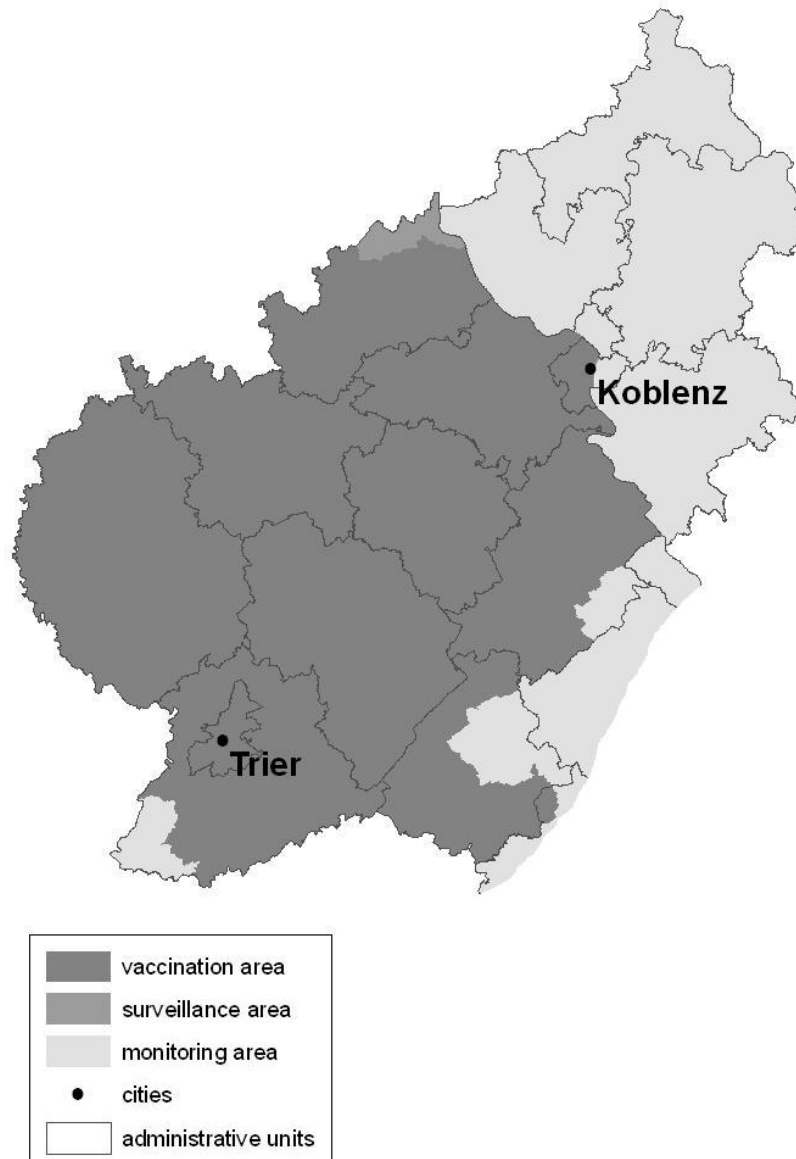
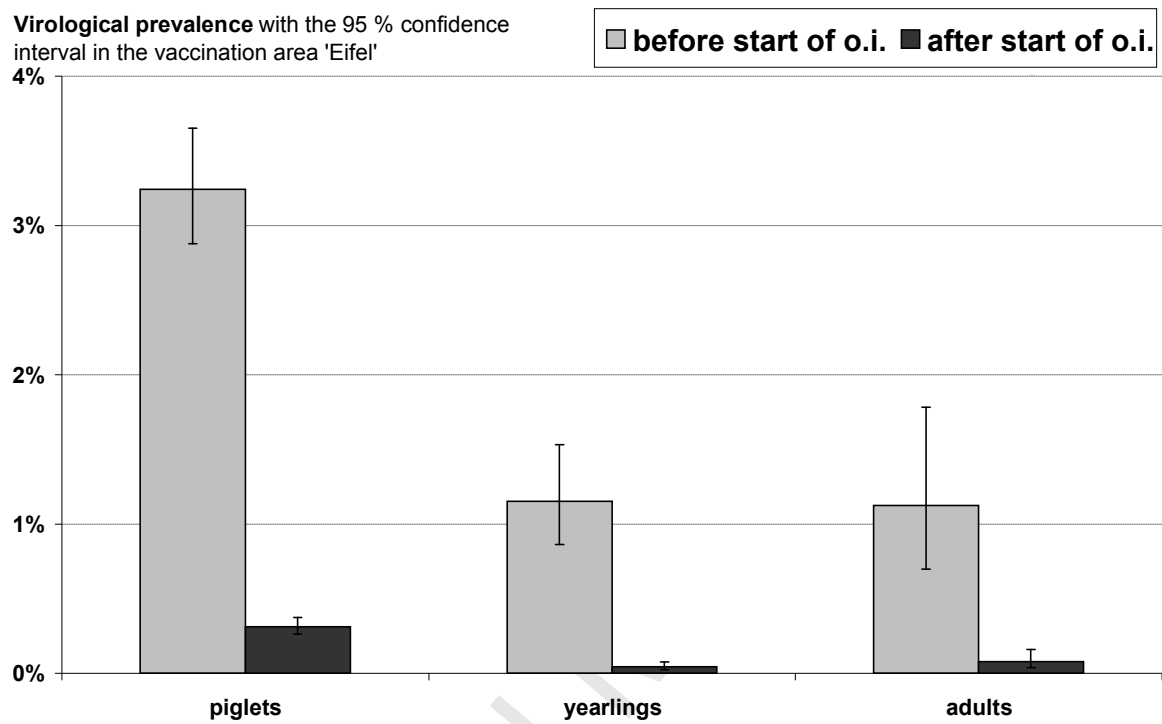


Figure 2: Distribution of CSFV prevalence by host age three years before and three years after start of o.i. Estimated VP (%) and the 95 %-confidence-intervals are shown.



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Figure 3: Distribution of CSF antibodies by host age three years before and three years after start of o.i. Estimated SP (%) and the 95 %-confidence-intervals are shown.

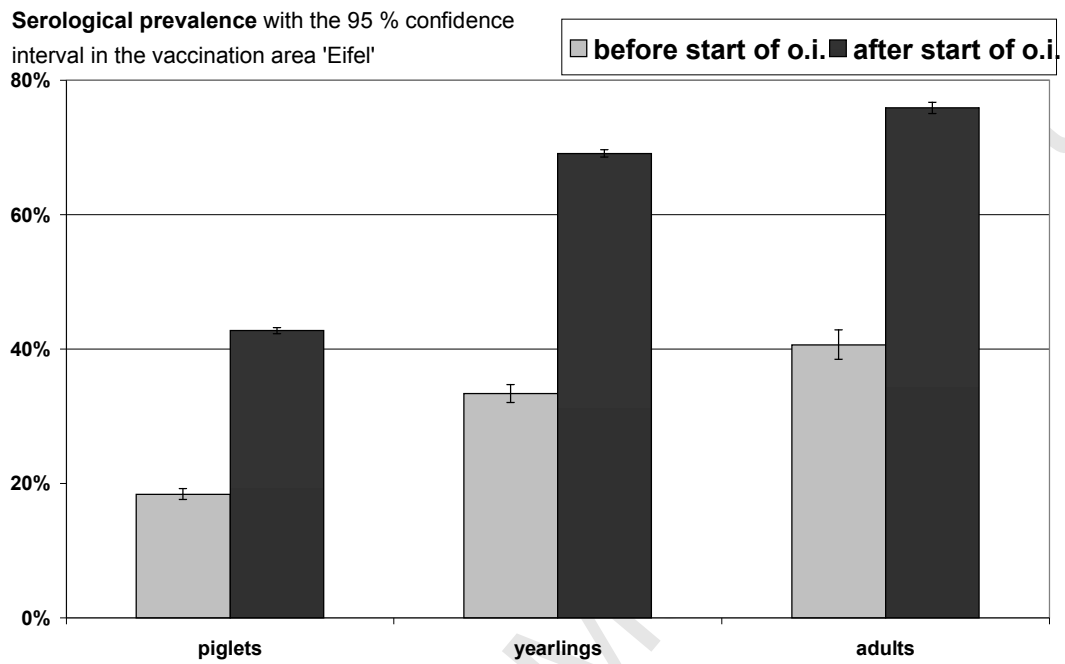


Figure 4: Serological prevalence per month in piglets, furthermore the number of shot animals in the vaccination area 'Eifel' *). Estimated SP (%) and the 95 %-confidence-intervals are shown.

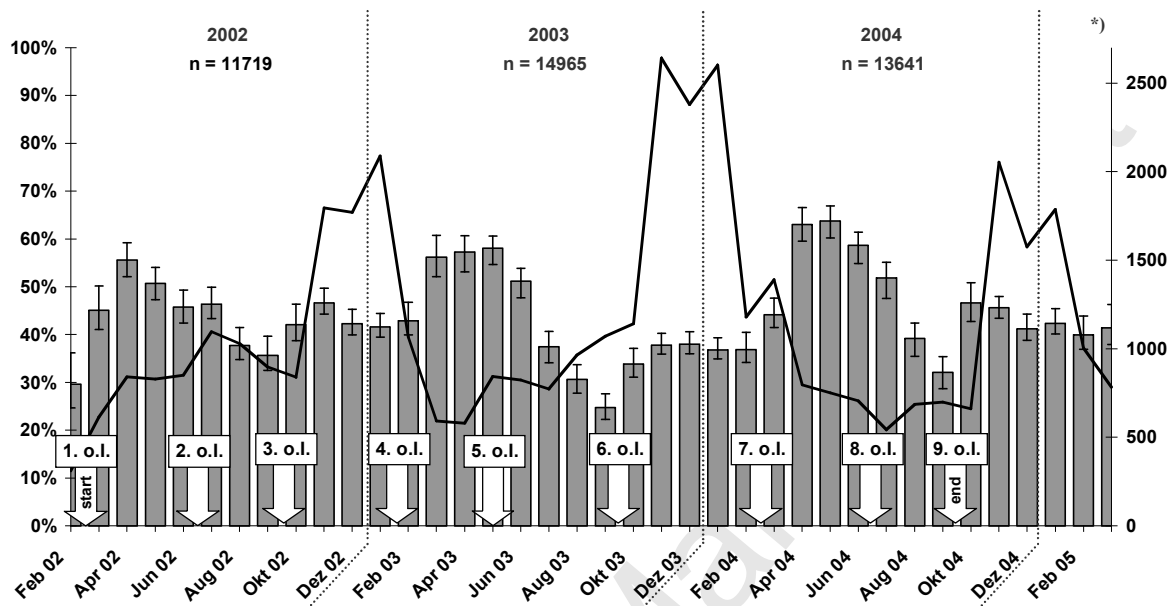


Figure 5: Serological prevalence per month. **Yearlings** and the number of shot animals in the vaccination area 'Eifel' *). Estimated SP (%) and the 95 %-confidence-intervals are shown.

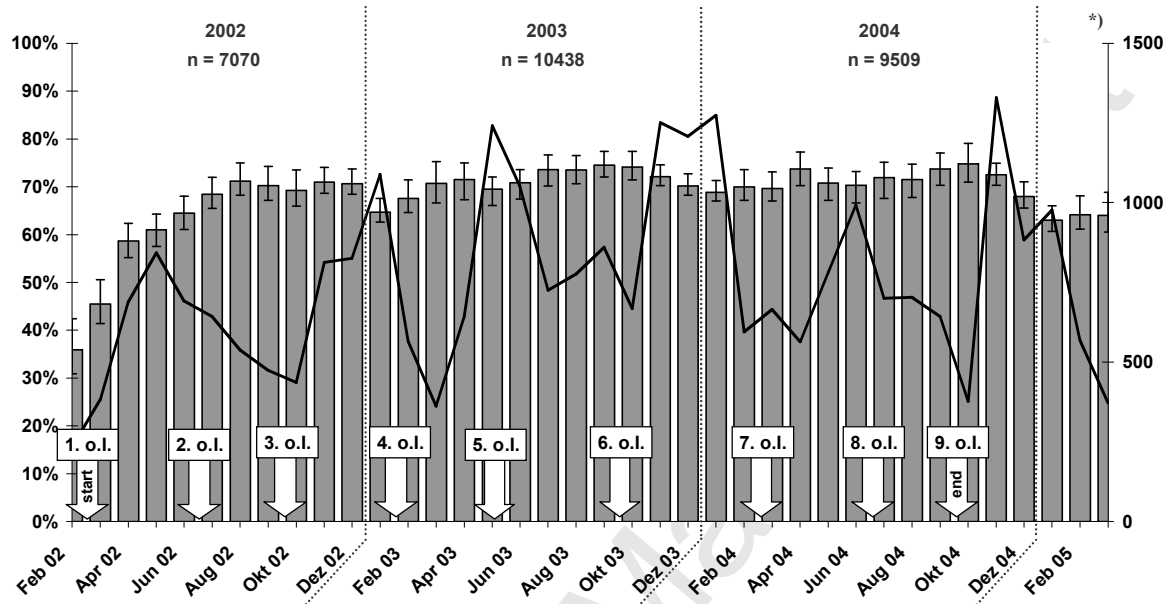


Figure 6: serological prevalence per month. **Adults** and the number of shot animals in the vaccination area 'Eifel' *). Estimated SP (%) and the 95 %-confidence-intervals are shown.

