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**Epidemiological survey of swine influenza A virus in selected wild boar
populations in Germany**

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Abstract

The aim of this study was to evaluate the epidemiological situation of swine influenza virus (SIV) infections in different wild boar populations in Germany based on a serological surveillance in some Bundesländer (federal states) in connection with virological investigations in wild boar shot in Northern Germany (Mecklenburg-Western Pomerania, district of Nordvorpommern). Altogether, 1,245 sera from wild boar were tested using the hemagglutination inhibition test. The established seroprevalence rate was low (on average 5.2 %). Antibodies were only detected against the subtypes H1N1 and H3N2 showing differences between wild boar populations and age classes. The virological investigation of samples derived from lungs of wild boar shot in Mecklenburg-Western Pomerania, district of Nordvorpommern ($n = 242$), revealed that the virus prevalence (two virologically positive animals, 0.8 %) was very low. Based on serological typing, the isolated SIV were identified as subtype H3N2. Molecular biological investigations of the hemagglutinin (HA) and neuraminidase (NA) genes confirmed this result. This study suggests that SIV infections in wild boar seem to be no serious threat for domestic pigs.

Keywords: wild boar, swine influenza, epidemiological survey, serological and virological investigations

1. Introduction

Swine Influenza (SI) is an infectious disease of pigs (*Suidae*) caused by type A influenza viruses of swine, human and avian origin. Influenza A viruses are enveloped negative-sense, single-stranded RNA viruses with a segmented genome. They belong to the family *Orthomyxoviridae*, genus *Influenza virus A* (Palese and

1 Shaw, 2007). Swine influenza A viruses (SIV) are classified into subtypes based on
 2 the antigenic properties of the surface glycoproteins hemagglutinin (HA) and
 3 neuraminidase (NA). Three subtypes of influenza A viruses (H1N1, H3N2, H1N2)
 4 are circulating in pig populations worldwide (reviewed by Olsen et al., 2006). These
 5 subtypes are widespread in several European regions and may cause clinical signs in
 6 infected pigs (Van Reeth, 2007).

7 SIV was first observed in 1918 during the human influenza epidemic in the USA
 8 and was characterised by Shope (1931). H3N2 virus infection in pigs has occurred in
 9 different European countries since the mid and late 1970s (Tumová et al., 1980;
 10 Aymard et al., 1985; Haesebrouck et al., 1985). Circulating porcine H1N1 virus in
 11 Europe was traced back to avian origin introduced from wild ducks into pig
 12 populations in 1979 (Pensaert et al., 1981; Van Reeth, 2007). This fact indicates that
 13 wild boar may also be affected by SIV. Although the disease may run as an acute
 14 infection in wild boar, a clinical detection of SI by hunters seems to be impossible
 15 and the detection of SIV, viral antigen or RNA is very difficult due to the very short
 16 course of the disease. Therefore, serological investigations generally dominate the
 17 epidemiological surveillance in wild boar. Serological diagnosis of SIV infections is
 18 based on hemagglutination inhibition (HI) test, virus neutralisation test (VNT),
 19 indirect immunofluorescence test (IFT) and enzyme-linked immunosorbent assays
 20 (ELISAs) (OIE Diagnostic Manual,
 21 http://www.oie.int/eng/normes/mmanual/A_00137.htm).

22 SIV infections in wild boar are reported in some European countries, e.g. in
 23 Poland (Markowska-Daniel and Pejsak, 1999), in Spain (Vincente et al., 2002), and
 24 in Slovenia (Vengust et al., 2006). In Germany, knowledge on the epidemiological
 25 situation of SIV infections in wild boar is more restricted. Therefore, we investigated
 26 sera derived from wild boar shot in different Bundeslaender for antibodies against

SIV. Furthermore, wild boar bagged in Mecklenburg-Western Pomerania, district of Nordvorpommern, were examined virologically. The aim of this study was to provide a preliminary overview on epidemiological data for SI in wild boar and to permit a risk assessment for SIV transmission from wild boar to domestic pigs.

2. Materials and methods

2.1. Investigation areas

Wild boar were investigated in five German Bundesländer (federal states). Altogether, 1,245 blood samples were collected for detection of antibodies against SIV. They were derived from animals shot in Mecklenburg-Western Pomerania during the hunting season 1997/98 ($n = 120$) and in 2006 ($n = 442$), in Brandenburg in 2004/05 ($n = 120$), in Saxony-Anhalt in 2001 ($n = 120$), in Baden-Württemberg in 2001/02 ($n = 81$), and in Rhineland-Palatinate in 2005 ($n = 117$). Furthermore, virological and serological investigations ($n = 242$) were carried out in wild boar bagged in the district of Nordvorpommern, Mecklenburg-Western Pomerania, from October 2005 to May 2006, in an area where also infections with highly pathogenic avian influenza virus (HPAIV) H5N1/ASIA were detected in wild birds.

2.2. Sample collection

Blood samples for the serological survey were collected from the pericardium or thoracic cavity immediately after shooting of the animals by the hunters. The samples were sent to the Local Veterinary Diagnostic Units where they were centrifuged and stored at -20°C until use. Except for sera derived from wild boar shot in Mecklenburg-Western Pomerania in 2005/06, all sera were sampled in regions where oral vaccination against classical swine fever took place (Kaden et al.,

2002, 2003). Samples were collected year-round, however, most of them during the main hunting season in winter. In total, 42 % of the evaluated samples were derived from young wild boar (≤ 1 year old), 45.6 % from sub-adults (aged >1 to 2 years) and 5.7 % from adults (>2 years old). 6.7 % of the collected blood samples could not be assigned to a specific age class. The gender of the animals was not analysed.

Organ specimens (lung) for virological testing were taken from wild boar shot in the district of Nordvorpommern in 2005/06. These samples were derived from animals of different age classes, however, most of them were ≤ 1 year old. First, samples were collected and stored at -20°C in the Local Veterinary Diagnostic Unit. Later, the sera and organ samples were transferred to the Friedrich-Loeffler-Institut on the Isle of Riems where the serological and virological investigations were carried out.

2.3. Serological investigations (antibody detection)

Sera were tested for antibodies using the HI test based on a slightly modified standard protocol (OIE Diagnostic Manual, http://www.oie.int/eng/normes/mmanual/A_00137.htm). All sera were checked for antibodies against SIV H1N1 (A/swine/Potsdam/1/81 and A/swine/Bakum/5/95), H3N2 (A/swine/Bakum/909/93), and H1N2 (A/swine/Bakum/1832/00). First, sera were adsorbed overnight with RDE (receptor-destroying enzyme, cholera filtrate from *Vibrio fetus*, SIGMA-ALDRICH Chemie GmbH) in a water bath at 37°C to eliminate nonspecific inhibitors. Then, $150\ \mu\text{l}$ 1.5 % sodium citrate solution (Roth) were added to $50\ \mu\text{l}$ of the pre-treated serum sample, and the mixture was heat inactivated for 30 min at 56°C . Afterwards, the pre-incubated sera were adsorbed on indicator chicken erythrocytes (50 % suspension of erythrocytes) collected from cocks from our own flock. The antigen suspensions of the H1N1, H3N2, H1N2

1 viruses were adjusted to 8 hemagglutination units (HAU) per 25 µl. HI tests were
2 carried out in duplicate in 96-well plates as described by the OIE Diagnostic Manual
3 (http://www.oie.int/eng/normes/mmanual/A_00137.htm). Serum dilutions of 1:10 to
4 1:2,560 were tested. One positive and one negative serum control were carried along.
5 The antibody titres were expressed as reciprocal values of the highest dilution of
6 serum inhibiting hemagglutination completely. Serum samples with no activity at the
7 1:20 dilution were considered negative for antibodies in the HI test.

8

9 **2.4. Virological investigations**

10

11 *2.4.1. Virus isolation in cell culture and embryonated hen's eggs*

12 Isolation of SIV from organ samples (lung) was carried out on confluent MDCK
13 (cell line no. 671) or permanent swine testis cell cultures (cell line no. 606), derived
14 from the Cell Line Collection in Veterinary Medicine, Isle of Riems, or on
15 embryonated hen's eggs (Lohmann Animal Health) after preparation of organ
16 suspensions (10 % w/v) in PBS (phosphate buffered saline). Tissue suspensions were
17 centrifuged (1,500-1,900 g for 15-30 min at 4 °C), and the supernatants were used
18 immediately for virological investigation or were frozen at -70 °C until use. For
19 virus isolation, undiluted and 1:10 dilutions of the suspensions (200 µl) of each organ
20 sample were inoculated into two wells of 24-well cell culture plates containing cell
21 monolayers. Sub-passages (normally five) were carried out into cell culture bottles
22 containing the same cells. The plates/bottles were incubated for 7 days at 37 °C
23 (plates in a CO₂ incubator) and CPE (cytopathic effect) was evaluated daily. At the
24 end of the incubation period, the plates or bottles were frozen at -70 °C. After
25 thawing, cell suspensions were centrifuged (see above) and the supernatants were
26 checked for the presence of virus by hemagglutination test as described in the OIE

1 Diagnostic Manual (http://www.oie.int/eng/normes/mmanual/A_00137.htm). In case
2 of a positive hemagglutination, the HAU of the isolate and, by cross neutralisation
3 with subtype specific SIV antisera, also the virus subtype, were determined.

4 At the beginning of the investigations, virus isolation was carried out in parallel in
5 embryonated hen's eggs (10 to 11 days incubation of eggs before inoculation). This
6 procedure is not described in detail as the virus isolations were mostly performed in
7 cell cultures which showed a sensitivity comparable to that of virus isolation in
8 embryonated hen's eggs (Lange, unpublished). However, allantoic fluid derived from
9 infected embryonated hen's eggs was used for sequencing of SIV isolates.

10

11 2.4.2. *Virological investigations by real-time RT-PCR*

12 For direct detection of SIV RNA from organs of wild boar, RNA was prepared
13 from pieces of lung samples using the RNeasy Mini Kit, a TissueLyser and
14 QIAshredder columns (all from QIAGEN GmbH). A universal internal control HEX-
15 labelled sample (IC) was included in each RNA preparation as described by
16 Hoffmann et al. (2006). Real-time RT-PCR for amplification of a conserved part of
17 the M protein coding gene was performed according to Spackman et al. (2002) with
18 slight modifications on a Mx3000P cyclor (Stratagene).

19

20 2.4.3. *Sequencing and phylogenetic analysis*

21 SIV RNA derived from wild boar WS169/06 and WS188/06 was prepared from
22 infected allantoic fluid using the High Pure Viral RNA Kit (ROCHE Diagnostics
23 GmbH) according to the manufacturer's instructions. The complete HA, NA, NP, M
24 and NS encoding gene regions were reverse transcribed and amplified in an
25 Eppendorf Mastercycler personal (Eppendorf AG) using primers as described by
26 Hoffmann et al. (2001) and the SuperScript III One-Step RT-PCR Kit (Invitrogen).

1 Primers were adjusted to a concentration of 50 pmol/μl. The program runs at 50 °C
2 for 30 min, at 94 °C for 2 min, 40x (at 94 °C for 30 sec, at 58 °C for 30 sec, at 60 °C
3 for 3 min) and at 58 °C for 5 min, at 8 °C, hold. RT-PCR products were eluted from
4 agarose gels and purified with the QIAquick Gel Extraction Kit (QIAGEN GmbH).
5 Full-length fragments of the two surface proteins as well as of the three internal
6 proteins were cloned into the pGEM-T Easy Vector by means of the pGEM-T Easy
7 Vector System (Promega Corp.). DNA was sequenced using the Prism BigDye
8 Terminator v1.1 Cycle Sequencing Kit on the DNA sequencer “3130 Genetic
9 Analyzer” (both Applied Biosystems). From each full-length RT-PCR product at
10 least 5 plasmid DNAs were selected and sequenced with vector-specific primers
11 from both sites. Sequence data were assembled and edited with GCG, Version 11.1
12 (Accelrys Inc.). Consensus sequences of the amplified, cloned and sequenced
13 fragments were generated. They were aligned with sequences of standard strains
14 contained in the GenBank (NCBI / Blast: <http://www.ncbi.nlm.nih.gov/BLAST/>).
15

16 *2.5. Statistical analysis*

17 The statistical analysis was carried out with the statistics program SigmaStat 3.0
18 (SPSS Science Software gmb, Erkrath, Germany). The results were considered
19 statistically significant when $p < 0.05$.
20

21 **3. Results**

23 *3.1. Serological survey*

24 By examination of 1,125 sera derived from wild boar shot from 2001 to 2006,
25 antibodies against SIV subtypes H1N1 and H3N2 could be detected in animals from
26 Mecklenburg-Western Pomerania, Brandenburg, Saxony-Anhalt, and Baden-

1 Württemberg as well as Rhineland-Palatinate (Table 1). No wild boar was diagnosed
2 seropositive for SIV H1N2. In addition, the investigated wild boar bagged in
3 Mecklenburg-Western Pomerania in 1997/98 were free from antibodies against SIV
4 H1N1 and H3N2. The highest percentage of positive sera was detected in wild boar
5 shot in Baden-Württemberg with 25.9 % followed by Rhineland-Palatinate (8.3 %)
6 and Saxony-Anhalt (8.3 %), Mecklenburg-Western Pomerania (2005-06: 3.1 %), as
7 well as Brandenburg with 2.5 % antibody-positive wild boar. Statistically significant
8 differences in the seroprevalence rates in wild boar were only found in Mecklenburg-
9 Western Pomerania with regard to both investigation periods as well as between the
10 seroprevalences established in Baden-Württemberg in relation to other regions.

11 The proportion of animals with antibodies against the H1N1 antigen (3.9 %) was
12 higher than that of animals with antibodies against the H3N2 virus (1.5 %). Three out
13 of 1,245 animals (0.2 %) had antibodies against both, SIV subtypes H1N1 and
14 H3N2. These three wild boar were diagnosed in Mecklenburg-Western Pomerania
15 (Table 1). With regard to seroprevalences against SIV in different age classes (Table
16 2) differences were observed. In general, the highest seroprevalence rate was found
17 in animals older than two years. The mean antibody titres were calculated against the
18 H1N1 SIV $1:65 \pm 64$ and against the H3N2 virus $1:221 \pm 283$.

19

20 3.2. *Virological findings*

21 Altogether, SIV was detected in two of 242 investigated wild boar (prevalence of
22 0.8 %) by virus isolation in embryonated hen's eggs. Both isolates were derived from
23 young wild boar (body weight after dressing: <25 kg) bagged in the district of
24 Nordvorpommern, Mecklenburg-Western Pomerania. One hundred and ten lung
25 samples from young animals were investigated by means of M-gene targeted real-
26 time RT-PCR. Except for a small number of suspect results (threshold cycle value

beyond 35) which, however, could not be confirmed by repeated testing, no positive results were achieved. However, more than half of the samples were also negative for HEX-labelled IC. This indicates that inhibitory factors contained in the investigated materials disturbed RNA preparation or the subsequent enzymatic reactions.

The analysis of the consensus sequences of the fragments derived from the HA, NA, NP, M and NS genes of both SIV isolates (WS169/06 and WS188/06) revealed that the sequences of the homologous gene fragments of both isolates were identical (data not shown). Therefore, only the sequences of wild boar WS169/06 were registered in the GenBank (Acc. Nos.: AM746616 - H3, AM746617 - N2, AM746618 - M, AM746619 - NP, AM746620 - NS). Table 3 summarises the relationship of the analysed genes with sequences of SIV reference strains from the GenBank.

4. Discussion

Wild boar may be a reservoir for many pathogens including SIV (Markowska-Daniel and Pejsak, 1999; Dedek et al., 1990; Vincente et al., 2002) and therefore are important not only for infections within wild boar but also for domestic pigs. From this point of view it is necessary to know the epidemiological situation regarding SIV in wild boar. Based on the serological survey in the Bundeslaender presented here, it can be considered that SIV is also present in our wild boar populations, even though on a relatively low level.

Regarding all sera investigated ($n=1,245$), the percentage of animals with antibodies against the H1N1 virus strains was higher (3.9 %) than that of animals with antibodies against the H3N2 virus (1.5 %). This result includes 120 sera from Mecklenburg-Western Pomerania which were sampled in 1997/98 and were all

1 diagnosed negative. Therefore, the seroprevalence within the investigation period
2 2001 to 2006 ($n = 1,125$) should provide a better overview of the recent
3 epidemiological situation. In this period, 5.8 % of the wild boar sera were diagnosed
4 serologically positive against SIV, 4.4 % against H1N1 antigens, 1.7 % against
5 H3N2 antigen. The highest rate of seropositivity against H3N2 virus was detected in
6 Baden-Württemberg (3.7 %, 3 out of 81 animals), followed by Mecklenburg-Western
7 Pomerania in 2005-2006 with 2.2 % (15 out of 684 wild boar) and Rhineland-
8 Palatinate with 1.7 % (1 out of 120 animals). In Brandenburg and Saxony-Anhalt, no
9 wild boar was tested antibody-positive against the H3N2 antigen. Antibodies against
10 subtype H1N1, however, could be detected in all five Bundesländer where wild
11 boar were investigated. In contrast, no antibodies were found against the H1N2 virus.
12 As the HA from H1N1 and H1N2 viruses are of different origin in Europe (Marozin
13 et al., 2002; Al Faress et al., 2005), nor or only slight cross-reactivity might have
14 been expected.

15 Seroprevalence rates were different in the individual wild boar populations and
16 ranged between 25.9 % in Baden-Württemberg and 2.5 % in Brandenburg. No reason
17 could be found for the high seroprevalence rate in wild boar in Baden-Württemberg.
18 It is possible that the small number of random samples collected from a small area
19 does not reflect the true situation in this federal state. Compared to the
20 seroprevalence rate against H1N1 antigens (22.3 % seropositive animals), the
21 proportion of wild boar with antibodies against the H3N2 antigen was relatively low
22 (only 3.7 %) in Baden-Württemberg. Interestingly, more animals were diagnosed
23 positive for the H1N1 virus A/swine/Bakum/5/95 (16 %) in comparison to 6.2 % of
24 wild boar detected seropositive for the H1N1 virus A/swine/Potsdam/1/81 in this
25 particular region. This suggests that an infection with a Bakum/5/95-like virus was
26 predominant in the investigated population of Baden-Württemberg. In contrast, the

1 proportion of wild boar carrying antibodies against the SIV A/swine/Potsdam/1/81
2 (H1N1) was predominant in all other regions investigated. Our serological results in
3 Baden-Württemberg differ from previous investigations carried out during the main
4 hunting season in 2003/04 (Polley et al., 2007). In the study of Polley et al. (2007),
5 only 3.4 % of the wild boar had antibodies against the subtype H1N1 whereas 13.4 %
6 showed a positive reaction with the subtype H3N2 and 9.9 % reacted with the
7 subtype H1N2. As our investigations in Baden-Württemberg in 2001/2002 did not
8 reveal animals with H1N2 specific antibodies, an infection of the population with
9 this virus subtype probably took place in this region during the last years.

10 In Mecklenburg-Western Pomerania (district of Nordvorpommern), no
11 seropositive animals were diagnosed during the hunting season 1997/98 ($n = 120$),
12 whereas a seroprevalence of 4.6 % (11 seropositive animals out of 242 wild boar
13 investigated) was detected in this district in 2005/06. This proportion of antibody-
14 positive wild boar is generally similar to that established by Dedek et al. (1990) in
15 the northern part of Eastern Germany. These authors diagnosed approximately 4.3 %
16 seropositive wild boar. The seroprevalence was slightly lower (2.3 % antibody-
17 carrying animals) in other regions of Mecklenburg-Western Pomerania in 2006.

18 In principle, SIV seroprevalences in wild boar in Germany agree with those
19 detected in wild boar populations of other European countries (Markowska-Daniel
20 and Pejsak, 1999; Vincente et al., 2002; Vengust et al., 2006). Saliki et al. (1998)
21 reported higher seroprevalence rates (11 %) in wild boar investigated in Oklahoma
22 (USA).

23 Our study established that 4.2 % of young wild boar ≤ 1 year old, 5.5 % of wild
24 boar in the second year and 14.1 % of adults (> 2 years old) were diagnosed
25 serologically positive. However, there were marked differences between the
26 individual wild boar populations investigated (see Table 2).

1 The mean HI antibody titre against the virus A/swine/Bakum/909/93 (subtype
2 H3N2) was higher ($1:221 \pm 283$) than against the H1N1 viruses
3 A/swine/Potsdam/1/81 and A/swine/Bakum/5/95 ($1:65 \pm 64$). The highest mean
4 antibody titres against the SIV subtype H3N2 ($1:270 \pm 301$) were detected in wild
5 boar shot in Mecklenburg-Western Pomerania in 2005-06. If these data in connection
6 with the detection of SIV H3N2 represent a current infection in this region or are
7 based on the higher immunogenicity of H3N2 than H1N1 virus cannot be estimated
8 exactly at this moment.

9 In the 1980s, H1N1 SIV outbreaks were reported in pig farms in Germany (Ottis
10 et al., 1981; Witte et al., 1981; Sinnecker et al., 1983; Kretzschmar et al., 1985;
11 Lange et al., 1985) and also in other European countries (summarised by Easterday
12 and Van Reeth, 1999; Van Reeth, 2007). H1N1 viruses isolated during this epidemic
13 were more closely related to avian H1, and virus transmission from ducks to pigs was
14 assumed (Pensaert et al., 1981; Van Reeth, 2007). Therefore, it was of strong interest
15 to know whether reassortants with avian genes exist in a local wild boar population
16 in the northern part of Germany, district of Nordvorpommern, where HPAIV
17 H5N1/ASIA infections in wild birds have been diagnosed. Two out of 242 wild boar
18 were diagnosed SIV positive during the hunting season in 2005/06. These
19 virologically positive animals were young ones (<25 kg body weight after dressing).
20 Both SIV isolates were characterised as H3N2 viruses by means of cross
21 neutralisation as well as by sequencing. Based on sequencing of five genes and their
22 comparison with sequences of SIV in the GenBank, we came to the conclusion that
23 the viruses isolated in Mecklenburg-Western Pomerania were reassortants of H3 and
24 H1 swine viruses occurring in Europe since the 1990s.

25 Additionally, our study revealed that all serum samples of wild boar shot in the
26 area where infections of wild birds with the HPAIV H5N1/ASIA occurred were

1 diagnosed free from hemagglutinating antibodies against this virus (data not shown).
2 Generally, we conclude that infections of wild boar with this virus should be
3 possible, especially since infections of pigs with low-pathogenic avian influenza H4,
4 H5 and H9 viruses were detected by serological investigations (Ninomiya et al.,
5 2002). Furthermore, Choi et al. (2005) tested 8 out of 3,175 sera (0.25 %) from
6 Vietnamese pigs positive for antibodies against H5N1 influenza viruses using VNT
7 and Western blot analysis. This indicates that infections of wild boar with the
8 HPAIV H5N1/ASIA might also be possible. However, the resistance of miniature
9 pigs against an HPAIV H5N1 strain isolated in Japan (Isoda et al., 2006) and the
10 negative serosurvey conducted in Korea (Jung et al., 2007) as well as further studies
11 revealed strong evidence that avian influenza viruses replicate much less efficiently
12 in *Suidae* than the typical swine influenza viruses (van Reeth, 2007). Thus, additional
13 factors may be needed for the establishment of avian influenza viruses in pigs (Kida
14 et al., 1994). Therefore, investigations of wild boar living in regions where the
15 HPAIV H5N1/ASIA was diagnosed in wild birds should be continued.

16 In conclusion, our findings suggest that SIV subtypes H1N1 and H3N2 are
17 circulating in our wild boar populations with a low prevalence. However, it seems
18 that the subtype H1N1 presently is more predominant in wild boar than the SIV
19 subtype H3N2. The virological investigations carried out in a small area in Northern
20 Germany underline the presence of SIV H3N2. Altogether, the recent SIV
21 seroprevalence rates in wild boar populations in Germany as well as in other
22 European countries indicate that wild boar infected with SIV should neither be the
23 basis for an epidemic in wild boar nor a dangerous virus reservoir for domestic pigs.

24

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26

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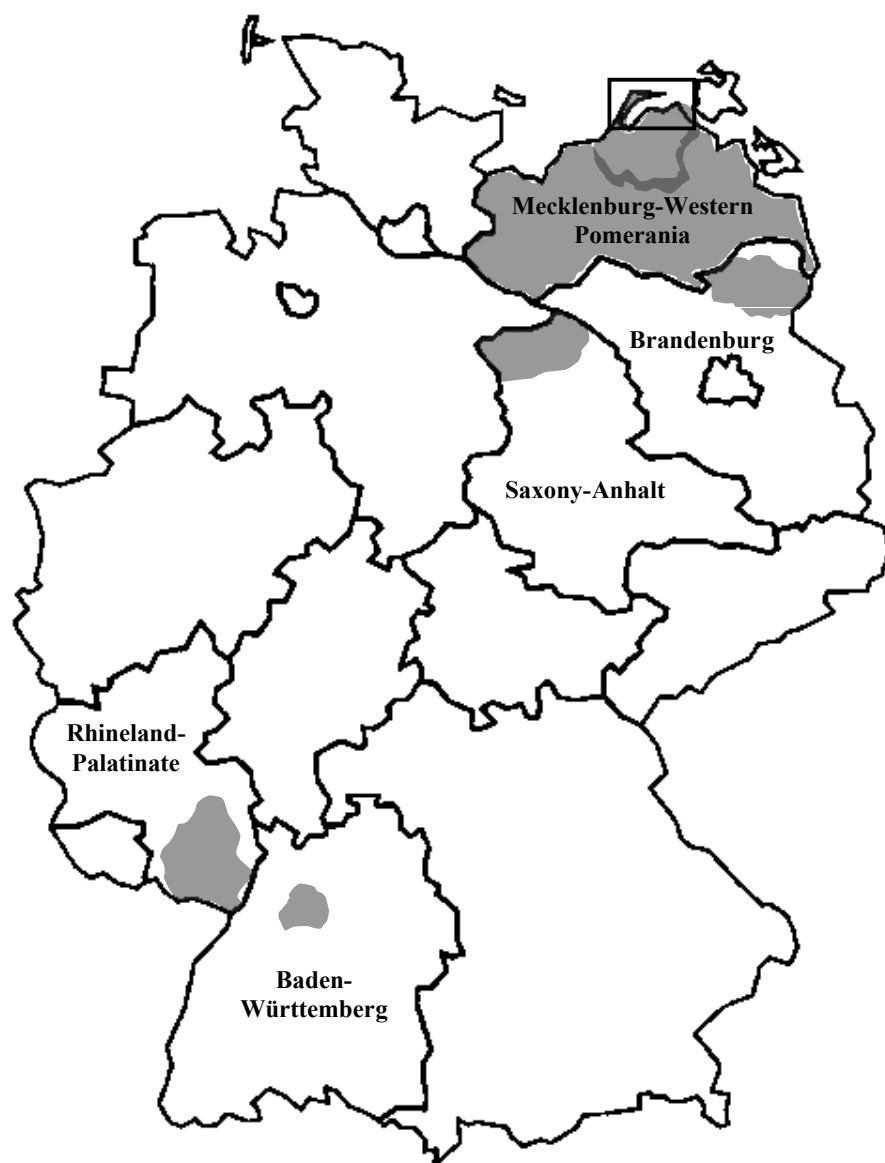
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- 15

1 Figure 1. Regions in Germany where samples were collected

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5 Grey areas - sampling areas vs areas with oral vaccination of wild boar against CSF


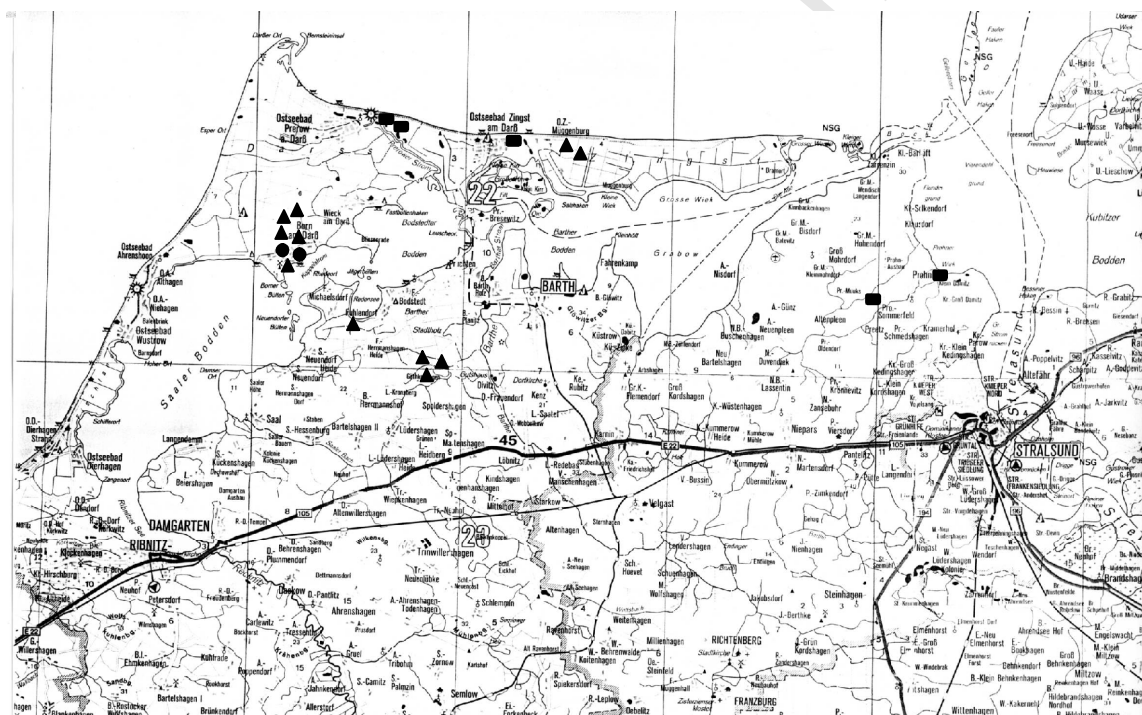
6  Excerpt from the district Nordvorpommern (see Fig. 2)

Figure 2.

Locations of serologically and virologically positive wild boar in the district Nordvorpommern (Mecklenburg-Western Pomerania)
including places of finding of H5N1/ASIA positive wild birds



- ▲ Wild boar with antibodies against SIV
- H3N2 positive wild boar
- H5N1/ASIA positive wild birds

Table 1.

Number of serologically positive wild boar in the investigated regions

Bundesland	Number of investigated animals	Number of antibody-positive animals per SIV subtype				
		H1N1			H1N2	H3N2
		/Potsdam/1/81	/Bakum/5/95	Σ		
Mecklenburg-Western Pomerania (1997/98)	120	0	0	0	0	0
Mecklenburg-Western Pomerania (2006)	442	6 ^a	0	6	0	5 ^a
Mecklenburg-Western Pomerania / Nordvorpommern (2005/06)	242	3 ^b	0	3	0	10 ^b
Brandenburg	120	1	2	3	0	0
Saxony-Anhalt	120	8	2	10	0	0
Baden-Württemberg	81	7	11	18	0	3
Rhineland-Palatinate	120	5	4	9	0	1

^aone animal with antibodies against both antigens, H1N1 and H3N2^btwo animal with antibodies against both antigens, H1N1 and H3N2

Table 2.

Age-dependent compositions of SIV antibody-positive wild boar

Bundesland (federal state)	Number of positive animals				Unknown age
	In total	Young wild boar	Sub-adults	Adults	
Mecklenburg-Western Pomerania 2005-2006	21/684 ^a	12/329	6/288	3/26	0/41
1997/98	0/120	0/48	0/53	0/15	0/4
Brandenburg	3/120	1/47	2/54	0/7	0/12
Saxony-Anhalt	10/120	0/7	8/91	1/6	1/16
Baden-Württemberg	21/81	7/40	8/24	6/11	0/6
Rhineland-Palatinate	10/120	2/52	7/58	0/6	1/4
In all	65/1,245	22/523	31/568	10/71	2/83
	(5.2 %)	(4.2 %)	(5.5 %)	(14.1 %)	(2.4 %)

^anumber of positive animals / number of animals per group

Table 3.

Most closely relatives to A/wild boar/Germany/WS169/06 determined by NCBI/BLAST/blastn

2.2.17 (www.ncbi.nlm.nih.gov/blast/Blast.cgi)

Segment	GenBank Acc.No.	Isolate	Subtype	Homology (%)
NS	Z26865	A/swine/Germany/8533/91	H1N1	98,26
	Z46440	A/swine/ Schleswig-Holstein/1/93	H1N1	97,92
	AJ344034	A/swine/Scotland/410440/94	H1N2	97,80
M	Z26861	A/swine/Germany/8533/91	H1N1	99,31
	DQ186981	A/swine/Leipzig/145/92	H3N2	99,21
	Z26859	A/turkey/Germany/3/91	H1N1	98,92
NP	Z26856	A/swine/Germany/8533/91	H1N1	98,07
	L40332	A/swine/England/195852/1992	H1N1	98,07
	M63772	A/swine/Italy/839/1989	H1N1	97,96
NA	EF409255	A/swine/Bakum/909/93	H3N2	99,72
	EF409257	A/swine/Lohne/1/97	H3N2	98,48
	AJ311455	A/swine/Belgium/220/92	H3N2	98,34
HA	AJ252130	A/swine/Bakum/909/93	H3N2	99,88
	EF409249	A/swine/Lohne/1/97	H3N2	98,68
	EF409248	A/swine/Jena/5/96	H3N2	97,14