# Surveillance of wild boar in Switzerland: prevalence of infections relevant to domestic pigs

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# Contents

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# Summary

Increasing wild boar population sizes throughout Europe gave rise to the concern that infectious diseases with relevance to domestic pigs will persist in wild boar over prolonged periods of time, and hence pose a threat to pig farming industries. The major concern is classical swine fever (CSF), a highly contagious viral disease due to which more than 11 million pigs were destroyed during the epidemic in domestic pigs in the Netherlands in 1997. Aujeszky's disease (pseudo-rabies, AD) is caused by an alpha-herpesvirus which induces severe economic loss due to a high lethality in young piglets and stillbirth. Porcine brucellosis, a bacterial infection causing abortion and birth of dead or weak young, was documented in wild boar throughout central Europe and has re-emerged in outdoor-reared pigs as a result of spillover from wild boar in France.

In Switzerland, wild boar populations increased since the 1970s. Concurrently, keeping cattle and swine in housings with open front became increasingly popular and is supported by the government since 1993. Therefore, we initiated a monitoring and surveillance system for contagious diseases in wild boar with relevance to domestic pigs, the aim of which was to provide a basis for (i) an early warning system, (ii) documenting the disease status for important pig diseases in Switzerland and (iii) assessing the success of interventions targeted at limiting disease outbreaks after disease introduction. Because of their economic or political importance we focused on CSF, AD and porcine brucellosis.

As a pre-requisite for a national monitoring and surveillance system in wild boar, we subdivided Switzerland into sampling units. A sampling unit was defined as a geographic area within which an outbreak of a contagious disease in wild boar would remain confined with a probability of 95% between two consecutive sampling rounds. In order to define such sampling units, we mapped the probability of wild boar occurrence, based on a regression analysis of the estimated wild boar population size on various geographic characteristics. We identified 2 sampling units: north and south of the Alps. Nevertheless, it was considered unlikely that a contagious disease would spread within the entire area north of the Alps within one year.

In order to explore the consequences of introducing one infectious pack into a population of susceptibles, we developed a spatially explicit transmission model, based on the map of the probability of wild boar occurrence. The model allowed to simulate different scenarios, whereby the wild boar poplation density, the home range size and the probability of transmission could be varied. The magnitude of the simulated outbreak was measured in terms of the number of infected packs, the duration of the outbreak (months) and the maximal distance of disease spread

within one year. The landscape induced considerable variability in magnitude of the simulated outbreaks. However, the latter was mainly determined by the wild boar's home-range size and the probability of transmission. The transmission model could contribute to a risk based surveillance system. In such a system, the model's predictions and additional data on the risk of spillover from wild boar to domestic pigs could be included into the decision of where to collect how may samples.

We successfully initiated a monitoring and surveillance program for contagious diseases in wild boar in Switzerland. Based on two survey rounds (November 2001 – February 2002; November 2002 – February 2003), we found no evidence for the occurrence of CSF, nor for the occurrence of AD. We did, however, confirm the occurrence of *Brucella suis* by both serology and bacterial isolation. The infection with *B. suis* was due to *B. suis biovar 2*, which prevails in Europe and was isolated from a wild boar near Geneva in 2001. This biovar is considered to be harmless in humans. We argued that the awareness regarding porcine brucellosis must be increased among farmers and veterinarians, since preventive measures, such as fences which protect outdoor-reared domestic pigs from the contact with potentially infected wild boar, may significantly reduce the risk of spillover to domestic pigs.

In order to provide a framework for analyzing the relationship between the prevalence of *B. suis* in wild boar and the number of wild boar killed by hunters per year, we developed a dynamic transmission model. Based on the limited data available, the model predicted that the current brucellosis prevalence of 14% in wild boar in Switzerland north of the Alps would decline to zero within 5 years, when a hunting rate of 50% of the total population would be achieved in the entire region. But the model's predictions need to be validated by additional data.

In addition to the diseases included in the development of a national monitoring and surveillance program, the presence of bovine tuberculosis was assessed in wild boar in cantons Ticino (southern Switzerland). This region was selected because it was closest to northern Italy, where *Mycobacterium bovis* was isolated from wild boar. We found no evidence for an infection with *M. bovis* in Ticino.

In conclusion, we did not recommend to formally prove the absence of CSF on an annual basis. Due to its high mortality, it is likely that hunters and veterinarians will discover the disease before it will be noticed by the monitoring system. It is thus of prime importance to maintain the currently high level regarding CSF in wild boar among hunters and veterinarians. Since AD is absent from both domestic pigs in Switzerland and wild boar in regions adjacent to Switzerland and due to the lack of evidence for the spillover from wild boar to domestic pigs, we did not recommend to formally prove the absence of AD in wild boar at an annual basis. For the same reasons, we did not recommend the further investigation of tuberculosis in wild boar in Switzerland. In contrast, we recommended the further monitoring of brucellosis in wild boar in Switzerland: (i) in order to determine whether the disease is spreading and (ii) in order to provide additional data on the course of the *B. suis* prevalence, which are needed to validate the transmission model. Finally, more reliable estimates regarding the wild boar population size, as well as data on wild boar demographics and migration are required to validate transmission models. Such data can be gained from a large-scale capture-recapture study, including the radio-tracking of individual wild boar.

# Zusammenfassung

In ganz Europa wächst die Wildschweinpopulation. Dadurch könnten infekziöse Krankheiten länger unter den Wildschweinen zirkulieren. Gleichzeitig steigt die Wahrscheinlichkeit des Kontaktes zwischen Wild- und Hausschwein und damit das Risiko einer Krankheitsübertragung. Von gösster Bedeutung ist dabei die klassische Schweinepest (KSP), eine hochansteckende Viruserkrankung. Aufgrund von KSP wurden während der Epidemie bei Hausschweinen in den Niederlanden 1997 über 11 Millionen Schweine gekeult. Die Aujesky'sche Krankheit (Pseudowut, AK) wird von einem alpha-herpesvirus verursacht und führt zu grossen wirtschaftlichen Einbussen aufgrund von Totgeburten und hoher Ferkelsterblichkeit. Porzine Brucellose, eine bakterielle Erkrankung, die Aborte und die Geburt lebensschwacher Ferkel bewirkt, ist beim Wildschwein in Europa weit verbreitet. Die Ansteckung von freilebenden Hausschweinen über den Kontakt mit infizierten Wildschweinen kam in Frankreich vor.

In der Schweiz nimmt die Wildschweinpopulationsgrösse seit den 1970er Jahren zu. Gleichzeitig erfreut sich die Offenstallhaltung von Hausschweinen wachsender Beliebtheit — in der Schweiz wird sie seit 1993 staatlich gefördert. Deshalb initierten wir ein Überwachungssystem für Krankheiten beim Wildschwein, die auch für Hausschweine bedeutend sind. Ziel dieses Überwachungssystems war die Schaffung von Grundlagen für (i) ein Frühwarnsystem, (ii) die Dokumentation des Krankheitsstatus betreffend wirtschaftlich bedeutender Schweinekrankheiten, sowie (iii) die Überprüfung ergriffener Massnahmen zur Krankheitseindämmung. Aufgrund ihrer wirtschaftlichen oder politischen Bedeutung konzentrierten wir uns auf KSP, AK und Schweinebrucellose.

Für ein nationales Krankheits überwachungsstem beim Wildschwein wurde die Schweiz in Probensammelgebiete eingeteilt. Um solche Probensammelgebiete zu definieren, stellten wir die Wahrscheinlichkeit für das Vorkommen von Wildschweinen in der Schweiz auf einer Karte dar. Die Wahrscheinlichkeit errechneten wir aufgrund des Zusammenhangs zwischen der geschätzten Wildschweinpopulationsgrösse und verschiedenen Landschaftseigenschaften (Regressionsanalyse). Aufgrund der Karte identifizierten wir 2 Probensammelgebiete: nördlich und südlich der Alpen. Wir erachteten es jedoch als unwahrscheinlich, dass sich eine ansteckende Wildschweinekrankheit innerhalb 2 aufeinander folgender Probensammelperioden (z.B. 1 Jahr) über das ganze Gebiet nördlich der Alpen ausbreiten würde.

Um die Konsequenzen der Einschleppung einer infizierten Wildschweinrotte in eine Population empfänglicher Rotten zu ergünden, entwickelten wir ein räumliches Ausbreitungsmodell. Dieses Modell berechnete verschiedene Szenarien von Krankheit-

sausbrüchen anhand von Populationsdichte und Ausbreitungswahrscheinlichkeit. Diese Szenarien konnten anhand der Anzahl infizierter Rotten, der Infektionszeit, sowie der maximalen Krankheitsausbreitungsdistanz innerhalb eines Jahres vergleichen werden. Unterschiedliche Populationsdichten, die aufgrund der Wahrscheinlichkeit des Wildschweinvorkommens (Karte) zustande kamen, bewirkten Variabilitäten in der Reichweite des simulierten Krankeitsausbruchs. Die Reich/-weite des simulierten Krankheitsausbruchs wurde hauptsächlich durch die Grösse des Wildschwein-Heimgebietes (home range) und die Ansteckungswahrscheinlichkeit bestimmt. Das Ausbreitungsmodell könnte Teil eines Risiko-basiertes Überwachungssystems bilden. Dabei könnten die Vorhersagen des Modells sowie zusätzliche Daten über das Risiko der Krankheitsübertragung zwischen Wild- und Hausschwein in die Planung der Probensammlung einbezogen werden.

Das nationale Krankheitsüberwachungsstem beim Wilschwein wurde mit Erfolg eingeführt. Aufgrund zweier Probensammelperioden (November 2001 - Februar 2002; Noevmber 2002 - Februar 2003) fanden wir keine Hinweise auf das Vorkommen der KSP oder der AK. Hingegen bestätigten wir das Vorkommen der Schweinebrucellose mittels Antikörpernachweis sowie mittels Isolierung der Brucella-Bakterien. Die isolierten Brucella-Bakterien waren *B. suis biovar 2*, dem in Mitteleuropa vorkommenden Biovar, der bereits 2001 aus einem Wildschwein in der Region Genf isoliert wurde. Für den Menschen ist die Infektion mit *B. suis biovar 2* harmlos. Um die Hausschweine vor einer Ansteckung durch Wildschweine zu schützen ist es wichtig, bei Schweinehaltern und Tierärzten das Wissen um diese Krankheit zu erweitern. Vorbeugende Massnahmen — wie das errichten von Zäunen, die den Kontakt zwischen freilebenden Hausschweinen zu Wildschweinen verhindern — können das Risiko einer Krankheitsübertragung entscheidend verringern.

Als Instrument um den Zusammenhang zwischen der Brucelloseprävalenz im Wildschwein und der Abschussrate zu ergründen, entwickelten wir ein dynamisches Transmissionsmodell. Das Modell sagte vorher, dass die gefundene Burcelloseprävalenz im Wildwschein in der Schweiz nördlich der Alpen innerhalb der nächsten 5 Jahre auf null reduziert werden könnte, wenn die Abschussrate 50% der Gesamtpopulation betragen würde. Jedoch muss das Modell anhand zusätzlicher Daten validert werden.

Zusätzlich zu KSP, AD und Schweinebrucellose untersuchten wir, ob die Rindertuberkulose beim Wildschwein im Kanton Tessin vorkommt. Diese Region wurde ausgewählt, weil sie an Norditalien angrenzt, wo die *M. bovis* beim Wildschwein gefunden wurde. Wir fanden keinen Hinweis auf das Vorkommen der Rindertuberkulose beim Wildschwein im Kanton Tessin.

Aufgrund der vorliegenden Studie erachten wir es als unnötig, die Abwesenheit von KSP jährlich mittels einer Stichprobenuntersuchung zu dokumentieren. Da die meisten KSP-Viursstämme beim Wildschwein eine hohe Mortalität verursachen erwarten wir, dass das Auftreten der Krankheit von Jägern und Tierärzten bemerkt wird, bevor dies durch das Überwachungssystem geschehen würde. Es ist daher von grösster Wichtigkeit, das derzeit gut etablierte Wissen über KSP bei Jägern und Tierärzten zu erhalten und zu erweitern (z.B. das Auftreten weniger virulenter KSP-Virusstämme in jüngster Zeit). Da die AK zur Zeit weder beim Hausschwein in der Schweiz, noch beim Wildschwein in einer an die Schweiz angrenzenden Region vorkommt, sowie die Übertragung des AK-Virus zwischen Wild- und Hausschwein

bisher nicht dokumentiert ist, erachten wir es ebenfalls als unnötig, die Abwesenheit der AK beim Wildschwein in der Schweiz mittels einer jährlichen Stichprobenuntersuchung zu dokumentieren. Aus den gleichen Gründen empfehlen wir keine weitere Untersuchung der Rindertuberkulose beim Wildschwein im Tessin. Hingegen empfehlen wir, die Untersuchung der Schweinebrucellose beim Wildschwein fortzusetzen, um (i) zu überprüfen ob sich die Krankheit ausbreitet und (ii) um zusätzliche Daten zur Validierung von Ausbreitungsmodellen zu generieren. Zusätzlich werden zur Validierung solcher Modelle genauere Schätzungen der Wildschweinpopulationsgrösse, sowie der Wildschweindemographie und Migration benötigt. Solche Daten könnten mittels einer nationalen Capture-Recapture Studie, und der Markierung von Wildschweinen mit Radiosendern gewonnen werden.

# List of Figures

# List of Tables

# Abbreviations List

| | |
|---|---|
| **AD** | Aujesky's disease (Pseudorabies) |
| **CSF** | classical swine fever |
| **OIE** | Office Internationale des Epizooties |
| **WTO** | World Trade Organization |

Cantons (administrative regions)

| | |
|---|---|
| **AG** | Aargau |
| **BL** | Baselland |
| **GE** | Geneva |
| **JU** | Jura |
| **SH** | Schaffhausen |
| **SO** | Solothurn |
| **TG** | Thurgau |
| **TI** | Ticino |
| **VD** | Waadt |
| **ZH** | Zürich |

Chapter 1

# Introduction

## 1.1 Transmission of infectious diseases between livestock and wildlife

Several diseases are transmissible between wildlife and domestic animals. On the one hand, wildlife can form a reservoir of infection for domestic animals — well known examples are badgers (*Meles meles*) and brushtail possums (*Trichosurus vulpecula*) representing a major source of infection for tuberculosis (*M. bovis*) in cattle in the UK (Phillips et al., 2003) and New Zealand (Coleman and Cooke, 2001; Corner et al., 1981; McInerney et al., 1995), respectively. On the other hand, wildlife species may be threatened by spillover of diseases from domestic animal. For instance, small and isolated African wild dog populations were driven to extinction by canine distemper, a common viral infection of domestic dogs, as a result of spillover (Ginsberg et al., 1995). Although less dramatic, (Giacometti et al., 2000) suggested that infectious keratoconjunctivitis in chamois (*Rupicapra rupicapra*) and ibex (*Capra ibex*) in the European Alps originated in places where infected sheep lived in close proximity during summer.

Increasing wild boar population sizes throughout Europe gave rise to the concern that the rate of disease-transmission between wild boar and domestic pig will increase. This in turn, is expected to allow diseases such as classical swine fever to persist in wild boar over prolonged periods (Kern et al., 1999) and hence pose a threat to pig farming industries. Wild boar populations increased throughout Europe since the 1970s. In Switzerland, the annual hunting bag increased from 60 to 6327 wild boar between 1970 and 2002 (Anonymous, 2002a). Similar trends were observed in Germany (Anonymous, 1999b; Briedermann and Rethwisch, 1992) and France (Artois et al., 2002). The increase in wild boar population density is attributable to the relatively frequent beech and acorn mast and mild winters in recent years, intensified agriculture and, partly, to additional feeding by hunters (Anonymous, 2004). At the same time, keeping cattle and swine in housings with open front became increasingly popular and is supported by the Swiss government since 1993 (Anonymous, 2002b). Therefore, we initiated a monitoring and surveillance system for contagious diseases in wild boar with relevance to domestic pigs, the aim of which was to provide the basis for (i) an early warning system, indicating the emergence of risk areas for pig

farming (ii) documenting the disease status of important pig diseases in Switzerland and (iii) assessing the success of interventions targeted at limiting disease outbreaks after disease introduction. Because of their economic or political importance we focused on classical swine fever, Aujeszky's disease and porcine brucellosis. In addition, we assessed whether tuberculosis was present in wild boar in Ticino (southern Switzerland). This region was selected because it is closest to northern Italy, where bovine tuberculosis was found in wild boar (Serraino et al., 1999; Bollo et al., 2000).

## 1.2 Diseases relevant to domestic pigs and wild boar

### 1.2.1 Classical Swine fever (CSF)

Classical swine fever (CSF) is one of the most important diseases in domestic pigs due to it's ethical dimension (large-scale culling) and economic losses (Artois et al., 2002). It can occur worldwide in countries with pig industry. After implementation of effective control measures, several countries, including Australia, New Zealand, Canada, USA, Switzerland and many member states of the EU have eradicated the disease from their domestic pig population. However, due to the complex international trade relations, the diseases can be transmitted over large distances and also be introduced into countries which were CSF-free for long time periods. During the CSF epidemic in domestic pigs in the Netherlands in 1997, more than 11 million pigs had to be destroyed and the economic loss was estimated to be US$2.3 billion (Meuvissen et al., 1999). Large-scale culling of pigs due to CSF was also conducted in other European countries between 1991 and 2001 (Austria, Belgium, Czech Republic, Germany, Italy and Spain, (Edwards et al., 2000)). The last case of CSF in domestic pig in Switzerland was recorded in 1993. Nevertheless, there was an outbreak of CSF in wild boar in the southern region of Switzerland (Ticino) in 1999, which was most likely caused by infected wild boar immigrating from Italy (Hofmann et al., 1999; Schnyder et al., 2002).

#### Causative agent and signs of disease

The causative agent of CSF is an enveloped, single stranded RNA virus of positive polarity which only affects Suidae. It is closely related to the bovine viral diarrhea virus and the border disease virus of sheep, all of them belonging to the genus Pestivirus, which in turn, belongs to the family Flaviviridae (Thiel et al., 1996). Domestic pigs and wild boar were shown to be equally susceptible to CSF (Brugh et al., 1964). The clinical symptoms are similar in domestic pigs and wild boar (Depner et al., 1995; Hofmann et al., 1999). The course of disease can be acute, subacute or chronic. The acute form is characterized by febrile disease with leucopenia, diarrhea, petechial hemorrhages, cyanosis of the skin, neurological symptoms (staggering and posterior paresis) and a high mortality rate in age group <1 year (90%, 5-10 days after disease onset). The subacute and chronic forms are characterized by similar symptoms, although milder than in the acute form. CSF-virus isolated during outbreaks in the 1990s were found to be of moderate virulence, causing a delay in the onset of disease, which in turn, complicated diagnosis. Further, the age and the im-

mune status of the infected pig was found to contribute significantly to the clinical course of disease (Floegel-Niesmann et al., 2003).

Infected sows (when infected late in pregnancy) can give birth to persistently infected piglets, which, although they do not show symptoms of disease, die after several months (Depner et al., 1995). Persistently infected piglets, in conjunction with a high population density, were shown to enable the maintenance of CSV-virus transmission in wild boar (Kern et al., 1999). In wild boar, reduced flight behavior and loss of natural shyness (e.g. entering farm buildings) were observed as additional symptoms (references in (Hofmann et al., 1999)).

#### Transmission and occurrence in wildlife

The CSF-virus is transmitted by ingestion of infected blood, tissue, saliva, tears, nasal mucus, feces and urine. The virus remains infectious in meat products for several months (Savi et al., 1965; McKercher et al., 1987) but appears to be inactivated within a few days in faeces and urine (Kaden, 1998). Disposal of pig carcasses in the woods was shown to have caused outbreaks of CSF in wild boar (Dahle and Liess, 1992).

CSF in wild boar became a problem in Europa (Germany, France, Italy, Austria, Luxembourg, Slovakia, Ukraine) in the last two decades. In most places, outbreaks of CSF in wild boar did not persist longer than a few years and wild boar are thus not regarded as a classic reservoir for CSF (reviewed by (Artois et al., 2002)). For instance, CSF disappeared in wild swine on Santa Cruz and Santa Rosa (two islands about 40km off the Californian coast) within a few years after the virus was deliberately introduced in the 1950s in attempts to eradicate these animals (Nettles et al., 1989). Nevertheless, wild boar were repeatedly identified as the source of infection in domestic pigs in Sardinia, (Laddomada et al., 1994; Biagetti et al., 2001) and Germany (Kaden, 1998). In Sardinia CSF was shown to persist in both wild boar and domestic pig in an area where free ranging pigs share their habitat with wild boar. In contrast, in Germany the direct transmission between infectious wild boar and susceptible domestic pig was considered to be of minor importance. Outbreaks in domestic pigs were often caused by indirect transmission via feeding of kitchen waste containing infectious swine or wild boar meat or via humans or animals (e.g.hunting dogs).

### 1.2.2 Aujeszky's disease (pseudo-rabies, AD)

Due to its high economic impact on pork production, many industrialized countries have implemented control programs with the aim of eradicating AD in domestic pigs. Nevertheless, AD still occurs in the EU at varying prevalence (Italy, France, Germany (Anonymous, 2003a)). The last case of AD in Switzerland occurred in 1990. Switzerland committed itself to demonstrating the freedom from AD in domestic pigs in the bilateral treaty with the European Union.

#### Causative agent and signs of disease

AD is caused by an alpha-herpesvirus (suid herpesvirus 1) that infects the central nervous system and other organs, such as the respiratory tract, in all mammals

except humans and tailless apes. In newborn domestic pigs, the AD-virus causes fever, neurological symptoms such as muscular trembling, ataxis, posterior paresis and epilepsy with a lethality rate of 100%. In older pigs (<4 weeks) the symptoms are similar, although less severe, and the lethality rate is lower. In fattening pigs, the AD-virus mainly causes respiratory symptoms while in pregnant sows it causes stillbirth and abortus. In other mammals, the AD-virus causes heavy itching and is always lethal (Kluge et al., 1999). In wild boar, the severe course of disease was similar when they were experimentally infected with an AD-virus strain isolated from domestic pigs, while mild disease (sneezing, slight nasal discharge, conjunctivitis) was observed in wild boar inoculated with an AD-virus strain isolated from wild boar (Müller et al., 2001). The same experiment demonstrated that AD-virus can be transmitted from wild boar to domestic pigs and vice versa.

### Transmission and occurrence in wildlife

In domestic pigs, the major route of transmission is the oral/nasal route (Kluge et al., 1999). In wild boar, oral shedding and transmission appears to be more common, as was suggested by virus isolations from the tonsils of free-living wild boar (Müller et al., 1998b) and by the observation that hunting dogs got bitten by a an infectious wild boar and sub-sequentially died of AD. Nevertheless, AD-virus was also isolated from nasal swabs of free-living wild boar in Germany (Müller et al., 1998b). Sexual transmission was identified as an important route of transmission in European wild boar and feral swine, respectively (Müller et al., 1998b; Romero et al., 1997).

AD was documented in wild boar in France (Bastian et al., 1999), Italy (Capua et al., 1997b), Spain (Anonymous, 1996; Gortàzar et al., 2002) and Germany (Müller et al., 2000). While AD caused mild disease (subclinical or respiratory symptoms) in wild boar in Germany (Müller et al., 2001), the virus caused severe disease (posterior paresis, tremor, incoordination) in wild boar in Spain (Gortàzar et al., 2002).

As the goal of eradicating AD in domestic pigs is achieved and attention is focused on keeping the AD-free status, concerns have been raised, that the AD-virus could be maintained in wild boar which in turn could represent a source of infection in domestic pigs. Encouragingly, the transmission of AD virus between wild boar and domestic pigs might be extremely rare. Nevertheless, antibodies against the AD-virus were detected in wild boar in an area where outbreaks of AD also occurred in domestic pigs (Lutz and Wurm, 1996). The transmission of AD-virus may occur more frequently from domestic pigs to wild boar than vice versa. For instance, AD was shown to be endemic in wild boar in an area in Eastern Germany which was free from AD in domestic pigs (Müller et al., 1998a). Furthermore, genetic comparisons of AD-virus strains isolated from domestic pigs over a 23-year period in 12 Italian regions revealed that all strains isolated between 1972 and 1984 belonged to group I, while from 1984 onwards group II prevailed. But AD-virus isolated from a wild boar in 1993 belonged to group I (Capua et al., 1997a). Similarly, genetic analysis by restriction-length-fragment polymorphism revealed substantial differences between the AD-virus isolated from wild boar and the AD-virus strains found in domestic pigs in Germany (Müller et al., 1998b). This supports the finding that AD was able to maintain itself in wild boar. Nonetheless, the increase in wild boar population density throughout Europe may enhance the maintenance of AD in wild boar and

thus increase the frequency of transmission between wild boar and domestic pigs. In Germany, the prevalence of antibodies against the AD-virus in wild boar increased over the past decade (Müller et al., 1998b). Similarly, (Guberti et al., 2002) found that the AD-virus was endemic in wild boar in Italy, in areas where there was a high wild boar population density.

### 1.2.3 Brucellosis

Brucellosis is one of the world's major zoonosis (Boschiroli et al., 2001). In industrialized countries, brucellosis in cattle, sheep, goats and pigs is under control and human brucellosis has become rare due to the widely applied pasteurization of the milk. Nevertheless, Brucella-infection is endemic in Asia, sub-Saharan Africa and Latin-America (Shaqra, 2000; Domingo, 2000; Mikolon et al., 1998). It also occurs in humans and lifestock in Mediterranean contrives (Perez-Avraham et al., 2001; Lithg-Pereira et al., 2001). Brucellosis can have a considerable impact on human and animal health, as well as wide socioeconomic impacts, especially in countries in which rural income relies largely on lifestock breeding and dairy products (Roth et al., 2003). Switzerland is officially free from brucellosis in cattle, sheep, goats and pigs.

#### Causative agent and signs of disease

Brucellae are Gram-negative, facultative intracellular bacteria. Genetic studies demonstrated that the genus *Brucella* is mono-specific (Verger et al., 1985). Nevertheless, the classic nomenclature is maintained (Anonymous, 2003b). Thus, six species are differentiated based on pathogenicity and host preference. The species, in decreasing order of importance in humans, are: *Brucella melitensis* (Malta fever, sheep and goats), *B. abortus* (Bang's disease; cattle), *B. suis* (pigs and wild boar), *B. canis* (dogs), *B. ovis* (sheep) and *B. neotomae* (desert rats). *B. ovis* and *B. neotomae* are not known to cause disease in humans. In *B. suis*, there are 5 biovars, of which biovar 1 (suides, Latin-America, Asia, Oceania), biovar 3 (suides, U.S.A., China) and biovar 5 (small ruminants, Russia) are highly pathogenic in humans. Biovar 4 (reindeer, U.S.A., Canada, Russia) causes moderate pathogenicity in humans. Biovar 2 (suids, central Europe) has only once been reported as the cause of disease in humans (Garin-Bastuji and Delcueillerie, 2001) and is thus considered harmless.

In mammals, the often unnoticed infection with *Brucella* bacteria causes abortion and birth of dead or weak young. In males, the most prominent sign is orchitis. In pigs, also bones, joints and tendon sheaths may be affected, causing lameness and sometimes paralysis. In humans, undulant fever, tiredness, night sweats, headaches and chills may be present initially, whereas anxiety and depression can occur in long-standing infection (Parnas, 1966).

#### Transmission and occurrence in wildlife

Transmission occurs orally, via skin injury, mucous membranes or infected sperm. Bacteria are shed through the sexual organs and the mammary glands. The major source of infection is infected placenta or aborted fetuses. In pigs, transmission during copulation is common. Human brucellosis is caused by direct contact with

tissues or fluids from infected animals and consumption of un-pasteurized milk and milk products. Human-to-human transmission does not occur (Krauss et al., 1996). However, humans may infect animals (Parnas, 1966).

*B. abortus* and *B. suis* have been isolated world-wide from a great variety of wildlife species, such as bison (*Bison bison*), elk (*Cervus elaphus*, wild boar (*Sus scrofa*), European hares (*Lepus europaeus*), foxes (*Vulpes vulpes*), African buffalo (*Syncerus caffer*), eland (*Taurotragus oryx*), waterbuck (*Kobus elipsiprymnus*), reindeer (*Rangifer tarandus tarandus*) and caribou (*Rangifer tarandus groenlandicus*, (Davis, 1990)). Since the first description of an abortion caused by Brucellae in a captive dolphin in 1994, several reports have described the isolation and characterization of Brucella strains from a variety of marine mammals, such as seals, porpoises, dolphins and whales (Cloeckaert et al., 2001; Godfroid, 2002).

Although *B. melitensis* was rarely reported in wildlife, cases were reported in chamois (*Rupicapra rupicapra*) and ibex (*Capra ibex*) in the Alps (Ferroglio et al., 1998; Garin-Bastuji et al., 1990). Despite numerous surveys which identified antibodies against *Brucella abortus* in free-ranging cervides in Europe, these infections were self-limiting or spillovers from cattle (Godfroid, 2002). In contrast, the infection with *B. abortus* is self-maintaining in bison (U.S.A), elk (Canada) and, possibly, African buffalo (southern Africa) (Dobson and Meagher, 1996; Godfroid, 2002). *B. suis* infections in feral pigs are regularly reported in Hawaii, the south-eastern states of the USA and Queensland (Australia). In the 1990s *B. suis biovar 2* was repeatedly reported in wild boar in Belgium, France, and Luxembourg (Godroid et al., 1994; Hars et al., 2000; Godfroid, 2002) but also in Austria, Germany, Portugal and Spain (Godfroid and Käsbohrer, 2002). *Brucella* bacteria were isolated from all age groups, suggesting that the infection with *B. suis biovar 2* was less pathogenic in wild boar than in domestic pigs (Godfroid, 2002). Porcine brucellosis has re-emerged in outdoor-reared domestic pigs as a result of spillover from infected wild boar in France (Hars et al., 2000) and was also described in Germany (Wilhelm and Zieris, 1985). Infection with *B. suis biotype 2* was also recorded in the European brown hare in many European counties (von Daemoser and Hofer, 1995; Godfroid, 2002) and also in Switzerland (Büttner, 1996; Haerer et al., 2001). Transmission between wild boar and brown hare occurs (Englert et al., 1964). However, the population of brown hares is decreasing in Europe due to changes in habitat, such as more intensive agriculture, and is therfore not considered to be an important risk factor for brucellosis in wild boar or outdoor-reared domestic pigs.

### 1.2.4 Tuberculosis

Tuberculosis, one of the most widespread infectious diseases, is the leading cause of death due to a single infectious agent among adults in the world. Someone in the world is newly infected with tuberculosis every second (Anonymous, 2002e). In industrialized countries, tuberculosis control and eradication programs in cattle, together with milk-pasteurization, have drastically reduced the incidence of disease caused by *M. bovis* in both cattle and humans. In developing countries, *M. bovis* remains widely distributed (Cosivi et al., 1995). Tuberculosis is a major opportunistic infection in HIV-patients (Raviglione and an A. Kochi, 1995).

### Causative agent

Tuberculosis is caused by acid-resistant, Gram-positive, aerobic bacteria. The genus Mycobacteria comprises 3 species: *Mycobacteria tuberculosis* affects humans; *M. bovis* has cattle as its main host, but also causes disease in humans and a range of wild and domesticated animals such as badgers, ferrets, cats, deer, hedgehogs, sheep, and Ilamaoids. *M. avium* has birds as its main host, but can also cause disease in pigs and humans (opportunistic infection in AIDS-patients).

### Signs of disease and transmission

Human tuberculosis (*M. tuberculosis*) is transmitted by droplet infection, i.e. through speaking, sneezing or coughing. Under normal immunity, the initial focus of infection, (in the lungs) encapsulates and can remain dormant for a lifetime (primary tuberculosis). In patients with reduced immunity defenses, e.g. due to AIDS or malnutrition, the initial focus of infection spreads in the lungs (open tuberculosis) or into other organs, in which case also the urine or excrement become infectious. The disease can also attack the skin, the skeleton and the brain. In cattle, the infection with *M. bovis* is often asymptomatic. Clinical signs of infection include weakness, loss of appetite, weight loss, cough and fever. Humans acquire the infection by inhaling cough spray from infected cattle. Such patients can infect other cattle. Evidence for human-to-human transmission is limited and anecdotal. In countries where bovine tuberculosis is uncontrolled, most human cases occur in children and result from drinking and handling contaminated milk (Cosivi et al., 1998). In humans, tuberculosis caused by *M. bovis* is clinically indistinguishable from tuberculosis caused by *M. tuberculosis*. Clinical signs of pulmonary tuberculosis in humans are tiredness, persistent cough, loss of appetite and chest pain.

### Occurrence in wildlife

Bovine Tuberculosis was found in free-ranging wildlife in many parts of the world (reviewed by (Lisle et al., 2001)). Badgers (*Meles meles*) and brushtail possums (*Trichosurus vulpecula*) are maintenance hosts of *M. bovis* which represent the principal source of infection in cattle in UK and New Zealand, respectively. *M. bovis* in wild boar was first detected in the 1930s in Germany (Kindinger, 1934) and was thereafter found in many countries, such as Italy, Bosnia-Herzegovina, Spain, New Zealand, Australia, USA (Bollo et al., 2000). Comparisons by spacer oligotyping of different *M. bovis* strains isolated from wild boar and cattle living in the same region, revealed the presence of a close genetic relationship between strains isolated from cattle and strains isolated from wild boar (Serraino et al., 1999; Aranaz et al., 1996). However, many studies showed that wild boar and feral pigs were not maintenance hosts for bovine tuberculosis and that there was no spillover from infected wild boar to cattle (Phillips et al., 2003; Coleman and Cooke, 2001; McInerney et al., 1995; Schulz et al., 1992). (Parra et al., 2003) argued that the transmission from infected wild boar to domestic pigs may have occurred in Spain.

## 1.3 Diseases monitoring and surveillance

The World Trade Organization (WTO) has the responsibility of implementing the various international agreements finalized through the Uruguay round of the General Agreement on Tariff and Trade (GATT, (Anonymous, 1994)). The WTO began operations in January 1995 and has adopted the codes of the Office International des Epitooties (OIE) to serve as guidelines for international trade in animals. The Agreement on Sanitary and Phytosanitary Measures (SPS agreement) of the WTO requires that, in international trade, the justification of measures taken to protect animal, plant or human health, needs to be based on scientific methods such as risk analysis (Anonymous, 1997). Its main intent is to avoid the use of SPS measures as unjustified barriers to trade. Many countries are working to eradicate trade limiting diseases such as rinderpest, tuberculosis, foot-and-mouth disease, classical swine fever, or enzootic pneumonia. Proof of the final success of such campaigns will need to be provided and will often be based on surveys. The OIE has developed standards for declaring freedom from rinderpest (Anonymous, 2000b) and contagious bovine pleuropneumonia (Anonymous, 2000a). However, such standards have not been developed for other economically important livestock diseases such as classical swine fever, Aujeszky's disease, bovine tuberculosis, or porcine reproductive and respiratory syndrome. Hence, individual trading partners decide on what data are necessary to substantiate their claims of disease freedom (Doherr et al., 2003).

The term 'survey' denotes an investigation or a study in which information is systematically collected for a specific aim or conceptual hypothesis (Salman, 2003). Surveys can be targeted at either the demonstration of freedom from disease or at the estimation of disease prevalence. In either case, absolute proof requires the examination of every animal in the population, using a perfect test. As this is not feasible, random sample surveys are used, and results are reported in terms of probability statements that the disease, if present, has a prevalence lower than a defined level which is determined by prior experience. When surveying to detect disease (or demonstrate freedom from disease), the conclusion of the survey will be that the disease is or is not present. This statement will be judged by a probability that it is correct. The probability is found by testing whether the prevalence detected by the survey is at or greater than the specified threshold prevalence (null-hypothesis), associated with a confidence level (usually 95%). Rejecting the null-hypothesis means that the prevalence detected by the survey is below the specified threshold prevalence with a probability of 95% (Cameron, 1999). Surveys to estimate disease prevalence will produce point estimates of the prevalence, the precision of which is judged by a confidence interval (i.e. the interval which encompasses the true prevalence in the population with a specified probability, usually 95%, (Armitage et al., 2002)).

The required sample size increases dramatically, as the threshold prevalence specified in a survey to substantiate freedom from disease — or the minimal prevalence a survey to estimate disease prevalence is desired to detect — approaches zero. Moreover, the required sample size for a given aim (e.g.substantiate freedom from disease at a 2% threshold level) is higher when the diagnostic procedure's sensitivity and specificity is low (Cannon and Roe, 1982; Levy and Lemeshow, 1991). Furthermore, a larger sample size is required when the surveyed population is clustered, than when a population of the same size is randomly distributed (Ziller et al., 2002; Cameron

and Baldock, 1998; van Schaik et al., 2003). When the study population is subdivided into several isolated sub-populations, the dynamics of disease transmission between the sub-populations is unrelated and hence, the disease-prevalence found in one sub-population can not be extrapolated to the other sub-populations. In a hypothetical population of 2000 individuals, a sample of 277 individuals would be required to assess freedom from disease at a threshold level of 1% and a confidence level of 95% (assuming a perfect test with 100% sensitivity and 100% specificity). When this population was subdivided into 2 isolated sub-populations of 1000 individuals each (leaving everything else equal), a sample of 285 would be required from each sub-population (Cameron, 1999). In contrast to domestic lifestock where the location and size of herds is registered, the spatial partition of wildlife populations is not known. In addition, the degree of separation is expected to vary in response to population density and the distribution of suitable habitat, both of which in turn may be subject to seasonal cycles (Begon et al., 1990).

In concept, a series of surveys can be considered as a monitoring system, which in turn, may become a surveillance system, if action is taken to prevent or control the disease. The term 'surveillance' was first used during the French Revolution, when it meant 'to keep watch over a group of persons thought to be subversive'. The term has been used extensively by epidemiologists and animal health professionals. Some authors have proposed the use of the term 'monitoring and surveillance system' to summarize the concepts (Stärk, 1996; Noordhuizen et al., 1997; Doherr and Audigé, 2001; Salman, 2003). In that context, monitoring describes a continuous, adaptable process of collecting data about diseases and their determinants in a given population, but without any control activities. Surveillance is a specific case of monitoring in which control measures are implemented whenever a certain threshold level related to the infection or disease status is exceeded. Surveillance is thus part of any disease control program (Noordhuizen et al., 1997).

Monitoring in wildlife allows to detect possible risk factors for both livestock industry and human public health. The concern of livestock industry is to prevent the re-introduction of an infection into livestock, when there are eradication programs in progress or when the region or state is officially declared free from the disease in concern. Human public health problems may emerge from expanding wildlife populations, which in turn allow zoonosis to persist at high population densities. For instance, in Queensland (Australia), feral pigs expanded in both numbers and geographic area within the past decades. Human brucellosis (*B. suis biovar 1*) is re-emerging due to recreational (hunting) and occupational exposure to feral pigs infected with *B. suis* (Robson et al., 1993). In 1994, *Brucella suis biovar 1* was isolated from a butcher in Belgium who had been handling imported feral pig meat (Godfroid, 2002; Godroid et al., 1994). Globalization in international trade may thus lead to new public health risks.

### Outline

ae considered the initiation of a monitoring and surveillance program in wild boar important in terms of (i) an early warning system to detect the emergence of diseases relevant to domestic pigs, (ii) an instrument for documenting the disease status for important pig diseases in Switzerland and (iii) as an instrument for assessing the

success of interventions targeted at limiting disease outbreaks after disease introduction. In Chapter 3, we assessed the spatial segregation of the wild boar population in Switzerland, based on the notion that the distribution of suitable habitat determines the distribution of the species. In Chapter 4, we carried out a survey to estimate the prevalence of classical swine fever, Aujeszky's disease and porcine brucellosis in Switzerland, based on the pattern of spatial separation of the wild boar population unraveled in Chapter 3. In Chapter 5, we investigated the effects of different hunting rates on the prevalence of brucellosis in wild boar, based on a dynamic transmission model. In Chapter 6, we assessed the prevalence of tuberculosis (*M. bovis*) in wild boar in Ticino, southern Switzerland. In Chapter 7, we present an overview of concepts and consideration relevant to a disease monitoring and surveillance system in wild boar in Switzerland. Finally, in Chapter 8, we highlight some of the insights gained in establishing a national monitoring and surveillance system in wild boar in Switzerland and suggest ideas for further research.

Chapter 2

# Goal and Objectives

## 2.1 Goal

To establish a monitoring and surveillance system for infectious diseases in wild boar in Switzerland

## 2.2 Objectives

- to define geographical regions suitable as sampling units in a national surveillance system for infectious diseases in wild boar
- to set up the organization/framework for the surveillance of infectious diseases in wild boar in Switzerland
- to document the disease status for classical swine fever, Aujeszky's disease and brucellosis in Switzerland
- to document the disease status for tuberculosis in wild boars in the region considered to be at highest risk

Chapter 3

# Defining sampling units for a national disease surveillance program in wild boar

## 3.1 Abstract

As a pre-requisite for a national surveillance program of contagious diseases in wild boar, we attempted to subdivide Switzerland into several geographic area within which an outbreak of a contagious disease in wild boar would remain confined with a probability of 95% within one year (sampling units). In order to define such sampling units we mapped the probability of wild boar occurrence per hunting area. This probability was assessed by the association between the configuration of the landscape and the number of wild boar registered in the annual hunting bag. The latter was considered as a surrogate for population density. Based on this map, we suggested the use of two sampling units for the current sampling protocol: north and south of the Alps. We considered it to be unlikely for a contagious disease in wild boar to spread across the entire region north of the Alps within one year.

In order to explore the consequences of introducing one infectious pack into a population of susceptibles, we developed a spatially explicit transmission model, based on the map of the probability of wild boar occurrence. The model allowed to simulate different scenarios, whereby the wild boar poplation density, the home range size and the probability of transmission could be varied. The magnitude of the simulated outbreak was measured in terms of the number of infected packs, the duration of the outbreak (months) and the maximal distance of disease spread within one year. The landscape induced considerable variability in magnitude of the simulated outbreaks. However, the latter was mainly determined by the wild boar's home-range size and the probability of transmission. The model predicted that a disease such as classical swine fever would spread less than 50 km per year, when an average population density of 1 – 2 wild boar $km^{-2}$, a home range size of maximally 25 $km^2$ and a probability of transmission of 40% was assumed. But the model needs to be validated by disease data and by data on the demography and migration of wild boar.

## 3.2 Introduction

A surveillance system for contagious diseases seeks to extrapolate from the prevalence in a sample population to the general population. This implies knowledge on the characteristics of the diagnostic procedure and on both size and spatial configuration of the target population. In wildlife, the degree of a population's spatial partition depends on the distribution of suitable habitat. The effects of habitat fragmentation on population persistence has been studied extensively (Turner, 1989; Hanski et al., 1995; Nee, 1994). With the advent of powerful geographic information systems, the development of predictive habitat distribution models has increased in ecology (reviewed by (Guisan and Zimmermann, 2000)). For instance, (Howells and Edwards-Jones, 1997) assessed the feasibility of re-introducing wild boar to Scotland, based on habitat analysis. Similarly, the feasibility of re-introducing European lynx was investigated, based on the analysis of landscape patterns in Germany (Schadt et al., 2002) and Switzerland (Zimmermann and Breitenmoser, 2002).

The meta population concept, which has been widely applied in theoretical ecology and conservation biology (Gilpin and Hanski, 1991), assumes that the distribution of many species can be described as a system of local populations, each of which may be subject to turnover as a result of extinction and subsequent recolonization by dispersing individuals (MacArthur and Wilson, 1967). Since the 1960s, a number of authors (Begon et al., 1990; Greenfell and Harwood, 1997) have pointed out that epidemiological and meta population theory share a common interest in the way in which the balance between extinction and recolonization affects the persistence of patchily distributed species. The minimum viable population of meta-population theory is — like the critical community size of epidemiology — a measure of the number of individuals required for an isolated population to have a given probability of surviving for a finite period. When a population is highly partitioned into small sub-populations, each sub-population is subject to stochastic events and extinction becomes more likely, the smaller the sub-population is (Lande, 1993). Analogously, for an infection to persist, there must be enough susceptible individuals to maintain a chain of transmission (Anderson and May, 2002). Thus, disease becomes established with greater difficulty when the degree of spatial partition of the population increases, and when the contact rate between individuals decreases (Rodriguez and Torres-Sorando, 2001).

A landscape can be viewed as an interacting mosaic of patches relevant to the organism under consideration (Dunning et al., 1992), (`www.umass.org/fragstats/help`). Wild boar movements are influenced by the spatial arrangement of preferred food types and breeding sites. Wild boar are predominantly herbivorous with vegetable matter constituting between 80% and 100% of their diet. Acorns and beech mast are the most significant natural resources, although cultivated plants (notably maize, oats and potatoes) form the staple diet of wild boar throughout Europe (Genov, 1981; Henry and Conley, 1997; Briedermann, 1986; Sjarmidi et al., 1992). The presence of water for wallowing and a dense understory for bedding and shelter are also important (Gerard et al., 1991).

The basic social units in wild boar is a group (pack) organized around a nucleus of two or three sexually mature breeding females. The rest of the pack consists of their most recent young and usually sub-adults (8-20 months of age) from the

previous litter. Males are expelled from the pack by the time they reach sexual maturity. Stable packs of subadult males and females in their wandering phase exist. In general, the average pack size varies between 5 and 10. Large packs (>40 members) split into two (Briedermann, 1986).

Estimates of home range sizes vary widely between locations. Packs covered 8-30 $km^2$ in the Jura-region of Switzerland, (Baettig, 1993), 5-15 $km^2$ in Germany (Stubbe et al., 1989), 2-40 $km^2$ in southern France (Spitz, 1992), 1-4 $km^2$ in Italy, (Boitani et al., 1994), and 11 $km^2$ in Australia. A pack defends its core area: 1-3 $km^2$ (Spitz, 1992), <1 $km^2$ (Boitani et al., 1994) while the rest of the home range overlaps with home ranges of neighboring packs. While packs use only a portion of their home range during any given month (Wood and Brennemann, 1980), the adult male, in contrast, has a single home range of up to 50 $km^2$, and may travel the entire length of this range in just one or two days (Spitz, 1992). Meanwhile, sexually immature subadult males and females often occupy the free space between established home ranges. During this nomadic phase, sub-adults typically range over 10 $km^2$ but have been shown to cover distances of up to 250 km (Oliver et al., 1993). Home ranges tend to be larger in winter than in summer (Spitz, 1992). Daily moving distances of packs were recorded to be around 8 km with a small percentage (3-5%) of boar moving long distances 20-60 km (Stubbe et al., 1989). Average population densities of 6 individuals $km^{-2}$, but up to about 24 individuals $km^{-2}$ after a good acorn crop, were described in Poland (Jedrzejewski et al., 1994). Similarly, (Hone et al., 1992) revealed a population density of 6 wild boars $km^{-2}$ in Pakistan, while lower population densities were found in Germany (about 3 wild boars $km^2$) (Kern et al., 1999) and Sardinia (1 $km^{-2}$) (Guberti et al., 1998).

Our objective was to identify geographic regions which are isolated enough from each other so that an outbreak of a contagious disease in wild boar would remain confined within the primarily infected sampling unit for the time between two consecutive survey rounds (1 year) with a probability of 95%.

## 3.3 Methodology

Based on the notion, that the occurrence of a species can be predicted from environmental parameters (Begon et al., 1990; Zimmermann and Breitenmoser, 2002), we related the estimated wild boar population density with various landscape variables. The statistical significance of these relationships was assessed by a multivariate logistic regression. Based on this model, the predicted probability of wild boar occurrence was mapped. Based on this map, we assessed the effect of the landscape (i.e. the probability of wild boar occurrence) on the consequence of introducing one susceptible wild boar pack into a population of susceptibles by means of a spatially explicit transmission model.

### Cartographic data

For cantons (administrative regions) where hunting areas corresponded to municipalities, the 'digital political and administrative boundary' dataset (GG25 version June 2002) was used. Municipalities, which consisted of several parts (e.g. enclaves) were treated as distinct spatial units. In cantons where hunting areas did not match

municipalities, the respective data were obtained from the cantonal authorities. We termed all spatial units 'hunting area', whether or not, in reality they were sutible as such.

The spatial composition and configuration of the landscape used in predicting the landscape's potential suitability for wild boar was based on the digital landscape model (Vector25, version May 2003) which includes 28 mutually exclusive ground categories such as forest, glacier, or lake, and the road and path network. The elevation above sea level was obtained from the digital height model (DHM25). All datasets were based on the National Map 1:25'000 with an accuracy in position of 3-8 m and were obtained from the Federal Office of Topography. In addition, the spatial data on maize cultivation area (ha per municipality, based on the National Map 1:25'000) was obtained from the Federal Office of Agriculture. The data on wildlife corridors (bridges across main roads, in order to allow the genetic exchange among wildlife sub-populations) was obtained from the Vogelwarte Sempach (Anonymous, 2001). An overview of the geographic regions in Switzerland is given in figure 3.1 on page 29.

### 3.3.1 Probability of wild boar occurrence

#### Landscape composition variables

For each hunting area, the average elevation above sea level, the variation in the elevation above sea level (standard deviation), the density of highways (meters per hectare) and the area (hectares) were calculated. The spatial composition and configuration of the landscape was assessed by two sets of metrics: class metrics and landscape metrics (FRAGSTATS3.2, `www.umass.org/fragstats`).

Class metrics treat one particular ground cover category (patch type, class) at a time while considering all remaining patch types as a uniform matrix in which the patches of the focal class emerge like islands. We focused on the three dominant ground cover categories: forest, open land and settlement areas. However, in 50% of the hunting areas for which we had data on the presence of wild boar, the sum of these 3 categories comprised over 95% of the respective hunting area. Hence, the variables 'percentage of forest', 'percentage of open land' and 'percentage of settlement' were correlated. Consequently, 'percentage of open land' could roughly be deduced from 'percentage of forest' and 'percentage of settlement'. We therefore omitted 'percentage of open land'. Thereafter, in 50% of the hunting areas, the-sum of 'percentage of forest' and 'percentage of settlement area' comprise over 38% of the hunting area's total area. Nevertheless, the category 'open land' was included in the calculation of the landscape metrics.

Landscape metrics quantify the spatial configuration of the entire landscape mosaic. This includes the total length of edge between adjacent patch types, or the extent to which patches of different types are interspersed. The following categories were included in the calculation of the landscape metrics: forest, shrub, open land (including agricultural area), settlement area, marsh, orchard, vineyard. Ground cover categories designating rock, glacier or lake were re-categorized as 'unsuitable'.

An overview of the variables included is given in table 3.1 on page 27. A thorough review of the different metrics is available at `www.umass.org/fragstats/help`.

#### Wild boar population density

In the absence of population data, we used the annual hunting statistics per hunting area as a surrogate. Wild boar were killed by hunters in 16 out of the 26 cantons existing in Switzerland (criterium: $\geq 5$ wild boar killed in 2002, (Anonymous, 2002a)). Data stratified by hunting area was available from 13 cantons for two years: 2001 and 2002. These data from 13 cantons were used in the regression analysis.

#### Regression analysis

Each of the landscape composition variables was related to the total number of wild boars killed per hunting area during the two-year period. These relationships were tested using a logistic regression (logit link and binomial error distribution; STATA 8.0, Stata Corporation 2003). Variables testing statistically significant ($p<0.05$) in the univariate analysis were subsequently tested by backward-selection (significance level for removal=0.05). The low p-value was chosen in order to achieve a reduction in the high number of variables.

In the regression model, we assumed that (i) the carrying capacity of wild boar was attained in all hunting areas, i.e. that the observed population sizes reflected longterm patterns (population sizes constant) and (ii) that the number of wild boar killed by hunters was proportional to the number of wild boar present.

The appropriateness of the regression model was assessed by the Hosmer-Lemenshov goodness-of-fit test. In addition, the deviance residuals (observed minus predicted) were mapped in order to check for unexpected spatial patterns.

Based on the final regression model, the predicted probability of wild boar occurrence was calculated for all hunting areas in Switzerland, whether they were used in fitting the model or not. The predicted probability probability of wild boar occurrence was mapped.

### 3.3.2 Transmission model

Based on the map of the probability of wild boar occurrence, we developed a spatially explicit transmission model in order to explore the consequences of introducing one susceptible wild boar pack into a population of susceptibles. The effect was measured in terms of the percentage of packs affected from the total population, the duration of the outbreak (months) and the radius within which the disease spread (km).

We focused on the pack (breeding females with their young) as the smallest epidemiological unit. This simplification may be adequate for diseases where the pack (or age group '<1 year') plays the key role in transmission, such as classical swine fever, (Kern et al., 1999).

At the beginning of each simulation, a number of packs populates the landscape described by the map of the probability of wild boar occurrence, whereby each cell (1 $km^2$) is chosen with its probability of wild boar occurrence. Each pack is assigned a square-shaped home range. The pack's starting position is at the center of its home range. In order to prevent packs from ending up in a single cell of acceptable habitat (i.e. the rest of the home range is unsuitable), the home range needs to be of a minimal quality. The home range quality is expressed as the percentage of the home range's maximal value (e.g. 9 cells with a probability of wild boar occurrence

of 50 each, would achieve a home range quality of 50%). If no home range can be established with the chosen starting position, then a new home range is searched.

All packs are susceptible initially. One additional pack, which is infectious, is placed in a randomly selected cell. The disease outbreak is then simulated by a sequence of 12 steps, each corresponding to one month in the packs' life. In each step, each pack behaves according to 2 rules: (i) if the pack is infectious, it infects all neighbors with a given probability, (ii) the pack moves one cell within the home range.

This procedure was repeated 100 times, leaving the position of the source of infection (initially infectious pack) constant. The average size of the outbreak (number of packs) and the average duration of the outbreak (months) was calculated over these 100 iterations (100 iterations = 1 simulation). In order to assess how the landscape (probability of wild boar occurrence) affects the outbreak resulting from the introduction of one susceptible pack, we run 10 simulations in sequence. Then, the mean outbreak size was calculated over these 10 simulations. Similarly, the mean duration of the outbreak was calculated over the 10 simulations. Finally, the outbreak-size was reported as the percentage of packs affected from the total population. In addition, a map showing each landscape cell's probability of hosting an infectious pack was produced such that, for instance, a cell being inhabited by an infectious pack in every iteration was assigned a probability of 100%.

### Parameters

The parameters used are presented in Table 3.2 on page 28. The disease related parameters were based on the literature for classical swine fever (CSF). The probability of wild boar occurrence (landscape), the wild boar population density, and the probability of transmission were estimated as follows:

**Landscape** The probability of wild boar occurrence per hunting area (revealed by the regression analysis) was extrapolated to the probability of wild boar occurrence per $km^2$ (Figure 3.3 on page 30). In order to achieve this, each variable for which the multivariate logistic regression revealed a statistically significant relationship with the presence of wild boars killed by hunters was re-calculated per $km^2$. The probability of wild boar occurrence per cell (1 $km^2$) was determined by multiplying each variable's value by its regression coefficient and adding up these products. The barrier effect of highways was taken into account as follows: For each cell, the number of bridges, tunnels and wildlife corridors (unless qualified as 'heavily disturbed', (Anonymous, 2001)) was added up and converted into the percentage of the maximal value (reduction factor). Then, each cell's probability of wild boar occurrence was decreased by that reduction factor. Thus, cells which did not contain any highway fragments were unaffected, while the probability of wild boar occurrence was reduced in the cells which contained highway fragments. A cell which contained a highway fragment but no bridges, tunnels or wildlife corridors was set 'unsuitable'.

**Population size** The wild boar population size (i.e. number of packs) was estimated from the hunting statistics (Anonymous, 2002a). The average annual hunting bag, $hb$, in Switzerland (data from 2001 and 2002) was 5'509 wild boar. According

to hunting practice, the annual hunting bag represents about 50% — 100% of the annual wild boar population increment, which in turn was estimated to be about 100% — 150% (maximally 200%) (Anonymous, 2004). In the following calculations, we assumed that (i) the increment in population size occurred at once, at the beginning of the year and (ii), that all hunting occurred at once, at the end of the year. The population at the start of the year was thus estimated by *hb*factor of annual population increment*. The minimal population size was calculated by assuming that (i) *hb* represented a population increment of 100% and (ii) that hunters killed 100% of the annual population increment. Then, the average population during the year would approximate $\frac{hb+hb*2}{2}$. thus, $\frac{5509+5509*2}{2}$=8264. Under this scenario, hunting would maintain the population size constant in time. The maximal population size was estimated by assuming (i) that the annual population increment was 200% and (ii) that hunters killed 50% of the annual population increment. Thus, *hb**2 approximates the population at the start of the year and $3 * (2 * hb)$ the population at the end of the year, just before the reduction by hunting would occur. The average population during the year would be $\frac{2*5509+3*2*5509}{2}$=22036. Under this scenario, the wild boar population would double every year.

The map of the probability of wild boar occurrence revealed that 70% of Switzerland (31'715 km$^2$ out of 42'653 km$^2$) was associated with a probability of wild boar occurrence greater than zero. The wild boar killed by hunters during the years 2001 and 2002 were restricted to an area of 9'100 km$^2$, which in turn corresponds to 30% of the area with a probability of wild boar occurrence greater than zero ($\frac{9'100}{31'715}$). The average population density in the area currently occupied by wild boar was estimated to be between $\frac{8'264}{9'100}$=0.9 and $\frac{22'036}{9'100}$=2.4 wild boar km$^{-2}$. These estimates are in range with the values provided by the literature (Kern et al., 1999), (Guberti et al., 1998). In the simulation, we were interested in the situation where wild boar occupy the entire area of suitable habitat in Switzerland. Therefore, the population size was estimated to be between 3*8'264=24'795 and 3*22'036=66'108. Assuming that packs consisted of 5-10 members (Briedermann, 1986), there would be between $\frac{24'795}{10}$=2'480 and $\frac{66'108}{5}$=13'222 packs.

**Probability of transmission** The probability of transmission, i.e. the probability of an effective contact between an infectious and a susceptible pack (transmission event) was estimated to be between 0.00063 $day^{-1}$ and 0.00121 $day^{-1}$, based on an a CSF-outbreak in free-living wild boar (Hone et al., 1992). Based on these estimates, we calculated the probability of transmission per month: 2% ($0.00063 * 30 * 100$) to 4% ($0.00121 * 30 * 100$).

## 3.4 Results

### 3.4.1 Landscape suitability map

In total, there were 2851 hunting areas identified in Switzerland. Hunting statistics for the years 2001 and 2002 were available for 1945 (68%) of these hunting areas. The number of wild boar killed per hunting area ranged from 1 to 154 (median=7). In 2001, at least one wild boar was killed by hunters in 679 out of the 1945 hunting ares. In 2002, at least one wild boar was killed in 690 out of the 1945 hunting areas.

In 550 out of the 1945 hunting areas there was at least one wild boar killed in both years. Thus, in 2002, wild boar were killed by hunters in 81% of the hunting areas where wild boar were also killed by hunters in 2001. Summing up both years, at least one wild boar was killed by hunters in 771(40%) out of the 1945 hunting areas. The 771 areas with at least one wild boar killed by hunters varied in size between 0.8 and 16'644 hectares (median=694) and in elevation above sea level between 193 and 1'595 meters (median=537). The areas where wild boar were killed by hunters were mainly situated along the Jura-mountains (Figure 3.1 on page 29).

### Regression model

Of the 1945 hunting areas we obtained hunting statistics for, 1876 (96%) were included in the multivariate regression analysis. Hunting areas in which only one ground cover category occurred, were omitted.

We identified 24 individual variables as being associated with the presence of wild boar killed by hunters. By backward-selection, the number of variables was reduced to 10 (likelihood $chi^2$=526.17, p=0.000). These variables were: diversity index, percentage of forest, percentage of settlement area, density of settlement area patches, density of forest patches, overall patch density, highway density, interspersion, maize cultivation area and mean elevation above sea level (for a description of the variables see Table 3.2 on page 28.

The Hosmer-Lemenshov goodness-of-fit test indicated that the model was appropriate ($chi^2$=6.4, Prob>$chi^2$=0.6). The model was able to classify 73% of the hunting areas correctly, according to their status (presence or absence of wild boar killed by hunters). However, mapping the residuals (observed minus predicted probability of wild boar occurrence) showed a better fit in areas with a moderate number of wild boar killed by hunters than in areas with a large number of killed wild boar (Figure 3.2 on page 29). In hunting areas with a very large number of wild boar killed by hunters, the model predicted a lower probability of wild boar occurrence than expected, whereas, in areas with few or zero wild boar killed by hunters, the model predicted a higher probability of wild boar occurrence than expected.

A high probability of wild boar occurrence was associated with hunting areas yielding a high percentage of forest (median percentage of forest in hunting areas with at least 1 wild boar killed by hunters=38.4%), a large maize cultivation area, a high overall patch density (number per 100ha) and a high diversity (probability that 2 randomly selected pixels would be of different patch types). The probability of wild boar occurrence decreased with an increasing main road density, an increasing percentage of settlement area, an increasing density of settlement forest patches, an increasing density of forest patches and an increasing degree of intermixing (chance that pixels adjacent to each other belong to different patch types).

Surprisingly, an increasing percentage of forest was associated with an increasing probability of wild boar occurrence, while an increasing density of forest patches was associated with a decreasing probability of wild boar occurrence. However, this may be explained by chance: as expected, the percentage of forest was positively correlated with the density of forest patches when all 2798 hunting areas with a percentage of forest greater than zero were considered (Spearman's r=0.11, p=0.000). However, the correlation was not statistically significant when only the subset of 1919

hunting areas we had also data on the number of wild boar killed by hunters from (Spearman's r=0.03, p=0.202).

Both the degree of interspersion and diversity tended to be high in densely populated rural areas. Diversity, in contrast to interspersion, measures the variety of patch types in addition to the degree of intermixing of the patch types present in a landscape. In conclusion, a high probability of wild boar occurrence was associated with hunting areas being comprised of a large percentage of forest and a large variety of highly intermixed patches.

#### Sampling areas

The predicted probability of wild boar occurrence was mapped (Figure 3.3 on page 30). The areas with a high probability of wild boar occurrence were along the Jura Mountains, in Klettgau and Ticino (geographical names are in Figure 3.1 on page 29). A high probability of wild boar occurrence was also predicted in eastern Mittelland, Napf, Ober-Aargau and lower Rhone Valley, where few or no wild boar were killed by hunters during the two-year period analyzed. The Alps constituted the only major barrier suggestive for a temporally stable subdivion of the population, although there was a small passage (Lukmanier pass). We therfore suggest to use 2 sampling regions for the current sampling protocol: north and south of the Alpes. This suggestion may be supported by the finding that wild boar in different regions north of the Alps (Switzerland, France) were not genetically different from each other, whereas genetic difference were found between wild boar north and south of the Alps (Boudry and Neet, 2001).

### 3.4.2 Transmission model

Different scenarios of introducing one infectious pack into a population of susceptibles are shown in Figure 3.4 on page 31. In each row, the population density increased from left to right, while the remaining parameters (home range size, probability of transmission) remained constant. For the following observations, we bear in mind that the existing epidemiological and demographic baseline data are weak and that a formal validation could not be be done yet. The probability of transmission estimated from the literature was too low to produce a remarkable outbreak (i.e. a spread of the infection to more than 1 susceptible pack).

**Scenario A:** When the probability of transmission was 10% and the home range size was 9 $km^2$, the percentage of packs affected from the total population was less than 0.01%. The increase in the population density from 2'500 to 14'000 packs resulted in a 1.3-fold increase in the average outbreak-size. The average duration of the outbreak was less than 2 months. Accordingly, the radius of disease spread was below 2 km.

**Scenario B:** When the home range size was increased to 25 $km^2$ (leaving the probability of transmission at 10%), the percentage of packs infected was $< 0.1\%$ when there were 2'500 packs and 0.7% when there were 14'000 packs. Thus, the increase in population size was associated with a 45-fold increase in the percentage of infected packs. Accordingly, the average outbreak lasted 1.3 months, when there were 2'500 packs and 8.7 months, when there were 14'000 packs. The radius of

disease spread was 7 km when there were 2'500 packs and 30 km when there were 14'000 packs.

**Scenario C:** When the probability of transmission was increased to a high value, such as 40%, the percentage of infected packs remained below 0.1%, when the home range size was 9 $km^2$. There was a 4.6-fold increase in the percentage of infected packs associated with the increase in population size from 2'500 to 14'000 packs. The average outbreak lasted 1.3 months when there were 2'500 packs and 4.7 months when there were 14'000 packs. The radius of disease spread ranged from 6 km to 20 km when there were 2'500 packs and 14'000 packs, respectively.

**Scenario D:** When the home range size was increased to 25 $km^2$ (leaving the probability of transmission at 40%), the percentage of infected packs from the total population increased from 0.3% to 7.1% when there were 2'500 and 14'000 packs, respectively. This corresponded to a 27-fold increase in the percentage of infected packs. Accordingly, the average outbreak lasted 4 months when there were 2'500 packs and 10.5 months when there were 14'000 packs. The radius of diseases spread was 20 km when there were 2'500 packs and 50 km when there were 14'00 packs.

Increasing the critical value for a suitable home range also increased the size and the duration of the outbreak. This was because the effect of increasing the minimal suitability value for home range acceptance lead to a concentration of the packs into areas of high wild boar suitability (e.g. central Mittelland, Napf, northern part of the Jura Mountains). Thus when an outbreak occurred in such a high density area, the outbreak was likely to be large.

The variation (standard error) in the percentage of infected packs increased with the average percentage of infected packs and was thus lowest in scenario A and highest in scenario D. In scenario A, the variation in the percentage of infected packs increased 3.5-fold, when the number of packs was increased from 2'500 to 14'000. In scenario C, the corresponding increase was 37-fold. In conclusion, the introduction of one infectious pack into a population of susceptibles at different locations resulted in a considerable variation in the percentage of infected packs, the duration of the outbreak and the radius of disease spread. But the magnitude of the simulated outbreak depended primarily on the size of the home range and on the probability of transmission.

## 3.5 Discussion

The composition of the landscape was used repeatedly in predicting the distribution of wildlife (Howells and Edwards-Jones, 1997; Schadt et al., 2002; Hausser, 1995; Zimmermann and Breitenmoser, 2002). We predicted the probability of wild boar occurrence — or the suitability of the landscape for wild boar — in Switzerland, based on a multivariate logistic regression of the estimated wild boar population density on various landscape characteristics. Wild boar live in home ranges of 8-30 $km^2$ in Switzerland (Baettig, 1993). Migrations exceeding 10 km are infrequent and are mainly performed by young males, when they reach sexual maturity (Geiser and Bürgin, 1998; Spitz, 1992). In contrast to other forest-dwelling wildlife species such as European lynx, the connectance of large forest areas (Schadt et al., 2002) may not be the major determinant of the probability of occurrence in wild boar.

Hence, our small-scale approach, based on geographic data at the scale of 1:25'000 and comparing landscape patterns in relatively small areas of around 700ha appears useful in the prediction of the landscape's suitability for wild boar.

The wild boar population density was estimated by the number of wild boar killed by hunters during a two-year period. Based on the resulting probability map, we explored the effect of introducing one infectious wild boar pack into a population of susceptibles in terms of the maximal radius of disease spread, and both size and duration of the outbreak using a simple, spatially explicit transmission model.

### Limitations of the regression model

A high probability of wild boar occurrence was associated with a large percentage of forest, a large maize cultivation area and a large variety of highly intermixed patches.

The use of hunting based data as a surrogate for population data might be confounded by factors which influence hunting intensity apart from wild boar density, such as the accessibility of hunting areas or socio-economic reasons. In some cantons, hunters have to pay for damages in corp fields caused by wild boar, while in other cantons such damages are payed from other resources (Anonymous, 2004). Further, the regression model was based on the assumption that the absence of wild boar killed by hunters can be explained by a combination of landscape variables. When there are few wild boar present in the hunting area, hunters may not kill a wild boar every year. But the probability of misclassification induced by small numbers of wild boar killed per hunting area was decreased by adding up the hunting bags over both years we had data from.

There was a large variation in the size of the hunting areas included in the regression analysis. Although 'size of the hunting area (ha)' was not statistically significant in the multivariate regression model, the size of the hunting area was likely to affect some of the landscape variables, such as 'diversity index' or 'interspersion'. This in turn is related to the fact that, in the analysis, we treated each hunting area as a separate landscape. This implied that a particular hunting area (landscape) with a small forest area at its edge did not 'see' whether it adjoined a large forest area in the adjacent hunting area. As (i) patch-sizes approximated the size of the hunting area and (ii) the distance between patches approximated the dimensions of the hunting area (e.g. west-east extension), the same patch appeared in several hunting areas. The subdivision of a region into many small hunting areas could thus yield a higher patch density, and possibly a higher diversity, than a single, large hunting would have yielded in the same region.

The regression model predicted a similar probability of wild boar occurrence in hunting areas where a large numbers of wild boar were killed by hunters as in hunting areas where moderate numbers of wild boar were killed by hunters. A larger observed probability of wild boar occurrence than was predicted by the model could result from the presence of other landscape elements, such as slope, the species composition of forests, or from other factors, such as the additional feeding of wild boar by hunters, which were not included in the model. In addition, hunting areas with neighboring hunting areas where large numbers of wild boar are present might have a larger probability of wild boar occurrence than hunting areas surrounded by neighbors where wild boar are absent. Thus, there might be variability among

hunting areas which is associated with their spatial location, in addition to their properties described by the included landscape variables. In other regions, the model predicted a higher probability of wild boar occurrence than was observed, mainly along the Jura Mountains, south of the highway crossing Switzerland from west to east. This may suggest that these regions are associated with a high probability of wild boar occurrence, but have not yet been colonized.

The area where wild boar were killed during the 2-year period analyzed corresponded to about 30% of the area which was associated with a probability of wild boar occurrence greater than zero. Thus, the carrying capacity for wild boar in Switzerland is probably not attained yet. This would be in accordance with the finding that wild boar are in the process of immigrating into the area between the Jura Mountains and the Alps and with the trend of increasing numbers of wild boar killed by hunters (Anonymous, 2002a). While the effect of colonizing additional areas can be unraveled by long-term monitoring, the predictions obtained by regression models, based on hunting statistics for a single year could be improved by incorporating the spatial location of hunting areas.

In Switzerland, highways are fenced and thus pose a severe obstacle to wild boar migration. On the map of the probability of wild boar occurrence, the course of the highways was vaguely apparent in places where the probability of wild boar occurrence was high on both sides of the highway (e.g. Ober-Aargau). However, in general, the barrier-function of the highways was underestimated by the regression model. This is because hunting areas containing main road fragments also contained other landscape elements. This effect increased as the size of the hunting area increased relative to the length of the main road fragment. In order to allow the connectance and genetic exchange among wildlife sub-populations, there exist several wildlife corridors (i.e. green bridges across main roads, (Anonymous, 2001)). The barrier-effect of main roads and the 'gaps' constituted by wildlife corridors was taken into account in the transmission model.

### Limitations of the transmission model

The map of the probability of wild boar occurrence which resulted from the regression analysis, was extrapolated to a regular lattice of 1 $km^2$ cells. This new map represented the landscape, based on which disease outbreaks caused by an infectious pack introduced in a population of susceptibles were simulated. On this map, the areas with a high probability of wild boar occurrence appeared more closely connected by intermediate habitat than on the original hunting area map. This can be explained by the fact that, in large hunting areas with a comparatively low probability of wild boar occurrence, the distribution of both patch types and patch sizes was highly inhomogeneous. This was most conspicuous in the pre-alpine and alpine regions, where hunting areas were large and contained a comparatively large proportion of unsuitable area. Therefore, the strip of unsuitable area caused by the Alps was narrower on the $km^2$-map than on the original hunting area map. In addition, the above mentioned limitation due to large variations in scale applies also here: the regression coefficients based on hunting areas may divert from the regression coefficients which would have been obtained from a regression based on a regular lattice of 1 $km^2$ cells instead of hunting areas.

The wild boar population estimates were extrapolated to the situation, where wild boar occupy the entire area where the regression model suggested a probability of wild boar occurrence greater than zero. This would be approximately three times the area occupied by wild boar in 2001/2002. This potential area also included areas in the pre-Alps which might be less intensively colonized by wild boar, than lowland areas. In addition, renewed efforts in the management of wild boar population in Switzerland may not allow a threefold increase in population size (Anonymous, 2004). The maximal population size assumed in the transmission model might thus over-estimate the potential wild boar population in Switzerland.

With the probability of transmission of 2%-4% estimated from the literature (Hone et al., 1992), our model did not produce any disease-outbreaks (i.e. the transmission to at least one susceptible pack). A reason for this may be that the estimated pack density was lower than the population density on which (Hone et al., 1992) based their estimate. In the simulation, packs were distributed across the landscape, according the the probability of wild boar occurrence, such that a higher than average pack density was possible in locations with a high probability of wild boar occurrence. However, the differences in the cell's probability of wild boar occurrence might not have been large enough to allow for the occurrence of sufficiently large local aggregations of wild boar packs for the chain of transmission to be maintained when the probability of transmission was below 10% and the home range was assumed to be 9 km $^2$. The radius of disease spread produced by scenario D (20-50 km, home range=25 km$^2$, probability of transmission=40%), was in range with the radius of disease spread described for rabies in fox in Switzerland, which was 25-60 km per year (Muller et al., 2000), whereby the distance between subsequent rabies cases was found to be less than 5 km.

In our preliminary model, the initially infectious pack was placed at a randomly chosen location. By repeating the procedure of placing an infectious pack at a random position and simulating the resulting outbreak under different conditions (probability of transmission, population density) in the area of Switzerland north of the Alps, it appeared that the entire area north of the Alps was connected, such that a contagious disease in wild boar could spread across the entire area within a few years. However, this hypothesis was based on the assumption that (i) wild boar crossed the highways by surpassing tunnels and wildlife bridges and under-passing bridges and that (ii) outbreaks started at every position with equal probability. In real life, it is likely that the risk of introducing a disease into free-living wild boar varies geographically (e.g. proximity to neighboring countries where the disease occurs). We therefore suggest to establish a risk-based surveillance system, in which additional data can be included to decide where to collect how may samples. The present transmission model could be extended to include additional data, such as the geographic location of outdoor-reared pigs and trade-routes (wild boar/pig meat and meat products). But more reliable estimates of the wild boar population size, wild boar demography, migration distances and migration routes are required in order to validate the model and thus, to get valuable predictions. Such data could be gained from a national capture-recaptures study including radio-tracking of individual wild boar. At a smaller scale, such a study is currently carried out in the region of Geneva (also including French territory) (Anonymous, 2002d).

The transmission model itself could be improved by allowing the home range size

to adapt itself to the landscape — i.e. a larger home range when the pack is placed in a location with a relatively low probability of wild boar occurrence Further, packs may be allowed to migrate long distances (e.g. 10-30 km, (Spitz, 1992)) within one month, instead of being restricted to a distance of 1 km (i.e. one of the adjacent cells).

## 3.6 Conclusions

Based on a multivariate regression of the estimated wild boar population size on various landscape characteristics and a simple, spatially explicit transmission model, we suggested the use of two sampling region regions for the current sampling protocol: north and south of the Alps. Nevertheless, since it is unlikely that a disease outbreak would affect the entire area north of the Alps within one year, we suggested to establish a risk based surveillance system. In such a system, the model's predictions and additional data on the risk of spillover from wild boar to domestic pigs could be included into the decision of where to collect how may samples. But better estimates of the wild boar population size, as well as data on wild boar demography and migration, as well as disease-related data are required to validate the transmission model.

Table 3.1: Variables used in the prediction of the probability of wild boar occurrence.

| Variable | Description |
|---|---|
| Altitude | Mean altitude above sea level (meters) |
| Streets | Street density (meters per hectare) |
| Maize | Maize-cultivation area ($kg^{-ha}$) |
| Area | size of the hunting area (ha) |
| Percentage of area(c) | Percentage of the area covered by the focal patch type (percent) |
| Patch density (c,l) | Number of patches per 100 hectares. Maximizes when (c): every other cell is of the focal patch type (i.e. in a checker board manner); (l): every cell is of a separate patch type |
| Edge density (c,l) | Sum of the lengths of all edge segments involving the corresponding patch type. Minimizes when the entire landscape consists of (c): the focal patch type: (l): a single patch type (m/ha) |
| Proximity | Median of size and proximity of all patches whose edges are within a search radius of 100m from the focal patch |
| Nearest-Neighbor (c,l) | Median of the Euclidean distance to the nearest neighboring patch of the same patch type (meters) |
| Clumpiness (c) | Frequency with which different pairs of patch types appear side-by-side on the map. It equals 0 when the focal patch type is distributed randomly; approaches 1 when the patch type is maximally aggregated and -1 when the focal patch type is maximally disaggregated |
| Interspersion (c,l) | (Interspersion and Juxtaposition index) The extent to which patch types are interspersed. (c): approaches 0 when the focal patch type is adjacent to only one other patch type and approaches 100 when the focal patch type is equally adjacent to all other patch types; (l): approaches 100 when all patch types are equally adjacent to all other patch types |
| Contagion (l) | Extent to which cells of a particular patch type are aggregated; approaches 0 when the patch types are maximally disaggregated (every cell is a different patch type) and interspersed (equal proportions of all pairwise adjacencies) and 100 when all patch types are maximally aggregated; i.e., when the landscape consists of single patch (0-100) |
| Diversity (l) | Probability that any 2 pixels selected at random would be different patch types (0-1) |

The predicted probability of wild boar resulted from a multivariate binomial regression of the above variables on the presence/absence of wild boars killed by hunters per hunting area.
(c): class metrics. Computed for every patch type (class) present in the landscape. Class metrics were computed for the ground cover categories 'forest' and 'settlement area'.
(l)=landscape metrics. Computed for the entire landscape mosaic, which consisted of the following patch types: forest, open land, settlement area, orchard, vineyard, marsh and river, unsuitable (rock, glacier, lake). Each hunting area was treated as a separate landscape.

Table 3.2: Variables in the transmission model

| **Parameter** | **Estimate (range)** | **Source or assumption** |
|---|---|---|
| Cell size (resolution of the map) | 1 $km^2$ | core area (Spitz, 1992; Boitani et al., 1994) |
| Critical habitat value | 30%[a] | set arbitrarily |
| Probability of wild boar occurrence | percent | regression of landscape variables estimated wild boar population density |
| Number of packs | 2'500-14'000 | extrapolated from the hunting statistics[b] |
| Home-range size | 9-25$km^2$ | (Baettig, 1993; Spitz, 1992) |
| Probability of transmission | 1-4%[c] | (Hone et al., 1992) |
| Latent period | 2-8 days[d] | (Hone et al., 1992) and references therein |
| Duration of infectiousness | 15 days[d] | (Hone et al., 1992; Dewulf et al., 2001) |
| Loss of Immunity | 0 | (Kern et al., 1999) |

[a]: the probability of wild boar occurrence in the home range needs to be of a minimal quality of 30% of the maximum. This prevents that packs are trapped in a single cell of possible habitat.

[b]: minimal: assuming (i) a 100% annual population increment and (ii) that hunters kill 100% of the annual population increment.
maximal: assuming (i) a 200% annual population increment and (ii) that hunters kill 50% of the annual population increment

[b]: (Hone et al., 1992) estimated the probability of an effective contact (transmission event) to be between 0.00063 $day^{-1}$ and 0.00121 $day^{-1}$. We estimated the probability of transmission per week to be between 2% (0.00063*30*100) and 4% (0.00121*30*100).

[d]: 1 month

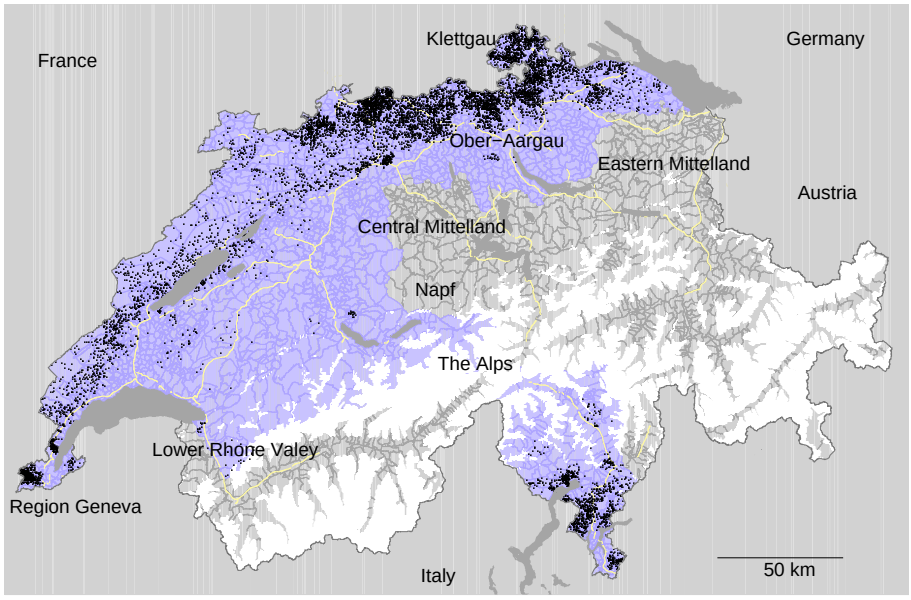


Figure 3.1: Number of wild boar killed by hunters area during two years (2001, 2002). black dots: wild boars killed by hunters per hunting area; white: the Alps (above 2000m above sea level); blue: administrative regions (cantons) from which hunting statistics were obtained.

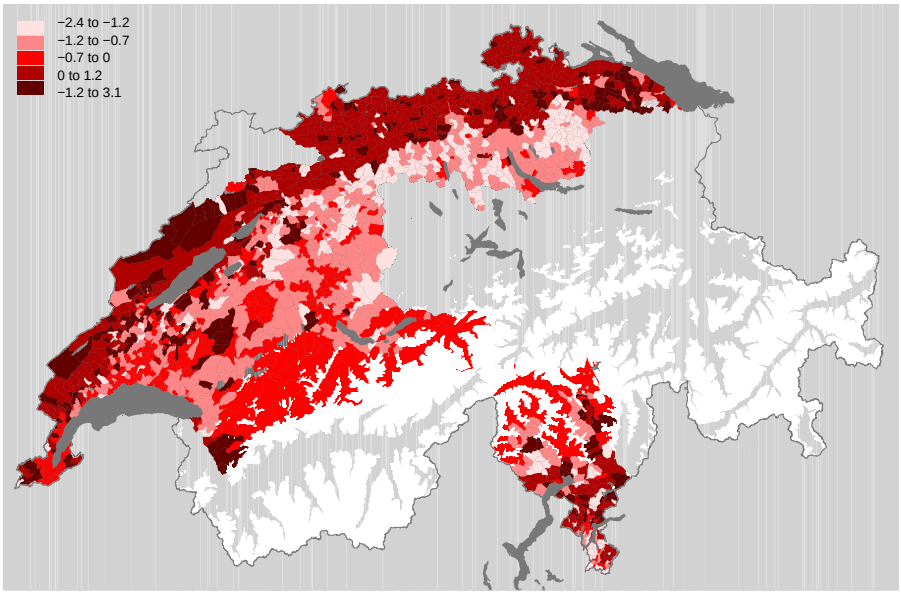


Figure 3.2: Multivariate logistic regression of various landscape characteristics on the estimated wild boar population density: deviance residuals. light colors=predicted value was greater than the observed value, dark colors=predicted value was smaller than the observed value. The values ranged from -2.4 to 3.1.

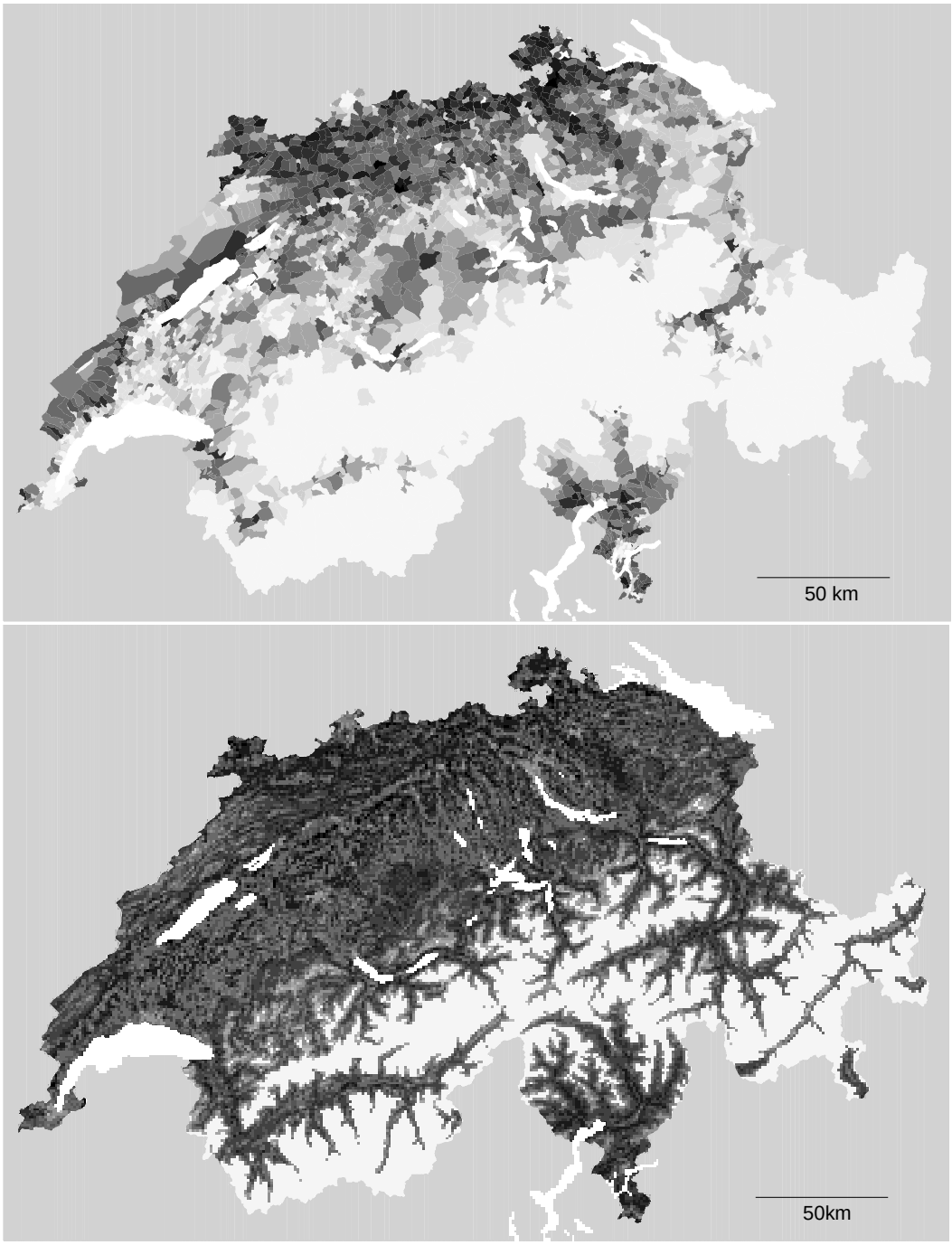


Figure 3.3: Predicted probability of wild boar occurrence. Above: per hunting area. Below: per $km^2$. The probabilities range from 0% (white) to 100% (black).

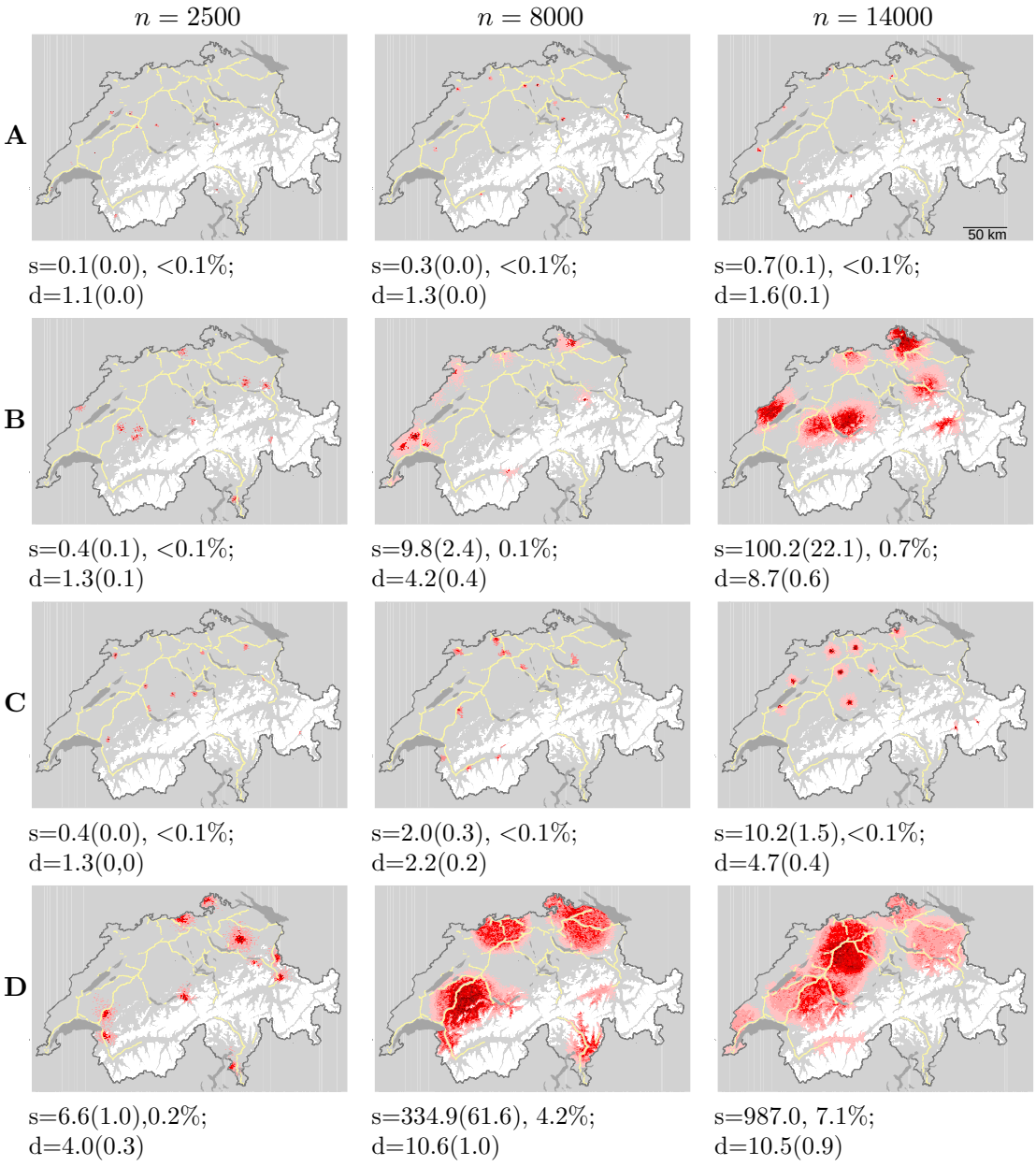


Figure 3.4: Simulated outbreaks resulting from the introduction of one susceptible pack into a population of susceptibles. One simulation consisted of 100 iterations of an outbreak starting from one particular cell. In each image, there are 10 simulations. The mean outbreak size and duration of the outbreak were calculated for each simulation. Then, the mean of the mean and the standard error of the mean was calculated over the 10 simulations. The second mean and standard error were also expressed as the percentage of the total population.
A: home range = 9 km$^2$, probability of transmission = 10%
B: home range = 25 km$^2$, probability of transmission = 10%
C: home range = 9 km$^2$, probability of transmission = 40%
D: home range = 25 km$^2$, probability of transmission = 40%
n: number of packs
s: outbreak-size: number of packs infected during the outbreak (standard error)
d: duration (months) of the outbreak (standard error)

Chapter 4

# Infection status of classical swine fever, Aujeszky's disease and brucellosis

## 4.1 Abstract

Increasing wild boar population sizes throughout Europe gave rise to the concern that infectious diseases with relevance to domestic pigs will persist in wild boar over prolonged periods of time and hence pose a threat to pig farming industries. Therefore, we initiated a surveillance system for such diseases in wild boar, the aim of which was to establish a basis for (i) an early warning system, (ii) documenting the disease status for important pig diseases in Switzerland and (iii) assessing the success of actions targeted at limiting disease outbreaks after disease introduction.

Based on two survey rounds (November 2001 — February 2002; November 2002 — February 2003), we found no evidence for the occurrence of classical swine fever, nor for the occurrence of Aujeszky's disease. We did, however, confirm the occurrence of *Brucella suis* by both serology and bacterial isolation.

## 4.2 Introduction

Several economically important diseases are transmissible between wild boar and domestic pigs. Of a major concern is classical swine fever (CSF), a highly contagious viral disease due to which more than 11 million pigs were destroyed during the epidemic in domestic pigs in the Netherlands in 1997. Wild boar may perpetuate foci of infection with CSF over long periods of time and thus pose a potential threat to the pig farming industry (Artois et al., 2002; Kaden, 1998). Aujeszky's disease (pseudorabies, AD), caused by an alpha-herpesvirus and leading to severe economic loss due to a high lethality in young piglets and stillbirth, is the focus of control programs with the aim of eradicating AD in domestic pigs in many industrialized countries. Although not yet documented under natural conditions, the transmission of AD-virus between wild boar and domestic pigs was shown experimentally (Müller et al., 2001). Occasional transmission from wild boar to dogs was described (Anonymous,

1998; Bastian et al., 1999). The spill-over of AD-virus from infectious wild boar to domestic pigs can thus not be excluded. AD was documented in wild boar in France (Bastian et al., 1999), Spain (Anonymous, 1996; Gortàzar et al., 2002) and Germany (Müller et al., 2000). Porcine brucellosis, a bacterial infection causing abortion and birth of dead or weak young, is wide spread in wild boar in central Europe (Godfroid, 2002) and has re-emerged in outdoor-reared pigs as a result of spillover from wild boar in France (Hars et al., 2000; Godfroid and Käsbohrer, 2002).

In Europe the wild boar population has been constantly growing since the 1970s and is still increasing (Anonymous, 1999b; Briedermann and Rethwisch, 1992). In Switzerland, the annual hunting bag increased from 60 to 6'327 wild boar between 1970 and 2002 (Anonymous, 2002a). At the same time, keeping swine in housing with open front is becoming more and more popular and is promoted by the Swiss government since 1993 (Anonymous, 2002b). The increasing wild boar population densities give cause for concern that newly introduced diseases will persist in wild boar for prolonged time intervals and that the risk of spill-over to domestic pigs will thus increase.

We hypothesize that major outbreaks of devastating contagious diseases in livestock may be prevented or limited in their effects by a surveillance system in wild boar. Such a system allows the early detection of risk areas, but also to assess the impact of actions targeted at preventing the further spread of such diseases. In addition, the need for documentation of the disease status — a prerequisite for countries participating in international trade with animals or animal products — may include wildlife in the future for diseases which persist in wildlife at endemic levels; for instance CSF: (Anonymous, 2000c; OIE, 2003).

Our aims in this chapter were (i) to document the disease prevalence for classical swine fever, Aujeszky's disease and brucellosis and (ii) to develop and apply a surveillance system for contagious diseases in wild boar.

## 4.3 Methodology

### 4.3.1 Sample collection

Blood samples were collected from wild boar which were killed by hunters: November 2001 to February 2002 (sampling round 1), November 2002 to February 2003 (sampling round 2). In sampling round 1, blood samples were collected in 5 out of the 26 administrative regions (cantons) of Switzerland. In sampling round 2, all cantons (n=10) where wild boar are abundant were included (criterium: >100 wild boar hunted on average during the years 1997 through 2000 (Anonymous, 2002a). Hunters were informed and provided with both numbered tubes and data sheets before the sample collection. They were asked to take a blood sample from each hunted wild boar, fill in the corresponding data sheet (date of hunting, hunting area, sex, estimated age and weight of the wild boar) and send both blood sample and data-sheet to the Institute of Veterinary Bacteriology, Berne, Switzerland, where the blood was centrifuged and the serum was stored at $-20°C$ until analysis.

In order to isolate *Brucella suis*, both the spleen and reproductive organs were collected in addition to the serum in sampling round 2. The collection of these

organs was confined to the western part of Switzerland where antibodies against *B. suis* were found in sampling round 1.

### 4.3.2 Diagnostics

#### Classical swine fever (CSF)

Antibodies against CSF virus were detected by an in-house indirect enzyme-linked immunosorbent assay (ELISA) at the Institute of Virology and Immunoprophylaxis (IVI), Mittelhäusern, Switzerland (Moser et al., 1996). In sampling round 2, samples testing positive in this ELISA were tested by a second ELISA (CHECKIT-CSF-SERO, Dr. Bommeli AG, Switzerland, which became available in the mean time. Samples testing positive in both the in-house ELISA and the commercial ELISA were confirmed by a virus-neutralization test (VNT) for CSF. In order to exclude CSF-positive results due to cross reactivity with antibodies against border disease or bovine viral disease, samples positive in the CSF-VNT were additionally tested by a VNT for border disease and a VNT for bovine viral diarrhea virus, respectively.

Since only samples testing positive in the first test (in-house ELISA) were tested by further tests, the sensitivity of the combined testing procedure equaled the sensitivity of the first test and the specificity of the combined testing procedure equaled the specificity of the last test. The sensitivity of the in-house-ELISA was estimated to be 98%. The specificity of the VNT was estimated to be close to 100% (estimates provided by the IVI).

#### Aujeszky's disease (AD)

Antibodies against AD virus were detected by a commercial ELISA (CHECKIT-Aujeszkytest II, Dr. Bommeli AG, Switzerland) at the IVI. AD-ELISA-positive samples were confirmed by a VNT. Samples positive in the VNT were further analyzed by Western blot at the German national AD reference laboratory (Bundesforschungsanstalt für Viruskrankheiten, Wusterhausen).

Since only samples testing positive in the first test (AD-ELISA) were tested by further tests, the sensitivity of the combined testing procedure equaled the sensitivity of the first test and the specificity of the combined testing procedure equaled the specificity of the last test (Western Blot). The sensitivity of the AD-ELISA was estimated to be 100%. The specificity of the Western Blot was estimated to be close to 100% (estimates provided by the IVI).

#### Brucellosis

Antibodies against *Brucella suis* were detected by a commercial ELISA kit (Dr. Bommeli AG, Switzerland) at the Institute of Veterinary Bacteriology, Berne, Switzerland. This test recognizes Ig-G1 (expressed at an advanced stage of infection), similar to the Rose−Bengal test and the complement fixation test. The sensitivity of the ELISA was recorded to be almost 100% in wild boar and thus higher than the sensitivity of both the Rose−Bengal test and the complement fixation test(Boué et al., 2002). The specificity of the ELISA was recorded to be 99.3% in domestic

pigs (Dr.Bommeli Diagnositcs AG, 2003) and almost 100% in wild boar (B. Garin-Bastuji, personal communication).

*Brucella*-bacteria were isolated from the reproductive organs and from the spleen using standard protocols (Institute Galli-Valerio, Lausanne, and Institute of Veterinary Bacteriology, Berne). All *Brucella*-like cultures were tested for *B. suis* by polymerase chain reaction (PCR) at the Institute of Veterinary Bacteriology, Berne. In the PCR reaction, four primers complementary to the sequence reported for *B. abortus* omp2 locus were used (Sifuentes−Rincón et al., 1997). The genetic variation at the omp2 locus, which is composed of two genes encoding for outer membrane proteins, was used to differentiate *B. suis* from *B. melitensis*. This was achieved by a restriction-fragment-length-polymorphism (RFLP) reaction using the Pst1 restriction enzyme (Ficht et al., 1990). The biovar of *B. suis*-positives was differentiated at the French OIE Reference Laboratory (Agence Francaise de Sécurité Sanitaire des Aliments, Cedex).

### 4.3.3 Statistical and geographical analysis

The spatial location of the blood sample collection sites was approximated by the hunting area recorded in the corresponding data sheet. In some cantons, the hunting areas corresponded to municipalities. The sampling locations (hunting area) were mapped using ArcView3.2 (Environmental Systems Research Institute, Inc 1999). The annual hunting statistics were obtained from all cantons where blood samples were collected (in Canton JU only for sampling round 1). The interval of a hunting year was standardized to 'April - March' . The percentage of samples obtained of the annual hunting bag with corresponding exact binomial 95% confidence intervals was calculated per canton (STATA 8.0, Stata Corporation, 2003).

The antibody prevalence (apparent prevalence) for CSF, AD and brucellosis was calculated with 95% binomial exact confidence intervals per canton (STATA 8.0, Stata Corporation, 2003). Differences between both sampling rounds in the proportion of ELISA-positive samples were assessed by the $ch^2$-test or the Fisher's exact test, when there were 5 or less observations per category. The corrected prevalence, which is a function of both sensitivity and specificity of the diagnostic test, was calculated based on the formula TP=$\frac{AP+Sp-1}{Se+Sp-1}$, where TP=true prevalence, AP=apparent prevalence, Se=Sensitivity, Sp=Specificity (Levy and Lemeshow, 1991; Cameron, 1999). We calculated the true prevalence separately for the two sampling regions identified in Chapter 3: north and south of the Alps.

## 4.4 Results

### 4.4.1 Samples

A total of 1'999 blood samples was obtained from 10 administrative regions (cantons) in Switzerland: 744 in sampling round 1 and 1255 in sampling round 2. The hunting area of origin was specified for 710 (95.4%) of the samples in sampling round 1 and for 1'239 (98.6%) of the samples in sampling round 2. In the subsequent analysis, we only included the 1'949 samples for which the hunting area of origin could be identified. Most of the samples (70%) were collected between December and January

(Figure 4.1). In 8 out of the 10 cantons the age categories '<1 year' and '1-2 years' were predominant (Figure 4.2 on the next page).

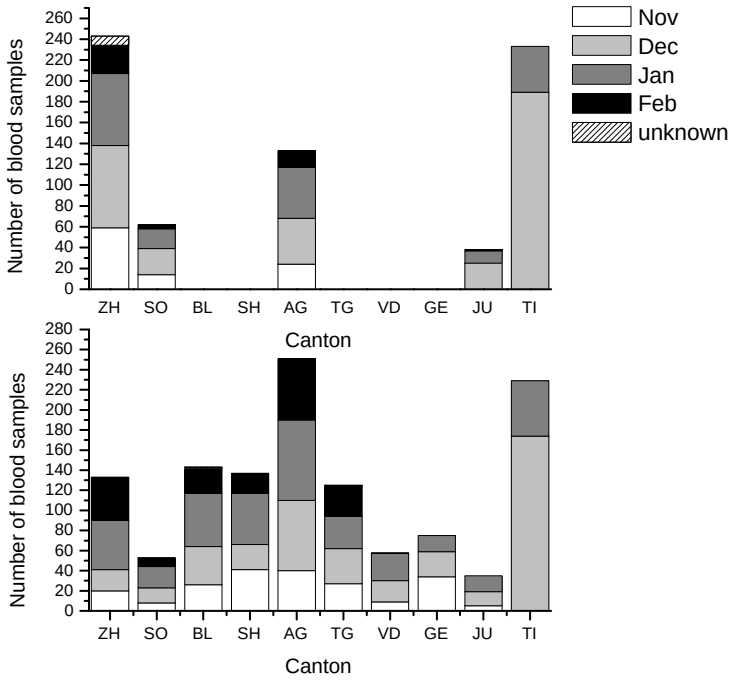


Figure 4.1: Month of blood sample collection per canton. Above: hunting year 2001/2002, below: hunting year 2002/2003.

The estimated weight of the sampled wild boars was on average 43.4 kg (95%CI: 41.9-44.8, n=670) in sampling round 1 and 42.2 (95%CI:41.1-43.4, n=1174) in sampling round 2. Age was correlated with weight (sampling round 1: spearman's r=0.82, p=0.000, n=649; sampling round 2: spearman's r=0.84, p=0.000, n=1123). The number of samples taken from female wild boar was similar to the number of samples taken from males (sampling round 1: females=358, males=343, not specified=9; sampling round 2: females=576, males=654; not specified=9).

On average, a blood sample was obtained from 16% of the annual hunting bag of the cantons where samples were collected in sampling round 1. In sampling round 2, a sample was obtained from 22% of the wild boar recorded in the corresponding annual hunting bag (Table 4.1 on page 39). Hunting areas ranged in size from 1 to 9'656 hectares (median=697) and in altitude above sea level from 193 to 2601 m (median=539). In sampling round 1, the samples originated from 204 out of 352 (58%) different hunting areas where wild boars were killed by hunters during the corresponding hunting year . In sampling round 2, the samples originated from 309 out of 611 (51%) of the hunting areas where wild boar were killed by hunters during the corresponding hunting year (Figure 4.3 on page 40).

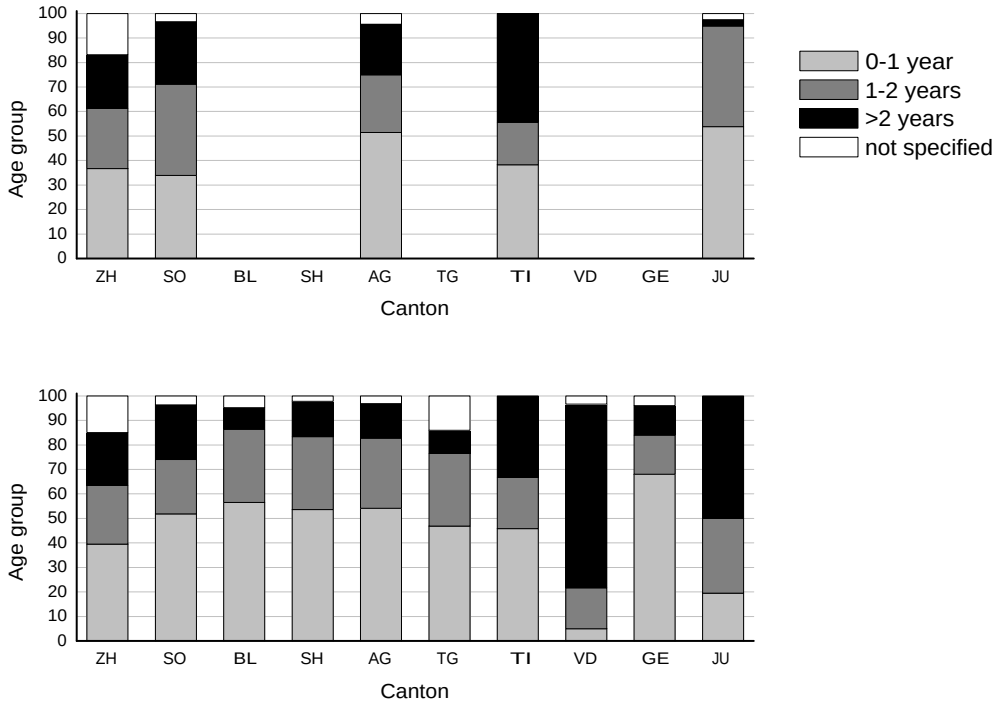


Figure 4.2: Wild boar blood samples collected per age group

### 4.4.2 Prevalence Estimates

#### Classical Swine fever (CSF)

The apparent prevalence assessed by the in-house-ELISA is presented in Table 4.2 on page 41. The apparent prevalence was higher in the southern region (canton Ticino) than in the southern region (remaining cantons) in sampling round 1 (Fisher's exact test, p=0.000), while the apparent prevalence was similar in both regions in sampling round 2 (Fisher's exact test, p=0.395).

In the southern region, the apparent prevalence was lower in sampling round 1 than in sampling round 2 (Fisher's exact text, p=0257). In contrast, in the northern region the apparent prevalence was higher in sampling round 2 than in sampling round 1 (no positives in sampling round 1; Fisher's exact test, p=0.001). The apparent prevalence was similar in males and females (chi2(1)=0.3, p=0.564).

In the southern region, the apparent prevalence was higher in age group '>2 years' than in both younger age groups, although the difference was statistically significant only in sampling round 1. In the northern region the apparent prevalence was similar in all age groups (Fisher's exact test, p=0.631).

The apparent prevalence was corrected for imperfect tests assuming the sensitivity and specificity of the combined testing procedure (ELISA followed by virus-neutralization test) to be 98% and 100%, respectively. The corrected prevalence in the southern region was 4.6(95%CI=3.1%-6.1%) and 2.3%(95%CI=1.3%-3.2%) in sampling round 1 and sampling round 2, respectively. In the northern region the corrected prevalence was 0.0%(95%CI=0.0%-0.0%) and 1.5%(1.2%-1.9%) in sampling

Table 4.1: Proportion of wild boar sampled in relation to the total annual hunting bag by canton.

| Canton | Sampling round 1 | | Sampling round 2 | |
|---|---|---|---|---|
| | **Number sampled** | **Percentage of annual hunting bag(95%CI)** | **Number sampled** | **Percentage of annual hunting bag(95%CI)** |
| ZH | 234 | 30.7(27.5-34.2) | 132 | 17.2(14.6-20.0) |
| SO | 62 | 22.4(17.6-27.8) | 53 | 14.7(11.2-18.8) |
| BL | — | — | 142 | 21.0(18.0-24.3) |
| SH | — | — | 138 | 24.4(20.9-28.2) |
| AG | 133 | 16.9(14.3-19.7) | 252 | 18.8(16.8-21.0) |
| TG | — | — | 125 | 33.4(28.7-38.5) |
| TI | 243 | 40.1(36.2-44.1) | 229 | 37.4(33.6-41.4) |
| VD | — | — | 58 | 11.2(8.6-14.2) |
| GE[b] | — | — | 75 | 21.1(16.9-25.7) |
| JU[c] | 38 | 29.2(21.6-37.8) | 35 | — |
| Total | 710 | 16.6(15.5-17.8) | 1239 | 22.3(21.2-23.4) |

Sampling round 1: November 2001 - February 2002
Sampling round 2: November 2002 - February 2003
Annual hunting bag: April 2001 - March 2002 and April 2002 - March 2003.
The annual hunting data were standardized to the months April-March.
[a]: hunting data June-July.
[b]: no data were available for 2002/2003.
[c]: hunting data January-December

round 1 and sampling round 2, respectively.

Of the ELISA-positives, 2 out of 8 (25%) were confirmed positive by the virus-neutralization test in sampling round 1, while 2 out of 20 (10%) were confirmed positive in sampling round 2. All the 4 positives originated from wild boar aged above 2 years (2 males, 2 females) in canton TI (Ticino).

### Aujeszky's disease (AD)

The apparent prevalence assessed by the AD-ELISA was higher in the southern region (canton TI) than in the remaining cantons (northern region; ($Chi^2$=28.0, p=0.000) in sampling round 2 but not in sampling round 1 (Fisher's exact test, p=0.629). In both the southern and the northern region, the apparent prevalence was higher in sampling round 2 than in sampling round 1 (Fisher's exact test; p=0.000 and p=0.003 in the southern and the northern region, respectively; Table 4.3 on page 41). The apparent prevalence was similar in males and females ($chi^2$=0.6, p=0.608).

In sampling round 1, 4 out of the 5 positives were assigned to age group '<1 year' (age not specified for the 5th sample). In sampling round 2, there was a lower apparent prevalence in age group '<1 year' than in age groups '1-2 years' and '>2 years' combined ($chi^2$=8.6, p=0.003).

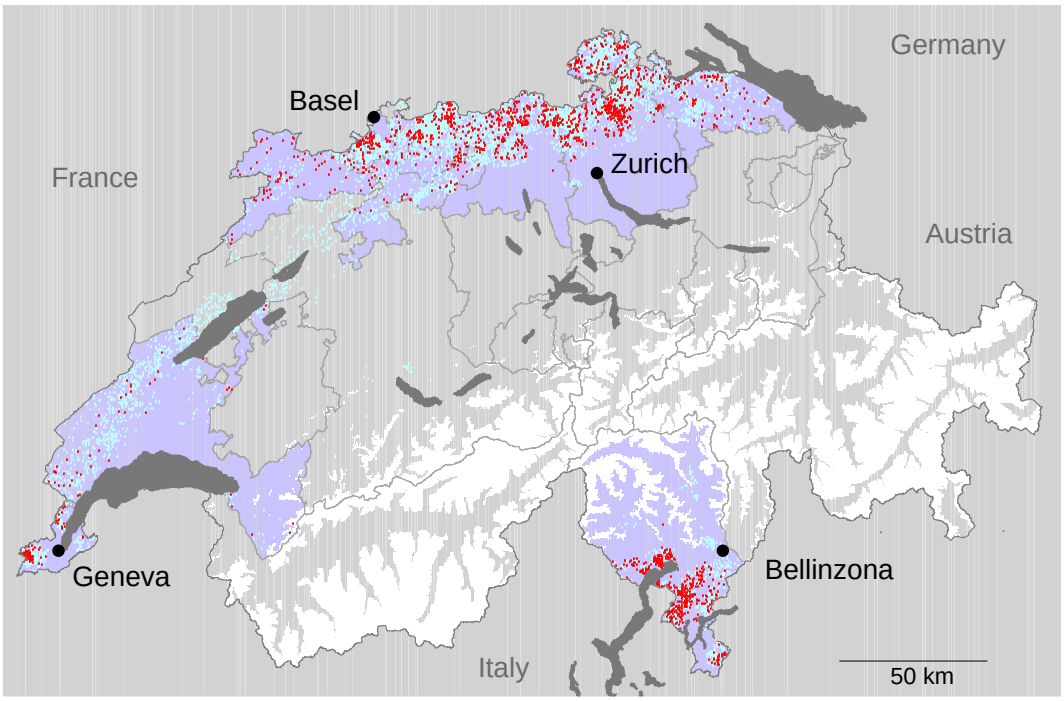


Figure 4.3: Wild boar samples collected during 2 hunting seasons and the annual hunting bags of the 2 corresponding years. Light blue dots: location of wild boar recorded in the annual hunting bag between April 2001 and march 2003. Red dots: locations of blood samples collected during 2 hunting seasons (November 2001-February 2002 and November 2002 - February 2003). Blue: cantons included in the study. White: above 2000m above sea level.

Assuming that both the sensitivity and the specificity of the combined testing procedure were 100%, the corrected prevalence equaled the apparent prevalence. In the southern region the corrected prevalence was thus 1.1%(95%CI=0.1-3.9%) and 11.4%(95%CI=7.4%-16.2%) in sampling round 1 and sampling round 2, respectively. In the northern region, the corrected prevalence was 0.7%(95%CI=0.1-2.0) and 0.6%(95%CI=2.2%-4.4%) in sampling round 1 and sampling round 2, respectively.

None of the 5 ELISA-positive samples in sampling round 1 was confirmed positive by the virus-neutralization test. In contrast, 9 out of 47 ELISA-positive sera (19%) were confirmed positive by the virus neutralization test in sampling round 2. For the remaining 11 ELISA-positive samples,there was not enough serum available to do the virus neutralization test. Of these 9 positives, 6 originated from the southern region, while 3 originated from the northern region. However, none of these 9 positives was confirmed positive by Western blot.

Table 4.2: Apparent prevalence of classical swine fever by canton.

| Canton | Sampling round 1 | | Sampling round 2 | |
|---|---|---|---|---|
| | Number positive /tested | Apparent prevalence (95%CI) | Number positive /tested | Apparent prevalence (95%CI) |
| ZH | 0/225 | 0.0(0.0-1.6)* | 0/132 | 0.0(0.0-0.3)* |
| SO | 0/59 | 0.0(0.0-6.0)* | 2/53 | 0.5(0.5-13.0) |
| BL | — | — | 1/142 | 0.7(0.0-3.9) |
| SH | — | — | 2/138 | 1.4(0.2-0.5) |
| AG | 0/131 | 0.0(0.0-2.8)* | 4/252 | 1.6(0.4-4.0) |
| TG | — | — | 2/125 | 1.6(0.2-5.7) |
| VD | — | — | 1/58 | 1.7(0.0-9.2) |
| GE | — | — | 2/75 | 2.7(0.3-9.3) |
| JU | 0/35 | 0.0(0.0-1.0)* | 1/35 | 2.9(0.1-14.9) |
| Total (north) | 0/450 | 0.0(0.0-0.8)* | 15/1010 | 1.5(0.8-2.4) |
| TI (south) | 8/178 | 4.5(2.0-8.7) | 5/229 | 2.2(0.7-5.0) |
| Total | 8/628 | 2.3(0.6-2.5) | 20/1239 | 1.6(1.0-2.5) |

Due to the separation of the wild boar population by the Alps, the results are given for both the northern and southern populations.
* one-sided Fisher's exact test

Table 4.3: Apparent prevalence of Aujeszky's disease by canton.

| Canton | Sampling round 1 | | Sampling round 2 | |
|---|---|---|---|---|
| | Number positive /tested | Apparent prevalence (95%CI) | Number positive /tested | Apparent prevalence (95%CI) |
| ZH | 2/225 | 1.0(0.1-3.2) | 9/133 | 6.7(3.1-12.5) |
| SO | 0/54 | 0.0(0.0-6.6)* | 2/53 | 3.8(0.5-13.0) |
| BL | — | — | 2/142 | 1.4(0.2-5.0) |
| SH | — | — | 5/138 | 3.6(1.2-8.3) |
| AG | 0/126 | 0.0(0.0-2.9)* | 0/251 | 0.0(0.0-1.5) |
| TG | — | — | 8/125 | 6.4(2.8-12.2) |
| VD | — | — | 3/58 | 5.2(1.1-14.4) |
| GE | — | — | 0/75 | 0.0(0.0-4.8)* |
| JU | 1/35 | 2.8(0.0-14.9) | 3/35 | 8.6(1.8-23.1) |
| Total(north) | 3/440 | 0.7(0.1-2.0) | 32/1010 | 0.6(2.2-4.4) |
| TI (south) | 2/178 | 1.1(0.1-3.9) | 26/229 | 11.4(7.6-16.2) |
| Total | 5/618 | 0.8(0.2-1.9) | 58/1239 | 4.7(3.6-6.0) |

Due to the separation of the wild boar population by the Alps, the results are given for both the northern and southern populations.
* one-sided Fisher's exact test

### Brucellosis

In both sampling rounds, the apparent prevalence of *B. suis* was lower in the southern region than in the northern region (Fisher's exact test, p=0.000 in sampling round

1; Pearson chi$^2$=21.0, p=0.000 in sampling round 2). Within the northern region, the apparent prevalence appeared to be higher in the western than in the eastern part (Figure 4.4 on the facing page, table 4.4 on page 44). The highest apparent prevalence was found in canton JU (where wild boars migrate between France and Switzerland): >30% in both years.

In both regions, the apparent prevalence was higher in sampling round 2 than in sampling round 1 (Fisher's exact=0.057 and Pearson chi$^2$=15.7, p=0.000 in sampling round 1 and sampling round 2, respectively). While in canton JU the increase in prevalence between both sampling rounds was not statistically significant (Pearson chi$^2$=1.8, p=0.179), there was a 3.1-fold increase in the odds of being sero-positive between sampling round 1 and sampling round 2 in the adjacent canton (SO).

In both sampling rounds the apparent prevalence appeared to be higher in females than in males, although the difference was not statistically significant in either sampling round.

The apparent prevalence was similar in all age groups when the data of both sampling rounds were aggregated (Pearson chi$^2$=3.3, p=0.188). However, in sampling round 1, the apparent prevalence was highest in age group '1-2 years' (odds ratio '<1 year' versus '1-2 years' = 2,6 in favor of the latter, chi2=4.9, p=0.027; odds ratio '>2 years' versus '1-2 years'= 4.3 in favor of the latter, chi$^2$=7.4, p=0.007). In contrast, the apparent prevalence (and the odds of being sero-positive) tended to increase with age in sampling round 2 (odds ratio '< 1 year' versus '> 2 years'=1.7, in favor of the latter, chi2=5.8, p=0.016; odds ratio '<1 year' versus '>1 year= 1.4 in favor of the latter, chi2=3.8, p=0.050).

Assuming the ELISA's sensitivity and specificity to be 100% and 99.3%, respectively, the corrected prevalence in the southern region was estimated to be 0.0% (95%CI=0.0-0.7%) and 2.8%(95%CI=1.6%-4.0%) in sampling round 1 and sampling round 2, respectively. In the northern region, the corrected prevalence was estimated to be 6.0(95%CI=4.8%-7.3%) and 14.1% (95%CI=13.0%-15.2%) in sampling round 1 and sampling round 2, respectively. The results were similar, when the specificity was assumed to be 100% instead of 93%.

*Brucella suis* bacteria were isolated from 4 out of 50 reproductive organs (1 uterus, 3 accessory sexual glands). *Brucella suis* bacteria were also isolated from 2 out of 62 spleens. All isolates were confirmed as *B. suis biovar 2*. For both isolates from the spleen, the corresponding ELISA was positive. Of the 4 isolates from the reproductive organs, the corresponding serum was available for 1 wild boar only. The ELISA-result of this sample was questionable.

## 4.5 Discussion

In a serological survey over two consecutive hunting seasons, we found no evidence for the occurrence of both classical swine fever, nor of the occurrence of Aujeszky's disease in wild boar in Switzerland. We did, however, confirm the occurrence of brucellosis (*B. suis*) in wild boar after the first case was recorded near Geneva in 2001 (Anonymous, 2002c).

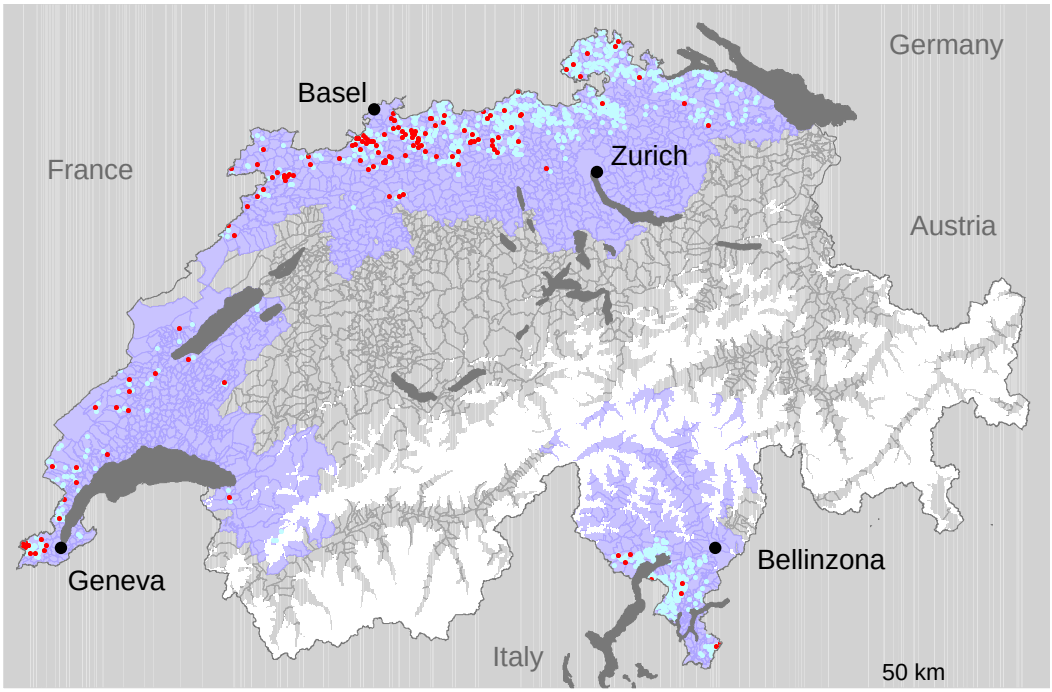


Figure 4.4: Geographic distribution of blood samples collected from wild boar in Switzerland which tested positive for antibodies against *B. suis*. The samples were collected during a survey over two hunting seasons (Nov. 2001-Feb. 2002 and Nov. 2002-Feb. 2003). Light blue dots: samples which were negative in the ELISA; Red dots: samples which were positive in the ELISA; White: area above 2000m above sea level; Blue: administrative regions (cantons) included in the survey.

### Surveys

There were more blood samples associated with a data-sheet in sampling round 2 than in sampling round 1. In addition, the data collected by data-sheets (hunting area and age of sampled wild boar) were more complete and the handwriting was more readable in sampling round 2 than in sampling round 1. This may suggest that the degree of information and motivation among hunters increased between both sampling rounds. However, the quality of the blood samples remained a problem in both survey rounds. Bacterial contamination was mainly a challenge in the diagnostics of AD. In addition, the quantity of serum obtained (1-2 ml) was insufficient when several tests had to be performed on them. Nevertheless, both the ELISA for CSF and the ELISA for brucellosis performed well.

During a sampling period of 4 moths (November – February) we obtained about 20% of the annual hunting bag. The percentage varied between 11% and 40% among cantons. In general, the percentage was highest in cantons which adopt the 'Patent'-hunting system. In these cantons, which are situated in the Eastern and the Southern part of the country, most hunting occurred during the winter months. In addition, less samples were missed due to the fact that hunters were obliged to present their

Table 4.4: Apparent prevalence of brucellosis (*B. suis*) by canton with associated binomial exact 95% confidence interval.

| Canton | Sampling round 1 | | Sampling round 2 | | |
|---|---|---|---|---|---|
| | **Number positive /tested** | **Apparent prevalence (95%CI)** | **Number positive /tested** | **Apparent prevalence (95%CI)** | **Difference (odds ratio)** |
| ZH | 1/181 | 0.6(0.0-3.0) | 1/122 | 0.8(0.0-4.5) | 1.5(0.1-23.9) |
| SO | 7/40 | 17.5(7.3-32.8) | 21/53 | 39.6(26.4-53.4) | 3.1(1.1-8.6)* |
| BL | — | — | 37/141 | 26.2(19.2-34.3) | — |
| SH | — | — | 6/138 | 4.3(1.6-9.2) | — |
| AG | 5/117 | 4.3(1.4-9.7) | 26/252 | 10.3(6.9-14.8) | 2.8(1.0-6.7) |
| TG | — | — | 3/113 | 2.7(0.6-7.6) | — |
| VD | — | — | 15/58 | 25.9(15.3-39.0) | — |
| GE | — | — | 18/75 | 24.0(14.9-35.3) | — |
| JU | 12/34 | 35.3(19.7-53.5) | 18/35 | 51.4(34.0-68.6) | 1.9(0.7-5.2) |
| Total(n) | 25/372 | (6.7(4.4-9.8) | 145/987 | 14.7(12.5-17.1) | 2.3(1.4-3.8)* |
| TI (s) | 2/239 | 0.8(0.1-3.0) | 8/228 | 3.5(1.5-6.8) | 4.3(0.9-20.7)* |
| Total | 27(611) | 4.4(2.9-6.3) | 153(1215) | 12.6(10.8-14) | 2.3(1.4-3.8)* |

The difference between sampling rounds was assessed by the odds of being sero-positive in sampling round 2 compared the odds of being sero-positive in sampling round 1. Statistical significance of the difference in the odds between sampling round 1 and sampling round 2 was assessed by the score test for the trend of odds (*= p-value $< 0.05$).
Total(n)=northern region. In the calculation of the odds ratio, only the 4 cantons which participated in both sampling rounds were included. In these 4 cantons, there were 66 positives (out of 462 tested) leading to an apparent prevalence of 14.3%(95%CI=11.2%-17.8%) in sampling round 2.
TI(s)=cantons Ticino (southern region).
Total: odds ratio calculated for the 5 cantons which participated in both sampling rounds. In these 5 cantons there were 74 positives (out of 690), leading to an apparent prevalence of 10.7%(95%CI=8.5%-13.3%) in sampling round 2.

prey at a check-point for cantonal registration. At these check-points, samples were collected by trained game keepers. In contrast, in the remaining cantons which adopt the 'Revier'-hunting system, samples were taken by individual hunters, and were thus more prone to bias due to regional differences in the hunters's training and motivation. Nevertheless, we did not detect any lacunae in the geographic area sampled when compared to the annual hunting bag by visual inspection (Figure 4.3 on page 40).

Apparent prevalences were calculated per canton because this allowed the results to be easily communicable to both cantonal authorities and hunters. However, cantons share much internal similarity (hunting times, hunting system, traditions), which may render the comparison of the apparent prevalence accros cantons difficult. In addition, these spatial boundaries are artificial and hence not relevant to

disease spread. Differences between adjacent cantons might thus be coincidencial. Nevertheless, since our aim was to unravel the prevalence of classical swine fever, Aujeszky's disease and brucellosis at the national level, we reported the final results (corrected prevalence) for the two sampling units identified in Chapter 3: north and south of the Alps. Wild boar do not cross the Alps and wild boar in Switzerland are thus separated into these two regions.

#### Classical swine fever (CSF)

We identified four samples (0.2%) positive for CSF. All of them originated from wild boar aged above 2 years in the southern region (Ticino), which suggests that these antibodies were probably acquired during the outbreak of CSF wild boar in 1999 in Ticino (Schnyder et al., 2002). No clinical or virological evidence for classical swine fever was found in Switzerland since 1999 and there were no young wild boar (<2 years) found to be sero-positive. Switzerland can thus be considered free from CSF in wild boar according to the Terrestrial Animal Health Code (OIE, 2003).

#### Aujeszky's disease (AD)

None of the 9 sera testing positive in the virus neutralization test was confirmed positive by the western blot. Moreover, the antibody titers obtained by the virus neutralization test were considerably lower than those found in a region where AD occurred in wild boar (<1:12 versus about 1:40, T. Müller, pers. comm.). Therefore, there is no evidence for AD in Switzerland.

While there were 0.8% false positives in the AD-ELISA, the percentage of false positives amounted to 4.7% in sampling round 2. Moreover, the VNT was discovered to be less specific than expected. Based on our results, its specificity was: 1-proportion false positive = 1-($\frac{9}{47}$)=0.809. In addition, the results of the VNT were difficult to interpret in both sampling rounds due to bacterial contamination. The testing procedure could thus be improved by using a second ELISA after the initial screening test, which in turn would reduce the number of VNTs and Western blots to carry out.

### 4.5.1 Brucellosis

The apparent prevalence of *B. suis* was 4.4%(27/611) in sampling round 1 and 12.6%(153/1215) in sampling round 2, respectively. This was lower than the apparent prevalence of *B. suis* found in France (20-25%, (Hars et al., 2000)) and Belgium (39% (Godroid et al., 1994)). Different diagnostic methods were used in both counties (Rose Bengale test and complement fixation test in France; an ELISA detecting wild boar-IgG in Belgium) and these results may therefore not be comparable. The prevalence assessed by bacterial isolation from reproductive organs was 8%(4/50) and 3%(2/62) from the spleen, which was in range with the bacterio-prevalences found in France (Hars et al., 2000) and Belgium (Godroid et al., 1994). The bacterio-prevalence appeared to be higher in the reproductive organs than in other organs. This may suggest that sexual transmission plays a major role in the transmission of *B. suis* in wild boar. The finding that the apparent prevalence of *B.suis* was highest in age groups '1-2 years' and '>2 years', which was also found in France

(Hars et al., 2000), may support this hypothesis. However, a higher prevalence in older age groups than in younger age groups might also suggest that *B. suis* bacteria are no longer circulating in the population.

The apparent prevalence of *B. suis* was higher in sampling round 2 than in sampling round 2. The difference between both sampling rounds was most pronounced in canton JU where wild boar are known to migrate between France and Switzerland and where the apparent prevalence of *B. suis* was recorded to be 30% on the French side of the border (Hars et al., 2000). The apparent prevalence was also above 20% in canton SO (adjacent to JU) and in the western part of Switzerland which also borders France. We had no evidence to assume that the increase in apparent prevalence of *B. suis* between both sampling rounds was due to an increased sensitivity in detecting antibodies. We thus hypothesize that the prevalence of *B. suis* in wild boar in Switzerland is increasing. Nevertheless, whether brucellosis is indeed in the process of spreading will need to be elucidated by future surveys.

Although *B. suis* is widely distributed in the world, the prevalence in domestic pigs is low, with the exception of South-East Asia and South America (Godfroid, 2002). In Switzerland, the apparent prevalence against *B.suis* was highest along the Jura mountains, an area where not many domestic pigs are reared. *B. suis* is less pathogenic in wild boar than in domestic pigs. The infection with *B. suis* in wild boar may thus rarely be noticed. Increasing the awareness among farmers with outdoor-reared pigs may efficiently reduce the risk of spill-over of *Brucella* bacteria from infectious wild boar to domestic pigs — the direct contact between wild boar and outdoor-reared domestic pigs may be reduced by effective fences.

In central Europe, brucellosis infection in both wild boar and brown hare was invariably due to *B. suis* biovar 2 (Godfroid and Käsbohrer, 2002; Büttner, 1996; Haerer et al., 2001). This biovar has only once been recorded as the cause of disease in humans (Godfroid, 2002) and is thus considered to be harmless.

## 4.6 Conclusions

Based on two survey rounds, we did not find evidence for the occurrence of classical swine fever, nor for the occurrence of Aujeszky's disease in wild boar in Switzerland. We did, however, confirm the occurrence of brucellosis *B. suis*) in wild boar. Antibodies against *B. suis* occurred predominantly in the northern and western part of the country, in areas bordering France (were the presence of *B. suis*) had been documented. We suggest to continue the surveillance of brucellosis, in order to determine whether the infection is indeed in the process of spreading. Further, awareness among farmers with outdoor-reared pigs should be improved, in order to prevent the spillover from infected wild boar — for instance by effective fences. Similarly, increasing the awareness for classical swine fever and Aujeszky's disease among hunters may be efficient in the early detection of these diseases.

Chapter 5

# Hunting based control of brucellosis in wild boar: a dynamic transmission model

## 5.1 Abstract

Porcine brucellosis, a bacterial infection causing abortion and birth of dead or weak young, is wide-spread in wild boar in central Europe. Increasing wild boar population sizes throughout Europe gave rise to concerns that the rate of transmission between wild boar and domestic pig may increase and hence pose a threat to pig farming industries. Apart from preventive measures, such as protecting outdoor-reared pigs from contacts with wild boar by fences, the risk of spillover could be reduced by decreasing the prevalence of *B. suis* in wild boar. In the absence of a vaccine, the regulation of the wild boar population size via hunting may be the only approach. We developed a dynamic transmission model, which provides a framework for analyzing the relationship between the prevalence of *B. suis* in wild boar and the number of wild boar killed by hunters per year. based on the limitaed data available, the model predicted that the current brucellosis prevalence of 14% in wild boar in Switzerland north of the Alps would decline to zero within 5 years, when a hunting rate of 50% of the total population would be achieved in the entire region. But the model's predictions need to be validated by additional data on the course of the *B. suis* prevalence, which will become available from future surveys, and by data wild boar demography.

## 5.2 Introduction

Brucellosis is one of the world's major zoonoses (Boschiroli et al., 2001). In industrialized countries, brucellosis in cattle, sheep, goats and pigs is under control and human brucellosis has become rare due to widely applied pasteurization of the milk. Nevertheless, brucellosis can have a considerable impact on human and animal health, as well as wide socioeconomic impacts, especially in countries in which rural income relies largely on lifestock breeding and dairy products (Roth et al., 2003).

In cattle, sheep, goats and pigs, *Brucella*-Bacteria cause abortion and birth of dead or weak young. Transmission occurs orally, via skin injury, mucous membranes or infected sperm. In wild boar, the major route of transmission appears to be during copulation.

*B. abortus* and *B. suis* have been isolated world-wide from a great variety of wildlife species (Godfroid, 2002). In the 1990s, *B. suis biovar 2* was repeatedly reported in wild boar in Belgium, France, and Luxembourg (Godroid et al., 1994; Hars et al., 2000; Godfroid, 2002) but also in Austria, Germany, Portugal and Spain (Godfroid and Käsbohrer, 2002). Porcine brucellosis due to *B. suis biovar 2* has only once been recorded as the cause of disease in humans (Godfroid, 2002). Nevertheless, porcine brucellosis has re-emerged in outdoor-reared domestic pigs as a result of spillover from infected wild boar in France (Hars et al., 2000).

Increasing wild boar population sizes throughout Europe gave rise to the concern that the rate of transmission between wild boar and domestic pig may increase and hence pose a threat to pig farming industries. In Switzerland, the annual hunting bag increased from 60 to 6'327 wild boar between 1970 and 2002 (Anonymous, 2002a). Similar trends were observed in Germany (Anonymous, 1999b; Briedermann and Rethwisch, 1992) and France (Artois et al., 2002). The increase in wild boar population density is attributable to the relatively frequent beech mast and mild winters in recent years, intensified agriculture and, partly, to additional feeding by hunters (Anonymous, 2004). Concurrently, keeping cattle and swine in housings with open front became increasingly popular and is promoted by the Swiss government since 1993 (Anonymous, 2002b).

In the absence of vaccines, regulating the wild boar population via hunting may be the only approach to reduce the prevalence of infection in wild boar. Targeted hunting proved highly efficient in reducing the prevalence of classical swine fever in Ticino (Schnyder et al., 2002; Hofmann et al., 1999). The effect of hunting on the prevalence of infection can be assessed by theoretical models.

There is a large body of literature on both the theoretical and empirical aspects of the transmission dynamics of infectious disease (Anderson and May, 2002; Edmunds et al., 1999) which is aimed at (i) the understanding of observed epidemiological patterns and (ii) predicting the consequences of planned interventions. (Hamer, 1906) postulated that the course of an epidemic depends on the rate of contact between susceptible and infectious individuals. This notion, the so called 'mass action principle', has become one of the most influencal concepts in mathematical epidemiology: the net rate of spread of infection is assumed to be proportional to the product of the density of susceptible individuals times the density of infectious individuals. The principle was originally formulated in a discrete-time model. In 1908 Ronald Ross (the discoverer of malarial transmission by mosquitoes) translated the problem into a continuous-time framework in his pioneering work on the dynamics of malaria (Ross, 1911). The ideas of Hamer and Ross were extended by (Soper, 1929) who deduced the underlying mechanisms responsible for the often-observed periodicity of epidemics, and by (Kermack and McKendrick, 1927) who established the threshold theory. According to this theory, the introduction of a few infectious individuals into a community of susceptibles will not give rise to an epidemic outbreak — i.e. the occurrence of health-related events in excess of normal expectancy (Last, 1988) — unless the density or number of susceptibles is above a certain critical value. From

an early stage, it became apparent that variation and chance were important determinants of the spread and persistence of infection. This led to the development of stochastic theories (Bartlett, 1955; Bailey, 1975).

Dynamic models, which are based on the mass action principle, were recently used to assess economic effects of vaccination campaigns in Mongolia (Zinsstag et al., 2003). Our aim here was to explore the consequences of different hunting rates on the dynamics of porcine brucellosis transmission in wild boar in Switzerland.

## 5.3 Methodology

We developed a deterministic model of porcine brucellosis transmission in wild boar in steps of one year, using the SIR (Susceptible - Infectious - Recovered) model structure. Because only data on sero-positive animals was available, we used one 'sero-positive' compartment instead of the 'infectious' and 'recovered' compartments. The simulated time period was 10 years. Stochasticity was incorporated by using probability distributions instead of point estimates for some of the input parameters.

### 5.3.1 Model

The model was implemented using the Vensim$^{TM}$ systems analysis software (Ventana System Inc. 60 Jacob Gates Road Harvard MA 01451, USA; `www.vensim.com`). The compartmental framework is presented in Figure 5.1 on the next page. The compartment $P$ (sero-positives) represents the estimated number of *B. suis*-positive wild boar in Switzerland north of the Alps. In this region, the majority of the *B. suis*-sero-positive wild boar in Switzerland was found (Chapter 4, Table 4.4 on page 44). The compartment $S$ (susceptibles) represents the estimated susceptible wild boar population in Switzerland north of the Alps. The rate of change in the number of susceptibles ($S$) over time is given by: plus the number born per unit time ($\alpha(S+P)$), plus the number loosing their immunity per unit time ($\delta * P$), minus the number who become sero-positive per unit time ($\beta\gamma SP$), minus the number dying of natural causes per unit time ($\mu S$), minus the number killed by hunters per unit time ($\varepsilon S$). The rate of change in the number of sero-positives ($P$) is given by: plus the number who become sero-positive per unit time ($\beta\gamma SP$), minus the number dying of natural causes per unit time ($\mu P$), minus the number killed by hunters per unit time ($\varepsilon P$), minus the number loosing their immunity ($\delta P$). This relationship was implemented by the following set of differential equations: number equations

$$\frac{dS}{dt} = \alpha(S+P) + \delta P - \mu S - \beta\gamma SP - \varepsilon S \quad (5.1)$$

$$\frac{dP}{dt} = \beta\gamma SP - \mu P - \varepsilon P - \delta P \quad (5.2)$$

where $S$=susceptibles, $P$=sero-positives, $\alpha$=birth rate. Births occur to both susceptible and sero-positive mothers, thus births occur in both the $S$ and the $P$ compartment, but all newborns are considered as susceptible. $\delta$= the rate of immunity-loss, $\mu$= mortality rate, $\varepsilon$=hunting rate, i.e the proportion of wild boar killed by hunters per unit time, $\gamma$=proportion infectious, $\beta$=contact rate, i.e. the probability of an effective contact between an infectious and a susceptible individual.

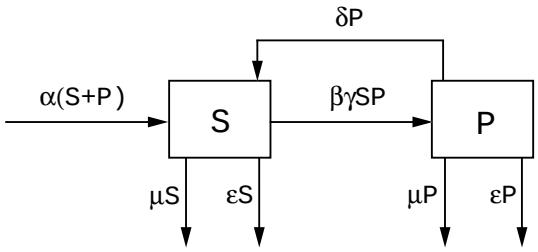


Figure 5.1: Compartmental model of the transmission of brucellosis in wild boar. $S$ = number susceptible, $P$ = number sero-positive, $\alpha$ = birth rate, $\beta$ = contact rate (probability of an effective contact between a susceptible and an infectious individual), $\gamma$ = proportion infectious, $\delta$ = rate of immunity loss, $\mu$ = mortality rate (due to natural causes), $\varepsilon$ = hunting rate (percentage of the total population).

### Parameters

The parameters used are shown in Table 5.1 on the next page. The mortality rate (due to natural causes, $\mu$), the loss of immunity rate ($\delta$), and the proportion infectious ($\gamma$) were based on the literature. The birth rate ($\alpha$), the number susceptible ($S$) and the number sero-positive ($P$) were estimated as follows:

**Birth rate:** According to hunting practice, the annual population increment in wild boar is between 100 and 150%. Maximal values of up to 200% may occur under favorable conditions, such as mild winters, acorn- and beach mast years, access to crop fields (maize, potatoes), and supplementary feeding by hunters (Anonymous, 2004). Nevertheless, such high values are unlikely to represent the general situation across the entire region in Switzerland north of the Alps. We thus based our model on values between 100% and 130%, in order to prevent an unrealistically rapid increase in population size.

**Number of susceptibles:** The initial number of susceptibles ($S$) was estimated from the hunting statistics. The average annual hunting bag, *hb*, in Switzerland north of the Alps was 4898 wild boar (data for 2001 and 2002, (Anonymous, 2002a)). According to hunting practice, the annual hunting bag represents about 50% – 100% of the annual wild boar population increment (Anonymous, 2004). In the following calculations we assumed that, (i) the increment in population size occurred at once, at the beginning of the year and (ii), that all hunting occurred at once, at the end of the year. The population at the start of the year was thus estimated by *hb * annual population increment*. The minimal population size was calculated by assuming that (i) *hb* represented a population increment of 100% and (ii) that hunters killed 100% of the annual population increment. Then, the average population during the year would approximate $\frac{hb+hb*2}{2}$. thus, $\frac{4898+4898*2}{2}$=7347. Under this scenario, hunting would maintain the population size constant in time. The maximal population size was estimated by assuming (i) that the annual population increment was 200% and (ii) that hunters killed 50% of the annual population increment. Thus, *hb**2 approx-

imates the population at the start of the year and $3 * (2 * hb)$ the population at the end of the year, just before the reduction by hunting would occur. The average population during the year would be $\frac{2*4898+3*2*4898}{2}$=19′592. Under this scenario, the wild boar population would double every year. In the model, we assumed an intermediate value of 15′000 minus the number sero-positive.

**Number of sero-positives:** The prevalence of *B. suis* was assumed to be 14%, in accordance with the results of a survey over 4 months in Switzerland in 2002 and 2003 (Chapter 4). The initial number of sero-positives was thus assumed to be $\frac{15'000*14}{100}$=2100.

The parameters were adjusted, such that an endemic equilibrium – i.e the basic reproduction ratio equals one – was attained as the baseline condition. The endemic equilibrium was attained when the hunting rate was set to be 30%. This corresponds to approximately 50% of the annual population increment, when the latter was assumed to be 130%. By the model, we investigated the effect on the brucellosis-prevalence of increasing the hunting rate to 50% and 75% of the population, respectively. Assuming the annual increment in population size to be 130%, a hunting rate of 50% of the total population corresponds to approximately 75% of the annual population increment and a hunting rate of 75% of total population corresponds to approximately 100% of the annual population increment.

Table 5.1: Parameters and definitions.

| **Parameter** | **Estimate** | **Source** | **Specification in model** |
|---|---|---|---|
| reproduction rate, $\alpha$ | 100-130% | (Anonymous, 2004) | Uniform(1,1.3) |
| number susceptible, $S$ | 12'900 | estimated population size minus number sero-positive | 12'900 |
| number sero-positive, $P$ | 2'100 | 14%, Chapter 4 | 2'100 |
| proportion infectious, $\gamma$ | 10-90% | analogous to (Roth et al., 2003) | Normal(0.1,0.5,0.9) |
| loss of immunity, $\delta$ | 0% | analogous to (Roth et al., 2003) | 0 |
| mortality rate, $\mu$ | 20-30% | (Briedermann, 1986) | Uniform (0.2,0.3) |
| hunting rate, $\varepsilon$ | 50-100% | hunting statistics (Anonymous, 2002a), current hunting practice (Anonymous, 2004) | (i) 30%* (ii) 50% (iii) 75% |

The rates are per year.
* baseline condition: brucellosis-prevalence remains at 14% (endemic equilibrium)

## 5.4 Results

The effects on brucellosis-prevalence and on total population size are presented in Figure 5.2 on the facing page. At baseline conditions (hunting rate=30%), the prevalence decreased slightly during the first 9 years, but started to rise rapidly thereafter. The population size increased continously, which, at the same time, resulted in a continuous increase in the number of susceptibles. After 9 years, the threshold was reached beyond which an epidemic was possible. Hence, maintaining the hunting rate at 30% of the population resulted in an approximately 4-fold increase in the total population size within 10 years.

Increasing the hunting rate to 50%, lead to an approximately 50% decrease in prevalence during the first year and a slower decrease during the subsequent 3 years which resulted in brucellosis fading out after 5 years. Accordingly, the shape of the curve of the number of susceptibles was very similar to the shape of the total population's curve. The total population remained almost constant during the 10 years simulated.

By increasing the hunting rate to 75%, the brucellosis prevalence was reduced to close to 0% during the first year and faded out during the second year. Similarly, the number of sero-positives was reduced to zero during the second year. However, also the number of susceptibles, and hence the total population, decreased — wild boar were extinct after 5 years.

## 5.5 Discussion

In a simple, dynamic transmission model, we assessed the effect of hunting rate on brucellosis-prevalence in wild boar in Switzerland north of the Alps over 10 years. A hunting rate of 30% allowed an endemic equilibrium during the first 9 years, while the model predicted a brucellosis epidemic in the $10^{th}$ year. At this time, the number of susceptibles crossed the threshold-population size, which is necessary for an epidemic to occur (Anderson and May, 2002).

In the model, we assumed that wild boar are homogeneously distributed in the landscape. This implies that every individual has the same probability of interfering with any other member of the population. Landscape elements such as highways constitute serious obstacles to wild boar migration. The probability of any two individuals interfering — and thus the probability of an effective contact between an infectious and a susceptible individual — is therefore unlikely to be equal across the study region. For instance, brucellosis may fade out after introduction in a small and isolated population, in which the number of susceptibles may be too small to maintain the chain of transmission, i.e. the susceptible hosts are depleted (become immune) faster than new susceptibles become available by birth or immigration. Nevertheless, the degree of spatial sub-division of the wild boar population in Switzerland north of the Alps is expected to be weak enough to allow diseases with a long duration of infectiousness, such as brucellosis, to spread within the entire area. Nevertheless, the wild boar's probability of crossing highways (by sur-passing tunnels and wildlife bridges or by sub-passing bridges) may not parallel an increase in population size on both sides of the highway. The spread of brucellosis may thus be slower than

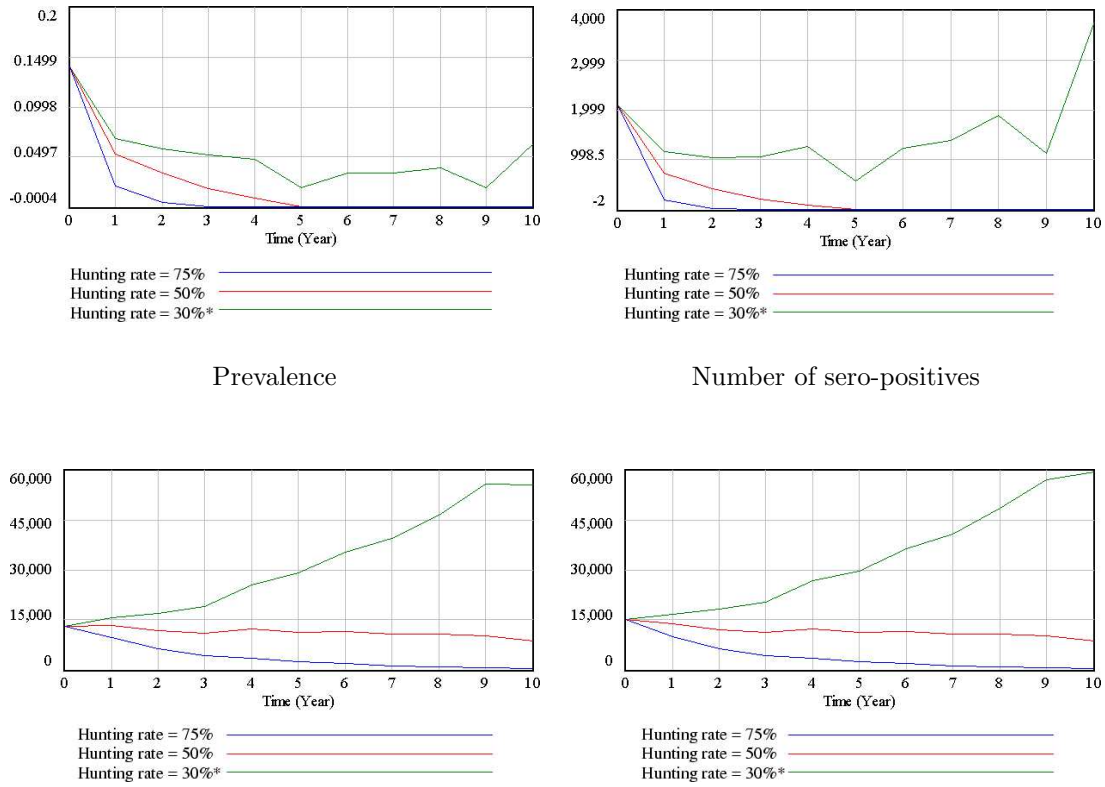


Figure 5.2: Results from a dynamic simulation of brucellosis in wild boar in Switzerland (north of the Alps).
* baseline condition: brucellosis-prevalence remains at 14% (endemic equilibrium).

expected in a homogeneously population of the same size. In addition, a brucellosis-epidemic may affect a smaller percentage of the total population, when the wild boar population is spatially sub-divided than when the population is not spatially sub-divided, Our model may thus over-estimate the effect of increasing population size on the increase in brucellosis-prevalence within 10 years.

For the following observations, we need to bear in mind that the existing epidemiological and deomgraphic baseline data are weak and that a formal validation could not be be done yet. Assuming a hunting rate of 30%, the population increased to approximately four times the initial value within 9 years. In Chapter 3, we found that wild boar occupied about 30% of the area in which wild boar were predicted to occur, based on the average hunting bag in 2001/2002 and on various landscape characteristics. The average population density in the area where wild boar were in fact present was estimated to be 0.9 — 2.4 per km$^2$. This is in accordance with the values provided by the literature: 3 km$^{-2}$ in Germany (Kern et al., 1999), 1 km$^{-2}$ in Sardinia (Guberti et al., 1998), although population densities of 6 or more wild boar km$^{-2}$ were recorded in highly suitable areas in Pakistan (Hone et al., 1992)

and Poland (Jedrzejewski et al., 1994). Thus, after a 3 to 4-fold increase in population size, the carrying capacity in Switzerland is likely to be attained. In 2112, the annual hunting bag would be approximately 18000 — twice as much as in 2002. This would be in accordance with the increasing trend: the annual hunting bag in Switzerland, north of the Alps, increased from 1065 wild boar in 1992 to 5715 wild boar in 2002 (Anonymous, 2002a). Nevertheless, the increase in the hunting bag is expected to reach a plateau, not only when the carrying capacity for wild boar is approached, but also when the hunting efficiency does not parallel the increase in wild boar population size (e.g. not enough hunters).

Increasing the hunting rate to 50% of the total population caused brucellosis to fade out within 5 years. At the same time, the population size remained stable at about 15'000, which is approximately corresponding to the population size in the northern region in 2002. To stabilize the wild boar population is targeted by agricultural and political institutions, in order to reduce the increasing cost due to destruction of crop fields by wild boar (Anonymous, 2004). A hunting rate of 50% translates into a hunting bag which represents approximately 75% of the annual population increment, when the latter is assumed to be 130%. The annual hunting bag is reported by the administrative regions (cantons) to represent 50% to almost 100% of the annual population increment. A hunting rate of 50% of the total population appears thus achievable within Switzerland north of the Alps. Consequently, a reduction in the prevalence of brucellosis by increasing the hunting rate such that the population size stabilizes at the current level may be possible, provided that the influx of infectious wild boar from outside (e.g. immigrating from France) does not exceed the current frequency.

## 5.6 Conclusions

The current model provides a framework for analyzing the relationship between the prevalence of *B. suis* in wild boar and the number of wild boar killed by hunters per year. The model's predictions can be validated when additional data on the progression of the *B. suis* prevalence will become available from future surveys. In addition, more reliable estimates regarding the wild boar population size would considerably improve the practical use of the model. Such data could be gained from a large-scale capture-recapture study.

Chapter 6

# Disease status of tuberculosis in wild boar in Ticino (Switzerland)

## 6.1 Abstract

The presence of bovine tuberculosis was assessed in wild boar in cantons Ticino (southern Switzerland). This region was selected because it was closest to northern Italy, where *Mycobacterium bovis* was isolated from wild boar. We found no evidence for an infection with *M. bovis* in Ticino.

## 6.2 Introduction

Tuberculosis (*M. bovis*) is found in free-ranging wildlife in many parts of the world. Badgers (*Meles meles*), brushtail possums (*Trichosurus vulpecula*), deer (*Odocoileus virginianus*), bison (*Bison bison*) and African Buffalo (*Syncerus caffer*) are maintenance hosts of *M. bovis* which can represent the principal source of infection in both domestic animals and protected wildlife species (Lisle et al., 2001; Morris et al., 1994; Phillips et al., 2003).

In domestic swine and wild boar, *M. tuberculosis* and *M. bovis* cause granulomatous-necrotizing lesions, which are mainly localized in the lymph nodes of the head (Mignone et al., 1991; Biolatti et al., 1992) but also occur in the lungs (Schulz et al., 1992). *M. bovis* in wild boar was first detected in the 1930s in Germany (Kindinger, 1934) and was thereafter found in many countries (Italy, Bosnia-Herzegovina, Spain, New Zealand, Australia, USA; reviewed by (Bollo et al., 2000). Comparisons by spoligotyping of different *M. bovis* strains isolated from wild boar and cattle living in the same region, revealed the presence of a close genetic relationships between strains isolated from cattle and strains isolated from wild boar (Serraino et al., 1999; Aranaz et al., 1996).

Tuberculosis (*M. bovis* and *M. tuberculosis*) was found in wild boar in northern Italy (Liguria) in 1998, after tuberculosis-like lesions in lymph nodes were reported by hunters (Serraino et al., 1999). Since it was shown that infectious diseases can be introduced into Switzerland via wild boar migrating between Switzerland and Italy (Schnyder et al., 2002), our aim in this chapter was to assess the presence of *M. bovis*

in Ticino (southern Switzerland). This region was selected because it is closest to Liguria.

## 6.3 Material and Methods

Submandibulary lymph nodes were collected from wild boar killed by hunters between December 2002 to January 2003 in Ticino (southern Switzerland). The lymph nodes were collected by a veterinarian at two checkpoints where hunters in canton Ticino are obliged to present their prey for official registration. In addition, a blood sample was collected from each wild boar carcass sampled as part of a national surveillance program for classical swine fever, Aujeszky's disease and brucellosis in wild boar (November 2001 - January 2003, Chapter 4). The samples (blood and, if present, the lymph nodes) together with the corresponding data sheet (date and place of hunting, age, sex and weight of the wild boar) were sent to the Institute of Veterinary Bacteriology, Berne, from where the lymph nodes were forwarded to the Institute of Fish- and Wildlife Medicine, Berne.

A Ziehl-Neelsen stain was done on histological sections of each lymph node, while the remaining lymph node material was stored at $-20°$C. Routine histological stains (Gram, Haematoxylin-Eosin, periodic acid Schiff reaction ) were used to better define the lesions suggestive of tuberculosis. Suspicious samples were then sent to the Institute of Veterinary Bacteriology in Zürich were Ziehl-Neelsen staining was repeated on the suspicious frozen lymph node material. Mycobacteria-positive samples were sent to the University hospital Zürich for identification of the mycobacteria species by culturing and polymerase chain reaction (Kox et al., 1995).

According to hunting practice in canton Ticino, the annual hunting bag represents about 80% of the annual wild boar population increment, which in turn is estimated at 100-150% (G. Leoni, personal communication, (Anonymous, 2004)). The average hunting bag in the years 2001 and 2002 was 600 wild boar (Anonymous, 2002a). Hence the estimated population size in the study period approximated 750 heads.

The adjusted prevalence, which takes into account both sensitivity and specificity of the diagnostic test procedure, was calculated based on the formula CP=(AP+Sp-1)/(Se+Sp-1), where CP=corrected prevalence, AP=apparent prevalence, Se=Sensitivity, Sp=Specificity (Levy and Lemeshow, 1991; Cameron, 1999). The specificity of the combined testing procedure was estimated to be 100% while we were unable to estimate its sensitivity. We therefore tested different scenarios using values between 10% and 100%. Since the prevalence of *M. bovis* was found to be above 4% in affected areas (Biolatti et al., 1992; Bollo et al., 2000), the probability for freedom from disease was calculated for a threshold level of 4% (Cameron, 1999). Thus , the population would qualify as 'free from *M. bovis*' when the prevalence detected by the survey was below 4%.

## 6.4 Results

Lymph nodes were obtained from 69 wild boar, which corresponds to 30% of the wild boar recorded from this area in the national surveillance program for classical swine fever, Aujeszky's disease and brucellosis (November 2002 to February 2003, n=233)

and to 37% of the corresponding annual hunting bag (Chapter 4), (Anonymous, 2002a)). Among the 62 wild boar for which a data sheet was obtained, the lymph nodes originated from 28 males and 34 females. Twenty-six (42%) of the samples originated from wild boars aged less than 1 year, 15 (24%) from age group '1−2 years' and 21 (34%) from age group '>2 years'. In Ticino, the geographic area in which lymph nodes were sampled was the same as the area covered by the national surveillance program for classical swine fever, Aujeszky's disease and brucellosis (figure 6.1).

The lymph nodes of 3 wild boar (4.3%) showed necrotic or calcified changes (2 males, '>2 years'and '<1 year', respectively and 1 female, '<1 year') from all of which *M. avium-intracellulare* was isolated. All samples were negative for *M. bovis*.

Assuming the diagnostic test procedure's specificity to be 100% and its sensitivity (since no estimate was available) to range between 1% and 100%, the corrected *M. bovis*-prevalence in a population of 750 wild boar was 0.0%(95%CI=0.0%-0.0%). The sample size was too small to distinguish a population with a prevalence of 4% from a disease-free population.

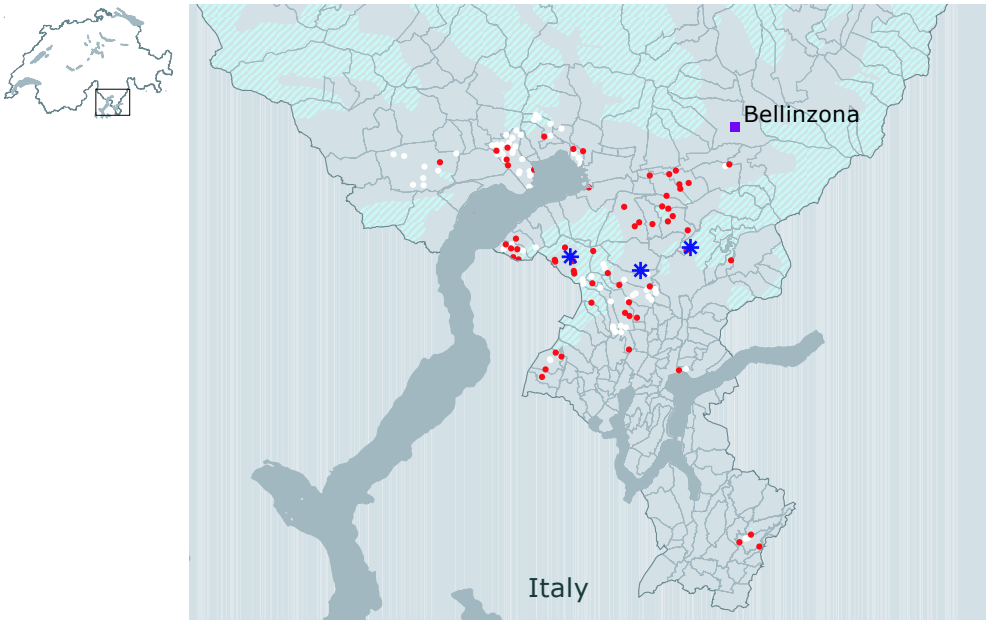


Figure 6.1: Geographic distribution of the mandibulary lymph node samples collected to assess the prevalence of *M. bovis* in wild boar in Ticino (southern Switzerland). Samples were spatially referenced by municipality (gray lines).
Blue stars: samples positive for *Avium−intracellulare*;
Red dots: lymph node and blood sample collected;
White dots: only blood sample collected;
Hatched: alpine pasture (unlikely area for wild boar to occur. The exact placement of the dot within a municipality was random.

## 6.5 Discussion

In a sample of 69 out of a population of approximately 750 wild boars in Ticino (Southern Switzerland) we found no *Mycobacterium bovis* positive samples. The sample size was too small to distinguish a population with a prevalence of 4% from a disease-free population.

In accordance with (Serraino et al., 1999), we restricted the Ziehl-Neelsen staining to the 3 samples where the lymph nodes showed necrotic or calcious lesions; because of the small probability of detecting Mycobacteria by Ziehl-Neelsen staining in lymph nodes without tuberculosis-like lesions. Therefore, while our testing procedure was highly specific, we expected its sensitivity to be considerably lower. Nevertheless, the sensitivity-value did not affect the apparent prevalence of 0.0%. Further, the percentage of lymph nodes with lesions suggestive for tuberculosis was higher in most surveys carried out in regions where an infection with *M. bovis* was present, than in our study population: 11% in Liguria (Bollo et al., 2000), 85% in feral swine in Australia before an eradication campaign in cattle (Corner et al., 1981) and 6% after the eradication campaign (McInerney et al., 1995). We thus concluded that it was highly unlikely for *M. bovis* to occur in canton Ticino.

There is no evidence so far, that the infection with *M. bovis* is self-maintaining in wild boar (Phillips et al., 2003). For instance, in Germany, no spillover of *M. bovis* to grazing cattle from infected wild boar occurred during a 6-year period of observation (Schulz et al., 1992). In New Zealand feral pigs were not capable of maintaining the infection in their own populations in the absence of infected possum populations (Coleman and Cooke, 2001; Corner et al., 1981) and (McInerney et al., 1995) demonstrated that the prevalence of *M. bovis* decreased in feral pigs after the prevalence had been reduced in cattle by an eradication program. Moreover, (Nugent et al., 2002) argued that wild boar could be used as an indicator for the presence of *M.bovis* in cattle.

In all 3 lymph nodes with gross lesions *M. avium-intracellulare* was isolated. *Mycobacterium avium-intracellulare* causes tuberculosis in birds and also sporadically tuberculosis-like lesions in mesenterial lymph nodes of slaughtered pigs. In humans, it is characterized by pulmonary disease, lymphadenitis in children, and systemic disease in AIDS patients (Anonymous, 2003c). The infection is most often acquired from contaminated water or food. *M. avium-intracellulare* survives for years in dried faeces or soil. *M. avium-intracellulare* is not considered relevant for veterinary public health.

## 6.6 Conclusions

Bovine tuberculosis is currently absent from cattle in Switzerland and from wild boar in regions adjacent to Switzerland in neighboring countries. In addition, despite the fact, that *M. bovis* was isolated from wild boar, there is no evidence that *M. bovis* is transmitted from wild boar to cattle. Therefore, we do not recommend to formally prove the absence of *M. bovis* from wild boar at an annual basis.

Chapter 7

# Concepts of disease monitoring and surveillance

## 7.1 Abstract

In the first part, the key concepts relevant to monitoring and surveillance are reviewed: active and passive monitoring, sample size, prevalence, freedom from disease and the consequences of imperfect diagnostic tests on the interpretation of survey-results. In the second part, important issues in the monitoring and surveillance of wildlife are summarized. The chapter closes with a brief review of monitoring and surveillance programs in wildlife in Switzerland.

## 7.2 Background

In 1924, the Office International des Epizooties (OIE) was established by an international agreement signed by 28 countries. By 2001, the membership had risen to 158 member countries. The OIE's main objective was to enable transparency of animal disease status worldwide by elaborating rules for international trade in animals and animal products (Anonymous, 2003e). The OIE is the most important internationl organization concerned with animal disease surveillance.All OIE member states have the obligation to report the occurrence of all infectious diseases, which are limiting international trade in animals and animal products due their socioeconomic or public health importance. Such diseases are classified as either List A, which have the potential for very rapid spread, irrespective of national borders, or list B, which are likely to remain contained within the country where it was detected (Anonymous, 2003d). Examples of List A diseases are foot-and-mouth disease, rinderpest, classical swine fever and Bluetongue. Examples of List B diseases are Aujeszky's disease, bovine brucellosis, bovine tuberculosis, anthrax, and rabies.

The World trade organization (WTO), who began operation in 1995, has adopted the codes of the OIE for guidelines in international trade. The WTO's Agreement on the Application of Sanitary and Phytosanitary Measures (SPS agreement) requires that, in international trade, the justification of measures taken to protect animal, plant or human health, needs to be based on scientific methods such as risk analysis

(Anonymous, 1997). Its main intent is to avoid use of SPS measures as unjustified barriers to trade. Efficient disease surveillance and monitoring systems are thus the basis for trust in international trade in animals and animal products. Animal disease surveillance activities have therefore gained in relevance over the past years.

## 7.3 Reasons for disease monitoring and surveillance

A disease which was absent from a country for generations(e.g. foot and mouth disease in Switzerland), can be introduced by importing infected animals, feeding infectious feed stuff or by spill-over from wildlife. Similarly, a disease may appear in a population for the first time, or increase rapidly in prevalence or geographic range in a area where the disease existed before (emergent disease). In either case, the time until detection will depend on the disease's clinical impact and on awareness, for instance on the presence of veterinarians, farmers and hunters with personal experience concerning the clinical signs of the disease. A disease causing high mortality or morbidity when introduced into an entirely susceptible population, such as avian influenza, will be detected earlier than a disease with a long incubation period or unspecific clinical symptoms, such as BSE. Disease awareness and disease detection are key prerequisites for any surveillance system.

Monitoring of infectious diseases in wildlife is conducted for several reasons: (i) it allows to detect possible risks of spillover to domestic livestock (Coleman and Cooke, 2001; Müller et al., 2001; Phillips et al., 2003) in areas where wildlife such as wild boar, badgers or bush tail possums, which can represent a source of infection for domestic animals, is abundant. The spill-over of diseases from wildlife is of particular concern for livestock industry and governmental authorities, when there is an eradication program in progress or when the region or state is officially declared free from a specific disease. (ii) monitoring is a prerequisite for the demonstration of freedom from disease. For instance, in order to maintain the 'freedom from classical swine fever in wild boar' status, member states are requested to provide serological evidence at an annual basis (Anonymous, 1999a). (iii) A monitoring system allows to detect important zoonoses, which might re-emerge due to spill-over from wildlife reservoirs. For instance, in Queensland (Australia), human brucellosis (*B. suis biovar 1*) is re-emerging due to recreational (hunting) and occupational exposure to feral pigs infected with *B. suis* (Robson et al., 1993). In 1994, *Brucella suis biovar 1* was isolated from a butcher in Belgium who had been handling imported feral pig meat (Godfroid, 2002; Godroid et al., 1994). Globalization in international trade may thus lead to new public health risks.

Monitoring may also allow to detect environmental or conservational issues. For instance, a monitoring program in Sweden revealed the problem of mercury poisoning of wildlife in the early 1950s (Borg, 1966).

## 7.4 Concepts

As mentioned above, the early detection of disease cases is an important determinant of the quality of data obtained form a monitoring system. A high level of awareness can reduce the time period during which an introduced disease may spread unde-

tected. Disease awareness can be achieved by information campaigns or targeted education. In Switzerland, the Federal Veterinary Office plays an active role in this respect by informing the public via print or electronic media as well as specific events, such as testing the concept for managing an outbreak of foot-and-mouth disease by a hypothetical outbreak, analogously to a fire fighting exercise (Geiser, 2002; Perler, 2001).

In the absence of specific control- or eradication programs, veterinary public health-relevant data are collected by routine investigations at the slaughterhouse and by the reporting of important diseases to a central (usually governmental) organization by veterinarians. In wildlife, the regular investigation of voluntarily admitted carcasses is be carried out by specialized laboratories, which also keep records of disease-causes. Such routine based systems, sometimes denoted 'passive monitoring', allow to detect an unexpected rise in disease-prevalence, or to investigate disease outbreaks retrospectively. For instance, the European brown hare syndrome was first observed in brown hares (*Lepus europaeus*) in Scandinavia in the early 1980s (Gavier-Widén and Mörner, 1991). It was not until 1989 that the infectious agent, a calicivirus, was described (Lavazza and Vecci, 1989) and retrospective serological studies demonstrated that the virus had been present in Europe since the 1971 (Moussa et al., 1992).

The detection of an emergent disease or the occurrence of an important infection in a neighboring country may cause an increased interest in a specific disease, which can result in information campaigns and the specific training of veterinary practitioners, laboratory personnel, farmers and hunters, if wildlife is considered. need to be collected in addition to disease-related data, such as serological results. Such systems, sometimes denoted 'active monitoring', also, allow to demonstrate the absence of economically important diseases, such as classical swine fever or Aujeszky's disease. This requires the collection of health-related data, usually collected by means of surveys, and data on both the population size and the population's spatial distribution.

The term 'survey' denotes an investigation or a study in which information is systematically collected for a specific aim or conceptual hypothesis (Salman, 2003). Surveys can be targeted at either the demonstration of freedom from disease or at the estimation of disease prevalence. When surveying to demonstrate freedom from disease, the conclusion of the survey will be that the disease is or is not present. This statement will be judged by a probability that it is correct. The probability is found by testing whether the prevalence detected by the survey is at or greater than the specified threshold prevalence (null-hypothesis), associated with a confidence level (usually 95% or more). Rejecting the null-hypothesis would thus mean that the prevalence detected by the survey is below the specified threshold prevalence with a probability of 95% (Cameron, 1999). Surveys to estimate disease prevalence will produce point estimates of the prevalence, the precision of which is judged by a confidence interval. A monitoring system is thus a series of surveys.

In order to control (reduce the prevalence of a specific disease) or eradicate (eliminate the disease below a specified threshold prevalence) specific measures to reduce the prevalence need to be implemented (treatment, vaccination, stamping out). Therefore, the above-mentioned data need to be collected over a period of time, in order to identify trends in prevalence and to test hypotheses (e.g. whether the preva-

lence decreased after an intervention). Thus, a monitoring system may transition into a surveillance system, if action is taken to prevent or control the disease.

The term 'surveillance' was first used during the French Revolution, when it meant 'to keep watch over a group of persons thought to be subversive'. The term has been used extensively by epidemiologists and animal health professionals. Some authors have proposed the use of the term 'monitoring and surveillance system' to summarize the concepts (Stärk, 1996; Noordhuizen et al., 1997; Doherr and Audigé, 2001; Salman, 2003). In that context, monitoring describes a continuous, adaptable process of collecting data about diseases and their determinants in a given population, but without any control activities. Surveillance is a specific case of monitoring in which control measures are implemented whenever a certain threshold level related to the infection or disease status is exceeded. Surveillance is thus part of any disease control program (Noordhuizen et al., 1997).

### Sampling considerations

A population may be broadly defined as a group of elements which share some characteristic. Examples of a population are thus all fish in the ocean or all wild boar in Europe. A target population (or population of interest) is the part of the population which is at risk of being affected by the condition being studied. Ideally, the target population is identical with the study population. However, for practical reasons, it might not be possible to include the entire population at risk in the study. In a study on foot-and-mouth disease in sub-Saharan Africa, for instance, it may not be possible to include pets or wildlife. Thus, the conclusions drawn from the study population cannot be automatically extended to the target population.

Absolute assessment of a condition or proof of a hypothesis requires the examination of every individual in the study population (census). In that case, there is no uncertainty about the results due to sampling error, other sources of uncertainty, such as measurement error, non-response, or data-management errors may still exist. While a census is appropriate in some circumstances (e.g. population census in a country), its main disadvantage is that it is slow and expensive. Further, it may be practically impossible; for instance sampling every wild boar in Europe. Hence, a random sample survey is usually used and results are reported in terms of a probability statement, which is judged by a confidence interval. This is the interval which encompasses the true prevalence in the population with a specified probability, usually 95% (Armitage et al., 2002).

When inference is made from a sample to the study population, the result is always subject to uncertainty because of the sampling process. It is desirable that, if an indefinitely large number of samples is taken from a population, the long run average of the statistic obtained (e.g. point prevalence) will equal the parameter being tested (e.g the true prevalence in the population). A statistic with such a property is an 'unbiased' statistic (or estimator). The difference between the true population value and the mean of repeated estimates is hence termed 'bias' (Armitage et al., 2002). Bias can occur by a systematic measurement error, by misclassification or confounding with additional factors.

### Sample size

In the process of estimating the disease prevalence in a population, the number of individuals required to be sampled depends (1) on the population's characteristics (degree of clustering), (2) the expected prevalence, or the desired minimal prevalence the survey should be able to detect, (3) the desired precision of the estimate (width of confidence interval) and (4) the accuracy of the diagnostic tests used. In addition, the population size needs to be taken into account when the sample represents an appreciable portion of the population size (e.g. 5%). Probabilities associated with 'sampling without replacement' (as is the case when in a finite population, i.e. a large proportion of the population is sampled) follow a hypergeometric distribution. When the population is very large compared to the size of the sample, then the result of sampling with replacement (which is assumed in the binomial distribution) is indistinguishable from that of sampling without replacement, and the hypergeometric distribution approaches — and is approximated by — the binomial distribution (Armitage et al., 2002). Increments in large populations (e.g. from 10'000 to 1'000'000) will thus have no effect on the required sample size.

Because of a range of biological, economic or management factors, animal populations are clustered into groups. For instance, the pig population of Europe is clustered into a number of highly intensive pig producing regions. Within these regions, the population is divided into farms. The probability of an effective contact between an infectious and a susceptible individual is greater within the cluster than between clusters. The animal-level prevalence in affected farms may thus be relatively high, but only a small proportion of farms are affected. Correspondingly, wildlife populations can be grouped into packs. In addition, wildlife populations are spatially subdivided by landscape elements which sustain large populations or, in contrast, prevent migration. In contrast to domestic livestock, the degree of a wildlife population's spatial subdivision is most often unknown and may be subject to seasonal variations.

When the study population is subdivided into several isolated sub-populations, the dynamics of disease transmission between the sub-populations is unrelated and hence, the disease-prevalence revealed in one sub-population can not necessarily be extrapolated to the other sub-populations. Therefore, in a hypothetical population of 2000 individuals, a sample of 277 individuals would be sufficient to detect a disease prevalence of 1% at a confidence level of 95% (assuming a perfect test with 100% sensitivity and 100% specificity). However, when the population was subdivided into 2 isolated sub-populations of 1000 individuals each (leaving everything else equal), a sample of 285 would be required from each sub-population (Cameron, 1999).

### Diagnostic tests

Most monitoring and surveillance systems are based on diagnostic tests, such as enzyme-linked immunosorbent assay (ELISA). The accuracy of a test is measured by its sensitivity (probability that the test result is positive, given the animal is truly infected) and specificity (probability that the test result is negative, given the animal is truly non-infected). Even if the entire population were tested, imperfect sensitivity means that any positive animal can have produced a negative result. The

survey does therefore not prove absolute freedom. Instead, the survey determines the probability of observing a given number of test-positive animal from a population that is diseased at a specified prevalence. Reasons for false positive ELISA results can be cross-reactivity with similar antigens (e.g *Yersinia enterolytica serotype 9* with *Brucella abortus*), or non-specific inhibitors which mimic the effects of antibodies in the latter's absence. Reasons for false negative results can be natural tolerance to antigens or low diagnostic sensitivity (i.e. ability to detect antibodies at low concentration) (Thursfield, 1995).

In monitoring or surveillance programs, a combination of different diagnostic tests is often used. Animals defined as diseased by an initial screening test are subjected to further tests to confirm their status. This is of particular importance if diseased animals have to be culled. Hence, on the one hand, re-testing positive individuals by an additional test increases the specificity and the positive predictive value of the testing procedure. (The positive predictive value is the probability of obtaining a positive test result when the animal is truly diseased). On the other hand, the sensitivity of the testing procedure decreases in comparison with a testing procedure which relies on a single diagnostic test.

## 7.5 Implementation of monitoring and surveillance in wildlife in Switzerland

In Switzerland, routine diagnostics in wildlife is carried out by several institutions, notably the Institute Galli-Valerio, Lausanne, the Center for Fish and Wildlife Health (FIWI), Berne and the Institute of Virology and Immunprophylaxis (IVI), Mittelhäusern.

The success of any monitoring or surveillance system crucially depends on the collaboration between different institutions and on efficient communication towards the public. The monitoring and surveillance of wildlife diseases relies on the collaboration with hunters and veterinarians. In Switzerland, both the management of hunting and the organization of veterinary services lies in the competence of the different administrative regions (cantons). In addition, there are three different hunting systems: In system (i), 'Reviersystem' hunters are organized in local groups, which are usually bound to one particular hunting area. Hunting is mostly due to individual hunting during the night. This system prevails in the north-eastern region of Switzerland; in system (ii), 'Patentsystem' hunters are not bound to specific hunting areas. Hunting is mainly due to group hunting in autumn and winter. This system is used in the alpine region, as well as in the western and southern part of Switzerland; in system (iii) hunting is not permitted. The wildlife populations are regulated by cantonal game keepers. These differences between cantons affect the process of sample collection. For instance, communication is usually faster and the sample collection process is more standardized in regions which adopt the 'Patentsystem'. In addition, regulations concerning the months during which hunting is allowed differ between cantons. A national concept for managing the rapidly increasing wild boar population in Switzerland was recently proposed and may facilitate the organization of sample collections across several cantons (Anonymous, 2004) — and thus the comparability of results.

Specific surveys of wildlife populations were implemented in geographically limited areas. For instance, the prevalence of small fox tapeworm *Echinococcus multilocularis* was monitored in fox and humans between 1993 and 1998 in canton Fribourg: no increase in alveolar hydatid disease in humans (caused by infection with the proliferative larval stage of *E. multilocularis*) was detected, despite the consistently high prevalence of *E. multilocularis* (47% to 56%) in fox and an increase in seroprevalence in human (Gottstein et al., 2001). Based on a prevalence survey, (Giacometti et al., 2002) hypothesized that infectious keartoconjunctivitis, an ocular infection which is common in domestic sheep and goats, occurred in ibex and chamois in eastern Switzerland as a result of spillover from infected sheep.

A national monitoring system was established to study the cause of mortality in European brown hare (*Lepus europaeus*) from 1997 to 2000, based on carcasses collected by hunters and game keepers (Büttner, 1996; Haerer et al., 2001; Fröhlich et al., 2001). Similarly, the causes of death were assessed from 1987 to 1999 in reintroduced Eurasian lynx in the Jura Mountains and the Swiss Alps, based on lynx found dead (Schmidt-Posthaus et al., 2002). A decrease in the prevalence of viral haemorrhagic septicaemia (VHS), infectious haemotopoietic necrosis and infectious pancreatic necrosis was documented in farmed and feral salmonids between 1984 and 2001, although the geographic distribution of VHS tended to have increased (Knuesel et al., 2003).

Surveillance programs were implemented in a national vaccination campaign for fox rabies in Switzerland in 1978, which resulted in the successful elimination of fox rabies by the end of the 1990s (Muller et al., 2000). At a smaller geographic scale, an outbreak of classical swine fever in wild boar was controlled by selective hunting (targeted at young wild boar, conserving older, immune individuals) in southern Switzerland (Ticino (Schnyder et al., 2002)) and the bordering region in Italy (Lombardia (Zanardi et al., 2003)).

## 7.6 Conclusions

Newly introduced diseases are most often detected by passive monitoring. The monitoring system may become active when the interest in a specific disease increases. Active monitoring implies the systematic collection of data (e.g. case reporting), usually by a sequence of surveys, and may require the training of veterinarians, hunters and laboratory personnel when the disease was absent for a long period of time. Active monitoring also allows to document the freedom from disease, which has become increasingly important in international trade, also for wildlife.

Monitoring and surveillance in wildlife is complicated by the fact that estimates of populations size and the population's spatial subdivision are rarely available. Monitoring and surveillance in wildlife relies often relies on the collection of samples by hunters and therefore on their awareness and motivation to collaborate.

Chapter 8

# Discussion and conclusion

Increasing wild boar population sizes throughout Europe gave rise to the concern that diseases such as classical swine fever will persist in wild boar over prolonged periods of time (Kern et al., 1999) and hence pose a threat to pig farming industries. In Switzerland, the wild boar population increased from 60 to 6'327 between 1970 and 2002. At the same time, keeping cattle and swine in housings with open front became increasingly popular and is supported by the government since 1993 (Anonymous, 2002b). Therefore, we initiated a monitoring and surveillance system for contagious diseases in wild boar with relevance to domestic pigs, the aims of which was to provide a basis for (i) an early warning system, (ii) documenting the disease status for important pig diseases in Switzerland and (iii) assessing the success of interventions targeted at limiting disease outbreaks after disease introduction. Because of their economic or political importance we focused on classical swine fever, Aujeszky's disease, porcine brucellosis.

As a pre-requisite for such a monitoring and surveillance system, we sub-divided Switzerland into two geographic areas (sampling areas) within which an outbreak of a contagious disease in wild boar would remain confined with a probability of 95%: north and south of the Alps. Nevertheless, we considered it to be unlikely that a contagious disease would spread within an entire sampling unit within one year, the most likely interval between two consecutive sampling rounds. Therefore, we developed a simple, spatially explicit transmission model, in order to explore the effect of introducing an infectious wild boar pack in a population of susceptible, in terms of both size and duration of the resulting outbreak and the geographic area affected.

## 8.1 Sampling areas

In order to define sampling units, we mapped the probability of wild boar occurrence per hunting area. This was based on the notion that the distribution of suitable habitat in the landscape is a crucial determinant of wildlife population densities (Begon et al., 1990; Schadt et al., 2002; Howells and Edwards-Jones, 1997) and is therefore likely to affect the dynamics of disease spread. Accordingly, the probability of wild boar occurrence per hunting area was revealed by the relationship between

the presence of boar and various landscape characteristics. The statistical significance of these relationships were tested by a multivariate logistic regression. A high probability of wild boar occurrence was associated with a large percentage of forest, a large maize cultivation area and a large variety of highly intermixed patches. A large percentage of forest, an the proximity to crop fields were also identified as predictors for a high wild boar abundance elsewhere (Briedermann, 1986; Howells and Edwards-Jones, 1997; Geiser and Bürgin, 1998).

### Limitations of the regression model

In the absence of population data, we used the number of wild boar killed by hunters during two years (2001 and 2002) as a surrogate. These estimates may be confounded by factors which influence hunting intensity apart from wild boar density. For instance, some hunting areas may be preferred by hunters because they can be reached easily by car, or hunter may be more active in hunting areas with a large percentage of crop fields — in some cantons (administrative regions) hunters have to pay for monetary compensation to farmers whose crop fields were devastated by wild boar.

The regression model was based on the assumption that the absence of wild boar killed by hunters could be explained by a combination of landscape characteristics. Nevertheless, there was a probability of 38% that a hunting area was classified as 'wild boar absent' when there were wild boar killed. A reason for that may be the presence of additional landscape elements or factors which were not included in the model, such as slope, the species composition of forests of the additional feeding of wild boar by hunters. Further, there was a probability of 20% that a hunting area was classified as 'wild boar present' when there was no wild boar killed. A reason for this may be that, when there are few wild boar present in the hunting area, hunters may not kill a wild boar every year. The probability of misclassification induced by small numbers of wild boar killed per hunting area was decreased by adding up the hunting bags over both years for which we obtained data at the scale of hunting areas. Hunting areas where the predicted probability of wild boar occurrence was lower than the observed probability, were mainly situated south of the highway crossing Switzerland along the Jura Mountains. An alternative explanation may thus be that the landscape was equally suitable on both sides of the highway, but that wild boar did not yet colonize the area south of the highway. Nevertheless, the 73% of the hunting areas were correctly classified by the model.

While the effect of immigration can be unraveled by long-term monitoring, the predictions obtained by regression models based on hunting statistics for a single year could be improved by incorporating the spatial location of hunting areas. This would account for the fact that hunting areas which are surrounded by neighboring hunting areas with large numbers of wild boar present are expected to yield a higher number of wild boar than hunting areas surrounded by neighbors without wild boar present.

Of the 28 mutually exclusive landscape categories available from the Vector25 data set (based on the National Map 1:25'000), we considered 14 as relevant to the occurrence of wild boar. The remaining categories, including rock, glacier, lake, were classified as 'background'. However, 'forest', 'open land' and 'settlement area' were the dominant categories in the majority of the hunting areas for which we had data

on the presence of wild boar: in 50% of these hunting areas (median), the sum of the 3 dominant landscape categories comprised over 95% of the respective hunting area. Hence, the variables 'percentage of forest', 'percentage of open land' and 'percentage of settlement' were correlated. Consequently, 'percentage of open land' could roughly be deduced from 'percentage of forest' and 'percentage of settlement'. We therefore omitted 'percentage of open land', which resulted in 50% of the hunting areas to comprise over 38% of the total area. Nevertheless, the category 'open land' was included in the calculation of landscape composition variables, such as the 'number of open land patches per hectare', 'diversity' (probability that 2 randomly selected pixels would be of different) or 'interspersion' (chance that pixels adjacent to each other belong to different patch types). Collinearity (i.e. correlations among the explanatory variables in a regression analysis), was thus present. Although collinearity (so long as is not perfect) does not violate the assumptions the logistic regression is based upon, a high degree of collinearity causes the confidence intervals for coefficients to be very wide. Consequently, it is harder to reject the null hypothesis (no association of the combination of explanatory variables with the predictor variable) when collinearity is present. However, for all variables remaining in the multivariate regression model after step-wise removal of insignificant variables, the two-tailed p-value for the z-test was highly significant. (The z-test tests the null-hypothesis that the odds ratio is 1; i.e. no change in the explanatory variable due to the predictor variable). Further, removing variables from the final model or adding variables which were significant in the univariate regression but were removed by the step-wise selection process, did not induce changes in the signs of the effects (e.g switches from positive to negative). We thus concluded that collinearity was not a problem in the present analysis.

There was a large variation in the size of the hunting areas included in the regression analysis. The size of the hunting area could affect some of the landscape variables, notably 'diversity index' and 'interspersion'. This in turn is related to the fact that, in the analysis, we treated each hunting area as a separate landscape. Consequently, a particular hunting area (landscape) with a small forest area at its edge did not 'see' whether it adjoined a large forest area in the adjacent hunting area. As (i) patch-sizes approximated the size of the hunting area and (ii) the distance between patches approximated the dimensions of the hunting area (e.g. west-east extension), the probability increased, that the same patch appeared in several hunting areas. This in turn caused the same patch to contribute to the 'diversity' or 'interspersion' of several adjacent hunting ares. Accordingly, adding up the values for 'diversity' or 'interspersion', respectively, over a particular region would yield larger overall-values when the region was subdivided into many small areas than when the region was hardly subdivided. The same limitation was also valid when the map of the probability of wild boar occurrence was converted to a regular lattice of 1 $km^2$ cell size, which was done in order to provide the basis for a spatially explicit transmission model (see below).

### Probability of wild boar occurrence

Based on the multivariate logistic regression model discussed above, the probability of wild boar occurrence was also predicted for the hunting areas which were not used

in fitting the model (because no data were available). Thus, from the 1867 hunting areas included in fitting the model, the predicted probability of wild boar occurrence was calculated for the remaining 984 spatial units (termed hunting area, although some of them may not be suitable as such). In general, the hunting areas which were used in fitting the model were situated along the Jura Mountains, where the hunting areas tended to be small, whereas no data were available from central and eastern Mittelland where hunting areas were larger. Thus, the area ($km^2$) which was associated with at least one wild boar killed by hunters during the 2-year period analyzed, encompassed 30% of the area in Switzerland where the the regression model predicted a probability of wild boar occurrence greater than zero. This implies that the wild boar population in Switzerland may further increase in size and geographic extension.

The map of the probability of wild boar occurrence showed a clear separation of the area which was predicted to be suitable for wild boar into 2 regions: north and south of the Alps. However, the barrier function induced by highways was underestimated by the regression model. This was because hunting areas which contained highways but highly suitable area otherwise, yielded a high probability of wild boar occurrence. The effect of highways was, however, taken into account by the transmission model.

### Transmission model

It is unlikely that an outbreak of an infectious disease outbreak would affect the entire area north of the Alps within one year. We therefore developed a spatially explicit transmission model, in order to explore the effect of introducing an infectious wild boar pack into a population of susceptible in terms of the geographic area affected by the outbreak, and both size and duration of the outbreak. The model was suitable for a disease such as CSF, where young wild boar, which live in packs with adult sows, play a key role in disease-transmission (Kern et al., 1999).

Assuming the home-range to be 9 $km^2$, the percentage of infected packs among the total number of packs (outbreak size) remained below 0.1% when the probability of transmission equaled 40% and below 0.01% when the probability of transmission between packs equaled 10%. The duration of the outbreak was less than 5 months and the spread of the disease did not exceed 10km from the source of infection. In contrast, the outbreak-size increased considerably with increasing population density (0.1%-7.0%) when the home-range size equaled 25 $km^2$. In accordance, the duration of the average outbreak size increased from 1.3 to 10.5 months and the radius of disease spread increased from below 10 km to 30 km when the probability of transmission equaled 10% and 40%, respectively. Wild boar were recorded to use home-range sizes of 8-30 $km^2$ in the Jura Mountains of Switzerland (Baettig, 1993), and 2-40 $km^2$ in southern France, depending on the season. An average home-range size of 25 $km^2$ appears thus realistic.

The probability of transmission was set higher than the estimates revealed by the follow-up of an outbreak of classical swine fever in free-living wild boar (Hone et al., 1992). However, (Hone et al., 1992) recorded a population density of approximately 6 individuals $km^{-1}$, whereas our simulation was based on an average population density of 1 to 2 wild boar $km^{-2}$, estimated from the annual hunting bag in 2001/2002.

However, packs were distributed across the landscape, according to the probability of wild boar occurrence, which allowed for a higher density of packs in locations with a high probability of wild boar occurrence. Nevertheless, the differences in the cell's probability of wild boar occurrence might not have been large enough to allow for the occurrence of sufficiently large local aggregations of wild boar packs, unless the probability of transmission was as high as 40%.

In our preliminary model, the initially infectious pack was placed at a randomly chosen location. By repeating the procedure of placing an infectious pack and simulating the resulting outbreak under different conditions (probability of transmission, population density) in the area of Switzerland north of the Alps, it appeared that the entire area north of the Alps was connected. However, this was based on the assumption that (i) wild boar crossed the highways by surpassing tunnels and wildlife bridges, under-passing bridges an that (ii) outbreaks start at every position with equal probability. In real life, it is likely that the risk of introducing a disease into free-living wild boar varies geographically (e.g. proximity to neighboring countries where the disease occurs).

The transmission model could contribute to a risk based surveillance system. In such a system, the model's predictions and additional data on the risk of spillover from wild boar to domestic pigs could be included into the decision of where to collect how may samples. But better estimates of the wild boar population size, wild boar migration distances and migration routes are required in order to validate the model and thus, to get valuable predictions. Such data may be gained from a national capture-recaptures study including radio-tracking of individual wild boar.

The model itself could be improved by allowing the home-range size to adapt itself to the landscape — i.e. a larger home-range when the pack is placed in a location with a relatively low probability of wild boar occurrence Further, packs may be allowed to migrate long distances (e.g. 10-30 km) within one month, instead of being restricted to a distance of 1 km (i.e. to one of the adjacent cells). It has been shown that wild boar migrate over 10 km within a few days, especially during the hunting period (Spitz, 1992). Such long-distance migration is likely to affect the spread of disease spread.

## 8.2 Infection status of wild boar in Switzerland

A national monitoring and surveillance system for contagious disease in wild boar was initiated in Switzerland. The apparent prevalence of classical swine fever, Aujeszky's disease and brucellosis (*B. suis*) was determined by two serological surveys over 4 months each. During the chosen sampling period ($1^{st}$ of November to $30^{th}$ of February) we obtained about 20% of the annual hunting bag. The percentage of samples obtained from the annual hunting bag depended on the cantonal regulations concerning the months during which hunting was allowed and on the hunting system.

As with any surveys in wildlife, the information and motivation of the hunters was a crucial determinant of the number of samples obtained and the sample's quality. The use of hunting based data might have induced bias, causing the prevalence estimates to be systematically over- or underestimated by factors which influence hunting intensity apart from wild boar density, such as the hunter's motivation to

contribute. This limitation was expected to be less prominent in cantons adopting the 'Patent'-hunting system than in cantons adopting the 'Revier'-hunting system. In the former, the samples were collected by game keepers at the check-points where hunters are obliged to present their prey for cantonal registration, where as in the latter, the samples were collected by individual hunters and was thus dependent on the hunter's training and motivation.

The quality of the data recorded by data-sheets (hunting area, age and weight of the sampled wild boar) improved between both sampling rounds: data-sheets became more complete, and the handwriting more readable. This may be attributable to the increased level of information and motivation among hunters in sampling round 2 (newsletters, articles in hunter's journals). However, the quality of the blood samples remained a challenge in both survey rounds. Bacterial contamination was mainly a problem in the diagnostics of Aujeszky's disease, in which the quantity of serum obtained (1-2 ml) was often not sufficient when several tests had to be performed on the same sample. Nevertheless, both the ELISA for classical swine fever and the ELISA for brucellosis performed well.

### Classical swine fever (CSF)

There were four samples (0.2%) positive for CSF. All of them originated from wild boar aged above 2 years in the southern region (Ticino). This suggests that these antibodies were most likely acquired during the outbreak of CSF wild boar in 1999 in Ticino (Schnyder et al., 2002). No clinical or virological evidence for classical swine fever was found in Switzerland since 1999 and there were no young wild boar (<2 years) found to be sero-positive. Switzerland can thus be considered free from CSF in wild boar according to the Terrestrial Animal Health Code (OIE, 2003).

### Aujeszky's disease (AD)

AD does not currently occur in domestic pigs in Switzerland, nor is there evidence for the occurrence of AD in wild boar in a region adjacent to Switzerland in any of the neighboring countries. In accordance, we did not find evidence for the occurrence of AD in wild boar in Switzerland.

### Brucellosis

Porcine brucellosis in wild boar (*B. suis*) is widely distributed in Europe (Godfroid, 2002). In Switzerland, *B. suis biovar 2* was isolated from a wild boar near Geneva in 2001 (Anonymous, 2002c). In the present study, we confirmed the occurrence of brucellosis (*B. suis biovar 2*) in wild boar: antibodies against *B. suis* occurred predominantly in the northern and western part of the country, in areas bordering France, were the presence of *B. suis* had also been documented (Hars et al., 2000), (Boué et al., 2002). The sero-prevalence of *B. suis* was higher in sampling round 2 than in sampling round 1.

By a simple, dynamic transmission model, we provided a framework for analyzing the relationship between the prevalence of *B. suis* in wild boar and the number of wild boar killed by hunters per year. The model suggested that brucellosis in wild boar in Switzerland north of the Alps would fade within about 5 years, when the

total population could be stabilized at about 15'000 individuals. This corresponds approximately to the population size in 2002. However, the model's predictions need to be validated by additional data on the progression of the *B. suis* prevalence and by data on the wild boar demography. Such data could be gained from a large-scale capture-recapture study.

**Tuberculosis (only canton Ticino)**

In Ticino (Southern Switzerland) we found no evidence for the occurrence of *Mycobacterium bovis* in wild boar. The percentage of lymph nodes with lesions suggestive for tuberculosis was higher in most surveys carried out in regions where an infection with *M. bovis* was present, than in our study population: 11% in Liguria (Bollo et al., 2000), 85% in feral swine in Australia before an eradication campaign in cattle (Corner et al., 1981) and 6% after the eradication campaign (McInerney et al., 1995). In contrast to brushtail possums and badgers, which can represent the principal source of infection in cattle (Phillips et al., 2003), there is no evidence so far, that the infection with *M. bovis* is self-maintaining in wild boar (Phillips et al., 2003; Schulz et al., 1992; Coleman and Cooke, 2001). Moreover, (Corner et al., 1981) and (McInerney et al., 1995) demonstrated that the prevalence of *M. bovis* decreased in feral pigs after the prevalence had been reduced in cattle by an eradication program. Thus, the finding of tuberculosis in wild boar in Liguria (Serraino et al., 1999; Bollo et al., 2000) is not regarded as reason for concern to Switzerland and we do not recommend future monitoring.

## 8.3 Conclusions

We successfully initiated a monitoring and surveillance program for contagious diseases in wild boar in Switzerland. Based on two survey rounds, we demonstrated the absence of both classical swine fever (CSF) and Aujeszky's disease (AD) in wild boar. We did, however, confirm the occurrence of *Brucella suis* by both serology and bacterial isolation.

For a highly contagious disease, such as CSF, a monitoring system based on annual sampling during the hunting season, is unlikely to be efficient as an early warning system. A CSF-outbreak is likely to be detected by hunters and veterinarians due to the high mortality caused by many CSF-virus strains, irrespective of a targeted sample collection. However, the presence of a monitoring and surveillance system may increase the level of awareness among hunters and veterinarians. Therfore, in order to detect the introduction of the CSF-virus into wild boar at an early stage, it is of crucial importance to maintain the currently high level of information concerning CSF — and thus awareness — among hunters and veterinarians. Nevertheless, we do not consider it the annual demonstration of the absence of CSF in wild boar in Switzerland necessary.

AD-virus does not currently occur in domestic pigs in Switzerland, nor in wild boar in regions adjacent to Switzerland in any neighboring country. Despite the evidence for the transmission of AD-virus from domestic pigs to wild boar , the spillover of AD-virus from wild boar to domestic has never been demonstrated. Therefore, it does not appear to be justified to demonstrate the absence of AD in wild boar at an

annual basis in Switzerland. Nevertheless, increasing the level of awareness among hunters and veterinarians may be crucial in the early detection of AD in wild boar, once the virus is introduced.

In contrast, we suggest to continue the surveillance of brucellosis in wild boar, in order to determine whether the infection is spreading. Further, awareness among farmers with outdoor-reared pigs must be improved, in order to prevent the spillover from infected wild boar — for instance by effective fences. Brucellosis due to *B. suis biovar 2* has only once been reported as the cause of disease in humans (Garin-Bastuji and Delcueillerie, 2001) and is thus considered to be harmless.

The routes of communication and organization opened within the current project can also be used and intensified when the implementation of interventions in order to minimize the spread of a newly introduced disease in wild boar becomes necessary. For any contagious disease in wildlife with relevance to domestic lifestock, the communication with veterinary services and hunting organizations in neighboring countries is of crucial importance in terms of both, the early detection of an infection in wildlife and the efficient implementation of interventions.

Data related to the risk of spillover from wild boar, such as the geographic location of outdoor-reared pigs should be collected in order to assure that surveys are performed in the regions at highest risk and to refine information campaigns. Data on wild boar the population density and migration patterns are required to validate transmission models. Such data could be collected by a national capture-recapture study, including radio-tracking of individual wild boar.

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# Appendix 1: public relations work

**2001**

- Wildtiere als Krankheitsreservoir, BVET-Magazin, 2001 (3):21-23.
- Annual meeting of the representatives of the cantonal hunting departments, Olten, June 6, 2001
- Annual meeting of the hunters in canton Zurich, Illnau, Oktober 11, 2001
- Meeting of the game-keepers in canton Ticino, Bellinzona, November 26, 2001
- Annual meeting of the group 'Monitoring', Liebefeld, November 29, 2001

**2002**

- Leaflet on sampling round 1, addressed to people involved in the sample collection, June 2002
- Article on the project in hunter's journals ('Schweizer Jäger', 'Jagd und Natur', 'la Caccia', 'Chasse et Nature'), September 2002
- Wildschwein - ein Ansteckungsrisiko? (by Christine Kuhn), BVET-Magazin 2002 (5):12-15
- Meeting of the game-keepers in canton Jura, St-Ursanne, November 26, 2002
- Contribution to TV-session 'Mensch-Technik-Wissenschaft', Swiss TV DRS, October 29, 2002
- Annual Meeting of the group 'Monitoring', Liebefeld, December 5, 2002

**2003-2004**

- Web-site: Monitoring and surveillance of diseases with relevance to domestic pigs in wild boar:`http://www.bvet.admin.ch/tiergesundheit/d/berichte_publikat/1_index.html`, August 2003
- Leaflet on sampling round 2, addressed to people involved in the sample collection, March 2004
- Article on the project in hunter's journals ('Schweizer Jäger', 'Jagd und Natur', 'la Caccia', 'Chasse et Nature'), in progress

**Following pages:**

1. Leaflet sent before the sample collection to representatives of hunting organizations, cantonal hunting departments and cantonal veterinary departments.
2. Data form, filled in for every wild boar blood sample
3. Leaflet on sampling round 1
4. Leaflet on sampling round 2

# Appendix 2: curriculum vitae

| | |
|---|---|
| **Name** | Regula Leuenberger |
| **Date of Birth** | $24^{th}$ of June 1969 |
| **Nationality** | Swiss |
| **Languages** | german (first language), english and french |
| **Education** | |
| 1976-1981 | Primary school, Füllinsdorf |
| 1981-1985 | Secondary school, Frenkendorf |
| 1986-1987 | College Medium Diploma (junior high school), Liestal |
| 1987-1989 | Art school, Basel and Zürich |
| 1990-1992 | College, Gymnasium (senior high school), Muttenz |
| 1993-1999 | Study of Biology, University of Basel |
| 2001-present | PhD in Epidemiology, Federal Veterinary Office and University of Basel |
| **Working experience** | |
| 1998 | March-October: laboratory works in analytics of vitamins in food and feed stuffs, F. Hoffmann-La Roche AG, Basel |
| 1999-2002 | December 1999 - March 2000: scientific assistence in the evaluation of health projects in Russia and the Ukraine, Support Centre for International Health, Swiss Tropical Institute, Basel<br>April 2000-August 2000: concept for a new data management tool, Kiebitz, Basel<br>December 2000 - June 2002: junior-programmer (java), Things Prime GmbH, Basel (part-time) |

During my studies I attended courses given by the following lecturers:

W. Arber, B. Baur, C. Boesch, T. Boller, R. Brun, W. Gehring, S. Jacomet, L. Jenni, C. Körner, C. Lengeler, D. Lüdin, G. Pluschke, U. Rahm, C.H. Rowell, G. Schatz, V. Schmid, D.G. Senn, U. Sequin, H. Sigel, T.A. Smith, S.C. Stearns, J. Stöcklin, M. Tanner, P. Vounatsou, N.A. Weiss, A.M. Wiemken.

### Conferences

Poster:

- Surveillance of brucellosis in wild boar in Switzerland. Brucellosis 2003 International Research Conference, September 15-17, 2003; Pamplona (Spain).

Oral Presentations:

- Surveillance des maladies transmissibles entre le sanglier et les animaux domestiques. Rencontre du Groupe d'Etudes sur l'Ecopathologie de la Faune Sauvage de Montagne (GEEFSM), June 6-8, 2003; Sampeyre (Italie).

1. Surveillance system for diseases in wild boar in Switzerland, $10^{th}$ International symposium for veterinary epidemiology and economics (ISVEE), November 17-21, 2003; Viña del Mar, Chile.

2. Developing a sampling protocol for the surveillance of diseases in wild boar, $10^{th}$ International symposium for veterinary epidemiology and economics (ISVEE), November 17-21, 2003; Viña del Mar, Chile.

### Publication

Tom A. Smith, Regula Leuenberger and Christian Lengeler (2001): Child mortality and malaria transmission intensity in Africa, TRENDS in Parasitology, 17(3):145-149.