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SITUATION-BASED SURVEILLANCE: ADAPTING INVESTIGATIONS TO ACTUAL EPIDEMIC SITUATIONS

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ABSTRACT: Surveillance approaches for wildlife diseases often are based on strategies devised for livestock diseases. Following standard protocols, surveillance sometimes continues after apparent disease elimination. However, in the case of recurrent wildlife diseases that cause decisive morbidity and mortality, efficient and effective surveillance strategies might need to be more dynamic and adaptable to the actual epidemic situation. Here, we evaluated existing surveillance schemes by reanalyzing historic data on three wildlife diseases in Europe: rabies, classical swine fever, and avian influenza. We analyzed the aims of different surveillance activities and the way in which they were performed. Our analyses revealed that static, nonadaptive surveillance was a suboptimal approach. Consequently, we propose and discuss a more adaptive alternative scheme of situation-based surveillance for recurrent wildlife diseases that cause readily recognizable morbidity and mortality.

Key words: Avian influenza, classical swine fever, CSF, disease surveillance, monitoring, policy, rabies, wildlife disease.

INTRODUCTION

The need for increased surveillance of diseases among wildlife is clear (Kuiken et al., 2005; Stallknecht, 2007). Because some wildlife diseases threaten humans (e.g., rabies in foxes [*Vulpes vulpes*]), livestock industries (e.g., classical swine fever [CSF] in wild boar [*Sus scrofa*]), or both (e.g., highly pathogenic avian influenza in wild birds), wildlife, livestock, and public health officials need to be informed about the disease situation in their respective jurisdictions. Accurate information on whether a disease of interest has been eliminated from a geographic region or jurisdiction, or whether it has been reintroduced, is crucial for decision making. Moreover, data from disease surveillance might be needed to meet local, national, and international guidelines. For example, maintaining "disease-free" status could require, among other requirements, the demonstration of an effective system of disease surveillance (Office International des Épizooties [OIE], 2008). Thus surveillance programs are established to routinely collect specimens for diagnosis and to obtain additional information on which administrative decisions in disease management are based (Mörner et al., 2002).

In the course of reviewing guidelines published by the World Health Organization (WHO), the OIE, or individual nations, we recognized that surveillance programs for wildlife diseases often are copied from livestock surveillance. These applied schemes are commonly based on continuing tests of quotas of the total population to demonstrate the absence of disease. In particular, these schemes involve continued testing after elimination (or even before arrival) of the disease of concern.

Obviously, however, sample designs for wildlife disease surveillance are more complex than for livestock disease (Artois

et al., 2001) because of the limited knowledge on wildlife species' abundance, limited access to the populations at risk, and limited ability to observe the susceptible populations closely throughout a jurisdiction (Guberti and Newman, 2007). Consequently, statistic certainty also might be limited unless the sample size is increased. Considering the economic burden caused by continuous testing of presumably healthy animals to ascertain freedom from a disease, we thought it worthwhile to reconsider common wildlife disease investigation efforts with regard to their aims, sample sources, and sampling designs.

Our evaluation was particularly motivated by 1) the need for European Union (EU) member states to specify their national surveillance programs for "notifiable diseases" in wildlife with respect to demonstrating freedom from disease according to existing EU regulations and 2) the urgent need for a surveillance scheme for rabies in foxes after elimination of this disease from several countries in Western and Central Europe.

Our evaluation was based on the premise that operating systems of surveillance (OIE, 2008) might not necessarily require the exclusive application of a single scheme, as is common practice. In particular, we focused on examples of contagious diseases that are characterized by high mortality or morbidity events (Mörner et al., 2002) once they are introduced into a susceptible wildlife population.

Our approach was to relate the epidemic situation in a region to specific surveillance aims, potential targets for sampling, and methods of sampling design. It turned out that changes in the epidemic situation (e.g., from "diseased" to "disease-free") resulted in changing aims for the accompanying surveillance that were ideally based on different sampling schemes. Therefore, we propose situation-based surveillance schemes for wildlife disease surveillance. We show that adapting surveillance to the actual epidemic situation provides a straightforward solution for the overall purposes of surveillance summarized above. The developed scheme could form the basis of cost-efficient surveillance of contagious diseases of noticeable morbidity in wildlife.

MATERIALS AND METHODS

Our study aimed at exploring recommended and standard disease surveillance and monitoring schemes for epidemic wildlife diseases. We focused on several legal guidelines (WHO, 1992, 2005; German National Directive on Rabies Control, 2001; European Commission, 2002a, 2006; OIE, 2008) related to three important diseases of wildlife in Europe.

Terminology

To make our evaluation as clear and consistent as possible by using concise terminology, we extracted four basic concepts from the epidemiology literature on surveillance activities and summarized their definitions. For a more in-depth analysis of concepts related to surveillance, see, for example, Thrusfield (2005).

Two broad kinds of activities are employed to assess the status of a disease or its control in wildlife populations: disease surveillance and monitoring of control:

Disease surveillance: This is the ongoing systematic use of routinely collected data to provide information that leads to action being taken to manage a disease of a highly contagious nature in a jurisdiction (e.g., on- or offset of control relative to case detection, following OIE [2008, appendix 3.8.1]). The aim of disease surveillance is the detection of infected animals. Hence, the logical source of information is the subpopulation of infected host individuals. Disease surveillance data are of interest whenever the disease is not known to be present.

Monitoring of control: This is the systematic assessment of disease control measures. Note that, in contrast to our understanding, some guidelines use the term "monitoring of control" to describe a mixture of both the ongoing disease surveillance during activated control and the performance evaluation of control measures (e.g., European Commission, 2002a; OIE, 2008, Chap. 1.4.7, "Surveillance for distribution and occurrence of infection"). A well-known example of control quality assurance relates to oral mass vaccination of foxes against rabies, wherein the efficacy of vacci-

nation was evaluated via seroprevalence or bait uptake (European Commission, 2002a). The aim of quantitative monitoring of control programs is to assess the efficacy of measures applied. The logical source of information is found in the uninfected subpopulation. These data are only of interest during active control.

In addition to these two motivations for assessing disease status, samples examined for surveillance generally are submitted for testing either because of some suspicion that the individual is a disease case or because of a systematic sampling plan; these sources are sometimes distinguished by terms like passive vs. active surveillance (OIE, 2008) or opportunistic vs. targeted sampling (Stitt et al., 2007). For our analyses, we used the classification according to the sample source of host individuals—indicator animals and hunted animals.

Indicator animals: Individuals suspected of having the disease includes animals killed because of clinical signs or suspicious behavior, those found dead or killed on roads, those belonging to high-risk species, or animals to which humans might have been exposed. For diseases that cause mortality or morbidity events, this sample source is, by definition, focusing the sampling effort in area and time toward the outbreak.

Hunted animals: Individuals suspected of not having the disease (i.e., not indicator animals) are, for example, animals sampled from regular hunting activity or specific sampling hunts or sampled alive (e.g., structured or nonrandom selection; OIE, 2008). This sample source is statistically assumed to be representative of the healthy population (i.e., susceptible or protected/treated) on large spatial and temporal scales.

Retrospective data analysis

We compared the performance of two different sample sources, hunted animals (HA) and indicator animals (IA), with respect to the information needed to perform disease surveillance. We calculated the effort required to find one diseased animal (effort per case) in samples taken from infected areas by dividing the total number of laboratory investigations by the number of sample units that were found positive. Epidemiologic odds ratios (ORs) were calculated (Sachs, 1992). Herein, the odds of testing positive are estimated from surveillance samples by the ratio of the number of sample units that tested positive when investigated in the laboratory to the number of sample units that tested negative. The quotient of the odds of two surveillance samples (e.g., IA vs. HA) forms the odds ratio, which is calculated to compare the sample sources. An odds ratio of 1 indicates no structural difference; hence the confidence interval (CI) is calculated to test whether the value 1 could be excluded at the specified confidence level (Bland and Altman, 2000). The odds ratio was calculated as

$$\mathrm{OR} = (\mathrm{IA}_{\mathrm{pos}}/\mathrm{IA}_{\mathrm{neg}})/(\mathrm{HA}_{\mathrm{pos}}/\mathrm{HA}_{\mathrm{neg}}),$$

where $\mathrm{IA}_{\mathrm{pos}}$ and $\mathrm{HA}_{\mathrm{pos}}$ denote the respective numbers testing positive among indicator and hunted animals and $\mathrm{IA}_{\mathrm{neg}}$ and $\mathrm{HA}_{\mathrm{neg}}$ denote the respective numbers testing negative among indicator and hunted animals, such that $\mathrm{IA}=\mathrm{IA}_{\mathrm{pos}}+\mathrm{IA}_{\mathrm{neg}}$ and $\mathrm{HA}=\mathrm{HA}_{\mathrm{pos}}+\mathrm{HA}_{\mathrm{neg}}$; the 95% CI for the OR was calculated as

$$95\%\,\mathrm{CI} = \exp\left[\ln(\mathrm{OR}) \pm 1.96\sqrt{1/\mathrm{IA}_{\mathrm{pos}} + 1/\mathrm{IA}_{\mathrm{neg}} + 1/\mathrm{HA}_{\mathrm{pos}} + 1/\mathrm{HA}_{\mathrm{neg}}}\right].$$

We performed the analysis with data on three example diseases.

Rabies in foxes: The database on rabies surveillance of red foxes during 1 January 1990 to 31 December 1995 in five German federal states situated in eastern Germany was explored (Table 1). This database is hosted by the Friedrich Loeffler Institut (FLI), the Federal Research Institute for Animal Health in Germany. The analyzed data comprised the results of 60,778 individual laboratory investigations of the infection status of submitted red foxes on the basis of fluorescent antibody test (FAT) and virus isolation in cell culture (RTCIT) (Dean et al., 1996; Webster and Casey, 1996). These data allowed classification according to sample source.

CSF in wild boar: The database on CSF surveillance in wild boar in the German Bundesland Rhineland-Palatinate during 1 January 1995 to 24 September 2007 was explored (Table 2). This database also is hosted by the FLI. These data comprised the results of 209,114 individual laboratory investigations of the infection or exposure status of wild boar on the basis of commercial ELISAs and virus isolation techniques (von Rüden, 2006; von Rüden et al., 2008). The data content enabled classification according to sample source.

Avian influenza in wild birds: Data from European surveillance of wild birds during Febru-

TABLE 1. Data on rabies surveillance in red foxes from 1990 to 1995 in five eastern federal states of Germany. (Data from Friedrich Loeffler Institut, Federal Research Institute for Animal Health, Germany.)

	Indicator animals (IA)			Hunted animals (HA)			$(IA_{pos}/IA_{neg})/(HA_{pos}/HA_{neg})$[a]	
Year	Pos	Total sample	Effort per case	Pos	Total sample	Effort per case	Odds ratio	95% CI
1990	93	289	3.1	186	2,155	11.6	5.0	(3.7–6.7)
1991	304	803	2.6	340	5,631	16.6	9.5	(7.9–11.3)
1992	38	2,750	72.4	90	6,390	71.0	1.0	(0.7–1.5)
1993	12	3,766	313.8	8	9,766	1,220.8	3.9	(1.6–9.5)
1994	3	3,848	1,282.7	1	7,592	7,592.0	5.9	(0.6–57.0)
1995	4	2,371	592.8	3	15,417	5,139.0	8.7	(1.9–38.8)
Total	454	13,827	30.5	628	46,951	74.8	2.5	(2.2–2.9)

[a] Pos=positive; Neg=negative.

ary to May 2006 was explored (European Commission, 2007). These data comprised 19,507 individual laboratory investigations of the infection status of wild birds of "risk species" from 13 EU member states that experienced cases of highly pathogenic avian influenza virus (HPAIV) H5N1 on the basis of polymerase chain reaction (PCR; European Commission, 2007). Data were classified according to the reason for investigation, corresponding to IA and HA definitions above.

RESULTS

Retrospective data analysis

Rabies in foxes: In Figure 1, the ratio of the diagnostic effort per detected rabies case in the HA sample relative to that in the IA sample is shown. On average, the effort required to detect a case in the HA sample was about 2.5 times (95% CI=2.2–2.9) the effort necessary to detect a rabies case from the IA sample (Table 1). Even after oral vaccination became effective and a systematic monitoring of HA was established (the years from 1992 onward), the chances of detecting a rabies case still tended to be higher among the IA sample.

CSF in wild boars: In contrast to rabies-infected foxes, not all CSF-infected wild boars die from the disease. Nevertheless, the effort to find a virus-positive animal among the sampled HA was much greater than finding a case among the IA sample (Table 2). Vaccination likely increased the investigation effort per detected case because of the general reduction in the number of infected animals actually available. The chances of detecting a virus-positive animal in the IA sample was about 55 times greater (95% CI=43–72) than the chances of finding the CSF virus in the HA sample before vaccination and nearly

TABLE 2. Data on classical swine fever investigations on wild boar in the German federal state of Rhineland-Palatinate from 1999 to 2007. (Data from Friedrich Loeffler Institut, Federal Research Institute for Animal Health, Germany.)

	IA			HA			$(IA_{pos}/IA_{neg})/(HA_{pos}/HA_{neg})$[a]	
	Pos	Total sample	Effort per case	Pos	Total sample	Effort per case	Odds ratio	95% CI
Virology								
Before vaccination	157	272	1.7	511	21,319	42	55	(43.0–71.8)
During vaccination	70	234	3.3	237	164,652	695	296	(217–403)
Serology								
Before vaccination	13	56	4.3	5,283	22,586	4.3	0.99	(0.5–1.9)

[a] HA=hunted animals; IA=indicator animals; Pos=positive; Neg=negative.

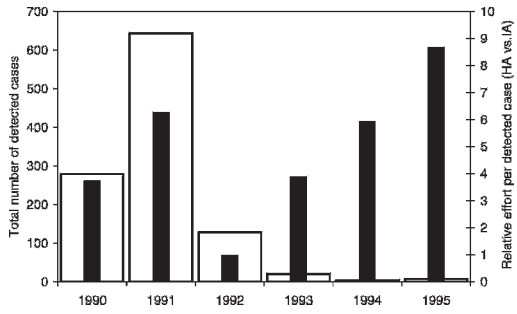

FIGURE 1. Rabies surveillance data from 1990 to 1995 from Germany. White bars represent the total number of detected rabies cases (left axis). Black bars represent the ratio of effort per case (tested samples per positive finding) in hunted animals (HAs) against effort per case in indicator animals (IAs, right axis). (See Table 1; data from Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health, Germany.)

300 times greater (95% CI=217–403) during periods of vaccination. In comparison, serology in animal samples before vaccination indicated survival of infection when the test was positive. Because the animal was no longer sick after seroconversion, one would expect no difference in effort to detect a seropositive animal from either sampling source. Indeed, the level of effort per detection was equal for the two samples (95% CI=0.5–1.9; Table 2).

Avian influenza in wild birds: A total of 16,780 wild birds from the IA sample source (risk species, dead or diseased; European Commission, 2007) were submitted for testing. Of these, 481 were found to be positive for HPAIV (H5N1), giving an effort of 34.9 investigations per case. In the HA sample, 2,997 wild birds from risk species were investigated, resulting in 39 HPAIV positives, for an effort of 76.8 investigations per case. The respective odds ratio was estimated at 2.2 (95% CI=1.6–3.1).

DISCUSSION

Improving the cost efficiency of existing surveillance and monitoring strategies is of general interest and recently has become a topic of discussion in livestock disease surveillance (Stärk et al., 2006). The purpose of our study was to evaluate common sampling schemes to derive an improved situation-based strategy. We focused on wildlife diseases that cause mortality or morbidity events. Our aim was not to discredit existing surveillance and monitoring schemes in general, but rather to relate these more closely to actual epidemic situations.

The financial burden placed on a society's economy by disease surveillance activities can be substantial on the basis of the amount of laboratory testing that might be required to ensure systematic sampling of unsuspicious animals (Tables 1, 2). Review of various guidance documents revealed that often a general sampling scheme is proposed for routine disease surveillance without prior reference to the actual epidemic situation. Particularly in wildlife, the need for continued observation seems questionable once the disease of concern has been eliminated by control.

Both after disease elimination and before its arrival, surveillance efforts typically focus on the discovery of a new outbreak of the disease. From our retrospective analysis of three independent historic surveillance data sets, we found that sampling of IA was more effective for detecting virus-positive animals than sampling of HA. These results support the notion that disease-related mortality or morbidity events are likely to be discovered through laboratory accessions and postmortem examination of IAs (Mörner et al., 2002).

In contrast, monitoring the efficiency of control measures shifts the focus of activities. To estimate the proportion of animals in the population that have been reached by the control (e.g., immunized) within a prescribed level of certainty, a predefined sample size must be investigated. This objective cannot be achieved by sampling the IA source because the sample would neither be target oriented nor guarantee the predefined sample quota (Guberti and

TABLE 3. Overview of aims and strategies and appropriate sample sources of surveillance measures with respect to particular knowledge corresponding to the disease status of an area. Once disease has been detected (second row), nothing further can be accomplished with disease surveillance per se; hence, there is no surveillance aim until the situation shifts to "control" (third row).[a]

Epidemic status	Knowledge of disease	Aim	Strategy	Source	Sample
Free	Absent	Detection of arrival	Disease surveillance (passive)	Infected (e.g., IAs)	amap
Diseased	Present	—	—	—	—
Control	Management because of presence	Assure performance	Monitoring (active)	Healthy (e.g., HAs)	Designed to estimate agreed figures
Finished	Management until absence	Proof of freedom	Stop and observe; inappropriate: rejection of design prevalence	Infected (IAs)	amap

[a] IAs=indicator animals; HAs=hunted animals; amap=as many as possible according to availability of samples and feasibility of laboratory investigations.

Newman, 2007). It follows that different aims in different epidemic situations encourage the adaptation of sampling to the actual epidemic situation. An adaptive approach would make long-term application of wildlife disease surveillance more efficient and cost effective.

Table 3 summarizes the attributes of surveillance referring to different phases typical of many epidemic wildlife diseases. These were combined in the schematic representation of an ideal adaptive scheme (Fig. 2). In the following sections, we first explain the adaptive scheme and the rationale of situation-based surveillance in general and then discuss whether the three example diseases fall under this scheme. We then discuss the three epidemic situations of the scheme (absence of disease, presence of disease, suspected freedom from disease) and its applicability in more detail.

Adaptive situation-based surveillance

Situation-based surveillance accounts for the current epidemic situation in an area (e.g., a country, province, state, or other sociopolitical jurisdiction). There are two distinct situations: absence or presence of disease (see Fig. 2, lowest level). If the disease of concern is absent (left and right vertical sections of Fig. 2), then the only information of interest is an occurrence (arrival or recurrence). A good surveillance strategy for such epidemic situations must be capable of detecting disease, but also should be suitable for long-term application whenever the disease of interest is absent. Although "disease-free" status is well defined in legal manuals (WHO, 1992; OIE, 2008), these guidelines seldom exploit knowledge about the disease status to adapt surveillance.

Indeed, as long as the disease is absent (sections labeled "no disease" in Fig. 2) surveillance is the only activity required and the aim is to detect a new arrival or occurrence. The situation assumes that there are no cases in the host population and information of disease arrival would be gained by rejecting this assumption (e.g., by finding one positive animal). This situation encourages observation of IAs, as supported by our findings that 1) the odds of finding a positive animal were higher for IA than HA samples (Tables 1, 2); 2) the numbers of samples submitted for diagnostic investigation were dramatically different, implying less laboratory effort and lower relative costs when detection effort focuses on IA; and 3) the increased number of IA sample units coming from exposed regions (i.e., areas where a newly intro-

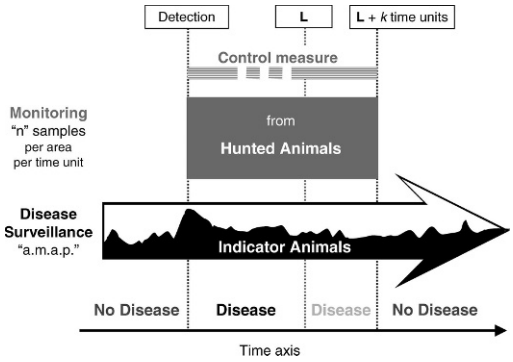

FIGURE 2. Schematic representation of situation-based surveillance. Time flow is represented on the bottom axis. Vertical lines represent points in time when knowledge on disease changes because of disease surveillance outcome (Detection; "*L*"=last case; "*L+k* time units"=safety buffer with continued control without case detection before control can be stopped). First horizontal level (from bottom) indicates the disease situation in the area. The second level symbolizes sample size of disease surveillance activity (black area within the arrow). The data are gathered from continuous sampling of indicator animals (arrow). The irregular shape of the black area symbolizes that a predetermined number is not useful; rather, "as many as possible" (amap) investigations are performed. The third level represents sample size over time required to systematically monitor efficiency of control measures. The upper level indicates the time when control measures are performed.

duced disease causes fatalities) will raise the number of laboratory investigations as needed. With these characteristics, the routine sampling of IA can efficiently meet disease surveillance needs in situations in which disease is absent (Fig. 2).

After detecting the first case in a region (first vertical line in Fig. 2), the epidemic situation changes because "presence of disease" is now known (section between the first and second vertical line in Fig. 2). In this situation, the search for disease is no longer useful (Table 3). However, appropriate control measures may be implemented if available (upper bold line in Fig. 2), and some form of monitoring becomes necessary to assess the protection or treatment rate in the uninfected part of the population. Because IA samples are biased toward infected animals and hence cannot give a true representation of the (uninfected) population, a systematic sampling of healthy animals (HA) appears appropriate to estimate population proportions or equivalent values with sufficient precision (middle rectangle in Fig. 2).

Toward the end of an outbreak (the section between the vertical lines labeled "*L*" and "*L+k* time units" in Fig. 2), either because of successful control or natural fade-out, the aim of disease surveillance again shifts to detecting diseased animals, if still present. As above, finding infected animals will be more efficiently fulfilled by testing IA (compare data of all three example diseases, particularly Fig. 1 and Table 2, virology during vaccination). It is known that the disease was in the control area at the time of the last case detection (label "*L*" in Fig. 2). Hence, control measures will still operate, and continued monitoring of control performance will be achieved by sampling from HA (middle rectangle in Fig. 2 stretched beyond "*L*"). Practically, it is not possible to rule out the detection of a new case until remnant foci or persistent low prevalence of the disease in the control population has been excluded (i.e., "suspected freedom of the disease"). Thus disease "freedom" (after label "*L+k* time units") cannot be reliably reached by data analysis alone but also must rely on epidemiologic arguments that specify the value of *k* for a particular disease. (A more pragmatic approach is discussed below.)

Example diseases

We considered three example diseases: fox rabies in Europe is an example of an established surveillance program (Baer, 1991); CSF transmitted from a wild boar population to domestic pigs (Fritzemeier et al., 2000) forced the routine inspection of the health of wildlife populations in transnational surveillance initiatives (Staubach et al., 2003); HPAI is an example of a wildlife disease surveillance scheme under discussion (Guberti and Newman, 2007). These examples were selected on the basis

of availability of relevant historic data bases (see Methods). The respective guidelines for disease surveillance and control monitoring serve to illustrate practical aspects of situation-based surveillance.

The example diseases have one thing in common: all of them propagate in an epidemic fashion. The relevance of rabies or HPAI for application of situation-based surveillance according to Figure 2 is clear because the numbers of clinical cases (i.e., morbidity or mortality events; Mörner et al., 2002) increase with the spread of HPAI or rabies. For example, rabies was reintroduced into Italy in 2008 and detected after laboratory investigation of a distinct IA sample, a fox that bit a human (De Benetictis et al., 2008). The follow-up detections of rabies-positive animals in the area came through IA submissions (OIE, 2009).

To consider the relevance of CSF in wild boar, further epidemiologic background is needed. Historically, the spread of CSF in wild boar was characterized by numbers of dead and morbid animals (Dahle et al., 1993; Paton et al., 2000), which would have put this example in line with the two others. Today, CSF infection in wild boar is still characterized by a mortality peak after the initial intrusion, whereas later in an epidemic, lower numbers of deaths occur mainly in very young animals (Artois et al., 2002). Consequently, disease surveillance for CSF in wild boar also uses serology (Artois et al., 2002; Rossi et al., 2005a), and efficiency of this approach was shown to be independent of the sampling source (Table 2). Nevertheless, for virus detection and isolation required for confirmatory diagnoses of outbreaks (Artois et al., 2002), laboratory analysis of the IA sample performed decisively better (Table 2; Rossi et al., 2005b). In support of the scheme in Figure 2, the CSF outbreak in Germany early in 2009 was detected after examination of a wild boar submitted because it showed clinical signs, a typical IA (ProMedMail, 2009). By comparison, a systematic investigation during the preceding 12 mo yielded about 11,000 HA samples that were negative for CSF infection and were not focused on the area of the new outbreak (data not shown).

Situation-specific considerations

Absence of disease: In jurisdictions that have not detected a disease of concern (historically free) or that have demonstrated freedom from disease after an outbreak ("disease-free" status according to legal guidelines), "absence of disease" is the relevant epidemic situation for the disease of interest. Following Figure 2, continuing disease surveillance in IAs will inform about an introduction or a recurrence of diseases that cause mortality or morbidity events. Sampling of IAs is done to see whether there are positives among the suspects in that group. As long as no true positives are found in the IA sample, the assumption of disease absence remains valid. However, the disease situation changes, as does the target of surveillance activity, on detection of a positive sample.

Field experiences with avian influenza surveillance in wild birds also support IA sampling as purposeful activity in cases of disease absence. For example, "sampling dead birds and investigation of mortalities" was regarded as an effective early warning system for HPAI (Pitman et al., 2007). Case reports of HPAI detection in wild birds in Germany in 2007 (FLI, 2008) provide field examples of how the IA sampling directed investigation efforts toward the localities of HPAI outbreaks in that country. Moreover, recent guidelines for HPAI surveillance explicitly leave the option of discontinuing designed sampling from HA (OIE, 2008, see §1.4.6.2).

An alternative concept for disease surveillance of epidemics in wildlife is repeated proof of absence via falsifying presence. For example, the investigation of 59 animals per annum (European Commission, 2002b; Guberti and Newman, 2007) suffices to reject prevalence greater than or

equal to 5% with 95% certainty (Cannon and Roe, 1982). Although intended for areas in which a disease of interest is suspected to occur (European Commission, 2002b), the approach is less helpful in areas where disease truly is absent and prevalence is zero. Further assurance of detection at a lower prevalence threshold might increase the required sample size to an impractical number (Guberti and Newman, 2007) but not solve the conceptual problem: The ultimate aim of this procedure is to "prove" that prevalence (p) is 0, and therefore $p>0$ has to be rejected on the basis of the sampling. Although the latter is difficult to achieve with the use of conventional statistics, the outcome is needed to assure disease absence. In this sense, the outcome is not particularly informative because absence of disease was already accepted before the survey. In contrast, by exploiting the assumed disease situation, one assumes no positives in the population and hence that prevalence is zero. The relevant disease surveillance activity then focuses on rejecting $p=0$ by providing a positive sample unit as evidence of disease introduction or recurrence. If rejection fails, however, the disease situation does not change. Although an ideal task for IA sampling, one might argue that sampling HA also can serve to search for the one positive of interest (i.e., to reject $p=0$). Although some jurisdictions might regard such efforts as worth the investment, as our data show, the odds of finding a positive are higher with IA sampling in these situations.

A recommendation for surveillance in the situation of disease absence that is very similar to the proposal presented in Figure 2 was included in the report of a WHO expert consultation on rabies (WHO, 2005, Chap. 9) in their consideration of rabies-free and provisionally rabies–free countries or areas. However, one difference remains: similar to recommendations in earlier documents, wherein particular sample size values for rabies surveillance were given (WHO, 1992), the more recent report recommends examining a "relevant number" of samples. However, for areas in which the disease is absent, the occurrence of IA (i.e., clinical suspects not affected by the disease of interest) might be rare or naturally limited; thus, a predetermined number of samples from IA cannot be guaranteed. An apparently pragmatic approach, often used by decision makers, is to supplement routine IA sampling with HA samples to meet sample size requirements (e.g., WHO, 2005). This strategy would seem logical only in cases in which the nominal sample size requirements are larger than the baseline intensity of IA submissions. However, because the majority of samples under this "supplemental" strategy will come from HA, shortcomings of this approach include 1) screening some proportion of animals that do not represent the subpopulation targeted for disease surveillance, 2) hampering the timeliness of outbreak detection, and 3) requiring continuous, relatively large diagnostic efforts in regions in which detection is unlikely. It follows that sample size specifications might force emphasis on HA that probably will not limit the overall effectiveness of disease surveillance but could be relatively inefficient under these conditions.

We speculate that the intent in requesting a minimum sample size in such guidelines was to ensure regular delivery and testing of samples. These recommendations seem mainly intended to counteract diminishing awareness in the case of disease absence. However, rather than setting a minimum sample effort to encourage submissions of IA, we encourage information campaigns explaining how outbreaks of epidemic diseases can be most effectively detected by continuing alertness for IAs (OIE, 2008).

In summary, if a disease is absent (Fig. 2) but known to be associated with mortality or morbidity events, then continuing to investigate as many occurrences of IA as possible (instead of imposing fixed

minimum sample sizes) should meet surveillance needs in this situation because 1) effort is lower when using IA sample sources during periods of disease absence, 2) a fixed sample size might not be guaranteed from collecting IA exclusively, and 3) the number of IA submissions likely will increase at the time and location of a disease outbreak.

Presence of disease: After detection, the epidemic situation changes (Fig. 2; Table 3) because the disease is now present and program emphasis may shift to monitoring or control. In cases in which an operable control option is available and applied, surveillance likely will focus on measuring progress toward disease reduction or elimination over time. Ideally, the adequacy of the control effort is monitored via estimating relevant parameters by sampling the healthy subpopulation and checking against standardized reference values. To make such estimates with necessary precision, a statistical design should guide decisions on sample size and spatiotemporal distribution (e.g., Cannon and Roe, 1982).

Rabies in foxes and control by oral mass vaccination programs provide a relevant example. This disease is lethal for infected animals, and mass vaccination has been shown to be an effective control measure. Because vaccinated individuals can be determined postmortem, a statistically designed sampling of HA will provide an estimate of the proportion of individuals that were vaccinated with specified precision. For example, in Figure 2, setting $n=8$ animals sampled per annum per 100 km^2 from an area of at least 5,000 km^2 (e.g., German National Directive on Rabies Control, 2001) allows an estimation of the percentage of vaccine-induced seroconversion in the population with 5% precision and 95% certainty because the total sample minimum of 400 animals exceeds the minimum sample size of 384 (Cannon and Roe, 1982). If the estimate of seroprevalence exceeded the prescribed minimum, then disease elimination would be expected after 2 yr to 4 yr of repeated vaccination campaigns (Aubert, 1994). During this period the search for rabies would no longer be necessary because it is reasonable to presume presence during control time; afterward, disease absence reasonably could be assumed on the basis of past experiences. Here, emphasis on HA sampling appears best suited to meet surveillance needs for the situation. In practice, however, these situations are rarely ideal, and the following examples illustrate why, for practical reasons, the scheme in Figure 2 proposes the investigation of both samples, IA and HA, simultaneously (although with different aims).

Continuing with the example of rabies, guidelines have been established prescribing sample sizes for monitoring the quality of oral vaccination programs in an area or country. Therein, the value of n in Figure 2 is set to either 8 (European Commission, 2002a) or 4 (WHO, 2005), reflecting the flexibility of the designed precision in accordance with perceived need. These samples are needed to assess control performance by estimating seroprevalence (or bait uptake if biomarkers were attached to the bait); however, the same sample size also is recommended for estimating disease incidence (European Commission, 2002a; WHO, 2005). Additionally, when sampling the n animals, priority was given to IAs.

Taking into account the situation for which the monitoring strategy was proposed, what information can be derived from such mixed samples? First, assessing control efficacy would be less rigid because IAs included in the sample do not represent the healthy subpopulation and should be excluded from percentage calculations. The remaining samples from the HAs might be too few to meet specified design quotas. Second, IAs, by their very nature, are not sampled from the general population but rather are "self-selecting" and therefore not repre-

sentative of the general population. Therefore, it is unclear what the base population would be for any resulting incidence estimate (Schoenbach et al., 2001). To our knowledge, inclusion of IA in the monitoring of rabies vaccination in foxes was aimed at detecting the disease as long as it was still present, which seems necessary only when the time until elimination is uncertain, the quality benchmark of control efficacy is not precisely known, or both. Thus the pragmatic, but not ideal, recommendation in the guidelines was based on uncertainty about the performance criteria of intervention measures; thus, some monitoring for disease presence was advised concurrent with control.

To address the problem, we again refer to Figure 2. The two samples proposed for simultaneous investigation have different objectives. First, the HA sample of prespecified design (not containing indicator individuals) provides the information needed for quantitative monitoring of control measures. Second, continued IA sampling aims at detecting diseased animals and rabies foci that still could be present. This approach can inform decisions on control termination: As long as rabies cases are detected (making the vertical line in Fig. 2 labeled "*L*" successively move forward), control should be continued.

The situation after detection of HPAI in wild birds can be taken as an example for diseases in which control is limited largely to preventive measures (e.g., increased security requirement or movement restriction in poultry). The resulting effect cannot be estimated via independent quantities from the wildlife disease host population. Consequently, the sample size designed for monitoring control in wildlife (height of middle rectangle or n in Fig. 2) is 0. Disease surveillance via IA sampling is applied to repeatedly confirm the presence of disease or, eventually, to motivate suspicion that the disease has disappeared.

Suspected freedom of the disease: As the time since last detected case lengthens, suspicion will eventually arise as to whether the epidemic situation is still classified as "presence of disease." As discussed before, the direct proof of disease absence by statistical testing alone does not work. Consequently, disease management guidelines incorporate plausibility arguments based on the epidemic character of the disease (most often some period of time without detecting a case via an operating disease surveillance program).

As depicted in Figure 2, the approach for achieving a conclusive decision on disease disappearance combines ongoing disease surveillance via IA with the logical consequence of disease elimination: if testing of IA does not provide evidence of presence over an epidemiologically reasonable disease-specific time period (symbol k in the upper right label of Fig. 2), then absence can be proved by stopping control and emphasizing routine disease surveillance in IA. After a finite time, the protective effect of control (e.g., proportion of immune animals) will diminish in the host population (e.g., OIE, 2008, see §1.4.6.1), and a persistent but unseen disease focus would resurge and eventually be found through disease surveillance.

An example of such a stop-and-observe strategy was found in legal guidelines for rabies mass vaccination. Mass vaccination has to be continued for 2 yr (Fig. 2, $k=2$ in label "$L+k$ time units") after the last detected case (Fig. 2, label "*L*"). Thereafter, the control area is declared rabies free if adequate disease surveillance is practiced (WHO, 2005; note that this declaration is based on the likelihood but not the "proof" of absence). This guidance was improved with observations that the proportion of immunized animals after the end of vaccination declines so rapidly that any persistent infection would likely recover into a detectable epidemic within 2 yr postvaccination (Schaarschmidt et al., 2002). Hence, by adding another 2 yr postvaccination to the man-

datory 2 yr without case detection required to stop vaccination, the time required to reasonably assure freedom from disease would be 4 yr after the last case was detected (Thulke et al., 2000; German National Directive on Rabies Control, 2001). It is noteworthy that under these recommendations, only testing of IAs is needed throughout the procedure (see Table 3).

Applicability of situation-based surveillance

We do not question the need and usefulness of "targeted monitoring studies" to improve scientific understanding about a disease and its spread. However, we have focused on applied management aspects of disease surveillance in cases of presence, suspected absence, or accepted absence of epidemic diseases that cause mortality or morbidity events. Facing hundreds of thousands of individual laboratory investigations of presumably unexposed animals throughout the years and throughout the European continent in the situation of absent diseases, our analysis urges a sit-and-wait approach while continuously testing IAs. In public health management, this proposal relates to the concept of syndromic surveillance (Henning, 2004; Mandl et al., 2004).

Logically, no other testing is needed to support decisions based on absence or presence of a disease of interest or concern. If further testing is done, the motivation seems most likely related either to research for improved scientific understanding or to allaying a decision-maker's fear of being uninformed. The latter is understandable but, as we have tried to show, seems unjustified. For example, the common expectation that earlier detection of disease arrival might improve the chances of successful control (which is derived from livestock disease management) is often used to argue for regular and systematic testing of HA in wildlife disease surveillance regardless of cost. However, we are not aware of examples in wildlife disease management where designed sampling of HA, or the mixture of both HA and IA, has provided earlier detection of an epidemic or mortality event than detected by IA sampling alone. In contrast, there are several historic examples in which sampling IA alerted responsible jurisdictions to outbreaks in the wild (e.g., Pitman et al., 2007; De Benetictis et al., 2008; FLI, 2008; OIE, 2009).

The greatest challenge in IA sampling appears to be maintaining willingness to acquire and submit IA. During periods of disease-free status, awareness is known to decrease (WHO, 2005) and proposals of fixed-size sampling appear aimed at countering decreasing awareness. The widespread perception that without permanent inflow of a high number of test results disease surveillance would be too uncertain might be fostered by calling on responsible professionals and various publics to intensify IA reporting and sampling when disease already has been detected in a region. In our opinion, such requests have created a false link between the need for suspect animals and a crisis situation. Rather than encouraging IA submissions only during crises, we recommend shifting emphasis to underscore the value of such samples in ongoing surveillance before and after outbreaks occur.

Extensive public awareness of the role of IA sampling in preventive management needs to be raised (Burgos and Burgos, 2007). In this context, guidelines should generally link awareness for "suspicious" wildlife to the prevention of harmful disasters rather than to documenting actual outbreaks. In this way, reporting and submitting IA would become the routine business of responsible people regardless of the disease state (see Fig. 2, second level), and motivation and awareness would become independent of the publicity of an outbreak (Mörner et al., 2002). Consequently, IA sampling would provide "an effective system of disease surveillance" for diseases causing pronounced mortality or morbidity patterns (OIE, 2008).

Conclusions

Careful consideration of the actual epidemic situation with respect to selected wildlife diseases in an area was found to be beneficial in constructing efficient, target-oriented surveillance or monitoring schemes. In particular, surveillance for epidemic wildlife diseases that cause highly visible mortality or morbidity events might be optimized with the use of situation-based schemes. Proving the absence of such diseases by systematic sampling of apparently healthy animals likely will be less effective and less efficient than the constant investigation of any available IAs. In areas of "disease free" status and with no control measures to assess, surveillance might be limited to a focused investigation of available IAs (OIE, 2008). These recommendations do not necessarily apply to efforts to improve the scientific understanding of epidemic wildlife diseases, but rather to the focused need of decision makers in health and resource management arenas to be informed on the occurrence of specific wildlife diseases as a basis for responsive management.

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