July 2019

Supplement to the Animal Disease Surveillance Report

Methods and specific information concerning the 2018 testing programmes

1/22

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1 Introduction

Animal disease surveillance serves to record and document the health status of farm animals in Switzerland. Every year the FSVO and cantonal veterinary services conduct disease-specific testing programmes to document the incidence of several <u>animal diseases and zoonoses</u> and assess the health status of Swiss farm animals. Official veterinarians take samples from farm animals on behalf of the national veterinary service. Recognised <u>diagnostic laboratories</u> test these samples for pathogens. Depending on the disease and animal species, samples are collected on farms, during milk collection and/or in slaughterhouses ("RiBeS" project). The results of this surveillance are crucial in deciding whether <u>control</u> measures need to be adopted or adjusted.

This supplement describes the methods used in the surveillance programmes and gives specific information for each disease concerned.

2 Methods

The surveillance programmes carried out in Switzerland pursue three different objectives, depending on the disease.

Demonstrating disease-free status: Animal diseases that have been successfully controlled and eradicated in Switzerland could be reintroduced at any time. Freedom from eradicated diseases has therefore been monitored since 1995. This monitoring is based on three pillars: investigation of clinically suspected cases, testing on import and an annual surveillance programme. Freedom from disease is monitored for the following diseases: IBR, EBL, BTV, AD, PRRS, brucellosis in small ruminants (*Brucella melitensis*), CAE. Freedom from disease is demonstrated by similar methods for several animal diseases. The similarities between methods are described in the following section, whereas aspects specific to each disease are discussed separately.

Monitoring the success of control: The control of animal diseases is a protracted process. Its success has to be documented regularly in order to determine any necessary adjustments to the control measures. Control is monitored for the following animal diseases: BVD, BSE, Salmonella infection in poultry. Depending on the disease, the control measures may vary: in this case, the monitoring methods are also different and are described in separate sections for each disease.

Early detection: The task of early detection is to continuously assess the risks posed by serious infectious diseases and to pass on the resulting information to decision-makers in a targeted manner. In the best scenario, the introduction of a disease can be prevented or the risk minimised. But in any case, early detection contributes to damage limitation. Early detection of animal diseases also depends on the methods used, and these are described in the individual sections.

2.1 General principles for demonstrating freedom from disease

2.1.1 Requirements for freedom from disease

"Demonstration of freedom from disease" relies on random testing, which in turn is based on statistical considerations. If all tests in the random sample are negative, the presence of the disease can be ruled out with a high degree of probability. In demonstrating freedom from disease, this probability is referred to as "confidence" and is expressed as a percentage. The maximum value is 100%. However, it can only be achieved in theory, as this would require all units of the population to be examined with a perfect test. However, 99% confidence can be achieved with comparatively small sample sizes.

Freedom from disease is generally demonstrated on an annual basis. In order to demonstrate freedom from an animal disease, there must be no prior evidence that the disease is present in the area concerned. Such a condition can only be met if there is an obligation to investigate cases of disease and suspected cases and to report them to the relevant authorities. If there is also a risk of introduction of a disease, this must be communicated and disease awareness increased in order to identify suspected cases: animals showing typical clinical signs of the disease must be examined accordingly.

If there is no evidence that the disease in question is present in Switzerland, the requirements for statistically based demonstration of disease-free status are met by means of random sampling. For some diseases, bilateral treaties with the EU stipulate that disease-free status must be demonstrated in order to export animals and derived products to EU countries which also have disease-free status. Diseasefree status also authorises countries to regulate the import of animals and derived products. Demonstrating freedom from disease therefore has economic advantages. Switzerland therefore demonstrates its freedom from disease "voluntarily" in certain cases, i.e. without being required to do so by treaties (one example is PRRS).

An important prerequisite for comparing disease-free status between individual regions and countries is that the quality of surveillance and the results obtained permit statistically sound conclusions and are therefore comparable. The scientific and statistical basis of the Swiss testing programme meets this condition.

2.1.2 Sampling

We select the sample based on statistical principles which are scientifically published and thus generally accepted. The principles rely on random selection of the farms to be tested. As a basic principle, it is only possible to draw conclusions about the total population if the farms in a sample are selected at random. This principle need not be observed if the distribution of risk factors in the population and the sample is known.

Based on these facts, the Federal Food Safety and Veterinary Office (FSVO) has developed and refined additional methods in recent years. The aim is to select samples as efficiently as possible. The two main methods it uses are:

- risk-based sample calculation: using this method, the annually calculated number of farms in the sample is lower than with the standard method;
- risk-based farm selection: farms which have an increased risk of the disease are specifically selected and tested.

Both methods reduce the number of farms tested. This enables us to lower the costs of the testing programme. The difference between the two methods is that the annual number of tests is smaller with risk-based sample calculation. This results in a lower confidence of detecting any cases of disease present. With risk-based farm selection on the other hand, despite the smaller number of annual tests, the confidence remains the same as there is more testing of high-risk farms.

Risk-based sample calculation: Switzerland's bilateral EU treaties call for the tests necessary to demonstrate disease-free status to be repeated annually. The reason for this is that the tests can only detect a prior outbreak of disease. They therefore provide meaningful results only for the past year. Based on the following consideration, it is possible to reduce the extent of repeat sampling: once freedom from disease has been successfully demonstrated there remains a constant, small possibility of the disease being introduced despite import rules and tests. As a result, the level of confidence decreases over time. This decrease is calculated using a quantitative risk assessment. The annual repeating of the testing programme therefore only needs to compensate for this decrease in confidence. Using this calculation method developed by the FSVO, we are able to reduce the number of farms tested annually and to do so on a scientifically sound basis.

Risk-based farm selection: Farms which have an increased risk of the disease are described as "sentinel farms". They are specifically included in the sample. Because these farms have a higher risk of the disease, the total number of farms in the sample can be reduced. However, the majority of farms are still selected at random, so the sample can still be regarded as a random selection.

In order to apply risk-based farm selection, we identify and quantify risk factors. These are used to gauge the probability of a disease occurring on a farm. This gives the relative risk of individual farms. This means that a farm with a high relative risk counts more heavily for surveillance purposes than another farm with a smaller calculated risk. For example, one farm with three times the relative risk can replace three farms with an average relative risk. This allows us to reduce the number of farms for testing.

Confidence of freedom from disease: The testing of samples collected according to statistical principles allows us to draw conclusions about the total population by means of probability calculation (stochastics). Using this method, we calculate how probable the sample result is if the population is composed of a particular species. In the case of demonstrating freedom from disease, we therefore determine how probable it is that the sample is negative if some cases of the disease did occur in the population. This probability is described as the confidence of freedom from disease. The requirement for the testing programme is that we need to be able to detect a certain assumed prevalence at herd level (the "design prevalence") with a defined level of confidence. Specifically: at least one contaminated farm - out of several farms assumed to be contaminated - would need to be found in the sample with a given probability. Based on this assumption, we calculate the necessary sample size. For IBR, EBL, brucellosis in small ruminants and Aujeszky's disease, the criteria to be met are set out in the bilateral agreements with the EU. For PRRS and CAE, Switzerland has defined these criteria independently (voluntary demonstration of disease-free status).

Two points often cause confusion and must therefore be addressed: first, the aim of the testing programme is to demonstrate freedom from a particular animal disease. This means that no cases should be discovered by other means, e.g. by testing suspected cases or investigating abortions. Second, the assumption that some contaminated farms or animals are present is made solely in order to calculate the sample. It is simply a calculation aid. Consequently, this assumption does not allow any contaminated farms or animals to actually be detected outside the sample. Were this to happen, Switzerland would lose its disease-free status for the disease in question.

Bayesian method: Since 2012, we have used a special statistical method to evaluate the samples, combining the results of the current sample with sample information from previous years (Bayesian method). To calculate the decrease in confidence for the previous year's sample, we have carried out an exhaustive quantitative risk assessment over many years. We include the resulting information in the Bayesian method, but without carrying out a risk assessment every time: in evaluating the current sample, we use a fixed value for the annual decrease in confidence for the previous year's sample, provided the number of imported animals is below a specified level.

2.1.3 Farm selection

Because livestock are kept on farms, the objective of a testing programme to demonstrate freedom from disease is normally a statement at farm level. If the sampling unit is the farm, we calculate, for each farm tested, how reliably we can rule out infection of the herd or flock. In doing so, we take the testing of individual animals as a diagnostic test for the farm.

Where requirements exist at animal level, the calculation is carried out – for simplicity – at animal level without considering the farm.

Risk factors and risk groups: In order to integrate farms into the sample on a risk basis in addition to random selection (see risk-based sample calculation and/or sentinel farms), disease-specific risk factors need to be determined in advance. Farms with the same combination of risk factors have the same relative risk of the disease; they belong to the same risk group. Farms with a very high risk of the disease (upper risk groups) are selected as sentinel farms. Farms with a lower risk (lower risk groups) are selected at random. The sample is stratified by canton. Distribution between the cantons depends on the number of farms with the species to be tested. This ensures that the cantons are properly represented in the sample.

Cattle: Farms from which milk samples are collected regularly for milk testing by Suisselab AG in Zollikofen are classed as dairy (milk supplying) farms; all other farms are classed as non-dairy.

Sheep and goats: A farm with sheep and goats is counted twice in the population and sample: once as a sheep farm and once as a goat farm.

Pigs: The target population comprises all pig farms, irrespective of whether fattening or breeding pigs are sampled. This is due to the special structure of pig farms in production (breeding pyramid). However, depending on whether breeding or fattening pigs are sampled, the population and sample may comprise only fattening, breeding or mixed farms. For example, random sampling is currently carried out only on breeding pigs, i.e. from breeding or mixed holdings, since they have a higher risk of entry of the diseases included in the sample.

2.1.4 Laboratory testing

Sample collection: Blood samples are collected on farms by authorised veterinarians. The samples are sent for testing to several FSVO-approved laboratories, where they are individually diagnosed. The veterinarian must complete a sampling report for each selected farm. If no blood samples could be taken, for example because the farm had stopped keeping the species in question or had no animals at the time of inspection, the reason must be given.

The collection of blood samples is increasingly being shifted to the slaughterhouses. Traceability of the animals sampled is important in this respect. Most sampling of cattle and pigs is now carried out at the slaughterhouse by the official veterinarians responsible for meat inspection.

In the case of cattle, traceability is ensured via the animal movements database (AMD); in the case of pigs, the official veterinarians responsible for meat inspection record the animals' farm of origin. For cattle samples, official veterinarians are supported by a web service of the animal movements database on cattle sampling at the slaughterhouse ("RiBeS"). This web service shows the veterinarian which animals to sample and creates the documentation. Shifting the sampling for non-dairy cattle from farm to slaughterhouse has made sample collection easier and less dangerous. However, the design of the testing programmes has had to be adjusted, as only a few animals per farm can be tested and the results depend on how many animals from individual farms are sent for slaughter.

The milk samples tested come from quality inspection at Suisselab AG (Zollikofen).

Since it is assumed prior to sample testing that Switzerland is free of the diseases in question, a negative test result is expected. Neither the competent veterinary authorities nor the keepers of the herds tested are sent a laboratory report if the results are negative.

Sensitivity and specificity: Any laboratory diagnostic method can yield false results, albeit very rarely and only under certain conditions. The result can be false-negative or false-positive. In the case of a false-negative result, an infected animal is not recognised as such. False-negative results reduce the sensitivity of a test. The sensitivity indicates the proportion of infected animals correctly detected as positive in the test. In the case of a false-positive result, a healthy animal is wrongly identified as infected. False-positive results reduce the specificity of a test. The specificity indicates the proportion of non-infected animals correctly detected as negative in the test.

To demonstrate freedom from disease, a serological test for specific antibodies is usually carried out. The first step is to carry out a screening test, usually an ELISA, that is as sensitive as possible. This ensures that no infected animals are missed. However, it may give a few false-positive results. Samples testing positive in the ELISA are then retested using a specific test to identify the false-positive samples. These confirmation tests on all positive samples are carried out by the national reference laboratory for the disease in question.

Evaluation of laboratory results: Here a distinction needs to be drawn between laboratory tests detecting antibodies and those detecting pathogens. In demonstrating freedom from disease, most samples are tested for the presence of antibodies. If antibodies are found, it means the animal was in contact

with the pathogen at some time and its immune system responded by producing antibodies. However, it can also mean that the animal was vaccinated, in which case it cannot infect other animals. Very rarely, animals may react positively to a serological test even though they have never had contact with the pathogen in question. These animals are described as "singleton reactors". Reasons for this response include non-specific immune reactions or cross-reactions with other pathogens. As a result, different initial situations can lead to the same positive test result. In the event of a positive finding, therefore, the situation needs to be clarified more precisely. Investigations are based on the measures prescribed in the Epizootic Diseases Ordinance (EzDO) in the event of an animal disease. It is only by conducting further tests on the animal and by investigating the affected farm and in-contact farms that we can differentiate singleton reactors from a real disease outbreak, identify the introduction route and tailor measures to the actual risk. At international level, singleton reactors do not lead to the loss of disease-free status.

2.2 Demonstrating freedom from disease: disease-specific information

2.2.1 Infectious bovine rhinotracheitis (IBR) and enzootic bovine leukosis (EBL)

Requirements: In parallel with clinical surveillance, testing for infectious bovine rhinotracheitis (IBR/IPV) is carried out on abortions, artificial insemination bulls and animals participating in shows or admitted to animal hospitals. For enzootic bovine leukosis (EBL), no further surveillance testing is conducted outside the sample. Lymph node changes detected during meat inspection and suggestive of EBL give rise to testing for EBL viruses. All of these tests must be negative in order to demonstrate freedom from disease for both of these diseases.

Based on the bilateral agreements with the EU, the sensible approach is to carry out a testing programme by random sampling in order to demonstrate freedom from IBR and EBL and be able to export bovine animals and derived products to other IBR- or EBL-free countries. In addition, imports of bovine animals and semen are regulated. In the case of IBR, imported animals must meet additional guarantees and are tested on import from non-IBR-free countries. Genetic products are also subject to special import conditions.

Sample calculation: The sampling procedure is identical for IBR and EBL, and largely the same farms and animals are tested. This keeps sampling and logistical costs low. Because testing using bulk-tank milk samples is much cheaper for a farm than testing using individual animal blood samples (although this is necessary for non-dairy farms), it seems attractive to focus on testing dairy farms. However, such an approach would violate the basic principle of random sample selection. We therefore regard dairy and non-dairy farms as separate sub-populations and demonstrate freedom from disease separately for each. Looking at the overall bovine population, these objectives go well beyond the EU requirements. There is therefore a greater probability of detecting any outbreaks of IBR or EBL. When this sampling procedure was introduced in 2013, care was taken to ensure that the costs remain unchanged.

The 2018 testing programme was designed in such a way that there is a 99% confidence of detecting the 0.2% design prevalence (corresponding to around 80 infected farms) in both sub-populations (dairy and non-dairy farms). Both requirements stem from the bilateral agreements with the EU and apply to the entire bovine population. Extrapolating from sub-populations to total population yields either a very high confidence at a 0.2% design prevalence or approximately halves the design prevalence at an existing confidence level of 99%.

Besides dividing farms into dairy and non-dairy, it is also necessary for risk-based farm selection to distinguish between sentinel farms and randomly selected farms in both sub-populations. Here we decided that half of the necessary confidence should come from the testing of randomly selected farms.

The other half comes from the testing of sentinel farms. Owing to the stochastic correlation, a confidence level of 90% corresponds to half of a 99% confidence level. At least 90% confidence must therefore come from each of the four types of farm. The EBL sample requires more sentinel farms than the IBR sample. Because we integrate EBL sentinel farms into the IBR random sample, slightly fewer randomly selected farms were tested for IBR than for EBL. However, since the number of farms involved is comparatively small and the organisational effort would be comparatively high, we do not compensate for this discrepancy and we test all farms and animals for both diseases. We also add a reserve to the calculated number of samples, since it may not be possible to sample individual selected farms. In the case of non-dairy farms, we do not use an additional reserve because evidence of freedom from disease must ultimately be provided for the total population and over-testing would cause comparatively high costs.

In calculating the sample, we forego the option of reducing the number of farms via risk-based sample calculation in favour of higher surveillance quality.

Selection and testing of farms: In the case of random sampling to test for IBR and EBL, all cattle sampled are screened for both diseases. These cattle are part of the cattle sampled for BVD. For the random sample, holdings are not selected in advance but animals are sampled as they arrive at the slaughterhouse. No random selection of the cattle and holdings to be sampled is possible in advance, as it is not known which cattle are to be slaughtered. However, the draw corresponds to a random selection, since there is no known distortion due to this procedure. Sentinel farms, on the other hand, are identified *a priori*. Sampling at the slaughterhouse is carried out with the aid of the "RiBeS" system, which triggers a signal at the slaughterhouse when a sample needs to be taken from a slaughtered bovine animal. The animal must meet certain selection criteria in the AMD and tests still need to be conducted for BVD on the farm of origin.

Animal disease	IBR and EBL				
Farm type	Non-dairy farms (blood samples)		Dairy farms (bulk-tank milk samples)		
Data basis	AMD as at 11.11. in the previous year		Milk testing as at 11.11. in the previous year		
Selection method	Random selection Sentinel farms		Random selection	Sentinel farms	
Random selection	Yes No		Yes No		
Stratification	Yes No		Yes	No	
Sampling period	January 1 to May 31		2 samples per farm, in January and April		

Table 2.2.1-1: Selection of farms and sample collection period

The bulk-tank milk sample is a pooled sample from all lactating cows on a farm. When testing bulk-tank milk samples, we need to bear in mind that only some of the cows on a farm are in lactation at any one time. we therefore test two samples with a three-month interval to cover all cows on a farm, or calculate the herd sensitivity for a sampling, reducing the test sensitivity by a factor corresponding to the proportion of non-lactating cows.

For IBR and EBL, in addition to random selection, we apply risk-based selection of sentinel farms.

IBR sentinel farms have one or more of the following characteristics identified in a survey of experts as IBR risk factors:

- Summer pasturing
- Farms with above-average animal movement (animal movements in the AMD)
- Farms that have imported cattle
- Farms close to the border (within 5 km of the border and cross-border roads)
- Farms in areas with a high farm density (farms/km²)

These five risk factors give 32 possible combinations or risk groups. Sentinel farms are selected from the upper risk groups.

In a separate survey of experts, we identified three EBL risk factors for the selection of sentinel farms:

- Summer pasturing
- Farms with above-average animal movement (animal movements database)
- Farms that have imported cattle

These three risk factors give eight possible combinations or risk groups. Farms with the highest relative risk are used as sentinel farms. Farms with a lower risk are not all required; sentinel farms from this group are therefore selected at random. The rest of the procedure the same as for IBR.

Animal selection: On non-dairy farms, blood samples are collected from bovine animals between six months and five years old and tested for IBR and EBL antibodies. For on-farm sampling only: If fewer than seven animals on a farm are over 24 months old, a total of seven blood samples are collected, including from younger animals. When sampling at the slaughterhouse using RiBeS, the number of animals per farm depends on the size of the group of animals to be tested for BVD. In most cases, the number of animals is five or fewer.

However, in the case of dairy farms, it is not known which cows supplied the milk contained in the bulktank milk sample. But by testing two samples with a three-month interval, there is a high likelihood that all lactating cows on the farm will be covered. Young stock and male animals are not covered by the testing of bulk-tank milk. In the case of dairy farms, two samples with a three-month interval enable us to achieve an over 99% probability of detecting any bovine animals infected with IBR or EBL. With only one sample, however, the herd sensitivity is only 78.8%.

Laboratory testing: In the case of bulk-tank milk samples, diagnosis is based on the remaining sample material after official milk testing has been carried out by Suisselab AG. All samples in the IBR selection are also tested for EBL. All laboratory methods are used to detect antibodies to BHV-1 or BLV.

The standard multi-step procedure can therefore be applied directly to the IBR blood samples. For bulktank milk samples and EBL blood samples, the procedure needs to be modified slightly as there are currently no suitable confirmation tests available. Because the bulk-tank milk tests are both highly sensitive and specific, the same ELISA test is repeated if the result is positive. If the second test is also positive, all bovine animals on the farm are tested by means of blood samples. If the second test is negative, the sample is tested a third time and this result is used (Table 2.2.1-2). The EBL blood test uses an ELISA test with higher specificity than the screening test employed as a confirmation test (Table 2.2.1-3).

Animal disease	IB	R	
Type of sample	Blood samples	Bulk-tank milk samples	
Screening method	ELISA test	ELISA test	
Sensitivity and specificity for in- dividual animal / farm	99.3% and 98.3% / depending on farm size and number of samples tested; sensitivity for randomly se- lected farms $Ø$ 30%; sentinel farms $Ø$ 68%; specificity 100%		
Confirmation testing method for positive samples	Serum neutralisation test	Blood samples on farm; all bovine animals > 24 months	
Sensitivity and specificity	Very good, 98.3-100% respectively	99% and 100%	
Reference laboratory	Institute of Virology of the Vetsuisse Faculty of the University of Zurich		

Table 2.2.1-2: Methods used to test for IBR, including sensitivity and specificity, and the IBR reference laboratory

Animal disease	EBL		
Type of sample	Blood samples	Bulk-tank milk samples	
Screening method	ELISA test	ELISA test	
Sensitivity and specificity	Almost 100% and 99.8% respec- tively	Both almost 100%; with one sam- ple, herd sensitivity 78.8%	
Confirmation testing method for positive samples	ELISA-Ab GP-51	Blood samples on farm	
Sensitivity and specificity for in- dividual animal / farm	Almost 100% and 99.5% respec- tively / farm as for IBR	-	
Reference laboratory	Institute of Virology and Immunology (IVI) of the Vetsuisse Faculty of the University of Bern		

Table 2.2.1-3: Methods used to test for EBL, including sensitivity and specificity, and the EBL reference

 laboratory

Case definition: The Epizootic Diseases Ordinance stipulates that in the case of IBR and EBL each antibody-positive bovine animal confirmed by the reference laboratory constitutes a case of disease and that measures must be taken on the farm concerned.

The additional investigations required in the event of disease make it possible to differentiate between a singleton reactor and an actual disease outbreak. If the findings are confined to a serologically positive result for an individual animal and no virus is found, the case is classed as a singleton reactor.

2.2.2 Aujeszky's disease

Requirements: Random sampling for Aujeszky's disease has been in place since 2001. Because Switzerland's neighbouring countries are also free of Aujeszky's disease and no live breeding pigs are imported, there is only a low risk of introduction. However, in recent years antibody-positive animals have been found in Switzerland during testing of wild boar. Based on the bilateral agreements with the EU, the sensible approach for Aujeszky's disease is to carry out a testing programme by means of random sampling. The testing programme is necessary in order to export live pigs and derived products to countries which also have disease-free status. This also allows us to regulate imports of live pigs and porcine semen.

Samples from the Aujeszky's disease testing programme are also tested for <u>Porcine reproductive and</u> <u>respiratory syndrome</u> (PRRS)<u>e</u> (PRRS). Synergies between the two programmes are exploited to the full, leading to low sampling and logistical costs.

Sample calculation: For Aujeszky's disease, we apply risk-based sample calculation. The reason is that the risk of introducing Aujeszky's disease is very low and there have been no further outbreaks in Switzerland since the introduction of random testing. The lower surveillance quality of random sampling associated with this procedure is not a concern for this disease and we are able to exploit the economic advantages. For Aujeszky's disease, according to the bilateral agreements, we need to be able to demonstrate in a random sample with 99% confidence that herd prevalence is below 0.2%. The testing programme from 2018 is only aimed at breeding farms, as these have a higher risk of entry in relation to the diseases in question (in particular PRRS), and an entry into the Swiss pig population can therefore be detected earlier than by testing the fattening farms downstream in the production chain. For organisational reasons, samples for Aujeszky's disease and PRRS are processed together.

We use the Bayesian method to **evaluate the samples**. Because Switzerland stopped imported breeding pigs several years ago, it is not possible to carry out a quantitative import risk assessment for Aujeszky's disease. We therefore use the simplified procedure, in which we integrate an annual 10% decrease in confidence into the calculation. This 10% figure is based on a management decision and is intended to encompass all conceivable import risks. The 10% decrease corresponds to a halving of the confidence level, i.e. the sample is around half as large as it is without this calculation method. The confidence level from the current sample must be 90% to compensate for the previous year's decrease and achieve an overall confidence level of 99 %.

Selection of farms and animals:

Sampling for Aujeszky's disease is carried out together with that for PRRS. The sample is identical. Details concerning the selection of farms and animals are therefore described in the section on PRRS.

Animal disease	Aujeszky's disease and PRRS
Animal category	Breeding pigs
Total samples	8,240 blood samples from individual animals (breeding animals)
Total number of pig breeding farms	2,000
Average expected samples per farm	Six samples per farm
Sampling period	1 January to 30 June each year

 Table 2.2.2-1: Total number of pig farms in Switzerland and the calculated sample size at sample level.

Laboratory tests: The blood samples taken by meat control personnel are sent to the designated diagnostics laboratories, where they are tested for antibodies against Aujeszky's disease and PRRS. Any laboratory diagnostic method can yield false-negative or false-positive results, albeit very rarely and only under certain conditions. The screening and confirmation testing methods for Aujeszky's disease, the respective sensitivities and specificities, and the reference laboratory for Aujeszky's disease are indicated below (Table 2.2.2-2).

Animal disease	Aujeszky's disease
Type of sample	Blood samples
Screening method	ELISA test
Sensitivity and specificity	99.5% and 99.9% respectively
Confirmation testing method for positive samples	Serum neutralisation test (SNT)
Sensitivity and specificity	Gold standard, over 99.5%
Reference laboratory	Institute of Virology of the Vetsuisse Faculty of the University of Zurich

Table 2.2.2-2: Methods used to test for Aujeszky's disease, including sensitivity and specificity, and the reference laboratory for Aujeszky's disease

Case definition: The Epizootic Diseases Ordinance stipulates that in the case of Aujeszky's disease each antibody-positive pig confirmed by the reference laboratory constitutes a case of disease and that measures must be taken on the farm concerned. However, because different initial situations can lead to a positive test result, it is important to investigate the situation more closely and to differentiate between singleton reactors and a real disease outbreak.

2.2.3 Porcine reproductive and respiratory syndrome (PRRS)

Requirements: In the case of PRRS, there is a high risk of introduction. The disease has spread rapidly in Europe since the mid-1990s and occurs in most European countries. It is therefore important to have a sound basis for the annual testing programmes. The basis for Switzerland's PRRS-free status was laid in 2001 when, following a minor outbreak, a mass screening programme was conducted in which over 40,000 pigs were serologically tested for PRRS. The results confirmed that Switzerland was once again PRRS-free after successfully controlling the outbreak. Since then, random testing has been sufficient to demonstrate freedom from the disease. However, a risk of introduction remains, as outbreaks in 2012 and 2014 show. And in recent years antibody-positive animals have been found in Switzerland during testing of wild boar. Strong disease awareness and effective early detection are therefore extremely important. Animals showing typical clinical signs must be investigated. For example, breeding sows in a herd with a noticeably high rate of abortions must be tested for PRRS. This is despite the fact that a different cause is generally assumed, as PRRS is not endemic in Switzerland. However, it could be introduced from another country at any time.

Unlike in the case of internationally regulated animal diseases, Switzerland cannot adopt import regulations for PRRS. Import organisations adhere voluntarily to their own strict rules. All pigs tested for swine fever due to a suspicion or for exclusion purposes will continue to be tested for PRRS as well, as the clinical symptoms are the same.

The PRRS testing programme is identical to the Aujeszky's disease testing programme; the samples collected are tested for both diseases. This makes the best possible use of synergies. In addition, the scientific and statistical basis of the PRRS testing programme is guaranteed.

Sample calculation: The PRRS testing programme is largely based on that for Aujeszky's disease. Risk-based sample calculation is therefore applied. A herd prevalence below 0.2% must be detected in a sample with 99% confidence. All other aspects of sample calculation are as described for Aujeszky's disease.

An obvious disadvantage of the smaller sample obtained by risk-based sample calculation is that the likelihood of finding contaminated farms - if any exist - is also reduced. Although this disadvantage can be tolerated for Aujeszky's disease due to the favourable international disease situation, it is not acceptable for PRRS, where there is a risk of introduction. In recent years, several cases of introduced PRRS and related outbreaks have been detected in Switzerland, sometimes as a result of the testing programme. To increase the likelihood of finding contaminated farms by using a testing programme, the latter's effectiveness was increased by testing breeding farms as from 2018. Breeding farms have a higher risk of virus introduction than fattening farms and are important for the spread of the virus. The testing of breeding pigs may also reveal the presence of the disease earlier than the testing of fattening pigs. To this end, over 3,000 breeding sows were tested for PRRS in 2015 in a pilot trial outside the scope of the testing programme.

Selection of farms and animals: In order to carry out screenings for Aujeszky's disease and PRRS. farms are selected by means of convenience sampling by the meat control personnel at 8 slaughterhouses. The meat inspectors decide independently from which animals and thus from which farms they will take samples. The FSVO specifies only the period and the total number of samples to be taken by the slaughterhouse, not the number of animals to be sampled per holding of origin. For logistical reasons, such a requirement is not possible for the sampling of breeding pigs. In addition, many breeding farms supply animals to different slaughterhouses, which means that animals from the same farm may be sampled at different slaughterhouses. Therefore, the number of samples actually submitted for testing may vary per holding of origin. To evaluate the sample, the average number of samples taken per holding is used. When determining the sample size, an average of six samples per holding of origin was assumed. By testing six animals, we achieve a herd sensitivity of 87% assuming a prevalence of 30% on an infected farm. To achieve a confidence level of 90% for the current sample with a breeding sow population of 2,000 farms and a 0.2% design prevalence, it is necessary to test 1,005 holdings, i.e. 6,030 samples. The assumption of six animals per farm results from the analysis of numbers of breeding sows slaughtered in the pilot trial carried out in 2015. However, since no reliable averages over several years were available for this purpose, a generous confidence level was taken into account in the sample size for 2018, which was therefore as high as in previous years, at 8,240 samples. The conclusion for each holding of origin is recorded in the meat inspection documentation.

Since practically no slaughter pigs come to the eight slaughterhouses from certain cantons because they have very few pig farms, additional samples are taken by veterinarians on three farms in the Cantons of Valais, Ticino and Glarus. This entails taking six blood samples from pigs over six months old on each pig farm.

Laboratory tests: All blood samples from the Aujeszky's disease testing programme are also tested for antibodies to PRRS. The procedure is as described for Aujeszky's disease. The screening and confirmation testing methods for PRRS, the respective sensitivities and specificities, and the reference laboratory for PRRS are indicated below (Table 2.2.3-1).

Animal disease	PRRS
Type of sample	Blood samples
Screening method	ELISA test
Sensitivity and specificity	94 % and 99.1 % respectively
Confirmation testing method for positive samples	Indirect fluorescence antibody (IFA) test
Sensitivity and specificity	96 % and 98.7 % respectively
Reference laboratory	Institute of Virology and Immunology (IVI), Mittelhäusern

Table 2.2.3-1: Methods used to test for PRRS, including sensitivity and specificity, and the PRRS reference laboratory

Case definition: The Epizootic Diseases Ordinance stipulates that in the case of PRRS two antibodypositive pigs on a farm confirmed by the reference laboratory constitute a case of disease. This special definition is necessary due to the comparatively low specificity of PRRS diagnostics. If the virus is detected, on the other hand, even a single pig constitutes a case of disease. If only one pig is confirmed as seropositive out of the six pigs sampled per farm, further samples must be taken on the farm of origin and submitted for testing. These results will determine whether or not a case of disease is recorded.

However, different initial situations can lead to a positive test result. It is therefore important to investigate the situation more closely and to differentiate between singleton reactors and a real disease outbreak. However, because antibodies to PRRS virus remain detectable for only a few months, prompt investigations are crucial in order to identify the actual cause of a positive serological PRRS finding.

2.2.4 Brucella melitensis

Requirements: In addition to clinical brucellosis surveillance, we also investigate elevated rates of abortion on a sheep or goat farm. This is despite the fact that a different cause is generally assumed, as brucellosis in small ruminants is not endemic in Switzerland. Based on the bilateral agreements with the EU, the sensible approach for brucellosis is to carry out a testing programme by means of random samples. The testing programme is necessary in order to export live small live ruminants and derived products to countries which also have disease-free status. This also allows us to regulate imports of small ruminants and their semen.

Sample calculation: For brucellosis, we apply risk-based sample calculation. The reason is that the risk of introducing brucellosis into Switzerland is very low and there have been no further outbreaks since the start of sampling. The lower surveillance quality of random sampling associated with this procedure is therefore not a concern and we are able to exploit the economic advantages.

For brucellosis, according to the bilateral agreements, we need to be able to demonstrate in a random sample with 99% confidence that herd prevalence is below 0.2%. Under EU Directive 91/68/EEC, sheep and goats can be combined in a single population for this purpose.

To constitute the sample for brucellosis testing, blood samples are taken from sheep and goats. On goat farms, additional samples are taken for the diagnosis of caprine arthritis-encephalitis (CAE). We use the Bayesian method to evaluate the sample. In evaluating the current random sample, we assume a decline in confidence for previous samples to 85%, provided that no more than 800 small ruminants were imported during the previous year. This standard decrease was deliberately chosen to be generous so as not to overestimate the resulting confidence level of the current sample. A total of 795 small ruminants were imported in 2016.

Farm selection: To constitute the sample for brucellosis testing, farms are randomly selected from the agricultural policy information system (AGIS). Goat farms must have registered at least three goats in AGIS and be recorded as a sheep or goat farm in the Animal Movements Database (AMD). In addition, they must not have been tested for brucellosis as part of a sample in the last three years.

Animal selection: Sheep over 12 months old and goats over six months old are tested for brucellosis. The different age thresholds are due to the combined sampling for brucellosis and CAE in goats. In larger flocks, a random sample of animals is used. The selection of animals in a sample is random and stratified according to epidemiological units on the farm. The number of samples taken on sheep and goat farms (Table 2.2.4-1) guarantees an appropriate flock sensitivity of 99%. The flock sensitivity is the probability of detecting an existing infection in a herd by means of random sampling. It depends on the sensitivity of the individual animal diagnostics used, the proportion of infected animals in the flock and the number of animals tested. The larger the sample, the greater the probability of detecting an infected farm.

Flock size (number of animals over 12 months old)	< 40	40-99	≥ 100
Number of blood samples	all	40	50

Table 2.2.4-1: Number of sheep and goats to be sampled for brucellosis testing

Laboratory tests: The laboratory tests the samples for Brucella antibodies. Any laboratory diagnostic method can produce false-negative or false-positive results, albeit very rarely and only under certain conditions. The screening and confirmation testing methods for brucellosis, the respective sensitivities and specificities, and the reference laboratory for brucellosis are indicated below (Table 2.2.4-2).

Animal disease	Brucellosis		
Type of sample	Blood samples		
Screening method	ELISA test		
Sensitivity and specificity	No information		
Confirmation testing method for positive samples	Complement fixation test and agglutination test		
Sensitivity and specificity	No information		
Reference laboratory	ZOBA, Institute of Veterinary Bacteriology of the Vetsuisse Faculty of the University of Bern		

Table 2.2.4-2: Methods used to test for brucellosis, including sensitivity and specificity, and the reference laboratory for brucellosis

The sensitivity and specificity of the laboratory test have not been scientifically published. Nevertheless, investigations by the reference laboratory and all experience to date show that the tests are very good and suitable for use in demonstrating freedom from disease.

Case definition: The Epizootic Diseases Ordinance stipulates that in the case of brucellosis each small ruminant confirmed by the reference laboratory as antibody-positive constitutes a case of disease and that measures must be taken on the farm concerned.

However, different initial situations can lead to a positive test result. It is therefore important to investigate such situations more closely in order to differentiate between singleton reactors and a real disease outbreak. Singleton reactors are rare in the case of brucellosis.

2.2.5 Caprine arthritis-encephalitis (CAE)

Requirements: After many years of control endeavours, CAE can be regarded as eradicated in Switzerland. Only a very few positive test results have been found in recent years. However, there is no evidence of clinical cases or of any link between the animals testing positive. Full-scale screening in 2014 showed that these are sporadic reactors and that no testing programme will be necessary in the medium term if the disease level remains very low. To complete the eradication, we are therefore conducting a three-year testing programme (2016–2018) designed to provide this evidence. It should be borne in mind that there is a significant risk of CAE being introduced into Switzerland because the disease is not regulated in neighbouring countries. This risk of introduction must be minimised by taking appropriate import measures.

Sample calculation: Conservative sample calculation is used for CAE (Canon and Roe, 1982). The sample should demonstrate with 99% confidence that flock prevalence is below 0.2%. To this end, the data from the three years 2016-2018 are compiled and calculated as a sample. This is possible in the case of CAE due to the long incubation period and slow spread of infection.

To constitute the sample for CAE testing, we use the blood samples taken from goats for brucellosis testing.

Farm selection: To constitute the sample for CAE testing, the same farms as for brucellosis surveillance are randomly selected from the agricultural policy information system (AGIS). Goat farms must have registered at least three goats in AGIS and be recorded as a goat farm in the Animal Movements Database (AMD). In addition, they must not have been tested for brucellosis as part of a sample in the last three years.

Animal selection: Goats over six months old are tested for CAE. In larger flocks, a random sample of animals is used. For details, see under brucellosis.

Laboratory tests: The laboratory tests the samples for antibodies to CAE virus. Any laboratory diagnostic method can produce false-negative or false-positive results, albeit very rarely and only under certain conditions. The screening and confirmation testing methods for CAE, the respective sensitivities and specificities, and the reference laboratory for CAE are indicated below (Table 2.2.5-1).

Animal disease	CAE		
Type of sample	Blood samples		
Screening method	ELISA test		
Sensitivity and specificity	No information		
Confirmation testing method for positive samples	Western blot, peptide ELISA, various other methods		
Sensitivity and specificity	No information		
Reference laboratory	Institute of Virology and Immunology (IVI) Vetsuisse Faculty of the University of Bern		

 Table 2.2.5-1: Methods used to test for CAE, including sensitivity and specificity, and the reference laboratory for CAE

The sensitivity and specificity of the laboratory test have not been scientifically published. Nevertheless, investigations by the reference laboratory and all experience to date show that the tests are very good and suitable for use in demonstrating freedom from disease. Differentiating between goat CAE virus (CAEV) and Maedi visna virus (MVV) infection poses particular diagnostic difficulties, notably because in many cases only the antibodies can be detected.

Case definition: The Epizootic Diseases Ordinance stipulates that in the case of CAE any goat confirmed by the reference laboratory as antibody-positive constitutes a case of disease and that measures must be taken on the farm concerned.

However, different initial situations can lead to a positive test result. It is therefore important to investigate such situations more closely in order to differentiate between singleton reactors and a real disease outbreak.

2.2.6 Bluetongue disease

Requirements: For disease-free status, the EU requires that in bluetongue (BT) free regions a prevalence of 20% at animal level must be excluded with 99% confidence by means of random sampling. In addition, the activity of the vector midges must be determined.

The sampling has to be divided into "BT areas", defined as areas of 2,000 square kilometres. However, this definition can be waived in favour of existing administrative boundaries. We used geostatistical methods to structure these BT surveillance areas in such a way that they correspond to the cantons as far as possible. This created a total of 16 BT areas for Switzerland, as several small cantons were combined into a single BT area. We also made sure that not only the land area but also the populations of susceptible species are approximately the same in each BT area. This enables us to test the same number of animals in each BT area. The Principality of Liechtenstein is listed as a separate BT area. However, its land area and animal population are much smaller than those of the other BT areas and in epidemiological terms it should be considered together with the adjacent BT area "AI AR SG".

Because the required prevalence at animal level is very high and already corresponds to an advanced epidemic, we decided that the Swiss testing programme should meet higher requirements. This allows us to detect an outbreak as early as possible and to take measures promptly. These requirements are in line with the observed prevalence in BT areas during the 2007/08 outbreak. Switzerland has therefore set the following new requirements for the testing programme: at national level, the finding of a 2%

prevalence at animal level with 99% confidence; in each of the 16 BT areas, the finding of a 20% prevalence at animal level, also with 99% confidence.

Sample calculation: As a first step, we calculate the sample size per BT area based on the average population size of a BT area. We multiply this number of samples per BT area by 16 to obtain the required number of samples at national level. We then calculate the predictive value of this required number of samples at national level. If it is not sufficient for the required 99% confidence level, we calculate the required number of samples at national level level directly as a second step and then divide this number between the 16 BT areas. In 2014, the sample size per BT area was 150 bovine animals. For the whole of Switzerland, 2,400 bovine animals had to be tested in order to meet the condition. Based on experience with previous testing programmes, we estimated the necessary reserve at 490 animals. In the case of BT, the reserve ensures that the testing targets are achieved in all BT areas.

Animal selection: Only bovine animals are tested in the testing programme. The blood samples are collected at seven large slaughterhouses with the aid of "RiBeS". Both the sampling and the selection of animals are carried out by the on-site meat inspectors. Animals must meet the following conditions:

- They must not be vaccinated. This means that only animals born after May 2010 are sampled.
- Animals must be at least eight months old. This allows us to rule out maternal antibodies and ensures that the animals have been exposed to potential transmission for as long as possible. Serological testing of older animals and of bulk-tank milk samples has shown that animals vaccinated against BT are still serologically positive 4–5 years after vaccination and can therefore pass on antibodies to their calves. These animals would thus be protected against BTV-8 infection and would be negative in a PCR test.

If possible, only single animals should be sampled from each farm. This limits the number of samples per farm in RiBeS. Samples for the 2018 programme were taken in November, corresponding to the end of the season with high midge activity. The PCR test is positive for up to 160 days in infected animals.

Laboratory tests: In random BT testing, blood samples from individual animals are tested (Table 2.2.6-1). The samples are sent to several FSVO-approved laboratories, where they are individually diagnosed. Traceability back to the farm of origin is ensured by meat inspection information and animal histories in the animal movements database.

Diagnosis focuses on detection of the BTV genome. Additionally in the case of BT, specific PCRs are used to determine the serotype.

Animal disease	ВТ	
Type of sample	Blood samples	
Screening method	PCR; pools of 5	
Sensitivity and specificity	99.99 % and 99.99 %	
Confirmation testing for positive samples	Serotype-specific PCR	
Sensitivity and specificity	No information	
Reference laboratory	Institute of Virology and Immunology (IVI), Mittelhäusern	

Table 2.2.6-1: Methods used to test for BTV, including sensitivity and specificity, and the reference laboratory for BT.

Case definition: The Epizootic Diseases Ordinance stipulates that in the case of BT any virus-positive animal constitutes a case of disease and that measures must be taken on the farm.

If only antibodies are found in the testing programme sample, it means that the animal had contact with the virus at some time. However, this can also mean that it was vaccinated, in which case it cannot infect other animals. If an antibody-positive animal cannot be shown to have been vaccinated, susceptible animals on the farm of origin must be tested for BT by a veterinarian. At least five blood samples

must be taken and tested for the BT virus. This procedure was designed to rule out a BT outbreak on the farm of origin. To this end, all samples must be negative for BTV and antibodies.

Midge surveillance: This is used to define the vector-free period. Animals can be moved more easily during this period because no fresh infections occur. Because sufficient data on midge activity has been collected in Switzerland in recent years, it can be used to define the vector-free period.

2.3 Monitoring the success of control: disease-specific information

2.3.1 Bovine virus diarrhoea (BVD)

Requirements: Freedom from disease is not demonstrated for BVD. As a result, there are no specific requirements for implementation of the testing programme. Nor is any random testing based on statistical principles carried out. No international requirements apply.

The BVD testing programme was carried out in the same way in 2018 as in 2017 and 2016. Together with 2018, both years are part of the second three-year BVD surveillance period which is based on the same concept as the first.

Farm selection: BVD-free cattle farms were divided into five groups according to Table 2.3.1-1. Dairy farms are farms which supply milk samples for regular testing at Suisselab AG.

Designation	Dairy farms		Non-dairy farms		Special farms ¹
	Without PI animals for 36 months	With PI ani- mals for 36 months	Without PI ani- mals for 36 months	With PI ani- mals for 36 months	
Group	1	2	3	4	5
Number of farms in 2018	21,467	151	18,916	98	777
Number of farms in 2017	21,467	172	18,657	77	589
Number of farms in 2016	21,563	102	18,893	68	809
Control testing	Bulk-tank milk testing	Bovine group	Bovine group	Bovine group	virological test- ing of newborn calves
Control frequency	Annual 2015:six- monthly	annual	annual, 1/3 of farms	annual	within 5 days after birth
1st follow-up test	Bovine group	virological herd testing	virological herd testing	virological herd testing	-
2nd follow-up test	virological herd testing	_	-	-	-

Table 2.3.1-1: Classification and testing schedule of BVD-free farms for BVD surveillance 2016–2018. ¹ Special farm: Farm classed as a "special farm" in the veterinary service information system (ISVet).

Animal selection: Serological testing of a group of bovine animals is subject to the following conditions: testing must cover 10% of the average number of animals kept in the herd, with a minimum of five animals, that are least six months old and no more than five years old. Animals have never tested serologically positive for BVD, have been kept exclusively in recognised BVD-free herds and have been in the current herd for a total of at least two months (on-farm samples six months) in the last 12 months.

This information is also stored in and can be retrieved from the veterinary service information system (ISVet). These criteria are also used to select animals for sampling at the slaughterhouse.

Laboratory tests: BVD diagnosis uses serological tests for samples of milk, bulk-tank milk and blood. Virological detection is performed by PCR or antigen ELISA. The reference laboratory also uses a range of additional tests, depending on the request.

Case definition: Detection of a persistently infected animal (PI animal) on a farm constitutes a case of disease. If additional PI animals are found on the farm, they are assigned to the previously identified case. If no PI animals are found but all test results indicate that one was present on a farm (it may have already been slaughtered, for example), the farm is classed as suspected of contamination.

2.3.2 Bovine spongiform encephalopathy (BSE)

Requirements: In the case of bovine spongiform encephalopathy (BSE), no random testing is carried out to demonstrate freedom from disease. There are therefore no specific requirements for implementation of the testing programme. Nor is any random sampling based on statistical principles carried out. Based on international (EU) obligations and in order to maintain the OIE (World Organisation for Animal Health) status of "negligible BSE risk", Switzerland is required to carry out an annual testing programme. The OIE transfers the BSE test results to a points system. In order to maintain the status of "negligible BSE risk", Switzerland status through surveillance every year.

Animal selection: In addition to suspected clinical cases, all fallen or culled bovine animals over 48 months old are tested for BSE.

Laboratory tests: Except for suspected cases, brain stem samples are investigated using a rapid test. In suspected cases, immunohistological procedures are used as well.

Case definition: A case is recorded if altered prion protein was detected and the result was confirmed by the reference laboratory.

2.3.3 Salmonella infection in poultry

Requirements: Salmonella infection in poultry is an animal disease to be controlled (EzDO Art. 255 *et seq.*). The aim of control is to prevent eggs or poultry meat from infected flocks from entering the human food chain. Control targets of <= 1% prevalence in breeders and broilers and <= 2% prevalence in layers have been set for this purpose. These targets relate to the serovars which most commonly pose a threat to human health. These are *S. enteritidis* and *S. typhimurium* (including monophasic strain 1,4,[5],12:i:-) and additionally *S. virchow, S. hadar* and *S. infantis* in the case of breeding flocks. If these serovars are detected in surveillance samples taken from the birds themselves, control measures are initiated.

Farm selection: Poultry farms with more than 250 breeders, 1,000 layers, 5,000 broiler chickens or 500 broiler turkeys must be tested for Salmonella in accordance with the <u>Technical directive on the collection</u> and testing of samples for Salmonella infection in domestic poultry. Samples are usually taken by the poultry farmers themselves.

Owners of poultry farms who test their poultry for Salmonella must register the stocking of each flock in the AMD. The test request form generated in the AMD must be used when testing these flocks. This form automatically incorporates important flock information such as the AMD number, flock ID, flock size and use (broiler, layer, etc.).

Data from this surveillance programme are evaluated via the Alis laboratory database. Tested flocks can be included in the evaluation only if the AMD request form containing all relevant flock information is sent to the laboratory with the sample material.

Animal selection: The <u>Technical directive on the collection and testing of samples for Salmonella in-</u> fection in domestic poultry specifies the testing times and sample materials. Surveillance is generally carried out by testing boot covers or dust samples or by serology in eggs or blood. If Salmonella or Salmonella antibodies are detected in environmental samples or during serological testing, or if illness occurs in the human population due to consumption of poultry meat or eggs, a suspected case is recorded. In a suspected case, the official veterinarian takes samples from 20 birds. If these samples also test positive, a case of disease is recorded.

Laboratory tests: Environmental samples are tested bacteriologically for Salmonella. Eggs and blood samples are tested serologically for Salmonella antibodies. Muscle, liver and spleen from the 20 animals sampled in the suspected case are tested bacteriologically for Salmonella.

Case definition: A case of disease is recorded if *S. enteritidis* or *S. typhimurium* (including monophasic strain 1,4,[5],12:i-) are detected in the muscle, liver or spleen of poultry (additionally S. *virchow*, S. *hadar* or S. *infantis* in breeding birds).

2.4 Early detection of animal diseases: disease-specific information

2.4.1 Low pathogenic avian influenza (LPAI) and Newcastle disease (ND) in commercial poultry

Requirements: Blood samples from Swiss commercial poultry have been tested for avian influenza viruses (AIV) H5/H7 and Newcastle Disease (ND) antibodies since 2006. In recent years, surveillance has been limited to free-range laying hens and broiler turkeys. Broiler chickens are not sampled because there is only a low probability of AIV infection due to their short lifespan. Ducks and geese are often kept outdoors and are therefore more likely to come into contact with AIV. However, the risk of spreading low pathogenic avian influenza (LPAI) is regarded as low because these largely small flocks kept by hobby-ists or pure-breed enthusiasts (<50 ducks/geese) rarely have close contact with commercial poultry farms. In addition, sampling these duck/goose flocks entails a great deal of effort.

Sample calculation: The number of flocks to be tested is set in such a way that at least one LPAIVinfected farm is found at a farm prevalence of at least 5% and 95% confidence. For Switzerland, with over 250 laying hen farms, this means a randomly and representatively drawn sample of 60 farms. In the case of broiler turkeys, all farms are sampled every year.

The number of birds to be tested per flock is set in such a way that at least one LPAIV-seropositive bird is detected at a prevalence of \geq 30% LPAIV-seropositive birds with 95% confidence. Ten birds per flock therefore need to be sampled.

Samples from the LPAI testing programme are also tested in the laboratory for Newcastle Disease (ND) antibodies. The samples are not taken for the purpose of demonstrating freedom from disease and are therefore not suitable for that purpose.

Farm selection: Free-range laying hen farms are selected by the FSVO on the basis of regularly updated slaughter lists from Gallo Circle, part of the Gallo Suisse egg producers' association. Flocks slaughtered in close succession are prioritised in order to minimise the effort entailed in sending samples from the German slaughterhouse. Due to the restriction (i.e. that only free-range laying flocks sent for slaughter are sampled), the selection of flocks is relatively limited and the farms tested each year are fairly similar.

Laboratory tests: Laboratory tests are carried out at the Institute of Virology and Immunology (IVI). The diagnostic procedures comply with the requirements of the World Organisation for Animal Health (OIE). All blood samples are tested using commercial, IVI-validated ELISA tests (competitive AI / blocking ND). Positive and inconclusive samples are retested using a confirmation ELISA (blocking AI / indirect ND). The haemagglutination inhibition test (HIT) is used as a definitive confirmation test for remaining ELISA-

positive sera in order to detect specific antibodies to AIV subtypes H5/H7 or to avian paramyxovirus serotype 1 (APMV-1).

Case definition: In infected flocks, we would expect to find antibodies in several birds. Flocks with only one bird showing an inconclusive test result are classed as negative and are not monitored further. Only if multiple birds in a flock give positive or inconclusive results is a farm classed as antibody-positive. Subsequent flocks or, in the case of farms with multiple age groups, the flocks remaining on the farm are tested serologically and virologically and epidemiological investigations are carried out.

2.4.2 Highly pathogenic avian influenza (HPAI) in wild birds

Requirements: Highly pathogenic avian influenza (HPAI, also known as <u>bird flu</u>) usually leads to significant clinical abnormalities and, depending on the virus subtype, wild bird species and weather conditions, may be fatal. HPAI viruses circulating in the wild bird population pose a risk of transmission to commercial poultry. To detect such circulation as early as possible, we test dead or diseased wild birds.

Animal selection: The public is asked to maintain increased vigilance. Findings of dead birds should be reported to the gamekeeper or police. Reported carcasses are collected and disposed of safely. Sampling should be carried out in the following cases:

A *wild bird finding to be clarified* is recorded if a swan, two or more waterfowl or birds of prey, or five or more other wild birds, are found diseased or dead at a single location within 24 hours, and there is no sufficiently substantiated connection to another cause of disease or death. Tests must always be requested using the NRGK's "Application for the testing of wild birds for classical avian influenza". It is especially important to give coordinates, the bird species and the number of dead birds found, as this provides an overall picture of the numbers of wild bird deaths.

Laboratory tests: The combined choanal and cloacal swabs are tested for influenza A viruses at NTGK using RT-qPCR.

Case definition: Detection of highly pathogenic influenza viruses is classed as a case of disease.

General information on animal disease surveillance

The principles of animal health surveillance are described online (in German) at: <u>https://www.blv.ad-min.ch/blv/de/home/tiere/tiergesundheit/ueberwachung.html</u>

The case numbers cited in this report are based on the FSVO's information system for disease reports (InfoSM). These can be found at: <u>https://www.infosm.blv.admin.ch/public/</u>

An annual compilation of cases per disease, per month and per canton is published on the <u>FSVO web-</u> <u>site</u>

This report and those from previous years can be found at: <u>https://www.blv.ad-min.ch/blv/en/home/tiere/publikationen-und-forschung/statistiken-berichte-tiere.html</u>

The monthly FSVO Radar Bulletins on the international animal disease situation can be found (in German) at:

https://www.blv.admin.ch/blv/de/home/tiere/tiergesundheit/frueherkennung/radar.html

Federal Food Safety and Veterinary Office FSVO Schwarzenburgstrasse 155 3003 Bern Website: <u>www.blv.admin.ch</u> Email: info@blv.admin.ch Telephone: +41-(0)58-4633033