	DEPARTMENT OF AGRICULTURE, LAND REFORM AND RURAL DEVELOPMENT DIRECTORATE: ANIMAL HEALTH
	EXEMPTION FROM DESTRUCTION OF
E	CHICKENS INFECTED OR IN-CONTACT
	WITH HIGHLY PATHOGENIC AVIAN
the state of the s	INFLUENZA (HPAI)
	Annexure to
TFE: XARRA INSC	Guidelines for control measures following a
	suspect/confirmed highly pathogenic avian influenza outbreak in chickens.
NO OF PAGES:	11 + 4

Maja. DR MPHO/MAJA DIRECTOR ANIMAL HEALTH DATE 08 November 2023

1. EXECUTIVE SUMMARY

The Director: Animal Health (the DAH) has determined that, under specific circumstances as outlined in this document, the controlled purpose will not be defeated by indefinite postponement from destruction of HPAI infected and in-contact chickens. This Exemption Annexure constitutes the required written approval from the DAH for exemption from destruction of chickens infected or in-contact with HPAI. No individual application for exemption is required, however the exemption is null and void if the responsible person does not comply with all of the conditions as outlined in this document. Please note that the lifting of outbreak quarantine remains subject to individual application, consideration and approval by the DAH.

Unless the requirements outlined in this document are met in full, the guidelines for control measures following a suspect / confirmed HPAI outbreak in chickens apply, the farm remains under quarantine and no live birds, eggs and waste can be removed from the farm and all infected and in contact chickens must be culled with immediate effect.

2. BACKGROUND

- 2.1 HPAI in chickens usually results in almost 100% morbidity and mortality of infected and in-contact chickens. The culling of such infected and in-contact chickens is thus merely accelerating the predetermined demise of such infected and in-contact chickens and serves welfare as well as disease control and eradication purposes.
- 2.2 In some outbreaks of HPAI infections, the above may not apply universally to some or all infected and in-contact chickens on an infected site, i.e. some or all chickens may survive despite being infected or in-contact and / or suspected to be infected or in-contact.
- 2.3 This scenario may arise under the following circumstances:
- 2.3.1 HPAI Vaccination: Vaccination protects against disease and not against infection (meaning it reduces the severity and duration of clinical signs, decreases mortality as well as amount and period of shedding. Vaccinated animals may still become infected with HPAI). This complicates surveillance because of the immune reaction following vaccination interfering with some laboratory tests. Given the resultant challenges associated with the increased risk of subclinical disease and the lower sensitivity of clinical surveillance and laboratory testing in vaccinated chickens, all of the houses and units on a vaccinated site that became infected with HPAI have to be considered infected and / or in-contact. Any references to "chickens separated from infected or in-contact chickens" in the HPAI Outbreak Guidelines are thus not applicable to HPAI vaccinated farms. Despite being accordingly infected or in-contact, some or all of the chickens on HPAI vaccinated and subsequently HPAI infected farms are likely to be sufficiently protected against the disease to be able to survive.

- 2.3.2 An outbreak with an unusual strain of HPAI and/or that for some reason displays lower pathogenicity and/or transmissibility on an infected site (first suspected to occur in South Africa with the HPAI H7N6 event in 2023). This scenario should be proven by both positive serology and positive PCR and/or virus isolation of samples taken from an infected house from chickens that showed some disease symptoms but did not die¹.
- 2.4 If, in the scenarios above, some or all chickens on an HPAI infected site are not severely sick and/or dying despite being infected or in-contact and/or suspected to be infected or in-contact, the disease risk will be substantially lower, because the amount of virus being shed tends to be proportional to the severity of the symptoms. It is thus possible, without defeating the controlled purpose that the productive and/or genetic value of these chickens be preserved by exempting them from the prescribed destruction in terms of Table 2 of the Animal Diseases Regulations.
- 2.5 However, for the Director Animal Health to grant such an exemption, the following associated risks would have to be addressed adequately, and confirmed in writing by the private veterinarian attending to the farm, namely:
- 2.5.1 The risk of prolonged and possibly subclinical circulation of virus that may increase the risk of spread of the disease and an increased risk of contact with other animals and humans these to be addressed by appropriately enhanced biosecurity conditions as detailed below; and
- 2.5.2 The risk of subclinical disease as well as the prolonged circulation of the virus resulting in the emergence of mutant strains that make it more difficult to rely on clinical passive surveillance alone and thus require an increased level of active surveillance, including increased deployment of laboratory agent identification methods, i.e. over and above those laid out in the main Guideline document, to satisfactorily determine the end of the outbreak; and
- 2.5.3 With the surviving chickens being partially immune, the continued risk of subclinical disease, should they become infected with HPAI again in future, ongoing intensified surveillance for the remainder of the lives of the surviving and/or vaccinated chickens as detailed below with the added requirement that these chickens will remain under lifelong movement restrictions to ensure that they are moved only for the purpose of direct abattoir slaughter or to a property on which the same biosecurity and surveillance measures will be honoured for the remainder of the lives of these chickens.

¹ Such suspicions should be treated with caution, as the most likely reason for chickens not dying from HPAI is that they were simply not exposed to the virus because of adequate separation and the most likely reason for lower than expected morbidity is that the chickens in question are infected with a disease other than HPAI. This scenario of such "chickens separated from infected or in-contact chickens" is already covered in the HPAI Guidelines as is the manner of dealing with such surviving non-exposed chickens. These "chickens separated from infected or in-contact chickens" do not require an exemption from the disease control prescripts in terms of Table 2 of the Animal Diseases Regulations and must be dealt with in terms of the HPAI Guidelines. The easiest manner of confirming that chickens have indeed not been exposed is to submit samples for appropriate serological tests – with due observance of the waiting period for seroconversion.

3. LEGAL BASIS

- 3.1 Exemption from destruction granted by the DAH for some or all chickens on an HPAI infected site that are not severely sick and/or dying, despite being infected or in-contact and/or suspected to be infected or in-contact, in terms of Regulation 11. (2) must satisfy and is subject to the following:
- 3.1.1 Full compliance with Section 11. of the Animal Diseases Act, 1984 (Act No 35 of 1984), that places certain obligations on the owner or manager of land on which there are animals and the owner of such animals to, amongst others, take all reasonable steps to prevent the infection of the animals with any animal disease and the spreading thereof from the relevant land or animals, or steps which are necessary for the eradication of animal diseases on the land or in respect of the animals which remains the full and sole responsibility of the owner and/or manager of the property or animals; and
- 3.1.2 Fulfilment of the highly intensified biosecurity and surveillance conditions as described in this document (even over and above those required for farms to qualify for HPAI vaccination) to ensure that the controlled purpose to prevent spread and control and eradicate the disease will not be defeated.
- 3.2 The owner or manager who takes advantage of this exemption from destruction of chickens infected or in-contact with HPAI, is not exempted from compliance with Section 11(1) of the Animal Diseases Act, 1984 (Act No 35 of 1984) and remains fully responsible for preventing the spread of and eradicating the disease with the conditions below representing the minimum reasonable measures that would apply for such exemption.
- 3.3 In terms of the control measures prescribed in Table 2 of the Regulations of the Animal Diseases Act, 1984 (Act No 35 of 1984), all infected poultry shall be destroyed by the responsible person under the supervision of an officer. All in contact poultry shall be isolated and destroyed by the responsible person under the supervision of an officer. This prescribed control measure remains the default for HPAI and, should the owner not take advantage of this exemption under the conditions described below, the destruction of the infected and in-contact animals must be implemented with immediate effect.
- 3.4 Any owner or manager of poultry who does not comply with the conditions for exemption as described below, and does not destroy HPAI infected and in contact chickens, will be transgressing the legal prescripts of the Animal Diseases Act (Act No 35 of 1984) and may be prosecuted for this criminal offence. In addition, any owner or manager of poultry who does not apply the biosecurity, record and surveillance conditions for exemption as described below, which are designed to ensure that the controlled purpose is not defeated, and who does not destroy HPAI infected and in contact chickens, may be subject to civil claims for damages should another party feel prejudiced by such actions that, in all likelihood of probability, contributed to their poultry property becoming infected with the disease or to any other material injury in relation to the disease.

- 3.5 This Exemption Annexure is issued in reference to Point 1.3. of "the guidelines for control measures following a suspect/confirmed highly pathogenic avian influenza outbreak in chickens" and constitutes the required written approval from the Director Animal Health for exemption from destruction of chickens infected or in-contact with HPAI under the conditions as outlined below. No individual application by the responsible person to the Director Animal Health for such exemption is required. However, the conditions below have to be fulfilled <u>AND</u> confirmed in writing by a private veterinarian within 48 hours of the disease being suspected on the farm for this exemption to be valid. Should the conditions and / or the private veterinary confirmation Not be in place within 48 hours after the disease has been suspected on the farm, irrespective of when confirmation is received from the laboratory, this exemption is null and void and all infected and incontact chickens have to be destroyed immediately. The exemption is null and void if the responsible person does not comply with all of the conditions as outlined below.
- 3.6 It is recommended that owners and managers of chickens envisaging that they may want to take advantage of this exemption in case of an outbreak of HPAI on their premises, timeously consult their private veterinarian and prepare for such an eventuality by ensuring that all the biosecurity and other conditions below are in place or can be implemented immediately, should this be required.
- 3.6.1 Immediate consultation with a private veterinarian upon suspicion of HPAI on a site is mandatory should an owner or manager consider taking advantage of this exemption under the conditions described below. The conditions below have to be fulfilled AND confirmed in writing by a private veterinarian within 48 hours of the disease being suspected on the farm for this exemption to be valid.
- 3.6.2 The private veterinary confirmation of the fulfillment of the conditions has to be delivered to the responsible state veterinarian responsible for the area of the establishment within 48 hours of HPAI being suspected on the site, irrespective of when the laboratory confirmation of the disease is received and irrespective of when the quarantine notice was issued. The private veterinary confirmation sent to the responsible state veterinarian has to be copied to the e-mail address epidemiology@dalrrd.gov.za at DALRRD.
- 3.6.3 Written acknowledgement has to be obtained from the state veterinarian and, together with the acknowledgement from the DALRRD e-mail address, has to be kept for at least 5 years as auditable proof of delivery of confirmation to the state veterinarian within 48 hours.
- 3.6.4 Should the conditions and / or the private veterinary confirmation Not be in place within 48 hours after the disease has been suspected on the farm, this exemption is nul and void and all infected and in-contact chickens have to be destroyed immediately.
- 3.7 Regulation 11 (2)(a) states that "a responsible state veterinarian may, if he is satisfied by the circumstances of a responsible person or his land or animals and that the achievement of the controlled purpose concerned will not be defeated thereby, and subject to the written approval of the director and for the time period as determined by the director and subject to the conditions as

determined by the director, grant to a responsible person written postponement from compliance with a particular controlled veterinary act,...".

- 3.8 The Director: Animal Health has considered the controlled purpose and determined that the controlled purpose will not be defeated by indefinite postponement from destruction of infected and in-contact chickens, for the duration of their production cycle, under the conditions detailed below.
- 3.9 All other sections of the GUIDELINES FOR CONTROL MEASURES FOLLOWING A SUSPECT/CONFIRMED HIGHLY PATHOGENIC AVIAN INFLUENZA OUTBREAK IN CHICKENS are still applicable for sites on which the owner or manager take the decision to apply the exemption from destruction and that the conditions as outlined below merely represent additional control measures that are applicable on such sites.

4. BIOSECURITY REQUIREMENTS

- 4.1 In order for the Director Animal Health to grant this exemption, the risks of prolonged and possibly subclinical circulation of virus that may increase the risk of spread of the disease and an increased risk of contact with other animals and humans, have to be addressed by appropriately enhanced biosecurity conditions.
- 4.2 There must be strict biosecurity in place that complies with the requirements specified in VPN44/2012-01 Standard for the Inspection of Poultry Farms for Export, or equivalent biosecurity measures must be implemented. Auditable records of compliance must be kept by the farm owner that can be produced on request by the State Veterinarian or any other legal entity.
- 4.3 As soon as HPAI vaccinated flocks or sites considering taking advantage of this exemption suspect HPAI infection, additional quarantine and biosecurity measures must also be implemented to limit the risk of spread of HPAI to other susceptible species and the exposure of humans and other mammals:
- 4.3.1 Section 2.3 Visitors (d) of VPN44/2012-01 has to be extended to confirm that all visitors to the facility have had no contact with poultry for at least 72 hours prior to the visit and ALL personnel and visitors have to shower in and out;
- 4.3.2 Personal protective equipment (PPE) has to be provided to all personnel and visitors as per the Guidelines for Control Measures Following a Suspect/Confirmed Highly Pathogenic Avian Influenza Outbreak in Chickens Version 1 May 2021, and include gumboots, overalls, gloves and face masks with the addition that these masks must be K95 masks and goggles must also be supplied.
- 4.4 These additional biosecurity requirements have to remain in place until the quarantine on the exempted farm is lifted with written approval from the Director Animal Health.
- 4.5 In the case of surviving chickens on seropositive exempted farms, after the outbreak quarantine has been lifted, these partially immune chickens will have to remain under intensified biosecurity

for the rest of their lives. The biosecurity measures applicable are the same as the requirements for compartmentalisation in terms of VPN 44 and have to remain implemented under supervision of a private veterinarian and / or under the supervision of an Animal Health Technician working under supervision of a private veterinarian.

5. SURVEILLANCE REQUIREMENTS

- 5.1 For the Director Animal Health to grant an exemption, the following associated risks would have to be addressed adequately, namely:
- 5.1.1 The risk of subclinical disease as well as the prolonged circulation of the virus resulting in the emergence of mutant strains that make it more difficult to rely on clinical passive surveillance alone and thus require an increased level of active surveillance, including increased deployment of laboratory agent identification methods, i.e., over and above those laid out in the main Guideline document, to satisfactorily determine the end of the outbreak; and
- 5.1.2 With the surviving chickens being partially immune, the continued risk of subclinical disease should they become infected with HPAI again in future, ongoing intensified surveillance for the remainder of the lives of the surviving and/or vaccinated chickens as detailed below is critical– with the added requirement that these chickens will remain under lifelong movement restrictions to ensure that they are moved only for the purpose of direct abattoir slaughter or to a property on which the same biosecurity and surveillance measures will be honoured for the remainder of the lives of these chickens.
- 5.1.3 All clinical monitoring and sample taking has to be done by a private veterinarian and / or by an Animal Health Technician working under supervision of a private veterinarian.
- **5.2** Surveillance must be carried out in terms of the attached Addendum on Surveillance. All affected parties must familiarise themselves with the entire content of the attached Addendum. For ease of reference, the surveillance requirements are summarised hereunder, however the additional requirements and procedures to apply for lifting of quarantine and allowing movements are detailed in the Addendum.

5.3 Surveillance to determine end of outbreak for the purpose of lifting quarantine.

Type of	Timing of	Samples	Number of	Outcome
surveillance	sampling	required	samples	required to move
				to next step in
				table
Clinical morbidity	28 day period	No samples	All houses and	No undue
and mortality	after the last	required, records	sites to be	morbidity or
	house became	to be checked	monitored	mortality for 28
	infected	daily		days
Post mortem	14 consecutive	Organ samples	Minimum 10	No positive PCR
examinations and	days, after the 28	from mortalities for	mortalities per	on organ samples
testing of dead	days above has	PCR testing	house	from 14
birds	lapsed		Minimum 60	consecutive days'
			samples per site	mortalities
			per week	
Sampling of live	Once PCR testing	Random serum	See table in	Negative results
chickens	of dead birds is	samples and	Addendum for	on PCR (please see
	negative	swabs collected	number of	Surveillance
		for serology and	samples	Addendum for
		PCR testing		positive serology implications)
Sentinels	Once PCR on live	If sentinels die or	Samples only	No deaths or
	birds is negative,	show clinical	required from	illness in sentinels
	place sentinels for	signs, organ	dead or sick	due to HPAI
	14 days	samples must be	sentinels	
		submitted for PCR		

5.3.1	Summary T	able (consult	Surveillance	Addendum for	details)
-------	-----------	---------------	--------------	--------------	----------

- 5.3.2 If all of the testing and monitoring as specified above indicates the end of shedding of HPAI virus from the remaining chickens, the evidence has to be submitted via the state veterinarian to the National Director Animal Health for consideration of lifting of quarantine.
- 5.3.3 If the serology of all the sampled chickens is negative, the DAH together with granting permission to lift quarantine, will also grant permission for all movement control and intensified biosecurity and surveillance to be lifted for the surviving chickens. However, if the serology of <u>any</u> of the surviving chickens is positive, these partially immune chickens will be subject to lifelong intensified biosecurity and surveillance and movement control, as below.

- 5.3.4 Expected normal production, morbidity and mortality parameters for the facilities involved must be provided when the application is sent to the DAH via the provincial SV authorities for consideration of lifting of the HPAI outbreak quarantine on sites on which the owner or manager take the decision to apply the exemption from destruction.
- 5.3.5 At the time of applying for the lifting of quarantine, the responsible person may also apply to the DAH for permission to release the table and hatching eggs produced after the cessation of virus circulation but before the lifting of quarantine for human consumption subject to pasteurization.

5.4 Lifelong surveillance on surviving chickens on seropositive exempted farms, after outbreak quarantine has been lifted.

Type of	Timing of reports	Samples	Number of	Outcome
surveillance		required	samples	required to move
				to next step in
				table
Clinical morbidity,	Weekly	No samples	All houses and	No decrease in
mortality,		required, records	sites	production or
production		to be checked		increase in
parameters		daily		mortality by more
				than 30%
Post mortem	Weekly	Organ samples	Minimum 5 per	No positive HPAI
examinations and		from mortalities for	house per week,	on PCR
testing of dead		PCR testing	minimum 30 per	
birds			site per week	
Sampling of live	Monthly	Random serum	Minimum 10 birds	No positive HPAI
chickens		samples and	per house per	on PCR (please see
		swabs for	month, minimum	Surveillance Addendum
		serology and PCR	120 birds per site	for positive serology implications)
			per month	implications <i>)</i>

5.4.1 Summary Table (consult Surveillance Addendum for details)

5.5 For all types of Surveillance above

- The submission forms to the laboratory must be completed in full and signed by the private veterinarian in charge of the facility.

 The laboratory must be instructed on the submission form to not pool samples across houses in order to ensure that every PCR result pertains to a specific house and that the collective PCR results represent all of the epidemiological units on the site.

Any suspect or positive result must be reported immediately to the responsible state veterinarian and a suspect outbreak investigation must be conducted to exclude the presence of HPAI. The fact that the laboratory is also obliged to report to the state veterinarian does Not exonerate anybody else, i.e. everybody involved, including the responsible owner or manager of the farm and the private veterinarian and private AHT are obliged to report such suspect or positive result immediately.

6. <u>MOVEMENT CONTROL APPLICABLE TO SURVIVING CHICKENS ON</u> <u>SEROPOSITIVE EXEMPTED FARMS, AFTER THE OUTBREAK</u> <u>QUARANTINE HAS BEEN LIFTED</u>

- 6.1 All chickens from seropositive exempted farms are under movement restriction and may not be moved from the exempted premises unless the results of the reinforced passive and active surveillance, implemented in accordance with Point 5. above, are negative for detection of infection with HPAI field virus throughout the duration of stay of the surviving poultry on the farm.
- 6.2 Chickens from seropositive exempted farms may only move to a slaughterhouse for immediate slaughter; or to other establishments that are approved to receive HPAI vaccinated / seropositive birds and/or that comply with the increased biosecurity and surveillance conditions as described in the HPAI vaccination strategy.
- 6.3 The owner of the sites on which the decision to apply the exemption from destruction was taken must keep auditable records with a tally of slaughter ready vaccinated or previously infected or incontact birds that are taken for slaughter and the abattoir must also keep in their slaughter records with the farm names, owners and numbers of previously exempted birds delivered to the abattoir for slaughter for audit purposes.
- 6.4 No live chickens from seropositive exempted farms with vaccinated or previously infected or incontact birds may be sold or given away for any other purposes, including individual sales of end of production chickens for home slaughter.
- 6.5 Disposal of dead chickens from seropositive exempted farms by feeding to pigs or any other animals will not be allowed.

7. EXIT STRATEGY

- 7.1 To regain NAI free list status, the farm must either be depopulated, cleaned and disinfected, or at least three months' serological testing at the cost of the owner will have to demonstrate that all houses on the exempted site are serologically negative.
- 7.2 The responsible state veterinarian must inform the National Director Animal Health once the normal requirements for listing on the NAI free list have been complied with, before the farm will be included on the NAI free list.

SURVEILLANCE ADDENDUM

to

Exemption from Destruction of Chickens Infected or In-Contact with Highly Pathogenic Avian Influenza (HPAI)

= Annexure to "Guidelines for control measures following a suspect/confirmed highly pathogenic avian influenza outbreak in chickens"

SURVEILLANCE REQUIREMENTS

1 Background

For the Director Animal Health to grant an exemption, the following associated risks would have to be addressed adequately, namely:

- 1.1 The risk of subclinical disease as well as the prolonged circulation of the virus resulting in the emergence of mutant strains that make it more difficult to rely on clinical passive surveillance alone and thus require an increased level of active surveillance, including increased deployment of laboratory agent identification methods, i.e., over and above those laid out in the main Guideline document, to satisfactorily determine the end of the outbreak; and
- 1.2 With the surviving chickens being partially immune, the continued risk of subclinical disease should they become infected with HPAI again in future, ongoing intensified surveillance for the remainder of the lives of the surviving and/or vaccinated chickens as detailed below is critical– with the added requirement that these chickens will remain under lifelong movement restrictions to ensure that they are moved only for the purpose of direct abattoir slaughter or to a property on which the same biosecurity and surveillance measures will be honoured for the remainder of the lives of these chickens.
- 1.3 All clinical monitoring and sample taking has to be done by a private veterinarian and / or by an Animal Health Technician working under supervision of a private veterinarian.

2 Surveillance required to determine end of outbreak for the purpose of lifting the HPAI outbreak related quarantine.

Monitoring and testing is required to determine the end of HPAI virus shedding from the surviving infected and/or in contact chickens.

- 2.1 Daily morbidity and mortality records have to show no undue morbidity and mortality for a period of 28 days (double the WOAH Incubation period) after the last house has become infected, with the information broken down per house and site.
- 2.2 Postmortem examinations must be conducted on every mortality for 14 (WOAH incubation period) consecutive days (after the 28 days as per point 5.2.1 above has lapsed). Organ samples must be collected from a minimum of 10 mortalities per house and not less than 60 samples per site and per week prioritize lung, trachea, spleen and caecal tonsil samples but other internal organ samples may also be taken. The collected organs samples for each individual bird must be placed in an individual sterile container labelled with the date, house and/or site details. These samples must be submitted to a DAH approved and SANAS accredited laboratory at the cost of the owner for avian influenza PCR testing. The person submitting the samples to the laboratory must inform the relevant laboratory in writing to store all the samples until all 14 consecutive days of samples have been collected and await written confirmation from the Director Animal Health as to how samples are to be pooled for PCR testing.
- 2.3 If the PCR testing as per point 2.2 above is negative, random serology samples and tracheal and cloacal swabs must be collected from the live chickens at a 5% prevalence per house according to the below table at the cost of the owner. Swabs must be submitted individually no more than 5 samples from individual birds may be pooled in the laboratory:

Population per house	5% MEP
<100	48
101-200	55
201-300	58
301-400	59
401-500	60
501-1000	62
>1000	63

- 2.4 If the PCR as per point 2.3 above is negative, sentinels must be placed for a period of 14 days. If any sentinels die, they must undergo postmortem evaluation and organ samples collected for PCR testing. Any sentinels still alive and showing suspected signs of disease should be euthanised for postmortem evaluation and sampling.
- 2.5 If all of the testing and monitoring as specified above indicates the end of shedding of HPAI virus from the remaining chickens, the evidence has to be submitted via the state veterinarian to the National Director Animal Health for consideration of lifting of quarantine.
- 2.6 If the serology of all the sampled chickens is negative, the DAH together with granting permission to lift quarantine, will also grant permission for all movement control and intensified biosecurity and surveillance to be lifted for the surviving chickens. However, if the serology of <u>any</u> of the surviving chickens is positive, these partially immune chickens will be subject to lifelong intensified biosecurity and surveillance and movement control, as below.
- 2.7 Expected normal production, morbidity and mortality parameters for the facilities involved must be provided when the application is sent to the DAH via the provincial SV authorities for consideration of lifting of the HPAI outbreak quarantine on sites on which the owner or manager take the decision to apply the exemption from destruction.
- 2.8 At the time of applying for the lifting of quarantine, the responsible person may also apply to the DAH for permission to release the table and hatching eggs produced after the cessation of virus circulation but before the lifting of quarantine for human consumption subject to pasteurization.

3 Surveillance applicable to surviving chickens on seropositive exempted farms, after the outbreak quarantine has been lifted.

- 3.1 The objective of ongoing life-long farm level AI virus surveillance in chicken flocks containing seropositive, partially immune birds is to promptly detect any circulation of AI field virus after introduction, to effectively control the disease and prevent further spread. Sero-positivity and partial immunity may lower the risk of introduction and reduce spread after introduction but will not prevent infection, which is why strict biosecurity is still required. The considerations and prescriptions below apply irrespective of whether the sero-positivity and partial immunity of a flock after an HPAI outbreak is due to the outbreak only or due to previous vaccination and the survived outbreak.
- 3.2 Given that the sensitivity of both passive clinical and post-mortem surveillance and laboratory tests, especially PCR tests, is highly dependent on the skills of the operator to detect possible symptoms of AI and ensure proper sample handling, it is mandatory that all the surveillance elements below are conducted either by the private consulting veterinarian personally or by the SAVC registered AHT who has been appointed for the farm to provide the required functions under supervision of the consulting veterinarian.
- 3.3 Possible subclinical circulation in sero-positive flocks makes passive surveillance less reliable and the immune reactions interfere with certain types of laboratory testing, especially serology. Thus, more intensive laboratory testing including more agent-identification testing has to be conducted on seropositive farms.
- 3.4 Given the endemicity of a LPAI H6N2 virus in commercial chickens and the sensitivity and specificity challenges with the AI serological tests, it has been decided to rather rely on agent

identification / PCR laboratory testing alone for the routine life-log surveillance required on seropositive farms.

- 3.5 In sero-positive partially immune flocks the expected prevalence after virus introduction will be lower than in sero-negative flocks. It is important to take this into account when calculating the sample sizes to be collected to provide for a high level of confidence that the survey system will detect any introduction of virus. In addition, prevalence assumptions must be tailored to the type of testing and, while longer periods of antibody persistence allow serological tests to detect higher cumulative prevalences, the short duration of virus shedding limits the detection of virus or virus particles to a lower pin-point prevalence for agent identification / PCR tests.
- 3.6 Introduction of an HPAI virus usually infects one house on a farm initially. Its spread to other houses will depend on the biosecurity between the houses and can take a considerable amount of time, Thus, in order to detect and introduction as soon as possible, it has been decided that a single house represents an epidemiolocal unit for surveillance purposes as detailed below.

3.7 Weekly enhanced passive targeted surveillance

- 3.7.1 Weekly enhanced passive clinical surveillance
 - The expected normal production, morbidity and mortality parameters must be established for the site and for every house if the subpopulations of chickens differ between houses.
 - These expected normal production, morbidity and mortality parameters must be monitored weekly under private veterinary supervision with production of a signed off weekly report.
 - Any decrease of production or increase of morbidity or mortality by more than 30% of the normal parameters must be reported immediately to the responsible state veterinarian and a suspect outbreak investigation must be conducted to exclude the presence of HPAI. The fact that the owner and manager are obliged to report to the state veterinarian does Not exonerate the private veterinarian and private AHT from their obligations in this regard.

3.7.2 Weekly enhanced passive targeted surveillance of mortalities

- Every mortality in every house must be subjected to post-mortem examinations under private veterinary supervision at least once a week with a weekly signed off post-mortem report.
- Mortalities can be classified as a targeted group with a higher expected prevalence and therefore it has been decided to sample for a ~50% minimum expected prevalence per house and for a minimum 10% prevalence per site.
- The number of samples to be collected amounts to samples from at least 5 dead animals per house to be submitted once every week while it has to be ensured that samples from at least 30 dead animals per site are submitted every week; i.e. in the case of less than 6 (populated) houses on the site, the sampled mortalities per house need to be increased to amount to 30 per site per week while in the case of more than 6 (populated) houses on a site, the total number per site will amount to more than 30 every week.
- Dead birds showing signs that could be suggestive of avian influenza must be prioritized for submission with the remainder of the sampled birds selected randomly for each house.
- Caecal tonsils, spleen, trachea, brain, and lung tissue must be collected in separate containers for every bird with the house identification / number clearly displayed on the container.
- Organ sample containers must be kept cool and transported in triple layer packaging to an accredited and approved laboratory for AI rt-PCR testing.
- A maximum of organ samples from 5 mortalities will be pooled per PCR test at the laboratory, this amounting to at least one PCR result from mortalities per (populated) house per week and a minimum of six PCR results per site per week.

3.8 Monthly active surveillance

- Apart from samples collected weekly as part of the enhanced passive surveillance system, active surveillance samples must be collected monthly from randomly selected chickens under private veterinary supervision.
- An expected prevalence of 5% per house, representing an epidemiological unit, is considered the internationally accepted baseline for active surveillance. This would amount to 60 birds sampled per (populated) house every month with pooling of samples from 5 birds at the laboratory resulting in 12 PCR results per (populated) house every month. In the interest of practicality and feasibility but allowing for scientific justification and reference to international guidelines, it has been decided to allow for an increase of expected prevalence to 25% per house but correspondingly lower the expected prevalence to 2.5% per site.
- This results in a rounded number of 10 birds to be sampled per month per house with a rounded minimum number of 120 birds to be sampled per month per site; i.e. in the case of less than 12 (populated) houses on the site, the number of sampled birds per house needs to be increased to amount to 120 per site per month while in the case of more than 12 (populated) houses on a site, the total number of sampled birds per site will amount to more than 120 every month.
- Veterinary oversight is needed with sampling to enable compliance. Private veterinarians will be responsible but must inform State Veterinarians of sampling events. This will allow State Veterinarians to attend should he/she wish to do so.
- Cloacal and tracheal swabs for PCR must be collected from the same birds and placed into a separate container / tube for every bird sampled with the house identification / number clearly displayed on the container / tube.
- Samples must be triple packaged and sent to accredited and approved laboratories for AI rt-PCR testing.
- Swabs of different birds must not be pooled on the farm but sent in a separate container for every bird to the laboratory where a maximum of samples from 5 birds will be pooled per PCR test at the laboratory, this amounting to at least two PCR results from monthly active surveillance per house per month and a minimum of 24 PCR active surveillance results per site per month.

4 For all types of Surveillance above

- The submission forms to the laboratory must be completed in full and signed by the private veterinarian in charge of the facility.
- The laboratory must be instructed on the submission form to not pool samples across houses in order to ensure that every PCR result pertains to a specific house and that the collective PCR results represent all of the epidemiological units on the site.
- Any suspect or positive result must be reported immediately to the responsible state veterinarian and a suspect outbreak investigation must be conducted to exclude the presence of HPAI. The fact that the laboratory is also obliged to report to the state veterinarian does Not exonerate anybody else, i.e. everybody involved, including the responsible owner or manager of the farm and the private veterinarian and private AHT are obliged to report such suspect or positive result immediately.