A Manual for the Control of Highly Pathogenic Avian Influenza (HPAI)

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Introduction

In response to the outbreaks of Highly Pathogenic Avian Influenza (HPAI) in Italy in 2000 and in the Netherlands in 2003 the Central Association of the German Poultry Industry (ZDG) formed a Poultry Disease Working Group in order to discuss and establish necessary measures for the control of Avian Influenza infections in poultry.

Members of the Poultry Disease Working Group of the ZDG were: W. Hoffrogge and K.P. Linn (ZDG) and seven poultry veterinary specialists representing poultry breeding, broiler, duck, laying hen and turkey production (J.J. Arnold, J. Bachmeier, K.-P. Behr, U. Löhren, M. Pöppel, G. Reetz and M. Voss).

The working group published a ***Manual for Avian Influenza (HPAI)***, covering the fields of epidemiology, diagnosis, personnel and transport vehicle hygiene, killing of poultry in the event of a disease outbreak, vaccination, measures for the protection of employees as well as the relevant German legislation in animal disease control. Although this manual has been basically a response to the 2003 outbreaks of HPAI H7N7 in Europe most of the chapters are still valid also for the current situation with HPAI H5N1 in Asia, threatening the world's poultry population.

This article represents a summary of the Manual for Avian Influenza. The full text has been put on the WPSA website, where it is available under
http://www.wpsa.com/downloads/ZDG%20Handbuch%20Gefl%FCgelpest%20E.pdf

1. Epidemiology

Ongoing epidemiological studies (avian influenza monitoring) in non-outbreak times, i.e. irrespective of a current HPAI outbreak, have been established in Germany as the LPAI Monitoring Program for slaughter turkeys, which was introduced and sponsored by the Association of German Turkey Producers in 2000 following the avian influenza epidemic in Italy. Such monitoring programs should be implemented as a self-monitoring system by the whole poultry industry in the form of spot checks in conventional holdings and on a flock basis in free-range holdings.

A continuous cultural avian influenza monitoring scheme should be introduced for wild fowl. This should be carried out in collaboration with bird protection/bird spotting stations and ringing centers and also involve the hunting community where appropriate (diagnostic shooting). In each federal state a representative number of wild fowl (wild waterfowl, coastal fowl and other wild fowl) should be sampled (faecal samples or cloacal swabs).

According to the Federal Catalogue of Contingency Measures for Avian Influenza, in an outbreak situation all animals, people and equipment potentially exposed to the virus on the affected holding for up to 21 days preceding the appearance of clinical symptoms shall be identified, recorded and monitored. This is done by the competent veterinary authorities in collaboration with representatives of the federal state concerned.

Depending on the risk of transmission of avian influenza by animals, people, equipment, vehicles, etc., such investigations and surveys shall distinguish between three types of contact holding:
K1 holdings are poultry farms/holdings which had exchanged live animals (including non-poultry species such as pigs) with the infected holding during the preceding 21 days. Once the suspicion of avian influenza has been confirmed on the original holding by molecular biological tests the infected flocks shall be immediately killed and safely disposed of.
K2 holdings are poultry farms/holdings where person-to-person contacts with the infected holding have taken place during the preceding 21 days. All persons who entered the poultry building/buildings, e.g. veterinarians, attendants/consultants, workmen, depopulation teams, should be identified. It should also be ascertained on which poultry holdings these persons worked subsequently.

The definition of a K2 holding also extends to farms with contact through vehicles, equipment and items that were used on the infected holding and might subsequently have been used on other poultry farms.
Once the suspected outbreak on the original holding has been confirmed by molecular biological tests, all flocks on K2 holdings with contact to the infected holding within a period of 5 days prior to the emergence of clinical symptoms must immediately be culled and safely disposed of. Flocks on K2 holdings with contact to the infected holding within a period of 6 to 10 days prior to the emergence of clinical symptoms and with inadequate hygiene and precautionary measures should also be culled and safely disposed of. Flocks on K2 holdings with contact to the infected holding which do not need to be emergency culled should be placed under official supervision for 21 days.

The definition of a K3 holding does not yet exist in animal disease control terminology. The term is suggested for the following types of contact:
K3 holdings are poultry farms/holdings which have had contact with the infected holding via people (not entering poultry buildings), vehicles, equipment etc. (e.g. feed vehicles, carcass disposal vehicles and their drivers, other social contacts by the poultry keeper) within a period of 48 hours prior to the emergence of clinical symptoms, in whose immediate vicinity (within about 500 m of the poultry building) manure from the infected holding was spread within 14 days prior to the emergence of clinical symptoms. K3 holdings should be closed by the veterinary authorities for a period of 21 days.

In case an outbreak of highly pathogenic avian influenza has been confirmed, the monitoring activities during non-outbreak times should be extended by epidemiological surveys of the wild fowl population, in particular the less susceptible pigeon and water fowl population in the protection and surveillance zone. In emergency situations shooting of huntable wild fowl species that are susceptible to avian influenza such as crows and wild pigeons should be stepped up.

2. Diagnosis

Directive 92/40/EEC, based on the existing definition of avian influenza, also lays down the diagnostic procedures to be applied for confirmation of the presence of avian influenza (HPAI – Highly Pathogenic Avian Influenza).

Previous outbreaks of avian influenza in the Netherlands, Belgium and Germany have shown that the disease does not present itself with a uniform clinical and gross pathological picture. While symptoms such as reduced water and feed intake, a drop in egg production in conjunction with swellings and blue discolorations on the head and legs and respiratory symptoms, followed by increased mortality rates with inflammations of the serous membranes and petechial haemorrhages of the internal organs provide initial indications of the presence of avian influenza, these symptoms can also be caused by other infections.

Before making a presumptive clinical diagnosis of avian influenza it is therefore necessary that, in addition to the attending veterinarian and the veterinary official, an independent veterinary poultry specialist with expertise in the particular species should be consulted in order to rule out other potential causes. It is advisable that once a certain mortality threshold without apparent explanation (in the Netherlands this was set at 2 % over 24 hours) has been exceeded, exploratory tests should be carried out (M-PCR or virus culture) before suggesting the presence of avian influenza.

Diagnostic procedures differ between those for the detection of antibodies and antigen assays.

When performing **antibody assays** a distinction has to be made between test systems which detect antibodies to all avian influenza viruses (group-specific antibodies) and those capable of differentiating between antibodies to specific haemagglutinin (HA) subtypes (subtype-specific antibodies). Detection of group-specific antibodies is done by the agar gel precipitation test (AGPT) or the ELISA assay. HA subtype-specific antibodies are detected by the haemagglutination inhibition (HI) test.

Antibody assays are capable of detecting, via a monitoring program, infections with low pathogenic avian influenza virus (LPAI). If an outbreak of highly pathogenic avian influenza (HPAI) is suspected, antibody assays are of little diagnostic value because there is insufficient time for birds to develop antibodies due to the peracute course of the infection.

Antigen assays for the detection of Influenza antigen are performed by classic virus cultivation in SPF embryo culture and by molecular biological procedures (PCR, sequencing). Virus cultivation in SPF embryos and the performance of PCR assays for detection of avian influenza genome in general (M-PCR to detect the group-specific matrix protein) are done in regional and private laboratories according to the method specified by the national reference centre. Nationwide facilities for this purpose must be created.

At least for the confirmation of an initial outbreak the subtype-specific PCR for viruses of subtypes H5 and H7 (H5/H7-HA-PCR) should only be conducted by the National Reference Laboratories for Avian Influenza because of potentially required confirmatory tests. In the event of an initial outbreak the national reference laboratory shall also determine the intravenous pathogenicity index (IVPI) in SPF birds.

Characteristic features unique to particular poultry species may require that only specific diagnostic procedures can be used. There is some doubt for example whether avian influenza virus is sufficiently invasive to induce antibodies in water fowl. In this instance direct virus isolation (virus culture, PCR) is probably the preferred method.

3. Personnel and transport vehicle hygiene

The avian influenza epidemics confirmed again that person-to-person contact and vehicle traffic are primarily responsible for transmission of the virus. The network of business relationships between poultry operations beyond state and administrative borders require correspondingly complex precautions against avian influenza. Animal disease contingency plans for a poultry holding or integration should be divided into two alert levels. The measures for level II are in addition to those of level I.

Alert level I should be in place when an outbreak of avian influenza occurred in a region without economic links (transport of feed, hatching eggs, live poultry, manure) to the holding/integration. An outbreak of avian influenza in a region with economic links (transport of feed, hatching eggs, live poultry, manure) to the holding/integration should result in **Alert level II**.

Personnel hygiene for alert levels I and II must include isolation of the farm and poultry buildings. Movement of people must be controlled and restricted to the minimum necessary (visitors' book). Only persons wearing the protective/disposable clothing are allowed into poultry buildings, disinfectant mats and foot dips at entrances to poultry buildings have to be used. Catching or loading teams must follow a strictly coordinated hygiene program (protective clothing, work only within regional borders). Access must be prohibited for individuals who have visited infected areas or had contact with infected holdings. Veterinary flock inspections should be reduced to a minimum. Medicine regulations, animal vaccine order and meat hygiene legislation should be amended to cover the specific outbreak situation in order to avoid unnecessary contact through movements of people and vehicles.

General rules for **transport vehicles**, like log books with proof of disinfection, use of compulsory routes, central washing and disinfection stations, installation of commercial facilities for cleaning and disinfecting vehicles in the vicinity of the farm and written training manuals for own drivers should be implemented.

General rules for cleaning and disinfection during **transport of live poultry** should be applied during alert level I. In addition, during alert level II disposable clothing and overshoes should remain on the premises and one-way packaging material should be used. Corridors between broiler farms and abattoirs should be created by the veterinary authorities and the poultry industry for the transport of slaughter poultry.

Similar requirements apply for the **transport of hatching eggs**, including compulsory disposable packaging at all alert levels, and the **transport of table eggs**.

Because of the high potential for disease spread transport of feed must include single delivery runs, use of disposable dust bags for capturing feed dust at the silo and daily cleaning and disinfection of feed vehicles. Compulsory routes should be established during alert level II.

Transport of litter material of any kind must be prohibited from protection and surveillance zones into uninfected areas. The removal of poultry manure always poses a considerable disease risk, especially if the manure is spread in close vicinity of other poultry holdings. All poultry manure transports, both local and remote, must be recorded in order to monitor the whereabouts of the material.

Transports of carcasses, hatchery residues and slaughter by-products to carcass disposal plants must include cleaning and disinfection of vehicles before each transport. Carcass disposal must be carried out in strict compliance with the system of clean/dirty separation. No carcass disposal vehicles are allowed into poultry facilities. Vehicles must drive straight to specific holdings, a compulsory route should be established and vehicles must have no contact with infected regions.

4. Killing of poultry in the event of an outbreak

The immediate slaughter of the flock suspected of being infected has top priority in disease control. As the infection is not necessarily associated with very high mortality it has to be accepted that this action is necessary to contain virus shedding. The personnel and equipment used in the culling, however, pose a major risk of disease spread. Once a buffer zone has been established around the infected holding, culling should start there from the outside in to prevent the virus from being carried outside the zone during the culling action. The granting of exemptions from a culling order, for instance in the case of vaccinated zoo birds or vaccinated individuals of rare poultry breeds, poses the risk of retaining virus shedders. If necessary, special rules may have to be introduced for handling these poultry categories.

Killing poultry outside closed housing systems in containers or mobile electrocution facilities poses the danger of windborne disease spread through feathers, faecal particles or dust. Moreover, not all poultry sites have outside yards that are sufficiently solid for adequate cleaning and disinfection. A closed-house culling operation is therefore preferable in order to contain the spread of the disease.

For any killing method animal welfare aspects have to be considered. Successful disease control is applied animal welfare. In addition, emergency culling for animal welfare reasons may become necessary if poultry cannot be transported because of official movement restrictions. To prevent broilers from growing beyond marketable body weight, it may be in the interest of bird welfare to kill flocks in affected areas.

Epidemiologists agree that culling actions within a restriction zone must be completed with 48 hours of taking the decision. The time factor in the implementation of stamping out is currently not covered by the German Poultry Disease Order. An amendment should therefore be considered.

Killing procedures

German legislation provides expressly that in the event of a contagious animal disease outbreak the competent authority is empowered to take discretionary decisions concerning slaughter procedures. The following procedures are suitable for killing poultry in the event of an avian influenza outbreak: Poultry house gassing with CO_2, container gassing with CO_2, poultry house gassing with CO, electrocution, poultry house gassing with HCN, killing by lethal injection (for example T 61) and neck dislocation.

Chemical procedures for killing poultry via the feed or drinking water have been tested and are currently considered unsuitable.

Poultry house gassing with CO_2 is the method of choice wherever possible but requires special equipment and the supply of sufficient CO_2. CO_2 may be pumped at high pressure (20 bar) into the poultry house and sprayed through numerous ultrafine nozzles spaced at about 20 m intervals. In layer hen cage systems the spray nozzles are positioned on top of the batteries so that CO_2 permeates the cages from top to bottom.

Container gassing with CO_2 requires special gas-tight containers with lids. The lids have openings for entering the chickens, which are then exposed to a CO_2 concentration of above 60 % within the container. The culling capacity per container lid is about 2,000 chickens/h, bringing the total number culled in two shifts to about 32,000 chickens per day.

To avoid the risk of explosions, **poultry house gassing with CO** may only be used by companies with specialized expertise and under the supervision of the local fire brigade. For the same reason the procedure has been ruled out for container gassing. The current version of the German Federal Catalogue of Contingency Measures expressly prohibits the use of this procedure. The Working Group suggested that this procedure should be added to the authorized list in the event of an avian influenza outbreak.

The first poultry **electrocution** facility of the Animal Diseases Insurance Fund of Lower Saxony has a capacity of 4,000 chickens or 1,000 turkeys per hour. The labor requirement is about 10 workers. Mobile electrocution units are usually so large that the culling operation takes place outdoors. Electrocution is the preferred method for killing water fowl because their biology is such that they can hold their breath for a very long time in gas stunning procedures. There are concerns whether the unit can be completely disinfected und hence worries that such devices might spread disease.

Because of the extreme toxicity of hydrogen cyanide poultry house gassing with HCN, too, should only be carried out by qualified personnel. Carcass disposal plants have expressed concern that they might not be able to dispose of carcasses killed by this method because of potential residues.

Killing by lethal injection, using injectable drugs such as T 61 or barbiturates is appropriate for killing individual birds, for instance on pedigree or hobby poultry farms.

As pointed out above, the suitability of any culling procedure depends essentially on the type of construction of the building and its location. Poultry farms should therefore be **categorized** and suitable culling procedures stipulated for each type of building; as well as the procedure of choice, fall-back procedures should be suggested in the event of technical, logistical or personnel constraints.

Selection of personnel for culling operations is one of the most critical factors. Since people are the most likely transmitters of the virus between holdings, especially over long distances; it is imperative that personnel carrying out the culling should have no contact with other poultry holdings. This means that catching/loading teams, which used to be the preferred choice for this type of work, are either ruled out or provisions must be made which ensure that some loading firms work exclusively in disease control, on a temporary basis, while others continue to work only outside the infected area in their standard business.

In all countries which have had outbreaks of avian influenza, the disposal of dead or culled poultry has always been done by burial in local landfills, as in Italy in 1999 and in the United States (Virginia) in 2001. This might be done not only because of a capacity shortage in carcass removal plants, but specifically in order to prevent the spread of the virus. For this reason the safe disposal of infected and suspected contaminated flocks by composting on site is also recommended. The temperatures generated during the composting process reliably kill the virus. The final disposal can be done later by incineration or burial. Ideally, composting should be done on the farm premises in order to avoid any risk of virus spread.

5. Audit sheets for large-scale culling of poultry in the event of an outbreak

Knowledge of the type of construction of a poultry house and its location is crucial for the selection of a suitable culling procedure. Poultry housing should therefore be categorized. The categorization should be the result of a visit by expert inspectors. An example is given in the full version of the manual.

6. Vaccination

Although Directive 92/40/EEC theoretically allows vaccination against avian influenza with officially approved vaccines to be carried out along with other control measures in the event of a disease outbreak, vaccination against highly pathogenic avian influenza is currently inadvisable for the reasons given below:

- Vaccinated flocks will continue to shed the virus, which is why vaccinated flocks must be considered potentially infectious. They consequently pose a non-controllable risk of spreading the virus.

- None of the vaccines currently on the market are capable of inducing a sufficient level of immunity in broiler flocks.

Vaccinated flocks must therefore be considered as potentially infectious. Consequently, vaccination of individual flocks against highly pathogenic avian influenza (HPAI) is inadvisable. Blanket vaccination of all poultry flocks, including all small flocks, is unrealistic and non-viable.

In order to protect against avian influenza the vaccine has to contain the same haemagglutinin subtype (H) which would potentially be encountered in the field. The neuraminidase subtype (N) is of lesser importance and can therefore differ from the field virus. This distinction has been exploited in recent years in Italy by applying the so-called “DIVA” principle (Differentiating Infected from Vaccinated Animals). The idea of the DIVA principle is based on the fact that by demonstrating antibodies to the neuraminidase subtype of the field virus, which differs from vaccine virus, it is possible to diagnose an infection in vaccinated flocks. But this requires that a sufficient quantity of **field virus (i.e. in this case highly pathogenic influenza virus!)** multiplies in the flock to trigger an immune response (antibody) in the birds. This may take three to four weeks after infection has occurred, especially if the poultry are protected by existing immunity so that antibody formation is delayed. During this period there is a **danger of undetected spread** of virus. The use of sentinel birds would also delay diagnosis of an infection because vaccinal protection of the vast majority of the poultry population reduces virus shedding and hence infection pressure on the sentinel birds. These would fall ill far later than if infection and virus replication could sweep through the flock unchecked. Using sentinels therefore poses the great danger, at least in vaccinated flocks, that infections are diagnosed too late and that the virus is transmitted to susceptible populations.

For the same reasons the success of ring vaccination is also highly questionable because it takes too long for sufficient vaccinal protection to be built up in susceptible populations and, in broiler flocks, is impossible anyway. Recent experiences from the Netherlands have shown that because of the extremely fast spread of the infection an area of several 100 kilometers around the outbreak would have to be vaccinated in order to hit immune poultry populations as the infection spreads.

8. Specific measures to protect workers from infections with highly pathogenic avian influenza

German rules on worker safety measures reflect current developments in safety technology, health at work, hygiene and occupational science when handling biological agents. One of these rules covers activities in livestock production where biological agents may be involved.

These measures apply to activities during which workers may come into direct contact with the virus of highly pathogenic avian influenza. Direct contact occurs when handling infected birds, during examination, treatment, care and transport of people who are considered to be either suspected or confirmed cases of avian influenza or when performing activities involving contact with bodily fluids or excretions of animals or humans, as defined in 1 and 2 above.

The causal agent of highly pathogenic avian influenza is an influenza A virus of the H5 or H7 subtypes and is categorized in risk group 2 for both humans and animals.

Infected poultry shed the virus in high concentrations with all body excretions (faeces, saliva, tears fluid), faeces in particular being highly infectious.

Transmission to humans can occur by the aerosol and smear infections via mucous membranes. Direct contact with infected poultry, their excretions or contaminated products and materials appears to be necessary for transmission. Indirect transmission by aerosol is also possible if a lot of dust is generated. The risk of human infection is generally considered low and has so far occurred only in the case of viruses of the H7N7 and H5N1 subtypes.

When handling infected (or suspected of infection) poultry and contaminated poultry materials (body parts, body tissues, blood, feathers, poultry excretions including used litter), when slaughtering infected birds and during cleaning and disinfection operations the generation of dust and other aerosols should be minimized. One way of achieving this is to kill the birds by flooding the poultry house with carbon

dioxide. The dead flock should be moistened with a fine water mist and the subsequent carcass collection and disposal should be done with as little dust exposure as possible.

Livestock areas containing infected (or suspected of infection) poultry should only be entered by personnel to do the necessary work, and their number should be kept to a minimum. When entering poultry areas special clothing and protective gear should be worn, which must be removed before leaving the area and stored in tightly closed containers for professional cleaning/disinfection or disposal in such a manner that the spread of virus is prevented.

After removing the workwear/protective clothing a whole body shower should be taken and the hands then disinfected. Specific legal requirements relating to animal disease control must be observed.

Health and safety at work rules do not require employers to offer their employees influenza vaccination with the current human influenza virus since this vaccination does not confer protection against infections with the virus of highly pathogenic avian influenza.

However, doing so could prevent double infections with human influenza viruses and avian influenza virus, which would pose the risk of emergence of new human pathogenic virus variants; it might therefore be advisable, in order to protect the population at large, to provide vaccination for employees who might potentially come into direct contact with infected poultry. Employers are required to offer prophylactic antiviral therapy with neuraminidase inhibitors to employees who might potentially come into direct contact with infected poultry. Sick people with flu symptoms should not be allowed to come into direct contact with potentially infected poultry.

9. Legislation related to animal disease control

Legislation related to animal disease control varies between countries. Within the European Community Directive 92/40 EC describes Community measures for the control of avian influenza to be taken in the event of an outbreak of highly pathogenic avian influenza.

This Directive and additional German legislation is available in the full version of the Manual for Avian Influenza.

Zusammenfassung

Ein Handbuch zur Kontrolle hoch pathogener Vogelpest

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Diese Übersicht gibt in verkürzter Form den Inhalt eines Handbuchs wieder, das der Zentralverband der Deutschen Geflügelwirtschaft (ZDG) in Zusammenarbeit mit namhaften Fachtierärzten für Geflügelkrankheiten herausgegeben hat. Die Grundsätze beruhen auf Erfahrungen in Italien (2000) und den Niederlanden (2003), sie gelten aber ebenso für die gegenwärtig im Mittelpunkt des Interesses stehenden Risiken von H5N1 Infektionen. Das Handbuch ist in deutscher Sprache vom ZDG zu beziehen.