ANNEX

DIAGNOSTIC MANUAL FOR AVIAN INFLUENZA

CHAPTER XI

Diagnosis of infection with AI viruses in pigs and other mammals

1. AI in pigs

AI viruses readily infect pigs and although replication is in most cases relatively restricted, there is a potential that infected pigs could transmit the disease to poultry and other susceptible animals. To date, there is no evidence from the field that infected pigs transmit AI viruses of H5 and H7 subtypes.

Experience gained during the outbreak in the Netherlands in 2003 indicated that H7N7 infected pigs did not show clinical signs that could be attributed to the H7N7-infection. Moreover, apparently no diseased pigs have been reported to date during the H5N1 outbreak in Asia and elsewhere.

Therefore, clinical signs may not be relied upon to indicate whether pigs are infected although clinical presentation due to infection of pigs with other influenza viruses of avian origin, can occur once a virus has become adapted to the host. The diagnosis of AI virus infections of pigs is essentially similar to diagnosis for avian species, relying on virus isolation, molecular techniques and detection of specific antibodies using haemagglutination inhibition tests. There are, however, certain differences and none of the tests are fully validated for the use in pigs to confirm infection with AI viruses.

2. Samples for virus isolation

AI virus infections in pigs are usually restricted to the respiratory tract and samples must be respiratory tract tissues and, if appropriate, oropharyngeal or nasal swabs, preferably taken from pigs showing signs of that disease. These samples and swabs may be processed for virus isolation or molecular detection of virus, using the same techniques described above for samples from birds. However, when using PCR techniques, proper controls must be used to ensure that the amplification is not inhibited by substances in the samples from pigs.

3. Inoculation and incubation of eggs

To isolate mammalian influenza viruses in 9- to 11-day-old embryonated fowls' eggs it is usual practice to inoculate each egg via the allantoic cavity and into the amniotic cavity. However, when testing pigs in contact with AI viruses, when virus has had little opportunity to adapt, allantoic cavity inoculation is probably sufficient.

Similarly, 35 ^{o}C is usually recommended for the incubation temperature for isolation of mammalian influenza A viruses, but again for viruses poorly adapted to pigs, 37 ^{o}C is not be detrimental to virus isolation.

4. Test for specific antibodies in HI tests

Virus isolation or molecular detection are likely to be the most sensitive for determining AI virus infections of pigs. However, serological responses in pigs have been detected in the absence of virus isolation or detection. HI tests, using pig sera, require some modifications to the test used for avian sera referred to in Chapter VIII.

Pig sera are notorious for their property of non-specific inhibition in HI tests and therefore each serum sample must be treated with a receptor destroying enzyme (RDE) to prevent this from occurring. The following method must be used:

(a) To 100 µl of pig antiserum add 400 µl RDE (predetermined working dilution) and mix thoroughly.

(b) Incubate at 37 ^{o}C for one hour.

(c) Then incubate for 30 minutes at 56 ^{o}C.

(d) Cool samples at 4 ^{o}C for a minimum of 15 minutes.

(e) Add 10 µl of 30 % (v/v packed cells) chick red blood cells and mix vigorously.

(f) Incubate at 4 ^{o}C overnight. Alternatively, if it is essential to use samples same day, incubate at 37 ^{o}C for one hour and centrifuge at 300 x g for five minutes.

The treated serum is then used in HI tests as described for avian sera in paragraph [..], the initial dilution is 1:10. A set of sera of pigs with a known sero-negative status regarding AI must be used to assess the specificity of the HI test for the virus strain to be used (see use of virus strain for serology derived from the outbreak; Chapter VIII). During the outbreak in the Netherlands in 2003 up to 2,6 % non-specific reactors were detected in the HI test using pig sera that were collected independently of the outbreak

5. Sampling of pigs

Particularly on farms that keep both pigs and poultry, either mixed or in separate houses, pigs are at risk of becoming infected with AI directly or indirectly via contact with poultry or poultry products. To exclude such infection, oropharyngeal or nasal swabs and blood samples must be collected according to the procedures described in point 8.21 of Chapter IV. Samples must be obtained from pigs that show clinical signs of the disease. However, when they do not show any clinical signs, samples may be collected at random over all sections of the house. If available in the laboratory, swabs must be tested in rapid molecular tests and/or virus isolation. The RT-PCR must have been appropriately validated and have a sensitivity at least equivalent to virus isolation in eggs for influenza A viruses.

Two to four weeks after culling of the AI infected poultry, at least 60 blood samples must be collected from pigs in such a way that at least some samples are obtained from groups of pigs that are in direct contact with each other. Samples must be tested in the HI test using virus derived from the poultry outbreak. Samples from both the acute and convalescent phases must be tested in the same test. Positive samples can be confirmed using virus neutralisation and/or Western blot analyses.

When any of those samples test positive, an epidemiological investigation on all pig farms located within the protection zone must be carried out, irrespective whether they are of mixed type or not.

6. **AI viruses in other mammals other than pigs**

Investigations in other mammals other than pigs which are susceptible to AI including cats must be undertaken. With specific reference to HPAI H5N1, the following must be carried out for testing cats:

Gross pathological lesions, associated with viral replication, concentrate on the lungs and liver, therefore samples for virological investigations must preferably be taken from these organs of

dead animals. In living animals, preferably tracheal/oropharyngeal swabs must be taken for virus detection. In addition, faecal swabs can be taken separately.

Blood samples to be examined in HI assays require heat treatment for 56 ^{o}C for 30 minutes and RDE treatment can be omitted.

Changes to legislation:
There are outstanding changes not yet made to Commission Decision of 4 August 2006 approving a Diagnostic Manual for avian influenza as provided for in Council Directive 2005/94/EC (notified under document number C(2006) 3477) (Text with EEA relevance) (2006/437/EC). Any changes that have already been made to the legislation appear in the content and are referenced with annotations.
View outstanding changes

Changes and effects yet to be applied to the whole legislation item and associated provisions

- Annex Ch. 5 para. 4(c) omitted by S.I. 2018/1410 reg. 10(7)(b)
- Annex Ch. 5 para. 4(d) omitted by S.I. 2018/1410 reg. 10(7)(b)
- Annex Ch. 5 para. 4(b)(iii) substituted by S.I. 2018/1410 reg. 10(7)(a)(i)
- Annex Ch. 14 para. 1 word substituted by S.I. 2018/1410 reg. 10(12)(a)
- Annex Ch. 1 para. 6(b)(i) words omitted by S.I. 2018/1410 reg. 10(6)(b)(i)
- Annex Ch. 1 para. 6(b)(ii) words omitted by S.I. 2018/1410 reg. 10(6)(b)(ii)(aa)
- Annex Ch. 1 para. 6(b)(ii) words omitted by S.I. 2018/1410 reg. 10(6)(b)(ii)(bb)
- Annex Ch. 5 para. 4(b) words omitted by S.I. 2018/1410 reg. 10(7)(a)(ii)
- Annex Ch. 1 para. 1 words omitted by virtue of S.I. 2018/1410, reg. 10(6)(a) (as substituted) by S.I. 2020/1388 reg. 25(7)(b)
- Annex Ch. 1 para. 1 words substituted by S.I. 2018/1410 reg. 10(6)(a) (This amendment not applied to legislation.gov.uk. Reg. 10(6)(a) substituted immediately before IP completion day by virtue of S.I. 2020/1388, regs. 1(2)(a), 25(7)(b))
- Annex Ch. 14 para. 5 words substituted by S.I. 2018/1410 reg. 10(12)(b)(i)
- Annex Ch. 14 para. 5 words substituted by S.I. 2018/1410 reg. 10(12)(b)(ii)
- Annex Ch. 14 para. 5 words substituted by S.I. 2018/1410 reg. 10(12)(b)(iii)(iv) (This amendment not applied to legislation.gov.uk. Reg. 10(12)(b)(iii) substituted and (iv) omitted immediately before IP completion day by virtue of S.I. 2020/1388, regs. 1(2)(a), 25(7)(c)(i)(ii))
- Annex Ch. 14 para. 5 words substituted by S.I. 2018/1410, reg. 12(b)(iii) (as substituted) by S.I. 2020/1388 reg. 25(7)(c)(ii)
- Art. 2a inserted by S.I. 2018/1410 reg. 10(3)
- Art. 2a(a)(ii)(iii) Art. 2a(a)(iii)(iv) renumbered as Art. 2a(a)(ii)(iii) in earlier amending provision S.I. 2018/1410, reg. 10(3) by S.I. 2020/1388 reg. 25(7)(a)(i)
- Art. 2a(a)(ii) omitted in earlier amending provision S.I. 2018/1410, reg. 10(3) by S.I. 2020/1388 reg. 25(7)(a)(i)
- Art. 2a(b) words substituted in earlier amending provision S.I. 2018/1410, reg. 10(3) by S.I. 2020/1388 reg. 25(7)(a)(ii)