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Diagnostic Tests for Crimean Congo Haemorrhagic Fever [CCHF] in Ratites

Report of the Scientific Committee on Animal Health and Animal Welfare

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Diagnostic Tests for Crimean Congo Haemorrhagic Fever [CCHF] in Ratites

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SCIENTIFIC COMMITTEE ON ANIMAL HEALTH AND ANIMAL WELFARE

Report

on the tests to detect the presence of Crimean Congo Haemorrhagic Fever (CCHF) virus or CCHF virus specific antibodies in infections of ratites.

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1. Request for an opinion

The Scientific Committee on Animal Health and Animal Welfare is asked for an opinion on testing for Crimean Congo haemorrhagic fever [CCHF] virus in ratites.

The Commission invites the Scientific Committee on Animal Health and Animal Welfare to deliver an opinion which covers:

a) a recommendation on the test or tests to be used to detect the presence of CCHF virus or the presence of CCHF virus specific antibodies in CCHF virus infections of ratites:

b) a description of the test or tests recommended.

2. Background

CCHF virus, which causes a severe haemorrhagic fever in humans with 30% mortality has been the subject of two recent detailed reviews by Swanepoel (1998) and Capua (1998 – Annex III); the following brief background information is taken from those reviews.

2.1. Virus

CCHF virus is a segmented [3], single strand, negative sense, RNA virus which is placed in the *Nairovirus* genus of the Bunyaviridae family. CCHF virus is placed in hazard group 4 (Directive 93/88/EEC) requiring containment level 4 facilities for laboratory and animal work with live virus.

2.2. Vectors

CCHF virus has been isolated from at least 30 species of ticks (2 argasids and 28 ixodids). All the evidence suggests that the most efficient vectors are members of the *Hyalomma* genus. It appears that ticks of other species were engorged with blood from a viraemic host and there is no evidence that they can serve as vectors.

2.3. Host range

Apart from the tick vectors, CCHF virus has been isolated from humans, cattle, sheep, goats, hares (*Lepus europaeus*), hedgehogs (*Erinaceus albiventris*) and a multimammate mouse (*Mastomys* spp). In addition antibodies have been detected in a

variety of wild and domesticated mammals. Surveys for antibodies in Africa have demonstrated their presence particularly in large herbivores [kudu antelope and larger], which are the preferred host of the adult *Hyalomma* tick, and small mammals [up to the size of a hare], the preferred host of the immature *Hyalomma* tick. Mammals of intermediate size are usually free of antibodies.

Infections of birds have been less well documented. Experiments suggested domestic fowl and passerine species were probably refractory to CCHF virus, but infected guinea fowl were reported to show a transient viraemia (Shepherd et al, 1987). Studies in West Africa showed ground-frequenting birds had antibodies to CCHF virus and were susceptible to experimental infection and passage of the virus to ticks. Ostriches appear to be a more important host for CCHF and surveys reported up to 24% of ostriches tested with antibodies to CCHF virus (Shepherd et al, 1987). In addition outbreaks of CCHF in humans in 1984 and 1996 were linked to ostrich abattoirs (Capua, 1998).

Experimental assessment of CCHF virus infection of ostriches was undertaken by Swanepoel et al (1997). The main findings were that there were no clinical signs, viraemia lasted 4 days, CCHF virus was isolated from blood and visceral organs, virus could not be isolated from muscle tissue but could be detected by RT-PCR, seroconversion was first detected between 5-13 days.

2.4. Geographical distribution

Capua (1998) lists 35 countries throughout Africa, the Middle East, Asia and some southern and eastern European countries where evidence of CCHF virus has been demonstrated. However, in some countries, especially France, Portugal and Turkey

the evidence is somewhat tenuous and requires confirmation. The distribution of CCHF virus appears to coincide with the geographical range of the chief vectors i.e. members of the *Hyalomma* tick genus. Countries where *Hyalomma* ticks are present, but are currently free of CCHF virus should be considered at risk. As suggested by Capua (1998), it would appear prudent for EU countries at risk to undertake surveillance of resident ratites for the presence of CCHF virus.

3. EU Legislation

Importation of ratites and ratite meat from African and Asian countries to EU Member States is regulated by Commission Decision 96/659/EC amended by Commission Decision 97/183/EC. Essentially the objective of this legislation is to ensure that viraemic animals are not slaughtered for meat or imported live into the EU. There is, therefore, a requirement that: a) for meat imports all birds are treated with acaricide and then kept in a tick-proof and rodent-proof environment for at least 14 days before slaughter; and b) for live bird imports a similar protocol is followed before movement of the birds and the acaricide treatment and 14 day quarantine is repeated in the importing country. As an additional measure, all birds should be tested for antibodies to CCHF virus with a negative result.

Serological testing is seen as an additional safeguard to the quarantine measures, although some authorities believe that if the acaricide treatment and the 14 day quarantine in a tick-free environment is rigorously practised birds with antibodies may present less of a risk than those without in view of the length of time a viraemia is present and the onset of detectable antibodies (see section 2.3). However, since

serological assessment is an integral part of the EU control policy it is important that the test(s) used are comparable and applied consistently in all member states.

4. CCHF antibody/virus testing in EU Member States

The National Laboratory for Newcastle disease of each Member State was contacted and asked if tests for CCHF were carried out in their country and what tests were used. A response was obtained from all Member States and these are summarised in Table 1.

Table 1. Response on serological testing for CCHF virus in ratites in EU Member States.

Member State	Serological test used for CCHF virus in ratites	Other tests used for ratite CCHF infections
United Kingdom	none	None
Germany	none	None
France	none	None
The Netherlands	none	None
Belgium/Luxembourg	none	None
Greece	none	None
Finland	none	None
Sweden	none	None
Denmark	none	None
Ireland	none	None
Austria	CELISA[a] available	None
Italy	CELISA	None
Spain	CELISA	None
Portugal	CELISA	RT-PCR[b]

[a]Competitive ELISA test for CCHF virus antibodies. [b]reverse transcription-polymerase chain reaction

Several representatives in countries not carrying out routine testing of ratites, usually because there was no call as trade was absent due to a lack of demand or banned, pointed out the presence of centres of excellence for human CCHF where assistance could be obtained if necessary. Others confirmed contingency plans for ratite testing which usually involved submitting samples to an approved laboratory in another Member State or obtaining commercially available CELISA kits.

All Member States either using or intending to use a test for antibodies for CCHF virus in ratite serum samples indicated that the test of choice is the competitive ELISA test developed and supplied by Prof. R. Swanepoel, National Institute for Virology, Sandringham, Republic of South Africa, which, in fact, is the only test available for testing ratite sera. The recommended protocol for this test is included at Annex I. None of the three laboratories reporting routine use of the CELISA test considered it posed any great problems. There were minor irritations such as the length of time the test took and what the expected OD values should be. It was also considered that there was little information on the sensitivity or specificity of the test and no opportunity to test it against another serological test. Overall the conclusion was that the test was simple to perform and usually gave clear-cut results.

In one laboratory (Portugal) a RT-PCR test was also used to detect the presence of CCHF virus RNA in serum samples. The protocol (Annex II) was based on that described by Burt et al (1998) and CCHF virus RNA obtained from Prof. R. Swanepoel, National Institute for Virology, Sandringham, Republic of South Africa. No problems were reported with the RT-PCR test and it was considered to work well. In humans positive RT-PCR results could usually be obtained from the serum of infected patients for a number of days after infectious virus could be detected (Burt et al, 1998). If this is also true of infections of ratites it could be considered a major

advantage over conventional virus isolation techniques for the purposes of screening imported birds.

5. Recommendations

5.1. Detection of antibodies to CCHF virus

Since the South African CELISA test is the only test available for detecting CCHF virus antibodies in ratite serum and no major problems have been reported, that test is recommended.

5.2. Detection of CCHF virus.

RT-PCR is recommended for the detection of CCHF virus RNA in serum samples.

6. References

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7. Annex I – Protocol for CELISA for the detection of antibodies to CCHF virus in ratite sera .

The following protocol is that supplied with the CELISA obtained from National Institute for Virology, Sandringham, Republic of South Africa.

7.1. Technique

This ELISA test is based on a competition technique in which the plates are coated with a capture monoclonal antibody (mAb) directed against the nucleocapsid protein of CCHF virus. The test serum and viral antigen are added to the wells. The capture mAb and antibodies in the serum specimen compete for the CCHF antigen. Captured antigen is subsequently detected using anti-CCHF horse radish peroxidase (HRPO) conjugate and ABTS substrate.

7.2. Materials

7.2.1. Coating buffer

Carbonate buffer: 1.59g Na_2CO_3 and 2.93g $NaHCO_3$ dissolved in 1 litre distilled water [check pH 9.6].

7.2.2. PBS

Phosphate buffered saline pH 7.4. [dissolve 10 tablets Oxoid cat no. BR14a in 1 litre distilled water.

7.2.3. Blocking buffer

10% w/v skimmed milk powder in PBS

7.2.4. Wash buffer

0.1% v/v Tween 20 in PBS

7.2.5. Sample diluent

2% w/v skimmed milk powder in PBS

7.2.6. Coating antibody

Capture antibody diluted in coating buffer [7.2.1]. [Optimal dilution for National Institute for Virology, SA reagent anti-CCHF 5G2 is 1/7000].

7.2.7. Test sera/controls

Dilute test sera and positive and negative controls 1/10 with sample diluent [7.2.5].

7.2.8. Antigen.

Dilute sucrose-acetone extracted antigen [SAAg] in sample diluent [7.2.5] [Optimal dilution for National Institute for Virology reagent R1 Batch no. 97001 is 1/400.].

7.2.9. Conjugate

Dilute anti-CCHF virus horse radish peroxidase conjugated serum in sample diluent [7.2.5] [Optimal dilution for National Institute for Virology reagent CCHF-HRPO 25.7.89 is 1:1000.].

7.2.10. Substrate

2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) [Kirkegaard & Perry Cat. no. 50-66-01].

7.2.11. Plates

Nunc Immunoplates F96 Maxisorb [Nunc Cat. no. 442404].

7.2.12. Washes

15 second washes with wash buffer [7.2.4].

7.3. Method

7.3.1. coat plates with 0.1 ml/well coating antibody [7.2.6] and place overnight [about 16 hours] at 4^{o}C.

7.3.2. wash x 3

7.3.3. To each well add 0.2 ml blocking buffer [7.2.3] and incubate for 1 hour in a moist chamber at 37 ^{o}C.

7.3.4. wash x 3

7.3.5. To each well add 0.05 ml diluted test serum [7.2.7] plus 0.05 ml diluted CCHF SAAg [7.2.8]. Incubate for 2.5 to 3 hours at 37 ^{o}C, shaking plates gently every hour.

7.3.6. wash x 3

7.3.7. To each well add 0.1 ml anti-CCHF virus HRPO conjugated serum [7.2.9] and incubate for 1 hour at 37 ^{o}C

7.3.8. wash x 3

7.3.9. To each well add 0.1 ml ABTS [7.2.10]. Incubate plates at room temperature in the dark for 20 minutes.

7.3.10. Read optical density [OD] at 405 nm.

7.4. Presentation and interpretation of results

Express results as the % inhibition:

100 – 100[OD test serum/OD negative control].

A positive result is >49 %.

8. Annex II - RT-PCR for CCHF virus

The methodology is described in detail by Burt et al (1998).

The method used in the laboratory in Portugal is essentially the same:-

8.1. Extraction

Total RNA is extracted from 0.1ml serum samples using Rneasy (QIAGEN) or RNAgents (PROMEGA) extraction kits. (If larger volumes are available these are centrifuged at 121,000**g** for 45 minutes and the RNA extracted from the pellet.

8.2. Primers

The primers are based on the nucleotide sequence of the nucleoprotein gene of CCHF virus strain 10200 and are designed to give a 538 base pair product.

Forward Primer: 5'TGGACACCTCACAAACTC3' nucleotide positions 135-153.

Reverse Primer: 5'GACAAATTCCCTGCACCA3' nucleotide positions 670-653.

8.3. RT-PCR

The single tube Superscript One-Step RT-PCR system [GIBCO BRL] or the Access RT-PCR system [Promega] is used with 200μM each dNTP, 50 pmol each primer and 1 μg sample RNA. The following thermocycler steps are used:

48 ^{o}C for 45 mins, 94 ^{o}C 2 mins. Then 30 cycles of: 94 ^{o}C for 30 secs, 47 ^{o}C 30 secs and 72 ^{o}C for 30 secs. These are followed by 72 ^{o}C for 5 mins before cooling to 4 ^{o}C.

CCHF RNA is included as a positive control for each RT-PCR.

8.4. Visualisation

10 μl aliquots of the PCR products were subjected to electrophoresis on 1.2% agarose gels with 1 μg/ml ethidium bromide and the DNA bands visualised on a UV transilluminator.

9. ANNEX III

Crimean-Congo haemorrhagic fever in ostriches by I. Capua.

A Review

Avian Pathology (1998) 27, 117-120

GUEST EDITORIAL

Crimean-Congo haemorrhagic fever in ostriches: a public health risk for countries of the European Union?

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Crimean-Congo haemorrhagic fever (CCHF) virus is the aetiological agent of a tick-borne zoonosis present in Africa, Asia and eastern Europe which causes human illness with an approximately 30% fatality rate The virus is a member of the *Nairovirus* genus of the family Bunyaviridae (Swanepoel. 1995)

In October 1996 there was an outbreak of 17 cases of CCHF among workers at an ostrich abattoir which employs about 400 people in the Oudtshoorn district, South Africa The South African authorities immediately reported the outbreak to the European Union (EU) Considering the severity of this zoonosis, the EU put a ban on South African ostrich exports (Decision 96/659/EC) in order to prevent the disease from entering the EU through live animals and meat. The ban was subsequently lifted on the basis of results obtained from the experimental infection of ostriches with CCHF (Swanepoel *et al*, 1997), and of the modifications in husbandry methods for export slaughter and live birds (Decision 97/183/EC). Since this is a serious disease for humans and there is a growing interest for the ostrich industry, background information, and a few considerations would be useful for scientists and veterinarians involved with ostrich diseases and breeding.

A **Brief Overview of the Disease**

History and distribution

The first outbreak of the disease was described in 1944 in the Crimean peninsula in people bitten by ticks while harvesting crops and sleeping outdoors, and therefore it was named Crimean haemorrhagic fever. The tick-borne virus aetiology was demonstrated in 1945 by inoculating filtered tick suspensions and blood into human subjects, but the virus was not isolated in a laboratory host until 1967 (Chumakov. 1974) In 1956. a virus named Congo was isolated from a sick child in what was then the Belgian Congo, and in 1969 it was demonstrated that the two viruses were, in fact, identical and from then on the two names have been used in combination (Casals, 1969, Chumakov *et al.*, 1969).

The disease is widely distributed in Asia, Africa and eastern Europe: evidence of the presence of the virus has been reported in Egypt, Ethiopia, Mauritania, Senegal, Burkina-Faso, Benin, Nigeria, Central African Republic, Zaire, Kenya, Uganda, Tanzania. Madagascar. Zimbabwe, Namibia, South Africa, Madagascar, Kuwait, Dubai, Sharjah, Iraq, Iran, Afghanistan, Pakistan, India, China, former USSR, Bulgaria, Turkey, Hungary, former Yugoslavia and, with reference to the European Union, in Greece, France and Portugal (Swanepoel, 1995). It should nevertheless be stated that the evidence for France, Portugal and Turkey is based on limited observations, particularly France. where antibodies were detected in two bats

Epidemiology

The outbreaks of disease in Eastern Europe and Asia have generally been linked to circumstances created by humans. In fact the original outbreak in Crimea was due to re-occupation of tick-infested areas during the war, and subsequent epidemics in the former USSR and Bulgaria were caused by abrupt changes in agricultural and animal husbandry practices In recent years. the outbreaks have generally been sporadic, with the majority of outbreaks being reported in Bulgaria (Swanepoel, 1995) iii the African continent, only 15 outbreaks were reported prior to 1981 (eight of which were laboratory infections). Since the first outbreak was diagnosed in South Africa in 1981, sporadic cases of haemorrhagic fever have been diagnosed each year. together with occasional common source no-

socomial outbreaks which have been reported throughout the years (Swanepoel, 1995)

The small number of cases diagnosed and the low incidence of seroconversion. suggest that the virus only sporadically infects humans, in contrast to the widespread infection which occurs in domestic and wild animals.

The virus has been isolated from cattle, sheep, goats, hares *(Lepus europaeus),* hedgehogs *[Erinaceus (Atelerix) albiventris]* and a multimammate mouse *(Mastomys* spp) and antibodies have been detected in a range of wild and domestic vertebrates (Watts. *et al.* 1989; Burt *et al.,* 1993).

Infection can be transmitted by ticks of several genera, and the virus has been isolated from 30 species of ticks (28 ixodid and two argasid species) (Hoogstraal 1979; Watts *et al.,* 1989, Camicas *et al,* 1990; Vesenjak *et al.,* 1991). Nevertheless, for several of these species there is no evidence that the ticks are capable of serving as vectors, since the virus could have been present simply because the tick had fed on a viraemic host. On the other hand, the trans-stadial and trans-ovarian transmission of infection has been reported for *Hyalomma marginatum marginatum, Riphicephalus rossicus* and *Dermacentor marginatus* Experimental evidence indicates that the most efficient vectors appear to be members of the genus *Hyalomma.* In fact, the world distribution of the virus coincides with the distribution of these ticks (Hoogstraal, 1979: Watts *et al.,* 1989).

The virus causes mild infection with transient viraemia in farm animals such as sheep and cattle which serve as hosts of adult *Hyalomma* ticks. Immature *Hyalommas* feed on small wild mammals, up to the size of hares, and ground-frequenting birds. The small mammal species that have been tested also appear to undergo mild infection with viraemia and they are thought to play an important role as a source of infection for ticks.

Little information was available on CCHF infection of birds prior to 1984 when a worker contracted the disease at an ostrich abattoir in Oudtshoorn district. South Africa: limited observations in the former Soviet Union had indicated that passerine birds and domestic chickens were refractory to the virus, although a low prevalence of antibody could be detected in wild birds (Hoogstraal, 1979; Shepherd *et a!.,* 1987). Transmission experiments were conducted in domestic chickens and guinea fowl, showing that the latter develop a transient viraemia (Shepherd *et al.,* 1987). Subsequently, it was found that a few species of wild birds tested in West Africa failed to develop demonstrable viraemia following experimental infection (Zeller *et al.,* 1994). The study conducted by Shepherd *et al.,* 1987 on free-ranging birds demonstrated that ostriches exhibit a much higher prevalence of infection compared to other birds.

Disease in man

The disease is a haemorrhagic fever Clinical symptoms are determined by liver and endothelial damage and impairment of haemostasis Platelet counts drop dramatically and there is evidence of widespread haemorrhages Disseminated intravascular coagulopathy occurs and contributes to further tissue damage.

The incubation period varies from 1 to 9 days following a tick bite and from 4 to 13 days in patients exposed to blood (or tissues) of livestock and humans. The onset is sudden with fever, chills, severe headache, dizziness, neck pain and stiffness, myalgia with intense backache and leg pains. Nausea, vomiting and abdominal pains with or without diarrhoea are generally present soon after onset. From the second to the fourth day fever is generally intermittent and patients may undergo changes of mood with feelings of confusion, aggression, and subsequently lassitude and somnolence. By this time petechiae may be present in the oral cavity, throat and tonsils. Petechial rashes develop on the limbs and trunk, and this may be followed by the development of large bruises and ecchymoses Bleeding begins commonly from about day 5: epistaxis, haematemesis, haematuria, melaena and gingival bleeding are common symptoms At times the only clinical symptom may be the oozing of blood from injection or venepuncture sites Internal bleeding such as retroperitoneal and intracranial haemorrhage may occur Recovery begins on day 9 or 10 of illness, but symptoms such as asthenia, confusion and conjunctivitis may continue for over a month Death generally occurs from day 5 to 14 of illness (Swanepoel *et al.,* 1987, 1989).

Diagnosis

Virus isolation from tissues or blood of ill patients should be performed in a maximum security laboratory. The most sensitive method is by intracerebral inoculation of suckling mice, although inoculation of susceptible cell lines such as VERO and CER may also be used The virus in infected cells or tissues may be detected by performing an immunofluorescence test (Shepherd *e al.,* 1986). A reverse transcriptase-polymerase chain reaction (RT-PCR) has also been recently developed (Burt *et al.,* 1997)

Antibodies (IgG and IgM) may be detectable by the indirect immunof1uorescence technique or by ELISA (Shepherd *et ali.,* 1989) and are present in an increasing proportion of patients from day *5* onwards All survivors of infection have demonstrable antibodies by day 9 at the latest. IgG antibodies remain demonstrable for at least 5 years. No vaccines are available at present for disease control in humans.

CCHF and Ostriches

Very little was known about CCHF in ostriches until an experimental infection was carried out recently (Swanepoel *et al.,* 1997). The susceptibility of ostrich was suspected because in a limited number of samples examined in South Africa. 24% of the birds contained antibody and there had been reports of human outbreaks linked to ostrich abattoirs. The experimental infection in ostriches was performed following the human outbreak of November 1996 (Oudtshoorn. South Africa) There was a concern that there may be a possibility of transmitting this disease to the EU ostrich product consumer. The trial yielded information on infection in ostriches which was useful for scientists and veterinarians who are involved with these birds. Scientific evidence obtained from the trial may be summarized as follows

- infection in ostriches is not characterized by clinical symptoms
- the birds undergo a viraemia which may last 4 days
- virus was isolated from blood and from a selection of visceral organs
- virus was not isolated from muscle but was detected by RT-PCR
- seroconversion begins on day 5 post-inoculation and was detectable in all the inoculated birds by day 13

The evidence enabled the EU to draft a decision which sets minimum requirements for the importation of live ostriches and of ostrich meat into Member States.

Decision taken by the European Union

Decision 97/183/EC, considering the results of the experiment supplied by the South African veterinary authorities, amends Commission Decision 96/659/EC on protective measures in relation to Crimean-Congo Haemorrhagic fever in South Africa

The first point made in Article I of Decision 97/183/EC is to extend protective measures to all African and Asian countries, since the disease is not present only in South Africa, but has also been reported in several Asian and African countries

The importation of ratites or ratite meat may be authorized by Member States if the provisions in the two annexes of the decision are complied with. The basic concept for both categories (live birds and meat) is that the EU does not accept for import birds which may be viraemic The only way to prevent viraemia is to avoid that birds get bitten by infected ticks during a set period of time before export or slaughter. Annex I deals with ostrich meat, and the basic requirement is that birds are to be treated with acaricide and kept tick free, in rodent proof areas for at least 14 days prior to slaughter. Considering that a viraemia may last up to 4 days after infection, complying with the requirements would prevent the slaughter of viraemic birds. In this way there should be no risk of infection for the consumer of ostrich meat or for abattoir workers

A similar requirement is stated in Annex 11 which deals with importation of live animals Birds must be treated for ectoparasites before entering tick-free surroundings in which they must remain for 14 days prior to departure Furthermore, all birds entering countries of the EU must be seronegative to CCHF virus The treatment for ectoparasites and the serological test is to be repeated on arrival in the Community These requirements should ensure that viraemic birds are not imported into the Community This is, as matter of fact, a double check: keeping the birds tick free should prevent them from getting infected, but should there be a leak in the system. by the time the birds reach their destination and get bled for serology, they would have seroconverted to CCHF and, therefore, would not comply with the EU requirements.

Is There a Risk?

The decisions undertaken by the member states of the European Union, if applied strictly, should be sufficient to avoid the introduction of the disease. The general rule that slaughter and export birds should be treated and kept in tick-free rodent proof areas for at least 14 days prior to slaughter or export should guarantee that the birds are not viraemic at slaughter or when they are loaded onto a plane. In this way, there would be no risk of contracting the disease handling meat, and of intro-ducing and possibly perpetuating the infection in Europe. With reference to this last point, it should be stated that the vectors of CCHF are widely distributed in several countries of southern and eastern Europe in which the presence of the virus has not been reported, and therefore environmental and ecological conditions for introduction of the virus already exist. For this reason, Italy has had specific requirements on CCHF since 1992 (O.M 24/10/1992), which necessitate that all ostriches originating from Africa imported into Italy from 1992 onwards must be tested for antibodies against CCHF virus Thus, the Italian ostrich breeder population is seronegative. This is probably not the case in other Member States in which the serologic test has not been performed on imported birds Moreover, this situation could easily represent a matter for dispute between Member States in intra-community trade.

One aspect which has not been considered by the Commission in Decision 97/I 83/EC is the sanitary situation of birds imported into the Community from eastern European countries. Bearing in mind that the infection is present in these

countries, it is advisable that ostriches imported from these states should have sanitary requirements with regards to CCHF.

Although imported and native birds should not represent a source of infection, considering the seriousness of the illness in man, precautions such as wearing protective gear should be taken when performing post-mortem examinations or bleeding ostriches. Furthermore, it would be very interesting to perform a serological survey on the ostrich populations of European countries in order to have data on the presence of antibodies to CCHF virus in these birds

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