TSE EURL

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TSE EU REFERENCE LABORATORY GUIDELINES FOR THE DETECTION OF CHRONIC WASTING DISEASE IN CERVIDS.

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1. BACKGROUND

Several commercial rapid tests are used for routine monitoring of BSE and scrapie within EU member states and are approved for use by the EU (Regulation (EC) No 1148/2014 - amending Regulation (EC) No 999/2001).

Rapid test approval and inclusion within the current EU TSE Regulations is based on the outcome of test performance from rigorous test evaluations. Previous EFSA opinions relating to the use of rapid tests for detecting BSE & scrapie comprise of several TSE test evaluation studies (most recently, EFSA, 2004[1], 2004[2], 2005[3], 2005[4], 2007[5], 2009[6], and 2012[7]).

The strength of formalised EFSA evaluations is based directly on comparable evaluation utilising a standardised set of samples. In contrast, rapid tests for detecting Chronic Wasting Disease (CWD) have not undergone such a full and directly comparable evaluation and therefore have not undergone equivalent validation.

1 EFSA. 2004. Scientific Report of the European Food Safety Authority on the Design of a Field Trial Protocol for the Evaluation of New Rapid BSE post mortem Tests. 1, 1-10.

2 EFSA. 2004. Scientific Report of the European Food Safety Authority on the Evaluation of Seven New Rapid post mortem BSE Tests. 18, 1-13.

3 EFSA 2005. Scientific Report of the European Food Safety Authority on the Evaluation of Rapid post mortem TSE Tests intended for Small Ruminants. 31, 1-17.

4 EFSA. 2005. Scientific Report of the European Food Safety Authority on the Evaluation of Rapid post mortem TSE Tests intended for Small Ruminants. 49, 1-16.

5 EFSA. 2007. Scientific Opinion of the Panel on Biological Hazards on a request from the European Commission on a protocol for the evaluation of new rapid BSE post mortem tests. The EFSA Journal. 508, 1-20

6 EFSA. 2009. EFSA Panel on Biological Hazards (BIOHAZ); Scientific Opinion on Analytical sensitivity of approved TSE rapid tests. EFSA Journal 7(12):1436.

7 EFSA. 2012. EFSA Panel on Biological Hazards (BIOHAZ); Scientific Opinion on the evaluation of new TSE rapid tests submitted in the framework of the Commission Call for expression of interest 2007/S204-247339. EFSA Journal. 10(5):2660

In the absence of a full EFSA evaluation, there are several peer-reviewed publications that provide evidence of the potential for rapid tests to detect CWD originating from North America and Canada. Additionally, two rapid test manufacturers have undertaken validation exercises to obtain United States Department of Agriculture (USDA) & Canadian Food Inspection Agency (CFIA) approval for the use of their rapid tests to detect CWD in North America & Canada.

Due to the lack of species specific CWD control material originating from Europe, it has not been possible to undertake a meaningful rapid test evaluation to date. Moreover, the current status is unclear as to the influence of factors such as different CWD strains, different cervid species present in the EU and different cervid genotypes on the capability of the tests to detect CWD.

This document aims to provide guidelines regarding the use of rapid tests for the detection of Chronic Wasting Disease (CWD) and is aimed primarily at EU member states undertaking targeted surveillance of cervids, as laid out in forthcoming EU regulations and the recent EFSA opinion on CWD (EFSA, 2017[8]).

These guidelines will be regularly reviewed and amended as required in response to the developing situation within the EU and the forthcoming EFSA Opinion on whether the conclusions and recommendations in the EFSA opinion of June 2004[9] on diagnostic methods for CWD are still valid.

2. SAMPLING

2.1. Sample Type

The TSE EURL currently recommends the testing of both obex and retropharyngeal lymph nodes to maximise the sensitivity of surveillance.

When undertaking sampling of both obex and retropharyngeal lymph nodes for rapid testing, consideration must also be given to the retention of sufficient and appropriate obex and retropharyngeal lymph node for undertaking confirmatory testing.

[8] EFSA. 2017. EFSA Panel on Biological Hazards (BIOHAZ); Scientific Opinion on Chronic wasting disease (CWD) in cervids. EFSA Journal 15(1):4667.

[9] EFSA 2004. EFSA Panel on Biological Hazards (BIOHAZ); Opinion on a surveillance programme for Chronic wasting disease in the European Union. EFSA Journal 70:1-7.

For lymphoid tissues, ensure that sufficient lymph node, ideally an anatomically orientable cross-sectional sample with good representation of the cortex, is retained for confirmatory testing in the event of a positive rapid test result. Confirmatory testing should, as a minimum, be performed on the tissue giving rise to the positive result. All collected tissues should be retained for possible further analysis from any animal test positive in any tissue. When undertaking rapid tests for obex and retropharyngeal lymph nodes, rapid test kit instructions for use (IFU) must be followed, as the IFUs are specific to different tissue types.

2.1.1. Sampling of the obex tissue

The TSE EURL provides guidance for the correct sampling approach required to collect brainstem (obex) tissue for undertaking TSE testing in cattle and sheep, which can be applied to cervids. Please note that cerebellum is not a primary sample from these species. This information can be found at:

ftp://ftp.izsto.it/EURL%20TSE/RAPID%20TESTS/Test%20protocols/

2.1.2. Sampling of lymph nodes of the head of deer

Barrel and Simpson-Morgan (1990[10]) described the lymph nodes and drainage of the tissues of the head of Fallow deer (*Dama dama*). They stated that the location of nodes was similar to that described for sheep and goats, with the sole exception that the medial retropharyngeal lymphocentre often comprised two nodes on either side (four in total). It therefore seems reasonable to assume that the location of lymph nodes in the head and neck of most species of deer will be more or less similar to those described for the Fallow deer and other ruminants.

Any method of sampling that results in collection of the lymph nodes with minimal contamination between samples is acceptable.

The following guidance and description of lymph node location is based on that provided by Barrel and Simpson-Morgan (1990) and Dyce, Sack and Wensing (2010[11]).

- Position the carcase or head in dorsal recumbency.

[10] Barrell, G. K., & Simpson-Morgan, M. W. 1990. Major lymph nodes of the head of the fallow deer (Dama dama) and lymphatic drainage of antlers. Aus Vet J, 67(11), 406-407.

[11] Dyce, K.M., Sack W.O. and Wensing C.J.G. 2010. Textbook of veterinary anatomy. Philadelphia, Pa; London: Saunders.

- Incise and reflect the skin from the ventral and lateral surfaces of the face and neck.
- The medial retropharyngeal LN lies embedded in fat between the dorsocaudal wall of the pharynx and the muscles overlying the base of the skull; medial to the stylohyoid bone. In fallow deer this lymph node often comprises a pair.
- Access to these lymph nodes is facilitated by incising the soft tissues between the tongue and mandibular rami and transecting the hyoid apparatus to reflect the tongue and larynx caudally to expose the soft palate and dorsal pharynx.
- Alternatively, once the head has been removed from the spine at the level of the atlanto-occipital joint, these lymph nodes can be assessed after lifting the trachea and dissecting the muscles rostral to the foramen magnum. The lymph nodes are located halfway between each angle of the mandible and the foramen magnum, caudal to the nasopharynx.

Specifically, for the testing of lymph nodes, sample from 2-3 different areas of the cortex of the lymph node to increase the likelihood of sampling a positive follicle.

3. DIAGNOSIS AND CHARACTERIZATION

3.1. Rapid tests

In the absence of rapid test data resulting from a full test evaluation of the performance for the detection of CWD in cervids, the TSE EURL currently suggest the use of rapid tests that have undergone validation and received USDA and/ or CFIA approval for use with cervid tissue.

The EURL also suggests that the test is manufactured by a company already marketing an EU-approved TSE test, this ensures that the manufacturer already meets the Quality requirements of current EU-approved rapid tests. These tests are:

• The Bio-Rad TeSeE SAP rapid test.

• The IDEXX HerdChek Bovine Spongiform Encephalopathy-Scrapie Antigen Test Kit, EIA.

3.2. Confirmatory testing

When the result of the rapid test is inconclusive or positive, the sample shall be subjected to confirmatory examinations using at least one of the following methods and protocols as laid down

in the latest edition of the Manual for diagnostic tests and vaccines for Terrestrial Animals of the World Organisation for Animal Health (OIE), namely:

• Immunohistochemistry;

• Western blot.

If it is not possible to confirm a positive rapid test result, an adequate quantity of target tissue is to be sent to the EURL for confirmatory testing to be undertaken.

3.3. Characterisation of isolates

In the event of a positive testing outcome, further isolate characterisation should be undertaken, in consultation with the TSE EURL. To this purpose, the NRL should contact the EURL to decide any further analysis of the isolate, depending on the available tissues. In the case that some methods (i.e. discriminatory testing, bioassay) will not be available at the NRL, an adequate quantity of target tissue is to be sent to the EURL.

4. TSE EURL CONTACT DETAILS

4.1. For TSE rapid test issues please contact:

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4.3. For TSE strain characterization (biochemical and biological typing) issues please contact:

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4.4. For prion protein genotyping issues please contact:

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This document is largely based on a previous one that originally was made available by APHA (UK) as EURL for TSEs. After the transition of the EURL to our consortium, in the documentation that we are making available, some minor changes were needed to update information regarding e.g. logistical aspects, sample flow, contacts and references, or changes carried out by the relevant companies in protocols and nomenclatures.