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VKM Report 2017: 26

CWD – update statement

Opinion of the Panel on Biological Hazards of the Norwegian Scientific Committee for Food Safety

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Assessed and approved

The opinion has been assessed and approved by Panel on Biological Hazards. Members of the panel are: Yngvild Wasteson (Chair), Karl Eckner, Georg Kapperud, Jørgen Lassen, Judith Narvhus, Truls Nesbakken, Lucy Robertson, Jan Thomas Rosnes, Olaug Taran Skjerdal, Eystein Skjerve, Line Vold.

Acknowledgment

The Norwegian Scientific Committee for Food Safety (Vitenskapskomiteen for mattrygghet, VKM) has appointed a working group consisting of both VKM members and external experts to answer the request from the Norwegian Food Safety Authority/Norwegian Environment Agency. Project leader from the VKM secretariat has been Danica Grahek-Ogden. The members of the working group Michael Tranulis (NMBU) and Georg Kapperud are acknowledged for their valuable work on this opinion. The Panel on Biological Hazards are acknowledged for comments and views on this opinion.

Competence of VKM experts

Persons working for VKM, either as appointed members of the Committee or as external experts, do this by virtue of their scientific expertise, not as representatives for their employers or third party interests. The Civil Services Act instructions on legal competence apply for all work prepared by VKM.

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Background and terms of reference as provided by the Norwegian Food Safety Authority/ Norwegian Environment Agency

Regarding the risk assessments on CWD (Phase1 and Phase2).

Canadian and American websites have published information about the results of a research project on experimental transmission of CWD to macaques. Links as follows:

https://thetyee.ca/News/2017/06/24/Chronic-Wasting-Disease-Research/

http://cjonline.com/outdoors/sports/news/2017-06-20/new-study-suggests-humans-may-be-susceptible-chronic-wasting-disease

The Norwegian Food Safety Authority and the Norwegian Environment Agency want a statement from VKM whether these or any other new information changes any of the conclusions in the risk assessments from VKM.

Literature and new data

Articles referring to the new studies were provided byt the Norwegian Food Safety Authority and the Norwegian Environment Agency and are listed in the references.

A phone conference between Stefanie Czub, head of the virology department at the Canadian Food Inspection Agency (CFIA) and Michael Tranulis was held 28th August to further clarify the results of the study presented earlier this year (Czub, 2017).

Findings in new studies

At the international prion conference held in Edinburgh, Scotland May 23-26, 2017, Dr. Stefanie Czub, head of the virology department at the Canadian Food Inspection Agency (CFIA), presented a progress report from a study initiated in 2009 (Czub, 2017). Although preliminary, the data reported were of great significance, since for the first time it was shown that CWD prions could, after experimental transmission, give rise to prion disease in Cynomolgus macaques (*Macaca fascicularis*), which is the closest relative to humans allowed for such transmission studies.

The study included 21 female macaques, 2.5 years old when recruited. All animals were of Mauritian origin and wild-type *PRNP* genotype, homozygous for Met at codon 129. Cynomolgus macaques from Mauritius are prone to development of type 2 diabetes (T2D), which particularly affects females. This feature of the animals was not taken into account when the present study was designed (Personal communication, Stefanie Czub). Several of the animals in the study have developed T2D of varying severity. Importantly, some of the features of this disease, such as weight loss is difficult to discriminate from the wasting seen in clinical CWD. Related to this, is the question of whether T2D could increase the susceptibility to CWD. Dysregulation of insulin signaling can increase neuronal vulnerability to different stressors. The putative importance of this for the results of this study is unknown and mentioned here for background purposes.

The principal objective of the study was to investigate the zoonotic potential of CWD. In order to achieve this, 18 animals were challenged with infectious material by four different routes: intracranial inoculation (8), oral (5), skin scarification (2) and through intravenous blood transfusion (3). Three animals were inoculated with non-infected material, serving as mock-infected controls.

Infectious material (inocula) were derived from two principal sources, namely CWD-positive brain or muscle tissue from preclinical white tailed deer (*Odocoileus virginianus*) and a brain-pool from three cases of clinical CWD in wapiti (*Cervus Canadensis*). Infectious material for blood transfusion was derived from non-clinical CWD infected macaques.

By the time the preliminary data were presented 11 animals had been sacrificed, one of which was a mock-inoculated control. Of the other 10 sacrificed animals, six were infected intracranially and four by the oral route. From this group of 10 animals, analyses were sufficiently complete for five animals: two from the intracranial group and three from the orally infected group. During presentation of the data, the focus was on these five animals. For the remaining five animals, the analyses are in progress (Personal communication, Stefanie Czub).

All five animals had prion specific histopathological changes, with accumulation of PrP^{Sc} deposits and/or amyloid seeding activity (RT-QuIC positive) in samples from brain and/or spinal cord. Thus, there could be no doubt that the animals had developed or were in the process of developing prion disease.

One of the animals (ID AU389) had been inoculated intracranially with CWD-positive brain tissue from wapiti and developed ataxia, anxiety, tremor and a wasting syndrome. This animal was sacrificed 4.5 years after inoculation due to the clinical symptoms. It tested positive on all diagnostic tests for prion disease (IHC and RT-QuIC). It was also shown that this animal suffered from T2D. The second animal in the intracranially infected group (AU519) was sacrificed according to experimental plan, 5.2 years after inoculation and was clinically healthy. This animal had been inoculated with CWD-positive brain material from white tailed deer and it tested positive for prion disease on all tests.

Two of the animals that were orally infected (AU501, AU385) developed ataxia, tremor, apathy and wasting to varying degrees and initially very subtle. These two animals had been fed raw meat from white tailed deer that were in the preclinical stage of CWD. These animals were from a flock of farmed deer that was stamped out due to occurrence of CWD. A total dose of 5 kg meat was given to the animals over a period of two years, with approximate doses of 200 gram per month. They were sacrificed 5.4 (AU501) and 6.3 (AU385) years after their primary inoculation. Both animals tested positive on all tests for prion disease. One of the animals (AU385) suffered from T2D.

The last animal (AU467) had been orally infected with brain material from white tailed deer and this animal died during anesthesia, 5.9 years after inoculation, without previous display of clinical symptoms. It tested positive on all tests for prion disease.

The presented results are remarkable for the following reasons:

1. For the first time it is shown that Macaque monkeys are susceptible to CWD, through both the intracranial and oral route of inoculation.

2. CWD inocula were from different species (white tailed deer and wapiti) and analysis of these indicated the presence of different CWD strains/types. The brain pool derived from three cases of CWD in wapiti was shown to be the CWD2 strain. The brain inoculum derived from the clinical case of CWD in white tailed deer was shown to the be heterogenic, consisting of two distinct CWD strains, demonstrating the co-

occurrence of CWD strains in a single diseased animal, as has previously been observed also in sheep scrapie (Masujin et al 2009, Arch Virol).

3. Incubation periods were relatively short. For comparison: Intracerebral inoculation of classical scrapie (see below) resulted in disease in a macaque after a 10-year incubation period (Comoy et al., 2015). A single oral dose of 5 grams of BSE infected material results in 100 % attack rate in macaques with a median incubation period of 4.7 years (Holznagel et al., 2013).

4. Oral challenge of two macaques with meat derived from CWD positive but preclinical white tailed deer, resulted in prion disease with only marginally longer incubation period than intracranial injection. This experiment was specifically designed to mimic a real-life human exposure to CWD through intake of venison.

It will take several years for the study to be finalized with all downstream analyses.

Another investigation (Race et al., 2014) observed that cynomolgus macaques did not develop CWD after intracranial (six animals) or oral (eight animals) inoculation. Animals were observed for more than 10 years after inoculations. The reasons for the discrepancies between ~~this~~ that study and the ongoing study discussed above are incompletely clarified and re-analysis of some of the materials derived from the study by Race et al is ongoing (Personal communication Stefanie Czub). For instance, the spinal cord must be carefully investigated and analysis of "prion converting activity" as assessed by RT-QuIC or equivalent would increase the sensitivity of analysis. The outcome of these studies will be published in due course.

Update on Norwegian situation

By 20. September 2017, a total of 4200 wild reindeer have been examined for CWD. Six of those (0,14%) tested positive, of which only one showed clinical symptoms of CWD. In three cases, the CWD agent was detected both in brain tissue and in lymphatic samples. Interestingly, brain samples from the other three animals (all tested in 2017) were negative, whereas lymphatic tissues were positive. This is not unexpected, as classical CWD is characterized by a prolonged asymptomatic incubation period, during which the agent may be detected in peripheral tissues including lymph nodes, and even in blood, prior to its detection in the CNS. It is important to realize that meat from such animals, as well as from their counterparts with clinical CWD, may represent a source for human exposure. In our previous opinion (VKM, 2017), we emphasized that "absence of clinical illness does not preclude human exposure, since cervids may harbour and shed the CWD agent for several months prior to onset of their symptoms". Thus, the findings in 2017 does not alter our conclusions.

Data from the ongoing surveillance of Norwegian cervids are frequently updated on the web-pages of the Norwegian Veterinary Institute; https://www.vetinst.no/sykdom-og-agens/chronic-wasting-disease

Uncertainties/data gaps

A growing body of scientific literature critically assessing the validity of animal experimentation generally, raises important concerns about the reliability and predictive value for human outcomes (Akhtar, 2015). Although the oral challenge experiments described above were specifically designed to resemble a real-life human exposure to CWD through intake of venison, there are considerable difficulties of reproducing the complexity of human diseases in animal models.

It is notable that the macaques were fed raw venison, while human consumption is restricted to cooked meat, although rare or medium steaks are preferred by many consumer. This, however, does not alter the conclusion since the prions retain their infectivity after exposure to the temperatures normally used in food preparation (Rutala, Weber, & Society for Healthcare Epidemiology of, 2010).

It needs to be underlined, that the results obtained from the experiments conducted by Czub et al. are preliminary data presented as a progress report from an ongoing study that will require several years to complete, including a number of additional analyses. The results have not yet been peer-reviewed and published.

It is also worth mentioning that a previous study failed to infect macaques orally after being observed for more than ten years post-inoculation (Race et al., 2014). As stated in the preceding section, some of the materials derived from that stydu are now subject to re-analysis using more sensitive techniques.

Moreover, in North America, human exposure to the agent via consumption of venison in the enzootic areas is well documented (Sigurdson, 2008). Data accrued to date provide no evidence of CWD causing disease in humans, despite a substantial consumption of meat from CWD-infected deer over several decades, and the large number of hunters being exposed to the agent through contact with infected deer. This, however, does not preclude that cases of human CWD may appear, given a sufficient incubation period and exposure frequency. Hence, the importance of reinforced surveillance and vigilance regarding Creutzfeldt-Jakob disease (CJD) suspect cases should be emphasized, including characterization of CJD subtypes using analyses enabling identification of CWD. It is important to realize that the clinical presentation of human CWD is unknown and may differ from CJD as well as from classical CWD in cervids.

Conclusions

The progress report by Czub and collaborators underlines that in prion diseases, the species barrier for disease transmission is rarely, if ever, absolute. Comoy and co-workers also showed this, when demonstrating that a case of natural classical scrapie in sheep could be transmitted to cynomolgus macaques by intra-cranial inoculation, after a 10-year incubation period (Comoy et al., 2015). This observation has not led to reassessment of the zoonotic risk of classical scrapie in sheep.

The uncertainty related to incomplete species barriers was carefully assessed in two previous VKM opinions, which concluded that the zoonotic potential is considered very low (VKM, 2016, 2017). The term "very low" was defined as "very rare but cannot be excluded.

Furthermore, the released data illustrate another aspect of CWD that was also discussed by VKM namely that infected animals can harbor significant levels of infectious prions in musculature while still appearing clinically healthy. According to Czub (Personal communication), this is particularly prominent in white tailed deer CWD and less so in wapiti CWD, in which levels of infectivity in peripheral organs seems somewhat lower. The data emerging from Norwegian cases of CWD in reindeer, although limited, indicate that this CWD strain is lymphotrophic, with significant involvement of peripheral lymphoid organs during the preclinical phase of disease. This is based on the observation of cases in reindeer with positive findings in lymph nodes while still negative/or very weak signals in the brain. These findings are in line with data from CWD in white tailed deer. Therefore, until investigated with sensitive methods, it should be considered likely that CWD infectivity is present also in reindeer musculature even at the preclinical stage of disease. Consequently, removal of the head and/or spinal cord may be a less effective measure for reducing human exposure for CWD prions than for other prion diseases.

The susceptibility of macaques to CWD after oral challenge with meat derived from CWD-positive, preclinical, white-tailed deer reinforces the need for precautionary and pro-active measure to reduce human exposure. At the present stage of knowledge, it cannot be excluded that CWD prions may cause human disease, but the risk appears very low.

In conclusion, the zoonotic risk of CWD is still considered to be very low, in accordance with our previous assessments.

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