Working Document on Camel Prion Disease (CPrD)

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Content:

I. Introduction

Camel prion disease (CPrD) is the last disease described in the family of prion diseases [1]. To date, it has been recognized only in Middle East of Algeria and in the neighboring region of Tunisia [2]. However, there are no known other initiatives of prion diseases surveillance in camels worldwide. CPrD might actually be limited to the already known geographic area in North Africa or spread undetected in other Countries, as a consequence of the movements of dromedaries along trans-Saharan commercial routes, the import/export trade flows of living animals and the traditional extensive and nomadic rearing systems.

According to the discussions in recent meetings of REMESA and OIE which indicated the need to extend the knowledge on CPrD spread in Countries where camels are extensively reared and considered as a part of the domestic livestock [3], and according to the initiative from CAMENET member countries to assess the risk in the CAMENET region, this working document aims to provide countries with the main technical and scientific knowledge necessary to implement surveillance programs on camel prion disease in its own territory.

Basic information contained in this document may also be helpful for the possible design of contingency plans.

The present working document is an 'alive' document. It should be regularly reviewed and updated as further information becomes available.

II. Camel prion disease[1]

Camel prion disease (CPrD) was diagnosed in 2018 in three adult camels showing clinical signs at the ante-mortem inspection at an abattoir in the region of Ouargla (Algeria) [1]. According to the published report symptoms suggesting prion disease occurred in 3.1% of dromedaries brought for slaughter to the Ouargla abattoir in 2015–2016. More recently, in 2019, the same disease was reported in the region of Tataouine (Tunisia) [2]. CPrD adds to the group of animal prion diseases,

[1] Modified from the OIE Bulletin: https://oiebulletin.com/wp-content/uploads/2019/12/OIE-News-December-2019-Camel-prion-disease.pdf?utm_source=World+Organisation+for+Animal+Health+%E2%80%93+OIE+Bulletin&utm_campaign=388d499799-EMAIL_CAMPAIGN_2019_12_05_09_06&utm_medium=email&utm_term=0_7694a173d1-388d499799-54758659

including scrapie in sheep and goats, chronic wasting disease (CWD) in cervids and Bovine spongiform encephalopathy (BSE) in cattle. As of today, very limited epidemiological information is available about the prevalence, geographical distribution and mode of transmission of the disease.

The involvement of lymphoid tissue in prion replication, observed both in the Algerian and Tunisian cases [1,2], is suggestive of a peripheral pathogenesis, which is thought to be a prerequisite for prion shedding into the environment. As with other animal prion diseases, such as scrapie and CWD, in which lymphoid tissues are extensively involved and horizontal transmission occurs efficiently under natural conditions, the detection of prion proteins in lymph nodes is suggestive of the infectious nature of CPrD and concurs to hypothesize the potential impact of CPrD on animal health. No evidence is currently available with which to argue for the relevance of CPrD for human health. However, no absolute species barrier exists in prion diseases and minimizing the exposure of humans to prion-infected animal products is an essential aspect of public health protection.

The worldwide camel population is ~35 million head, 88% of which is found in Africa [4]. The camel farming system is evolving rapidly, and these animals represent vital sources of meat, milk and transportation for millions of people living in the most arid regions of the world. This makes it necessary to assess the risk for animal and human health and to develop evidence-based policies to control and limit the spread of the disease in animals, and to minimize human exposure. As a first step, the awareness of Veterinary Services about CPrD and its diagnostic capacity needs to be improved in all countries where dromedaries are part of the domestic livestock.

Since the first description of CPrD, the OIE promoted discussions on the impact of this new disease through the OIE Scientific Commission for Animal Diseases (Scientific Commission). It evaluated if CPrD should be considered an ‘emerging disease’ based on the criteria listed in the Terrestrial Animal Health Code. The OIE Scientific Commission noted that limited surveillance data were available on the prevalence of CPrD and that the evidence was not enough to measure, at that time, the impact of the disease on animal or public health. Therefore, it was concluded that, with the current knowledge, CPrD did not currently meet the criteria to be considered an emerging disease. Nonetheless, it was emphasized that CPrD should be considered as a new disease not to be overlooked and called for the collection of further scientific evidence through research and surveillance in the affected countries and in countries with dromedary camel populations to

measure the impact of the disease. As new scientific evidence becomes available, the OIE Scientific Commission will reassess whether this disease should be considered as an emerging disease.

At the regional level, CPrD was first discussed in the 18th Joint Permanent Committee of the Mediterranean Animal Health Network (REMESA) held in Cairo, Egypt, in June 2019 and at the 15th Conference of the OIE Regional Commission for the Middle East in November. During this conference, the CAMENET launched a wide-ranging proposal for training, coordinated surveillance and research on CPrD. In addition, the ERFAN (Enhancing Research for Africa Network), a platform aimed at enhancing scientific cooperation between Africa and Italy, during its 2nd ERFAN meeting for North Africa, presented a project on CPrD with the objective of increasing CPrD coordinated surveillance in North Africa.

The OIE, through its Reference Laboratories for prion diseases, and by involving the above scientific initiatives, is keeping a close watch on the evolution of the disease to gather scientific evidence and to allow a proper and more thorough assessment of the risk associated with this novel disease.

III. Case definition

Clinical criteria

The clinical manifestations of CPrD cases from Algeria included weight loss, behavioral abnormalities and neurologic symptoms, such as tremors, aggressiveness, hyper excitability, abnormal and excessive movement of the neck and head, hesitant and uncertain gait, ataxia of the hind limbs, occasional falls, and difficulty getting up as the disease progresses.

As of today, in Algeria, CPrD has been reported in animals over 9 years of age [1]. However, two CPrD-affected animals aging 3 years have been recently diagnosed from Tunisia [Agrimi, personal communication].

Therefore, animals of ≥3 years of age, with abnormal behavior and neurological symptoms, in which rabies and other diseases causing neurological symptoms have been ruled out, should be considered as clinically suspects.

Laboratory criteria

Although the vacuolation of neurons and neutrophil (spongiform degeneration) is frequently detected, it is not an obligatory neuropathologic feature of prion disease. The presence of astro-gliosis and micro-gliosis, although not specific histologic alteration to the prion diseases, are more constantly seen. The lack of a lymphocytic inflammatory response is also an important characteristic. In the CPrD cases studied to date spongiosis is faint. Therefore, CPrD diagnosis cannot rely purely on histopathology, but PrP^{Sc} detection in the brain is crucial for a proper diagnostic assessment [5]. The detection of disease specific PrP (PrP^{Sc}) is obtained by means of:

- Western blot
- Immunohistochemistry

In addition to the detection of PrP^{Sc} by immunohistochemistry (IHC) and Western blot, the so-called "rapid tests", mainly based on the ELISA technique, have been developed and extensively used in Europe for the surveillance of prion diseases in cattle and small ruminants. Rapid tests are used as screening approaches in active surveillance and usually require confirmation of positive samples by confirmatory Western blot or IHC [5].

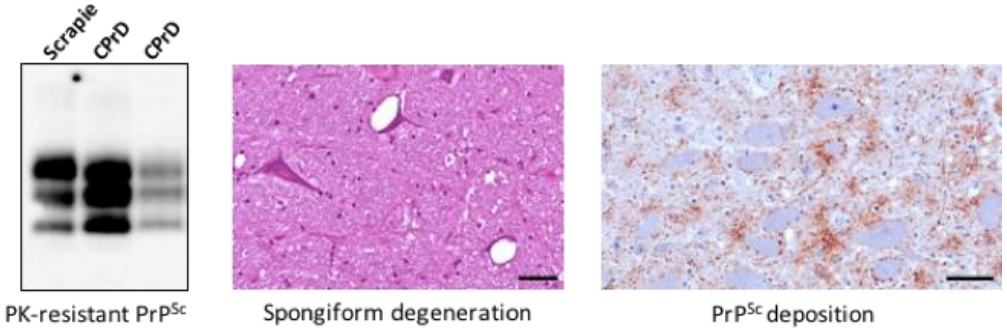

Figure 1: Western-blot, histology and immunohistochemistry on brain samples from CPrD cases (L42 MAb). A case of sheep scrapie was used for comparison in W-b.

Epidemiological criteria

Epidemiological criteria to be considered include: i) reporting in the area of neurological signs in camels for which diagnostic investigations for other diseases causing nervous symptoms were negative or inconclusive; ii) import of camels from areas where CPrD cases have been reported.

Case classification

It is still early for a formal case classification of CPrD. Nevertheless, a preliminary classification is required for the time being and can be refined gradually as knowledges progress.

1. **Possible case**: any dromedary of ≥3 years of age with one of the following clinical signs:
    a. Behavioral abnormalities including aggressiveness and tendency to kick and bite
    b. Nervous signs such as tremors and hyper excitability
    c. Abnormal and excessive movement of the neck and head, hesitant and uncertain gait, ataxia of the hind limbs.
    d. Downer camels, defined as any animal of ≥3 years of age that is recumbent, lying down on chest or side and unable to get up or stand unassisted. There are many possible reasons for an animal staying down. However, diagnostic investigations for prion diseases in downer cattle represented a crucial step of BSE surveillance in Europe. The relevance of downers animals for CPrD surveillance is not known, but it deserves to be investigated.
2. **Probable case**: any camel of ≥3 years of age, meeting the clinical criteria and with epidemiological link to a known infected area.
3. **Confirmed case**: any camel meeting the laboratory criteria for case confirmation, whether it fulfils the clinical criteria or not.

IV. Epidemiological surveillance

Different types of surveillance do exist. Regular reporting of disease cases by competent authorities is called passive surveillance. It involves passive notification by surveillance sites and

there is no active search for cases. Active Surveillance occurs when competent authorities proactively look for disease cases.

The type of surveillance for a particular disease depends on the attributes of that disease (e.g. risk for animals and humans) and the objectives of the surveillance.

Until 1999, BSE surveillance in Europe was limited to the notification of clinically suspected cases by farmers and veterinarians to the veterinary authorities (passive surveillance). However, because passive surveillance relies solely on the reporting of clinical suspects and is dependent on many factors, including perceived consequences on the farm and diagnostic competence, it is not necessarily consistent or reliable. In Europe, underreporting has been an important constraint in the passive surveillance of BSE. To optimize the identification of positive animals, improve the surveillance data and increase the consumers' confidence, those populations of cattle that were identified as at increased risk of having BSE were actively targeted within national surveillance systems. In Europe, the population of all healthy slaughtered cattle over 18 months of age were submitted, for several years, to active surveillance by diagnostic rapid tests.

Although active surveillance on healthy slaughtered animals is able to increase the sensitivity of surveillance and to provide a more complete estimate of disease frequency, it is costlier and more labor intensive. In the context of CAMENET Countries, its implementation can be possibly considered, if needed, as a further step.

In the framework of the present program, CPrD surveillance should be targeted to:

1. suspected cases found at farms, pastures or slaughterhouses
2. animals "at risk" of ≥3 years of age, such as:
    - ✓ fallen stock which have died or been killed, not in the framework of an epidemic
    - ✓ emergency slaughtered animals, downer dromedaries.

V. Biosafety

In prion-affected animals, the highest concentration of prions is found in the central nervous system (CNS), therefore caution must be exerted when handling CNS samples. In BSE-affected cattle, more than 90% prion infectivity is found in the CNS, while in scrapie and CWD, prions are spread in the cerebrospinal fluid, spleen/lymph nodes, lung, liver, kidney, placenta, etc. [6]. Preliminary results in CPrD show that, beside the CNS, prions are detected also in lymphoid tissue [1].

Depending on the country, animal prions are classified in the risk class 2 or 3, with scrapie always included in class 2 and BSE in 2/3 or 3 [6,7]. Prions are normally, not transmitted via respiratory route [7].

Risk assessment is required to work with prions and biosafety protocols need to be developed for both laboratory work and sampling activity in the field.

The main risks are wounds from cutting, inoculation or accidental ingestion. Personal protective equipment and ad hoc procedures need to be developed to minimize these risks.

Prions are resistant to chemicals and procedures traditionally used for decontaminating classical infectious agents. They are very resistant to chemical and physical agents and are very persistent in contaminated environments [7].

Therefore, working area for prions should be separated from other activities and frequently decontaminated. Cleaning and decontamination procedures of equipment and work surfaces, as well as waste management, take on strategic importance in protecting workers' health and the environment [7].

The absence of a complete and formally certifiable decontamination procedure due to the unavailability of analytical methods capable of detecting traces of the agent in the work environment, makes incineration still the safest method for the elimination of prion-contaminated material. Where possible, therefore, disposable materials should be used, disposed of by incineration. Instruments and other material should be dedicated to prions and left in the prion area

[7]. As for non-disposable materials, such as laboratory equipment, refrigerators, computers, these should be dedicated and appropriately and necessarily decontaminated through the use of NaOH, NaClO or autoclaving at high temperatures, before being disposed of. Decontamination protocols suggest using solutions of NaOH 2N or NaClO with 20.000 ppm of active chlorine for the decontamination of laboratory surfaces, instruments, etc. Alternatively, heat-resistant materials can be submitted to autoclave with gravity replacement or steam input at 134 ° C for at least 30 min [8] (Table. 1).

Equipment
1. Immerse the equipment in a solution of 1N NaOH (40 g per liter of water) or NaClO with 20,000 ppm of free chlorine for at least one hour; remove the equipment from the solution and put them in gravity replacement or steam injection autoclave at 134 °C for at least 30 min.
2. Immerse the equipment in a solution of 2N NaOH or NaClO with 20,000 ppm of free chlorine for at least one hour. Wash the equipment thoroughly in water.
Surfaces (lab benches, hoods, etc.)
Use 2 N NaOH solution (80 grams per liter of water) for at least one hour or, alternatively, NaClO solution with 20,000 ppm of free chlorine for at least one hour. It is always advisable to protect the surfaces with absorbent and waterproof material as a precaution to limit contamination.
Histological preparations
The tissues to be used for histological examination are decontaminated by immersing them in 96% formic acid for 1 h. This precaution reduces the risk of infection resulting from accidents during microtome cutting procedures.

Table 1. Decontamination procedures for instruments (in decreasing order of efficiency), surfaces and samples for histology.

VI. Capacity building

In the present context, capacity building is addressed to build and strengthen organizational and technical capacities for laboratory staff and field veterinarians, in its own role, to recognize CPrD, take part in surveillance activities and carry out laboratory diagnosis. At the same time, personnel

from Competent Authorities can acquire basic information and knowledge for the design and implementation of possible contingency plans.

Training is a crucial component of capacity building. It aims at:

- improving the capacity of field Veterinarians to identify CPrD suspect cases
- building and strengthening the capacity of laboratory diagnosis of CPrD
- providing National Veterinary Services basic knowledge for risk analysis, early warning and contingency plans development
- providing practical experience on laboratory methods for the diagnosis and investigation of CPrD

Training programs can be done in-country or in a reference laboratory such as ISS and/or IZSPLVD. Training consists of two components:

Training programmes will include:

1) Courses for field veterinarians, laboratory staff and veterinary services personnel
    a) Prion diseases of humans and animals
        - Basic concepts on prions and prion diseases of humans and animals: nature of the causative agents, pathogenesis, risk for humans and animals
        - Epidemiology and surveillance of animal prion diseases
        - Diagnosis of animal prion diseases
        - Biosafety guidelines to protect both the personnel and the environment (biocontainment)
    b) Camel Prion Disease
        - Clinical diagnosis of CPrD and recognition of suspected cases
        - Surveillance
        - Sampling at post-mortem in slaughterhouses, incinerator facility, farm or other collection site, storage and transport to the lab
        - Data collection by using standardized forms
        - Audiovisual aids for CPrD
        - Communication protocols

2) Courses for laboratory staff

- Laboratory methods and techniques for CPrD diagnosis and research (OIE-approved methods, rapid tests and others)
- Characterization of prion strains: laboratory techniques and interpretation of results
- Analysis of the PrP gene (PRNP)* [1,9]
- Laboratory equipment
- Biosafety and biocontainment procedures under laboratory conditions

VII. Early warning and response

Due to its geographical location, the Middle East is under risk of transboundary animal diseases from Africa and Asia. No information is available on CPrD in the region but the presence of camels and their import from neighboring countries suggest extending the knowledge on CPrD spread also in this geographic area.

Based on the current limited knowledge, CPrD does not meet the OIE criteria to be considered an emerging disease. However, the suspect of its transmissibility under field conditions makes emergency preparedness and contingency planning important tools for its control, in case CPrD is found.

In the present context, only the basic principles of early warning and response planning for CPrD are provided.

Emergency preparedness planning for emergency diseases introduction is comprised of two main components: 1) early warning and 2) early response [10].

* Sequence analyses in prion diseases cannot be applied for diagnosis since prions are devoid of nucleic acid. However, PrP sequence analysis is important because in sheep, goat and deer, PRNP polymorphisms have a strong influence on prion susceptibility/resistance.

1. Early warning.

   It includes all actions (disease surveillance, reporting and epidemiological analysis) aimed at the rapid detection and assessment of the introduction of the disease. In particular, an effective early warning system should comprise:

   - updated diagnostic capacities
   - effective surveillance systems
   - access to and analysis of real-time data
   - efficient epidemiological support
   - efficient and multidirectional reporting systems
   - suitable and efficient organization of the various components of the system

2. Early response.

   It comprises the effective and rapid implementation of all measures needed to contain the outbreak and to eliminate it progressively. This goal is achieved through the development of national contingency plans. These include:

   - national coordination - Strategical operation plan with an effective control center, well identified key roles and efficient/effective communication
   - risk assessment - assessing the risk associated with importation and spread of the disease
   - efficient access to and analysis of continuously updated data
   - communicating with veterinary and food sector services as well as with public health sector (if public health issues arise)
   - when capacity building is not available at national level, a regional capacity-sharing system should be established
   - animal health management and public health management (if needed) - Involvement and participation of all stakeholders, including breeders' associations. Information and education are crucial

- public information and the media - Providing a transparent information and establishing a strong emergency response system is key in improving the confidence and reassurance of public
- rapid and efficient communicating with OIE and international organizations also for possible foreign technical and economic support.

VIII. Risk Factors

Prion diseases present in animals with different origins:

- as putatively spontaneous diseases, such as atypical/Nor98 scrapie of sheep and goats and in atypical BSE (in its L-type and H-type forms)
- as infectious, but not contagious diseases, such as classical BSE, where the disease is only transmitted via infected feedstuff and cattle behave as dead-end hosts being not able to transmit the disease to healthy animals
- as infectious and contagious disease, such as scrapie or CWD, where the disease spreads from infected to healthy animals in the flock.

Our limited knowledge on CPrD prevents to definitely classify this new disease into the previous categories. However, the involvement of lymphoid tissue observed in the first cases is suggestive of the infectious and contagious nature of the disease.

No information is available about possible relationship between CPrD and other animal prion diseases. Although the origin of CPrD from another prion disease cannot be ruled out, as of today, the presence of other prion diseases is not considered a risk factor for CPrD.

No information is available about the distribution of infectivity in tissues of CPrD-affected animals. Although the detection of PrPSc in the nervous system and lymphoid tissue is reminiscent of what is usually seen in sheep scrapie, no conclusion can be drown at this stage about the risk of CPrD-affected animals.

Given the limitless of current knowledge, the following list of risk factors for CPrD should be only regarded as indicative and needed to be updated and refined as knowledges progress.

1. Potential import risk factors
    a. Import of dromedaries from infected areas.
    b. Incursion of free-ranging infected dromedaries through permeable country borders.
    c. Import of dromedaries' products, included meat and bone meal produced with dromedaries' offals, from infected areas.
    d. Potential incursion of disease by importation of contaminated camels feed with meat and bone meal produced with dromedaries' offals.
2. Potential risk factors within the country
    e. Presence of camels (susceptible animals).
    f. Absence of an effective surveillance system for prion diseases
    g. Type of production system:
        - ✓ extensive breeding systems with different dromedary herds sharing common pastures and limited effectiveness of surveillance
        - ✓ nomadic movements of animals along distances with limited effectiveness of surveillance
        - ✓ intensive breeding system with the use of processed camel proteins in feedstuff
    h. Processing of camel's offal for the production of animal by products
    i. Absence of animal identification and traceability systems.

Potential risk factors for humans

To date, no information is available on the risk of CPrD for humans.

Given the limited knowledge of the molecular basis of the "barrier" existing in the transmission of prion diseases between different species, measures have been adopted in Europe to minimize the exposure of humans to any prion diseases.

Therefore, the consumption of central nervous system and lymphoid tissue from CPrD-infected camels should be avoided as precautionary measure.

IX. Knowledge gaps

Multiple areas of understanding and knowledge of CPrD need to be investigated to fill the many existing gaps. Among the others:

- Origin of CPrD - Is CPrD a newly emerged disease or it is a long existing but unrecognized disease of dromedaries?
- Geographic distribution of CPrD.
- CPrD strain characterization - Does CPrD have any relationship with other animal prion diseases? Similarities and differences with other animal prion strains.
- Is CPrD sporadic or infectious? This is of relevance for animal health and strongly affects the control measures.
- Pathogenesis and prion/infectivity distribution in dromedary tissues - This is of relevance for animal and human risk.
- Risk for other camelids and other animal species
- Risk for humans
- CPrD epidemiology and risk factors
- Others

X. References

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