

PRIONS AND ANIMAL TRANSMISSIBLE SPONGIFORM ENCEPHALOPATHIES

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Received 02 March 2017; Accepted 11 April 2017

Published online: 21 April 2017

Abstract

Background. Transmissible spongiform encephalopathies (TSEs) or prion diseases are a unique group of neurodegenerative diseases of animals and humans, which always have a fatal outcome and are transmissible among animals of the same or different species.

Scope and Approach. The aim of this work is to review some recent data about animal TSEs, with the emphasis on their causative agents and zoonotic potential, and to discuss why the surveillance and control measures over animal TSEs should remain in force.

Key Findings and Conclusions. We still have incomplete knowledge of prions and prion diseases. Scrapie has been present for a very long time and controlled with varied success. Bovine spongiform encephalopathy (BSE) emerged unnoticed, and spread within a few years to epidemic proportions, entailing enormous economic consequences and public concerns. Currently, the classical BSE epidemic is under control, but atypical cases do, and probably will, persist in bovine populations. The Chronic Wasting Disease (CWD) of the cervids has been spreading in North America and has recently been detected in Europe. Preventive measures for the control of classical BSE remain in force, including the feed ban and removal of specified risk materials. However, active BSE surveillance has considerably decreased. In the absence of such preventive and control measures, atypical BSE cases in healthy slaughtered bovines might persist in the human food chain, and BSE prions might resurface. Moreover, other prion strains might emerge and spread undetected if the appropriate preventive and surveillance measures were to cease, leaving behind inestimable consequences.

Key Words: BSE, CWD, scrapie, TSE, zoonotic potential

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INTRODUCTION

Transmissible spongiform encephalopathies (TSEs) or prion diseases are a unique group of neurodegenerative diseases of animals and humans with an invariably fatal outcome. Prions (PrP^{Sc}), unconventional infectious agents that do not contain nucleic acids, are responsible for these diseases (Prusiner, 1982). Prion diseases can develop spontaneously as a sporadic disease, as an inherited disease or by infection. In the case of classical bovine spongiform encephalopathy (BSE), transmission by the oral route with ingestion of contaminated meat and bone meal (MBM) was essential for the spread of the disease. In any case, prions become transmissible to other hosts of the same or several other species, where they recommence their pathogenic activity and develop a clinical disease. Several known TSEs in animals include scrapie in sheep and goats, BSE in cattle and in certain captive wild bovines, chronic wasting disease (CWD) in farmed and free-range cervids, transmissible mink encephalopathy (TME) in farmed mink, and feline spongiform encephalopathy (FSE) in domestic cats and large wild cats in zoo gardens. Active screening for TSEs in bovine, ovine and caprine populations revealed new forms of animal TSEs: two atypical BSE types in cattle, L-type (low molecular weight) and H-type (high molecular weight) BSE, and atypical scrapie in sheep and goats (OIE, 2016a; OIE, 2016b). The aim of this work is to review our current knowledge of animal TSEs, focusing on causative agents and zoonotic potential of scrapie, BSE and CWD, and to substantiate that, although classical BSE is successfully controlled, the current surveillance and control measures for animal TSEs are still necessary (EFSA, 2016).

CELLULAR PRION PROTEINS (PrP^C) AND INFECTIOUS PRIONS (PrP^{Sc})

Prion proteins (PrP^C) are normal cellular proteins found in humans (Kuczius et al., 2011), animals (Kuczius & Groschup, 2013) and yeasts (Alberti et al., 2009; Li et al., 2004). Proteins with similar characteristics were described in plants (Chakrabortee et al., 2016), and most recently found in bacteria (Yuan & Hohschild, 2017). In animal cells, PrP^C is encoded by *Prnp* gene. PrP^C is highly expressed in the brain, eye, placenta, pregnant uterus, testis and the lymphoreticular system, but its physiological role is still obscure, and no widely accepted opinion on its function has been accepted (Bakkebo et al., 2015; Watts & Westaway, 2007). It may have a role in copper regulation, in signal transduction, in immune system and/or protection from programmed cell death; it probably has a role in the functions of the central nervous system, might have a potential role in cancer development and progression, and could have a role in development of Alzheimer's disease (Atkinson et al., 2016).

Prions (PrP^{Sc}), on the other hand, are misfolded cellular prion proteins (PrP^C) encoded by the same gene and having the same amino acid sequence as PrP^C; however, they obtain different spatial conformation, which causes different biochemical and biophysical characteristics and pathological behaviour. According to the protein-only hypothesis, PrP^{Sc} is the only component of the infectious particle found in TSEs and replicates

by recruiting molecules of PrP^c and templating their conversion into additional PrP^{Sc} molecules (Prusiner, 1982). Prions are capable of self-replication and turning all available PrP^c molecules into a pathogenic PrP^{Sc} form. Studies of prion diseases have many limitations; they are time consuming, difficult and expensive. However, one of the more recently developed methods, protein misfolding cyclic amplification (PMCA), enabled *in vitro* studies, as it is possible to mimic prion replication in the test tube (Morales et al., 2012). PMCA is able to detect very small amounts of infectious prions, the equivalent of just a single molecule, and then propagate prions that maintain high infectivity, strain properties and species specificity. At present, this method is mostly used in research, but it could be important, for instance, for the diagnosis of human and veterinary prion diseases, and for detection of prions on instruments or in the environment. Intracellular mechanisms involved in neuronal degeneration are not completely known, but they include accumulation of insoluble proteinase-K resistant aggregates and fibrils of PrP^{Sc} within neurons, vacuolation in neuronal perikarya and grey matter neuropil, synaptic alterations and finally neuronal death and neuronal loss (Soto & Satani, 2011). Prions can also form deposits around neuronal bodies and processes, in glial cells and around blood vessels, while amyloid plaques are rare in animal TSEs. Location, pattern and quantity of prions within different anatomical locations of the brain, other parts of the nervous system, in lymphatic organs and other tissues can differ widely according to the animal species, *Prnp* genotype, strain of the agent, animal age and clinical stage of the disease (Spiropoulos & Simmons, 2017; Langeveld et al., 2016; Masujin et al., 2016; Konold et al., 2012; González et al., 2010).

PRION STRAINS AND TRANSMISSIBILITY

As there are differences in amino acids in the PrP^c among animal species, there are also differences in the strains of prions among and within animal species (Kupfer et al., 2007; Morales et al., 2007). We recognise several strains of ovine classical scrapie, atypical scrapie in sheep and goats (Fast & Groschup, 2013), the classical form of BSE and two atypical BSE strains, H-type and L-type (Langeveld et al., 2011), and several strains of CWD (Benestad et al., 2016; Velásquez et al., 2015), all with distinct biochemical and clinical differences. Prion strains differ in many aspects: by their biochemical profiles and characteristics, like differences in band pattern in electrophoresis, resistance to proteinase-K digestion and altered amino-terminal proteinase-K cleavage sites, also resulting in different antibody binding patterns. Additionally, they differ regarding their efficiency of transmission, differences in species barrier, the length of incubation time, pattern and intensity of brain lesions and clinical symptoms, different immune response, and stability (Solforosi et al., 2013; Šnajder et al., 2012; Hamir et al., 2011; Černilec et al., 2007; Telling, 2004). In sheep, for instance, we recognise at least ten ovine scrapie strains (Moore et al., 2016; Benestad et al., 2008; Morales et al., 2007; Bossers et al., 2000). Barria et al. (2014) described the species barrier as difficulty in transmitting a prion disease from one species to another in a primary transmission. In other words, this means restriction in the capability of the agent to adapt to the

second species. It is assumed that prion strain specificity is determined at the level of protein conformation and tertiary structure of prions (Morales et al., 2007; Tanaka et al., 2004; Peretz et al., 2002). Inefficient transmission between animals is, in some cases, due to different *Prnp* genotypes, which can influence the susceptibility within a species, as proved for ovine and less so for caprine scrapie (Zabavnik Piano et al., 2016; Bucalossi et al., 2011; González et al., 2010). Transgenic mouse models are frequently used in transmissibility studies and studies of biology of prions to override species barriers (Barria et al., 2014). In addition to species barrier, many other factors can influence successful infection with prions, e.g. the age of the animal at exposure, strain and amount of the agent, genetic susceptibility, the potential of other concomitant diseases, and other probable factors.

SCRAPIE

Classical scrapie was first described centuries ago (Schneider et al., 2008). Its spread is global, with the exception of Australia and New Zealand (OIE, 2016b), and it is quite common in European countries (EFSA, 2016). It affects sheep and goats (OIE, 2016b; Fast & Groschup, 2013), and six cases were reported in moufflons (Wood et al, 1992). The biology of ovine scrapie is closely associated with *Prnp* genotype (Hunter, 2007). Atypical scrapie was detected for the first time in Norway in 1998 (Nor98) (Benestad et al., 2003) and later in many other countries after introduction of more sensitive diagnostic methods (EFSA, 2016; Juntas et al., 2010; Fediaevsky et al., 2008). Atypical scrapie affects genotypes that are usually more resistant to classical scrapie. Between 2002 and 2015, around 8.4 million sheep and goats were tested for TSEs in the European Union (EU) as part of surveillance according to Regulation (EC) No 999/2001 (EFSA, 2016). In 2015, for instance, 319,638 sheep and 135,857 goats were tested in the EU Member States, and 641 ovine and 1,052 goat scrapie cases were reported. In addition, 40 scrapie cases in sheep were reported by two non-member states: Iceland 30 cases and Norway 10 cases. Of the 641 scrapie cases in sheep, 124 were atypical scrapie (19.4 %), 498 were classical scrapie (77.7 %) and 19 were of unknown type (2.9 %). In goats, only 14 cases (1.3 %) were declared atypical. It is evident from this European Food Safety Authority (EFSA) report that some EU Member States detected only classical scrapie cases, and others detected classical and atypical or only atypical cases. In Slovenia, for example, goats with classical scrapie were found in one mixed flock of sheep and goats in 2005, during scrapie eradication (Zabavnik Piano et al., 2011), and one case of goat atypical scrapie was found in 2015, during routine testing of dead animals with post-mortem rapid tests. Some EU countries, for instance, France, Germany, Ireland, The Netherlands, Slovenia, and the United Kingdom (UK) (Melchior et al., 2010; Gorjanc et al., 2008; Zabavnik Piano et al., 2004), are using breeding programmes based on selection of breeding animals with resistant *Prnp* genotypes to reduce classical scrapie, and have been successful with these programmes, whilst in other countries, e.g. Greece, Italy and Romania, the number of classical scrapie cases persists over time. At the EU level, the resistant genotype group

accounts for 65 % to 75 % of the total number of sheep genotyped over the past five years, whereas the susceptible group accounts for less than 20 %, after excluding Cyprus. In the context of individual countries, these ratios vary significantly (EFSA, 2016).

BOVINE SPONGIFORM ENCEPHALOPATHY (BSE)

The first BSE case was reported in the UK in 1987 (Wells *et al.*, 1987). Since then, 190,608 BSE cases have been confirmed in Europe. The majority of BSE cases were diagnosed in the UK, 184,627 in total, and of those, 3,872 since 2001 (OIE, 2017a; OIE, 2017b) after the introduction of active surveillance with rapid post-mortem tests according to Regulation (EC) 999/2001. Active surveillance commenced in 2001 in most European countries, and approximately 114 million cattle were BSE-tested in the EU between 2001 and 2015 (EFSA, 2016); as from 2001, 4,204 cases were identified in the other European countries, excluding the UK. Only a small number of BSE cases were found in the Southeast European countries, most of them in Slovenia, where eight classical BSE cases and one atypical H-type BSE were diagnosed. The first classical BSE case in Slovenia was found in 2001 (Juntas *et al.*, 2002), the last one in 2007. In 2015, one atypical BSE case was found during routine post-mortem rapid testing. Beside Slovenia, Greece, with one classical BSE case found in 2001, and Romania, with two cases in 2014, one classical BSE and one atypical L-type BSE, were the only countries with positive cases in this region. The number of BSE cases diagnosed per year shows clear decline in the number of cases over time, and only seven cases were detected in Europe in the past two years (OIE, 2017a; OIE, 2017b). Six cases were detected in 2015 and one in 2016: Ireland, Slovenia and Spain had one case each, the UK had two cases, Norway had one case and one case was found in France. Two 2015 cases (Ireland and the UK) and a 2016 case in France were classical BSE, and the remaining were atypical BSE cases (three H-type, and a single L-type) (OIE 2017a; OIE 2017b). All the cases originated from fallen stock, i.e. animals found dead or killed on farm. Atypical BSE cases are currently considered a spontaneous disease randomly occurring in bovine populations and in a low number, similarly to the sporadic CJD (sCJD) in humans. BSE-typing of archived and currently diagnosed BSE cases showed that some BSE cases belonged to these atypical forms, and 100 of the 3,540 BSE cases diagnosed between 2003 and 2015 have now been confirmed as atypical BSE cases (45 H-type and 55 L-type) (EFSA, 2016). Classical BSE cases detected between 2015 and 2016 gave rise to concern as to their origin, as they occurred in younger animals born in 2009, 2010 and 2011, long after the feed ban enforcement of 2001. Detection of such cases may constitute an early warning of a potential re-emergence of a BSE epidemic or persistence of BSE in cattle population. A recent study by Masujin *et al.* (2016) further showed that the H-type BSE can transform into a novel type of BSE (BSE-SW) through serial subpassages in bovinised transgenic mice (TgBoPrP). This different type has a shorter incubation period and different phenotype than the original H-type, and a similar biochemical profile as classical BSE.

Results of this Japanese study suggest that intraspecies transmission of H-type BSE in cattle allows the emergence of a novel BSE strain.

CHRONIC WASTING DISEASE (CWD)

This transmissible disease of captive and free ranging cervids was recognised in North America in 1960 in captive mule deer in Colorado, but was first described as a spongiform encephalopathy 20 years later by Williams & Young (1980). CWD affects several species of cervids including mule deer (*Odocoileus hemionus*), white-tailed deer (*Odocoileus virginianus*), elk (*Cervus elaphus nelsoni*), and a subspecies of moose (*Alces alces sibirasi*) (Haley and Hoover, 2015). Since early cases detected in Wyoming, CWD spread to over 20 states within the United States of America, some provinces in Canada, and was transferred to the Republic of Korea with infected animals imported from Canada (Saunders et al., 2012; Kim et al., 2005). In 2016, CWD was detected for the first time in Europe, in Norway, being found in moose (*Alces alces*) and reindeer (*Rangifer tarandustarandus*). The origin of these cases is unknown (Benestad et al., 2016; VKM Report, 2016).

In contrast to BSE, CWD prions are not only transmissible, but also infectious. Prions are present in the nervous system (Williams, 2005), lymphatic tissues (Race et al., 2007), faeces (Tamgüney et al., 2009), blood, saliva and urine (Haley et al., 2009; Mathiason et al., 2006), antler velvet and skin (Angers et al., 2009), in placenta, amniotic fluids and milk (Nalls et al., 2013), and in skeletal muscles (Angers et al., 2006). They can survive in soil for a very long time and remain infectious (Kuznetsova et al., 2014; Gough & Maddison, 2010). Decomposing carcasses of animals diseased with CWD severely contaminate the soil and are a source of new infections. Prions were even found in the water from CWD endemic areas (Nichols et al., 2009). Testing of cervids for CWD is not obligatory in the EU, and for this reason, only small numbers of animals were examined for CWD on a voluntary basis. Pilot surveillance was conducted in the EU between 2005 and 2007, and no positive cases were found. Upon detection of CWD in Norway in 2016, surveillance for CWD is now planned for the following three years in Norway, Sweden, Finland, Iceland, Estonia, Latvia, Lithuania and Poland, i.e. the countries with reindeer and/or moose populations. As recommended by EFSA, selection of animal species for testing was extended to seven wild, semi-domesticated, and farmed cervid species (Eurasian tundra reindeer, Finnish (Eurasian) forest reindeer, moose, roe deer, white-tailed deer, red deer, and fallow deer) (EFSA, 2017).

ZOONOTIC POTENTIAL OF ANIMAL TSEs

The variant Creutzfeldt-Jakob disease (vCJD), described for the first time in the UK by Will et al. in 1996, is the only form of human prion diseases which is linked with the animal TSEs (Will et al., 1996). It was linked to the BSE agent indirectly by epidemiological, neuropathological and biochemical analyses, which showed that both the diseases were caused by the same strain of prion. vCJD engendered numerous

speculations about the impact of BSE on human health, provoked further studies, and strengthened the causative link between the two diseases (Diack *et al.*, 2014). Studies raised additional concerns due to the fact that many persons were infected with BSE agents during the years of BSE epidemic and harboured prions in their lymphatic systems, as discovered in retrospective studies of human appendices and tonsils in the UK (Gill *et al.*, 2013; Hilton *et al.*, 2002), and further concerns were raised by the evidence that prions can be transmitted by blood (Andréoletti *et al.*, 2012). Over the years it seemed that only individuals homozygous for methionine at codon 129 of PrP (129MM) would develop disease after infection with bovine prions, but that belief changed very recently, when a patient with the *Prnp* genotype 129 methionine/valine (129MV) died of vCJD (Mok *et al.*, 2017). This patient, with a more common *Prnp* genotype, showed that many more persons might develop the disease.

BSE is the only animal TSE that is currently considered a zoonotic disease (OIE, 2016a). The concerns about the zoonotic potential of animal TSEs (BSE, scrapie, CWD, TME) had been raised in the past, and EFSA prepared a Scientific Opinion on these topics (EFSA, 2011). This EFSA opinion states that at present there are no epidemiological evidences that classical scrapie is zoonotic, and that there are not enough epidemiological data to conclude that atypical scrapie has zoonotic potential. However, it also states that transmission experiments to human PrP transgenic mice or primates suggest that some TSE agents other than classical BSE in cattle, including L-type atypical BSE, classical BSE in sheep, TME and CWD agents, might have a zoonotic potential that appears similar to, or even higher than, that of the classical BSE agent. In 2014, a group of researchers published an article with evidences of the zoonotic potential of ovine scrapie prions (Cassard *et al.*, 2014). This group showed that a panel of sheep scrapie prions, when transmitted to several transgenic mice genetically modified to overexpress the human prion protein (tgHu), showed transmissibility comparable to that of BSE. They also showed that the serial transmission of different scrapie isolates in these mice led to the propagation of prions that were phenotypically identical to those causing sCJD in humans. Their conclusions opened up new questions about the possible link between animal and human prions, but they also concluded that even if the zoonotic potential of scrapie were confirmed, it did not constitute a new major public health risk. EFSA reacted with a new Scientific Opinion (EFSA, 2015), where they deduced that the results of that study were based on experimental transmission and did not provide comparable results under field conditions. However, Comoy *et al.* (2015), the same year, published a study reporting the direct transmission of a natural classical scrapie isolate to cynomolgus macaque after a 10-year silent incubation period and with features similar to those reported for human cases of sporadic CJD. Their study showed that scrapie was actually transmissible to primates with incubation periods compatible with their life expectancy.

Another question that became acute recently concerns CWD prions, which are spreading among cervids. In a recently published EFSA Scientific Opinion (EFSA, 2017), the authors summarised that the human species barrier for CWD prions did

not appear to be absolute. Prions are present in the skeletal muscles and other edible tissues, and humans can consume infected material in areas where CWD is present. So far, epidemiological studies have proved no association between the occurrence of sporadic CJD in humans and exposure to CWD, though some experimental studies have given contradictory results (Wilson et al., 2012; Béringue et al., 2008).

An important issue was emphasised by Morales et al. in their work published in 2007. They wrote that the dynamic nature and interrelations between strains, and the potential for the generation of a large number of new prion strains created a perfect recipe for the emergence of extremely dangerous new infectious agents (Morales et al., 2007). With that in mind, and because of the characteristics of prions and prion diseases and all the unknowns about them, the current surveillance and control measures should remain in force to protect animal and human health. Many important BSE-preventive measures, including the feed ban and removal of specified risk materials, still apply (Regulation (EC) No 999/2001), although some measures were lifted when the BSE epidemic subsided and, with few exceptions, active BSE surveillance is now conducted in the risk group animals only, including fallen stock, emergency slaughter and sick-ante-mortem bovines. These complex preventive and control measures are indispensable as they are precluding the (classical or atypical) BSE cases in healthy slaughtered bovines from entering the human food chain, and consequently, the BSE prions from recirculation.

CONCLUSION

Scrapie has been present for a very long time, and controlled with varied success. BSE emerged unnoticed and spread in a few years to epidemic proportions, entailing enormous economic consequences and public concerns. Currently, the classical BSE epidemic is under control, based on effective preventive and control measures, whilst the atypical BSE cases do, and probably will, persist in bovine population. CWD has been spreading in North America and has recently been found in Europe. Some other prion strains could emerge and spread undetected and, for this reason, prevention and surveillance measures are still indispensable for precluding the inestimable consequences.

Acknowledgements

This work was supported by the Slovenian Research Agency (Research group P4-0092; Animal Health, Environment and Food Safety) and the Administration of the Republic of Slovenia for Food Safety, Veterinary Sector and Plant Protection (AFSVSPP). We thank Ms. Tatjana Šterk for proofreading the English text.

Authors' contributions

PJ has made substantial contribution to the conception and design, acquisition and interpretation of data, drafted the manuscript and prepared final version for publication.

JZP has made critical revise of the concept, has given substantial contribution to analysis and interpretation, and been involved in drafting the manuscript and revising it critically.

IA has made substantial contribution by critically revising the content and data included in the manuscript, and has been involved in drafting the manuscript.

All authors read and approved the final manuscript, and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy and integrity of any part of the work are appropriately investigated and resolved.

Declaration of conflicting interests

Hereby we disclose any financial and personal relationships with other people or organisations that could inappropriately influence our work.

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PRIONI I TRANSMISIVNE SPONGIFORMNE ENCEFALOPATIJE ŽIVOTINJA

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Kratak sadržaj

Uvod. Transmisivne spongiformne encefalopatije (TSE) ili prionske bolesti su jedinstvena grupa neurodegenerativnih bolesti životinja i ljudi, koje uvek imaju fatalan ishod i mogu se prenijeti među životinjama iste ili različitih vrsta.

Cilj i pristup. Cilj ovog rada je razmatranje nekih novijih podataka o TSE kod životinja, sa naglaskom na uzročnike i njihov zoonotski potencijal, kao i da se raspravi o razlozima zbog kojih mere nadzora i kontrole nad životinjskim TSE treba da ostanu na snazi.

Ključni nalazi i zaključak. Još uvek imamo nepotpuno znanje o prionima i prionskim bolestima. Skrepi je prisutan veoma dugo i kontroliše se sa različitim uspehom. Pojava spongiformne encefalopatije goveda (BSE) je počela nezapaženo, a onda se za nekoliko godina proširila do obima epidemije sa ogromnim ekonomskim posledicama i javnim problemima. Sada je epidemija klasičnog oblika BSE pod kontrolom, ali atipični slučajevi su još uvek, i biće i nadalje, prisutni u populaciji goveda. Kod jelena u Severnoj Americi širi se hronična bolest mršavljenja (CWD), a nedavno je pronađena i u Evropi. Preventivne mere za kontrolu klasičnog oblika BSE, kao što su mere zabrane određene hrane i uklanjanje specifičnih rizičnih materijala još su uvek na snazi, međutim, aktivni nadzor za BSE se znatno smanjio. Bez ovih preventivnih i kontrolnih mera atipični BSE slučajevi bi kod zdravih zaklanih goveda ostali u lancu ljudske ishrane, a to bi značilo da bi BSE prioni mogli ponovo da cirkulišu. Mogli bi se pojaviti i neki drugi sojevi priona i proširiti neprimetno, a u slučaju, da se preventivne mere i nadzor ne bi više sprovodili, posledice bi bile nepredvidive.

Ključne reči: BSE, CWD, skrepi, TSE, zoonotski potencijal