Final Progress Report

2020 Creutzfeldt-Jakob Disease Foundation Research Grants

Title: Exploring the zoonotic potential of porcine-derived materials from animals exposed to infectious prions.

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The responsible agent in prion diseases is an unusual transmissible pathogen known as prion, which is considerably smaller and simpler than other infectious agents such as viruses or bacteria. Prions impair memory, motor functions and behavior, and cause a progressive degeneration of the central nervous system that invariably leads to death.

In 1986, a devastating prion disease known as bovine spongiform encephalopathy (BSE) or "mad cow disease" was reported. This disease importantly impacted the cattle industry due to its proven zoonotic potential. Considering this, animal prion diseases such as scrapie (affecting sheep and goats) and chronic wasting disease (CWD, affecting cervids) are treated with caution and contaminated meats derived from prion infected animals are removed from the human food chain.

Among animal prion diseases, CWD is of particular concern. This animal prion disease has been increasingly expanding in North America and has been recently identified in Northern Europe. CWD is extremely problematic as it affects not only captive deer populations but also wild animals. This has resulted in relevant economic costs to the deer and hunting industries, and several efforts from federal and state agencies have been implemented to control and eradicate its massive spreading. An additional concern for CWD includes the interaction of infected animals and derived-materials (e.g. meats and carcasses) with humans. Hunters, veterinarians, taxidermists and the general public eating CWD-contaminated meats are exposed to this particular prion agent. CWD also interact with environmental components, as well as with animals and parasites that cohabit with infected cervids.

Another relevant animal prion disease is scrapie afflicting sheep and goats. Scrapie was the first prion disease ever described. As other animal prion diseases, scrapie has generated significant losses in livestock production. Importantly, several researchers suggest this disease as the origin of BSE. In turn, experimental evidence demonstrate that sheep and goats are susceptible to the infamous BSE agent. Worrisomely, sheep-adapted BSE prions induce disease in rodent models of human prion diseases demonstrating their zoonotic potential. However, it is very important to note that there is no evidence that sheep are naturally affected by BSE. Along the same line, no cases of humans affected by scrapie or CWD have ever been reported; however, the zoonotic potential of these animal prionopathies is still questioned.

It is important to note that the transmission of prions between different animal species (inter-species transmission) can be risky and alarming due to the potential generation of new prion variants that could be transmissible to humans. For that reason, several research groups have performed inter-species prion

transmission studies on domestic swine. This has been done mostly by two reasons: i) pigs share habitats with prion susceptible animals in farm and wild settings, and ii) pigs are a common source of human food. Even though no evidence describing naturally occurring prion disease in pigs exists, these animals are susceptible to BSE, scrapie, and CWD under experimental conditions. This brings up the question: can prions from deer, sheep or cattle induce disease in pigs? If so, are the newly generated pig prions transmissible to humans? Answering these questions is highly relevant for three reasons: first, domestic pigs are an important contributor in the human food chain; second, they are exposed to farmed prion-infected ruminants (e.g., cows, deer, sheep); and third, feral swine cohabit with animals exposed to CWD in wild settings.

The objective of this study was to detect disease-associated prions in the brains and muscles (meat) collected from domestic pigs exposed to scrapie, CWD and BSE prions. This was achieved using an ultrasensitive technique named protein misfolded cyclic amplification (PMCA) that amplifies very low quantities of prion particles contained in a given sample. The relevance of this project was to examine prion infectivity in pork meat cuts that are commercially available and commonly used to cook. In addition, to study whether pig-derived prions have the potential to infect humans, we are using the same PMCA technique with settings that models inter-species transmissions in humans.

Our findings showed that brains from pigs experimentally exposed to BSE, scrapie and CWD contain infectious prions. These results confirmed previous reports demonstrating that domestic pigs are susceptible to a variety of animal prionopathies. We also demonstrate that prions in the brain of these animals can be replicated using the PMCA technology. Moreover, we observed that brains from BSE-infected pigs carry higher levels of prions than those of pigs exposed to either scrapie or CWD. However, these conclusions need to be considered with caution as BSE-infected pigs were exposed to higher quantities of prions compared to their scrapie and CWD counterparts. Regardless, this data further demonstrates the susceptibility of pigs to a variety of animal prion diseases. The apparent high susceptibility of pigs to BSE goes in line with the previously reported “promiscuous” nature of cattle prions, as they have been transmitted with relative ease to several animal species. Importantly, a relevant conclusion from our research was to demonstrate that CWD, BSE and scrapie prions adapted in pigs correspond to different prion strains. Previous reports suggested that the pig prions derived from BSE, scrapie, and CWD presented shifts in their electrophoretic mobilities after proteolytic digestion as well as differences in their glycosylation profiles. We confirmed these results by showing that PMCA products also display the above-mentioned differences. Specifically, PMCA products derived from scrapie-, BSE- or CWD- pig prions manifest with specific biochemical features that included different electrophoretic mobilities and glycosylation patterns. PMCA products derived from these porcine prion strains are currently being investigated for other features including protease resistance and conformational stability.

Importantly, our results demonstrate the accumulation of disease-associated prions in pigs’ muscles, albeit in considerably lower quantities compared to those found in brains. In fact, disease-associated prions in muscles were only visualized after *in vitro* prion replication using PMCA. The specific muscle tissues analyzed in our experiments included the semitendinosus (found in the pork leg), masseter (cheek meat), psoas (tenderloin or chain), and the diaphragm (skirt meat) muscles. The semitendinosus muscle of pigs exposed to

BSE presented higher quantities and consistency (reproducibility across pigs) in prion detection compared to the masseter, psoas, and diaphragm muscle from swine exposed to scrapie and CWD. The lower quantities of prions present in muscles compared to brains were expected considering the lower expression of the endogenous prion protein in the former tissues.

We are currently executing the second part of this project, that includes testing if prions accumulated in pig meat are able to induce the misfolding of the human prion protein. This research is relevant, as it will indicate a zoonotic potential on prion exposed pigs, and suggest whether the properties of scrapie and CWD prions (both acknowledged to possess low or none zoonotic potentials) are altered after adaptation in the pig host. This study is being done by performing PMCA assays mixing brains and muscles homogenates from prion-exposed pigs with physiological human prion proteins. These results are expected to be completed in the following months and will conclude the experiments listed in this project. After completion, all data will be compiled and prepared for communication in a specialized journal. Our next steps include bioassays. Specifically, we plan to inoculate pig brain and muscle extracts in genetically modified mice expressing the human prion protein. This will allow us to validate the PMCA data and understand whether the low quantities of prions found in pork meat represent a zoonotic threat. Unfortunately, these experiments are expensive and we are currently looking for funding to execute them. On a related note, it is important to mention that we are collaborating with the USDA to study the possible transmission of CWD prions to feral swine that are in contact with infected cervids in the wild.

In conclusion, our studies contribute to the knowledge dealing with prion transmission from domestic animals to humans. This information is highly significant for the current effort that regulatory agencies are making to prevent and control prion transmissibility, and protect consumers from emergent diseases.