CIC bioGUNE
Biozientzietako Ikerkuntza Kooperatiboko Zentroa
Centro de Investigación Cooperativa en Biociencias

PRESS RELEASE

Research published in "PNAS"

Mad rabbit disease

- *The Biogune research centre has shown that rabbits, which were previously thought to be resistant to prion diseases, can also develop such infections.*

- *This research, led by Dr. Joaquín Castilla, has been published in the prestigious journal Proceedings of the National Academy of Sciences (PNAS) and has resolved a mystery that has been studied for several decades.*

- *However, the authors of this study consider that an epidemic of mad rabbits similar to that seen in cows in the 1990s is highly unlikely.*

(Bilbao, March 13th, 2012).- Rabbits have been considered to be resistant to prion disease for more than 40 years. This certainty was based on the experimental inoculations with prions from various sources to which this species was submitted, which proved to be negative. Furthermore, there is no evidence of transmissible spongiform encephalopathies in these animals in Nature, and no prion diseases have been diagnosed in any of the rabbits from those zoos that used feed contaminated with the agent known to cause this disease in cows, which is able to infect the majority of species in captivity.

All these findings appeared to confirm an exception to the rule that essentially all mammals could develop an infectious and transmissible prion disease. In other words, rabbits were safe from prion infection.

This has led dozens of research groups to study the reasons for such strange behaviour, work which has led to the publication of more than 60 scientific papers in this field.

However, research in the Prion Laboratory at the Biogune research centre, led by Dr. Joaquín Castilla and published in the specialised journal *Proceedings of the National Academy of Sciences (PNAS)* on 12th March has shown that, although rabbits are unusually resistant to prion diseases in comparison with other mammals, they can nevertheless still become infected.

In light of this new discovery, Dr. Castilla's group has decided to study whether prions can jump even higher barriers and infect birds or fishes, for example. In this sense, the Biogune team aims to untangle the molecular mechanisms that explain the different susceptibilities shown by species

towards prions in order to mimic nature and develop new therapeutic strategies to combat transmissible spongiform encephalopathies.

- **The research**

According to Joaquín Castilla, Head of Biogune's Prion Laboratory, "*we mainly focus on in vitro studies, i.e. in test tubes, where we reproduce the prion replication process that occurs in animals, but faster and more efficiently. During these studies, we attempted to determine whether the apparent resistance shown by rabbits was insurmountable or not. The results were surprising as essentially all the prions used in the test tube experiments were able to generate a rabbit prion in vitro, irrespective of their origin (cow, sheep, pig, etc.) This was the first time that a leporid (rabbit) prion had been seen*".

After the laboratory phase, Dr. Castilla's team went on to study the infectivity of the product generated *in vitro* in rabbits themselves. In accordance with previous studies over the past few decades, rabbits were found to show a strong resistance, although this resistance was not complete. Thus, almost 800 days post-inoculation, a single rabbit was found to be infected, although the remaining animals remained completely healthy after four years.

One of the main characteristics of a prion is its ability to self-replicate through its congeners, therefore the definitive proof that a rabbit prion had been obtained was achieved by inoculating it into two animal models, namely wild type rabbits and transgenic mouse, the latter of which produced a rabbit protein that was able to replicate instead of the corresponding mouse version. It took around a year for transgenic mice, and a year and a half for rabbits, to demonstrate the ability of the rabbit prion to transmit itself in the same species.

Although these findings confirm that rabbits cannot be considered to be a prion-resistant species, the long incubation times required for replication suggest that an epidemic similar to that seen in cows is highly unlikely.

- **Debate**

This study opens up the debate regarding the suitability of feeding various species with animal proteins that may be contaminated with prions, even those that for many years have been considered to be resistant. "*The ability of prions to adapt during their passage through various species suggests that any mammal is susceptible to infection and that the only safe means of preventing this is to avoid using feed that has come into contact with animal proteins"*, concludes Dr. Castilla.

❖ Supplementary information Prions

"*Prions are the pathogens responsible for TSEs, which are also known as prion diseases. TSEs belong to a group of fatal neurodegenerative diseases that affect both humans and animals and for which there is no available therapy. These diseases may be hereditary, sporadic (supposedly spontaneous) or infectious in nature",* explains Prof. Castilla.

The bovine transmissible spongiform encephalopathy epidemic in Europe, better known as "mad-cow disease", resulted in an increase in the number of research teams dealing with this kind of infectious agent. As a result, the last decade has witnessed major progress in our understanding of prions. However, there is still much to be discovered.

According to Castilla *"prions are probably one of the most intriguing pathogens in nature as their proposed single-protein composition and the appearance of clearly differentiated strains give them an unprecedented scientific value".* Their replication mechanism, which resembles that of Alzheimer's or Parkinson's disease, amongst others, makes them unique pathogens. *"In addition, as the nature of prions remains a mystery, many researchers, including those in my team, find their study completely irresistible"*, concludes Castilla

One of the greatest mysteries in the field of prion diseases, and one in which Prof. Castilla's group is particularly interested, is how a single protein is able to infect some species but not others. Although a great deal of progress has been made in this respect as a result of new techniques that have been developed recently, a great deal of work still lies ahead.

One of the techniques currently used to study prions is known as *in vitro* replication. In the 1980s and 90s, the only techniques available for the study of prions and their transmissibility were based on inoculating experimental animals with samples containing the infectious agent. Subsequently, however, other *in vitro* techniques that allowed prions to be amplified in test tubes for the first time were developed. "*Although bioassays using transgenic animals replaced many in vivo studies, much greater effort is now being directed towards in vitro studies. Indeed, we can now generate prions for different species starting from those from basically any species. The PMCA* (*Protein Misfolding Cycling Amplification) technique, which was developed by our group, allows these infectious agents to be amplified in the laboratory*", notes Castilla.

This technique is key to diagnosis and the study of the molecular mechanisms that allow prions to replicate.

Although scrapie, the fatal degenerative disease found in sheep and goats, is probably the oldest prion disease, BSE (Bovine Spongiform Encephalopathy) has attracted much more public attention due to its involvement in a new human disease and its proven transmission to the majority of other species. Likewise, Creutzfeldt-Jakob disease, which has a very low annual incidence of 1-2 cases per million inhabitants, also remains very much unknown, especially as regards those sporadic cases whose origin is still a mystery. A very uncommon situation is found in the Basque Country, which hosts a significant percentage of all global cases of fatal familial insomnia, a prion-related human disease of genetic origin.