Improvement of goat TSE discriminative diagnosis and susceptibility based assessment of BSE infectivity in goat milk and meat.

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Thematic Priority: Food quality and safety

Final activity report (publishable)

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1 This project has been initially planned as a four year project with expectation that request for extension would become necessary which was approved after reporting period 4.
Goats cleaning the grape waste from the wine production
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dear reader:
when encountering an abbreviation or term, check for explanation at the page with "ABBREVIATIONS"
PROJECT EXECUTION

FOOD-CT-2006-36353

goatBSE

Proposal for improvement of goat TSE discriminative diagnosis and susceptibility based assessment of BSE infectivity in goat milk and meat.

Project Objectives

In light of the known ability of the BSE agent to cross the animal/human species barrier, the evidence establishing the presence of BSE in goat is especially alarming, as it represents a potential risk of food-born contamination to human consumers of goat milk and meat products. The main objective has been to determine the tissue distribution of BSE after oral exposure of goats while simultaneously generating indispensable data on genetic susceptibility in the most commonly used production breeds. This proposal aims:

(i) at providing data to allow evaluation of human risk associated with BSE passaged in goat,
(ii) at providing pathogenesis data and biological material from first and second passage BSE in goats,
(iii) at evaluating the possibility of BSE self-maintenance in goats by maternal/horizontal transmission,
(iv) at validating and improving our ability to detect and discriminate caprine BSE from goat scrapie.

Our approach integrates the predicted influence of PrP gene polymorphisms on scrapie and BSE susceptibility so that it could potentially be used for the control of field TSE outbreaks in goats. We will document European field TSE strain variability in goats by recruiting a large number of TSE goat isolates from affected European countries. Already established or specifically created animals models (strain typing) and biochemical tools (PrPSc typing) are being investigated for their ability to efficiently discriminate BSE from scrapie in goats. Finally, by measuring infectivity in various tissues (including skeletal muscle) and secretions (milk), collected from goats at different stages of BSE infection, we will provide indispensable data for quantitative risk assessment.

The intended work contains five work packages which setting is shown in the pert diagram on the next page. Then, after discussing a number of scientific end results (page 7-14), the level of fullfillment of these main four project objectives will be presented on pages 15-18.
CONTRACTORS INVOLVED

This consortium consists of 10 partners from 7 EU Member states;
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END RESULTS

Projects: GoatBSE and GOAT-TSE on the internet:

www.goatbse.eu

logo of the GoatBSE project (FP6)

logo of the GOAT-TSE-FREE project (EMIDA ERA-NET)
View on some results combined from different work packages

Finding TSE-protective prion protein (PrP) alleles in goats
Data from field studies in different countries and laboratories (Italy, Greece, France) pointed out that the 222K PrP allele is a serious candidate for TSE resistance breeding. Data from challenge studies with scrapie (intracerebral) and BSE (1st and 2nd oral passage) confirm this choice, though some carriers show sub- or pre-clinical signs of disease at the end of the experiment (>1900 days post infection), while goats with the wild type, 142I/M or 211R/Q genotype nearly all went much earlier down with disease. Bioassay studies in newly generated mice transgenic for wild type, 142M or 222K goat-PrP challenged with goat scrapie, bovine BSE and goat BSE showed resistance in the 222K mice for bovine BSE and goat scrapie; however in these mice, the resistance for BSE was not absolute since goat-derived BSE (obtained by experimental infection with bovine BSE in goats) appeared well transmissible. Similarly, in vitro assays using PrP^C from either prokaryotic or eukaryotic origin as substrate indicated that goat scrapie and bovine BSE were very poor converters of normal PrP^C to the disease associated form PrP^Sc, while goat BSE was an efficient converting agent. In short, the PrP 222K allele is a powerful protective allele for scrapie and BSE, though not absolute. This imperfect situation however is also known for sheep 171R allele carriers (ARR sheep). This allele is a highly successful target in sheep TSE-resistance breeding programs (see Figure 1).

| 222K the preferred allele within protective potential candidates (142M, 154H, 211Q, 222K) |
|---|---|---|---|
| 1. in goat field studies | 2. in goat challenge studies | 3. in rodent bioassay | 4. in vitro conversion assays |
| 222K most protective | 222K orally with bovine BSE & goat BSE; i.c. with goat scrapie i.e. with BSE running till ± 2017 | 222K resistant for scrapie & bovine BSE, not for goat BSE | 222K poorly converted by scrapie & bovine BSE, converted by goat BSE |

Figure 1: The outcomes of a large part of two work packages summarized in four items has revealed that the 222K PrP allele is an outstanding candidate for TSE-resistance breeding initiatives in goats. Abbreviations i.c. = intracerebrally challenged with agent indicated. The 154H allele was only partially tested i.e. under panels 1 and 4.
In seven EU countries the GoatBSE consortium has searched widely in the goat population for the variability of the *PRNP* gene. This gene is coding for the PrP protein. This was carried out for two reasons:

1. estimating the genetic variation within the protein, and concomitant estimating the frequencies of the most interesting resistance associated variants coding for PrP with either 142M, 146S, 146D, 154H, 221Q or 222K,

2. finding a relation between disease and DNA sequence variation within the regulatory (or promotor) region of *PRNP*.

### PrP-gene analysis: frequencies of most interesting alleles related to TSE protection

With respect to genetic variation, a review was published in 2009 that reported the number of coding variants at 29, however since then and largely due to the consortium efforts this number has increased to over 40 based on more than 6000 analyses (these variants were G61S, G74D, G96D, G97D, S106R, M112T, V125I, L141F, D147N, Y153S, Q215R, Q220L). The frequencies of the most important alleles related to potential TSE resistance are displayed in figure 2, together with their occurrence in the seven countries involved. The 171R allele used for sheep TSE resistance breeding has not been encountered in goats. The 222K variant is occurring at low frequencies of 0.5-15%, and is absent in some breeds.

![Figure 2: Allele frequencies for PrP codons wt, 142M, 146S, 146D, 154H, 211Q & 222K: overall averages per country. The codon 240 polymorphism in goats is different from that in sheep: in sheep the codon is a serine (S) while in goats this can be a proline (P) or a serine (S) with a majority for P in most breeds. Therefore, for each specific allele at other codons than 240, the 240 polymorphism is specified. The bars above “rest alleles” do reflect the remainder of polymorphisms together, which can be associated with either 240P or 240S.](image-url)
Goat breeds investigated for the amino acid sequence of prion protein (PrP) and frequency of occurrence of scrapie-resistance associated variations.

Alpine (Gr, Fr, NL, Sp)  Saanen (Fr, It, Nl, Sp)  Corsica (Fr)  Skopelos (Gr)  Damascus (Cyp)

Valdostana (It)  Roccaverano (It)  Camosciata delle Alpi (It)  Rossa Mediterranea (It)  Maltese (It)

Garganica (It)  Murciano Granadina (Sp)  Retinta (Sp)  Pyrenaica (Sp)  Poitevine (Fr)

Moncaina (Sp)  Boerbok (SA, UK, NL)

**PrP-gene analysis: regulation**
Analytical results from studies of the PRNP gene regulatory region compared with those in other reports on the non-coding regions in goats and other species allowed the identification of putative transcription factor binding motifs. The preliminary results suggest that at least two transcription factors bind to sequence motifs in the caprine PRNP gene intron 1 between position 5840 and 5865. Binding of these factors could represent a novel regulatory mechanism for PRNP gene expression in tissue. Further work is required to prove this possible link.
Scrapie in small ruminants does exist already for a long time. The nature of the agent in goats was however not well studied. Initially the project considered more than sixty cases over a large geographical area of Europe, and ultimately selected twenty four of them (see Figure 3) for in-depth analyses by means of biochemical, microscopic and rodent bioassays. Some bioassays are still running. Parallel to these assays, a goat BSE brain sample was tested; this was derived from a goat experimentally infected with bovine BSE.

Four main aspects have been investigated on these sample sets (Figure 4). None of the field cases yielded a BSE suspicion. Studies by microscopy, biochemistry and bioassays have been performed. For the latter two studies brain stem materials were first centrally collected, homogenized to 50% in water and then finally distributed to partner laboratories. For microscopy, fixed tissue blocks were available.

1. Rapid testing. Three rapid EU tests approved for active surveillance performed with 100% specificity on a set of negative samples, while sensitivity for the positive sample set ranged between were respectively 76%, and 95%.

2. By microscopy, all TSE isolates behaved as classical scrapie, except one case from UK which in the brain was like CH1641, using suitable site-specific antibodies. From Italy an atypical/Nor98 case was recognized.

3. New biochemical techniques were developed to identify the PrPSc type. These techniques used the protease susceptibility of PrPSc in two approaches: A, removal of the N-terminal side of PrPSc protein, B, degradation of the whole PrPSc molecule.
Approach A was able to discriminate between classical scrapie from Italy (5 cases from Italy, 1 case from France), 1 case of CH1641-like scrapie (from UK), and classical scrapie as usual with relatively high resistance to protease digestion (remaining 17 field cases), while the parallel BSE sample was most susceptible.

In approach B however, BSE behaves as the most protease resistant PrP^Sc, while the other isolates were variable in their resistance, but this could not be related to any origin or typing parameter.

By further available biochemical parameters the TSE status of most samples could also be confirmed for the protease resistant moiety of PrP^Sc (PrP^res) prepared under standard protocols. PrP^res parameters were: molecular weight of lower band, glycoprofile, antibody binding, and indications for a dual population as in CH1641 scrapie (only UK case). BSE was excluded in any of them. One case of atypical/Nor98-like scrapie (from Italy) and one of CH1641-like scrapie (from UK) were discovered. The remainder behaved like classical scrapie.

4. By bioassay rodent lines: Atypical scrapie (only transmissible in Tg338 ovPrP_{VRQ} mice) and experimental BSE (typically transmissible in all lines, with stable features) could be recognized in each line with consistent features as expected for these two TSE types. Regarding the remaining field goat isolates in the geographical study, none of the strains isolated in rodents was similar to BSE and in all rodent models there was evidence of strain variability. Italian classical scrapie cases and one French case were detected as a homogeneous type of scrapie in different rodent models ( provisionally named: Italian scrapie). The other isolates (from Greece, Cyprus, Spain, Netherlands, France and UK) gave a divergent picture, interpreted as presence of different “sub-strain” components in some sample: i.e. in conventional mice and bank voles, these isolates resulted in classical scrapie strains previously
identified in sheep, including CH1641-like scrapie. One UK goat case with CH1641-like molecular properties - as mentioned above under point 3 approach B - was a clear example of a phenotype from which both CH1641 and classical scrapie could be isolated.

**Distribution of agent in goat lymphoid, nervous system and muscle tissues upon oral infection**

A large set of goats have been subjected to challenge through the oral route with either bovine BSE or goat BSE. In some of these goats, spreading of disease in the body was more closely followed until the end of the experiments (about 45 months post challenge). This was accomplished by two ways: by microscopy with PrP-specific antibodies, and by infectivity in the most susceptible mouse line (Tg110 mice, transgenic for bovine PrP).

By microscopy, all genotypes tested except 222Q/K were clearly positive in the brain in both 1st and 2nd passage, with nerve cells of the enteric system (ENS) also positive but the lymph nodes were usually negative or sparsely positive (Table 1).

<table>
<thead>
<tr>
<th>genotype</th>
<th>1st oral passage</th>
<th>2nd oral passage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CNS (brain)</td>
<td>ENS (gut nerves)</td>
</tr>
<tr>
<td>wt</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>142I/M</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>142M/M</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>211R/Q</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>222Q/K</td>
<td>±</td>
<td>N</td>
</tr>
</tbody>
</table>

Table 1: goats challenged with BSE from cattle or goat through the mouth exhibit positivity in their gut nerves when positive in the brain. Brain positive goats were also positive by visibly clinical signs. The involvement of lymph nodes was very limited and not always present though locally the ENS was positive. The 142M/M goats remained negative after 2nd oral passage like nearly all 222Q/K goats in both passages. Y=positive, N=negative, ± = a slight positivity only in some case(s) per group.

By infection in mice, samples collected from goats in end stage of disease or at end of the experiment showed that tissues tested (brain, muscle and popliteal lymph nodes) were positive in wt and 211R/Q genotype animals, and that both muscle and lymph node are carrying infectivity at a level 100 – 1000 times lower that in brain, which means that still a rather high infectivity level can be present in the body. The muscles tested were from the eye and thigh bone muscle, lymph nodes were from the knee joint. Thus infectivity will spread to the periphery at late stage of the infection process, and the 222Q/K genotype carrying goats are only at non-clinical stage at about 4 years after challenge positive in only the brain. Apparently, after oral infection infectivity moves from gut through body nerve cells to brain after which it can spread to muscle and lymph nodes. To find arguments whether or not these tissues are infective to humans, infection studies in transgenic mice expressing human PrP are on-going.
Production of goat milk and delivery of goat kids and infectivity testing

Oral challenge of goats with goat BSE leads to appreciable levels of infectivity in consumption related tissues of wild type and 211R/Q goats, while 222Q/K goats are protected to a high level. Experiments are still ongoing. It is not yet clear whether there is infectivity present in milk products derived from these goats (for a summarizing plan of these experiments see Figure 5).

With respect to placenta and the kids derived from infected wild type goats: timing of mating and challenge periods were well chosen since dams became clinically positive in the susceptible genotypes around weaning. By microscopy, goat kids and placentas were negative, and by infectivity tests (only placenta tested in Tg-shpIX mice). The limited number of animals involved in the experiments does not allow to definitely conclude that vertical transmission does not occur.

![WP 4: Evaluation of a potential risk of goat BSE for humans](image)

1. goat-adapted BSE transmission barrier between species including humans
2. infectivity of tissues and secretions from orally BSE infected goat in human PrP transgenic mouse models
3. abnormal PrP and infectious titre in consumable tissues and milk from orally BSE infected goats

Figure 5: Overview of research activities in Work package 4 to obtain data on risks of goat adapted BSE to human consumers.

Susceptibility of different species for goat BSE and scrapie

For BSE transmission to other species there was hardly any barrier in the studied rodent models and goat derived BSE even showed an increased transmissibility in Tg-Bov, Tg-Po and Tg-Hu mice compared to cattle BSE. In the same study, scrapie did not transmit in the presence of human (129M) or porcine PrP. These results suggest that the risk in humans for a potential goat-BSE agent should not be underestimated.
Degree objectives reached

As appeared from the above paragraphs, the objectives stated at the beginning of this report on page five have been reached in many ways by performing the different work packages.

The work will be detailed in relation to the main aims posed in the proposal DOW v41 which were:
1. providing data to allow evaluation of human risk associated with BSE passaged in goat,
2. providing pathogenesis data and biological material from first and second passage BSE in goats,
3. evaluating the possibility of BSE self-maintenance in goats by maternal/horizontal transmission,
4. validating and improving our ability to detect and discriminate caprine BSE from goat scrapie.

The degree of objectives reached is summarized at the end of this paragraph in Table 2.

1 Providing data to allow evaluation of human risk associated with BSE passaged in goat

In work packages WP1, WP2 and WP4, data have been generated to evaluate factors that can be used for human risk assessment if an eventual event of BSE in goat would occur. These data were generated by: raising goats of different prion protein (PrP) polymorphisms or genetic genotypes using pre-existing knowledge about field studies where a resistance association was found for a number of alleles. In the first two years of the project challenges in these animals could be started. In work package 1 transgenic mice were generated containing different goat PrP variants. Humanized mice were stationed at two consortium member institutions. For in vitro test-development PrPs were produced for a set of goat variants and the human 129V and 129M variants, and conditions for new types of conversions have been developed, including conversion substrates production being non-glycosylated PrPs from pro-karyotic cells and glycosylated PrPs from mammalian cells. Some of the goats were used for breeding goat kids to establish any indication of presence of infectivity in the milk and tissues generated for studies in this objective. Important conclusions could be drawn from the experiments, while testing of milk fractions is still underway (in humanized and bovinized mice).

The main achieved conclusions are:
- Transmission experiments in transgenic mice expressing goat-PrP showed that, as expected, all goat BSE isolates, in contrast to scrapie isolates tested, are well transmitted, without apparent transmission barrier. Thus, also humanized 129M Tg mice are susceptible for goat BSE. Testing of infectivity in goat mother milk samples is still on-going in humanized transgenic mice (expected to be ready around 2013/2014).
• In all transmitting goat BSE samples, the molecular signature of the PrPres generated in the mice was similar to that observed in the original inoculum.

• The in vitro tests assessing conversion efficiency associated with various caprine PrP sequences reflect the in vivo susceptibility of transgenic mice and goats (as studied in the deliverables D1 and D2 series). Goat-passaged BSE is well capable to convert all goat PrPs relatively efficiently except for the ovine 171R PrP allelic variant which only showed a very limited conversion, while conversion of the human PrP allelic variants 129M and 129V have to be repeated for a definite interpretation which is pending.

• The results suggest that the risk in humans for a potential goat-BSE agent should not be underestimated.

2 Providing pathogenesis data and biological material from first and second passage BSE in goats

In work packages WP2 and WP4, information has been generated about the spread of infectivity in goats challenged with BSE at first and second passage, either after intracerebral or oral administration. Goats from different genotypes (wt, 142I/M, 142M/M, 211R/Q and 222Q/K (only oral administrations) have been challenged. A broad set of tissues were collected such as: brain, spinal cord, extra-neuronal nerves, lymph nodes from head and gut, lymph nodes including intestinal samples, spleen, and muscles. These are available for further investigations from the partner’s laboratories in case of interest. Most interesting was that oral infection of goats with goatBSE leads to appreciable levels of infectivity in consumption related tissues (muscles), while it is not yet clear whether in milk products derived from these goats there is infectivity present. Infectivity tests on the goat tissues in the Tg mouse bioassays are still ongoing. It also appeared from the mouse studies, that infectivity will spread to the periphery at late stage of the infection process in all diseased wt, 142I/M, and 211R/Q goats; only one 222Q/K genotype carrying goat was positive only in the brain in still healthy (non-clinical) stage at 4 years after challenge. Thus, a relatively strong protection to BSE infection exists in 222Q/K goats. Furthermore apparently after oral intake, BSE infectivity moves from gut through body nerve cells to brain after which it can spread to muscle and lymph nodes.

With respect to goat scrapie: goats (genotypes: wt, 142I/M, 154R/H, 211R/Q, 211Q/Q, 222Q/K, 222K/K) were investigated after intracerebral challenge and all genotypes became positive, but none of the 222K allele carriers except one out of five 211Q/K animals; lymph node positivity (by microscopy) did appear only in the wt, 211R/Q, and 142I/M animals. Thus, only the 222K polymorphism carriers demonstrate strong resistance to intracerebral scrapie infection, though in heterozygous condition the resistance was not complete.
3 Evaluating the possibility of BSE self-maintenance in goats by maternal/horizontal transmission.

Work packages WP2 and WP4 used two different approaches to study potential transmission of BSE horizontally and maternally (vertically). With respect to horizontal transmission, during the oral and intracerebral BSE challenges -either 1st or 2nd passage- always one or two goats were present in the same pen as a kind of sentinel animals. In none of the experiments these animals turned out to be TSE positive by microscopy or clinical signs up till 48 months post challenge of the experimental infections. Another horizontal route of infection could have been the drinking from mother goats by other goats, including by the kids. This route was not possible to investigate. In most cases placenta was removed from the delivering mothers at birth; it must be mentioned here that the placentas investigated appeared negative by IHC microscopy. With respect to maternal/vertical transmission, goat kids from wild type PrP mothers have been drinking during the most susceptible period since there was clinical positivity within 1-2 months after delivery with respect to a wild type mother goat; mother goats with PrP genotype 211R/Q and 222Q/K were probably too early mated to have a heavy spread of disease at the time kids were drinking from them. The kids from 1st passage BSE infected mothers have been euthanized early (5-7 months old). Detecting signs of infection by drinking should not be expected in these cases, and all kids were negative at euthanasia by IHC microscopy and biochemistry for PrP\textsuperscript{Sc}. All the kids from the 2nd passage BSE infected mother goats remained negative by tonsil biopsy and none have shown any signs at time of euthanasia (27 months old). Nevertheless, the limited number of animals involved in the experiments does not allow to definitely conclude that vertical transmission does not occur. Milk samples of the mothers during early lactation are under investigation still in both ovinized (TgshpIX, ovPrP\textsubscript{ARQ}; not planned in the DOW v41) and bovinized (Tg110, boPrP, as planned in WP4) transgenic mice. Here part of the milk at early and late lactation were fractionated into cells, whey serum and immunoprecipitated prion material from whey and cream. These infectivity studies are underway at about 400-600 days (per January 2013) and will be finished in 2013.

4 Validating and improving our ability to detect and discriminate caprine BSE from goat scrapie

Activities from WP3 together with WP1 and WP2.
To know the use of diagnostic tools for goat TSEs means to know the way to differentiate strains and isolate types, and also BSE if it were present. That it can be present is clear from the detection of two cases of BSE in goats, one in France and one in United Kingdom\textsuperscript{9}. This project has carried out a unique activity to reach this aim.
A wide geographical collection of >60 goat scrapie/TSE isolates was made available to:
1. check three current EU approved tests for rapid testing in the active surveillance,
2. find specific tools for TSE analysis in goats,
3. use and improve discriminatory confirmation diagnostics that are currently used for small ruminants but which have been mainly developed using goat TSE materials,
4. to analyze the isolates in rodent models for their strain type properties.

To perform all these 4 tasks a selection from this collection was made to have as much variability factors involved as possible: thus taking into account that sufficient material was available for distribution to all partners involved, diversity in geographical origin, pre-existing knowledge, PrP genotype and results from an initial analysis in a CEA discriminatory ELISA-test that recognizes classical scrapie, BSE and atypical scrapie. Ultimately 24 isolates were subjected plus one experimental BSE sample (Fig. 3). Included was also a DEFRA approved inclusion of samples from the United Kingdom where potentially BSE could be involved.

All above four points have been applied on these 24 samples. Results have been accumulated in several deliverables for WP3. Seven rodent lines including mice transgenic for goat PrP generated in WP1 (line Tg501) were used, and about 10 experimental BSE samples from WP2 and a reference goatBSE sample from INRA were used to confirm the BSE status if present in the geographical set. Some conclusions were drawn for each of these points and have been discussed in the preceding part of this report.

Summarizing the degree of achievement in the obtained results with respect to the objectives is 100% in nearly all aspects except for the ongoing mouse assays to further validate the potential spread of infectivity to milk and the potential risk of milk products for humans. Other issues can be considered as being fully achieved. This is illustrated in a time scale table (see Table 2). Nevertheless, also these two details of objectives 1 and 3 are underway, and come to an end before halfway 2014.

| degree of achievement of efforts that were planned to investigate the objectives (in %-age) |
|----------------------------------|---|---|---|---|---|---|---|---|
| 1 Providing data to allow evaluation of human risk associated with BSE passaged in goat | a | a | a | a | a | a | a |
| 2 Providing pathogenesis data and biological material from first and second passage BSE in goats | a | a | a | a | a | a | a |
| 3 Evaluating the possibility of BSE self-maintenance in goats by maternal/horizontal transmission | a | a | a | a | a | a | a |
| 4 Validating and improving our ability to detect and discriminate caprine BSE from goat scrapie | a | a | a | a | a | a | a |

Table 2: Degree of achievement of objectives initially planned (i.e. activities performed for obtaining the intended information).
Methodologies and approaches; relation to the state-of-the-art

Methods/techniques of special interest to approach scrapie and BSE issues and answering questions which these agents might pose to the European goat holding and policy makers (with relation to the state-of-the-art underlined and in bold):

1. Creation of transgenic mice carrying goat variant PrP alleles to obtain information about the susceptibility for scrapie and BSE in goats hosting different genetic PrP variants. These are new for the TSE scientific world and have significance for science in small ruminants field as a means to acquire information of susceptibility of small ruminants for TSEs, especially BSE.

2. Design and use of in vitro systems to convert healthy prion protein (PrP\textsuperscript{C}) into the disease associated form (PrP\textsuperscript{Sc}). Here two new \textit{in vitro} PrP production systems have been generated: a \textit{prokaryotic system without application of physical forces} has been introduced as well as cell lines that supply the \textit{variant mammalian polymorphic PrPs} to study species barrier of TSE transmission.

3. \textbf{Consolidated protocols for sequencing goat PrP gene} especially for the coding region as well as for studies in the promotor region; this has allowed the analyses on more than 6000 goats in 7 different countries and showed that goat PrP is quite polymorphic (>40 PrP variants are now known, compared to 29\textsuperscript{footnote 7} at the start of the project).

4. A goat nucleus is used and further under development in France where the PrP gene is being monitored for further breeding towards healthy animals including with respect to TSE agents (unique in the world).

5. During this project probably the \textbf{largest scrapie association field studies} (performed in France) have been added to the already existing studies.

6. Challenge studies – both intracerebral and oral - with BSE and scrapie have been carried out in goats of different PrP genotype to confirm expected TSE resistance association as found in field studies:. Such a broad and \textbf{international challenge study in small ruminants} is quite unique.

7. Goat kids were raised in dams when incubating BSE to study maternal transmission.

8. Collected milk from infected dams was separated into cream and whey and cellular fractions. Prion material was isolated by immunoprecipitation efforts.
9. Techniques separating milk fractions have been worked out and applied to investigate distribution of **infectivity between cells, whey, cream and whole milk**.

10. An *in vitro* technique for evaluation of the distribution of prions during cheese production has been designed in a prototype procedure. This is **a new development** in TSE research and food safety.

11. Many tissue samples have been analyzed by microscopic technique of immunohistochemistry using the best antibodies available for a broad impression of prion spread and prion type in infected goats.

12. **One of the largest geographical** collections of natural and experimental TSE samples have been created for the aims of the project; this was necessary to employ current standards of diagnosis to find out about strain variety in a small ruminant species which was not well investigated before in goats.

13. EU-approved rapid screening assays for small ruminants have been investigated on **diagnostic specificity and sensitivity in goats** (not performed before in such a scale on goat brains).

14. Several **new biochemical prion (PrP\textsuperscript{res}) parameters** have been added to the already existing diagnostic array of PrP\textsuperscript{Sc} characteristics.

15. Discriminatory biochemical & microscopic techniques focusing on classical scrapie, BSE-like and CH1641-like isolates have been applied **in one study** (Figure 6).

16. Transgenic mice carrying bovine, murine, human, porcine, and polymorphic goat PrP variants, as well as bank voles and wild type inbred mice have been used for different applications: species barrier estimation, human goat scrapie- and BSE-susceptibility testing, tissue and milk infectivity distribution, titration of infectivity, goat TSE-variability in the field situation. This served points 1, 5, 6 and 9 of this paragraph.

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Figure 6: at left a biochemical Western blot picture of different triple band PrP\textsuperscript{res} patterns of respectively scrapie (S) & BSE (B) in brain (antibody L42), and at right immunohistochemical microscopy of prion positive gut nerve cells (brown means positive; antibody 6C2). Courtesy of Jorg Jacobs CVI NL and Kerstin Tauscher FLI GE.

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Impact on research and small ruminants sector

The project will have a number of important outcomes with immediate relevance to the “European” problem of BSE in goats:

1. There was hardly any species barrier observed for transmission of goat-BSE. Similar low barrier observations have been obtained with sheep BSE (BSE-in-sheep project, FP5 project EU QLK3-CT-2002-01309, coordinator O. Andréoletti). This suggests that TSE safety measures for goats could be comparable to those taken for sheep.

2. BSE prion distribution and infectivity titres in goat tissues and milk have been evaluated. This is yielding useful information on risk estimations. It is clear that also outside the brain infectivity in tissues like muscle can be present, while for maternal transmission through milk still more research has to be done. Anyway, all goat kids from positive dams until now have remained negative by microscopy.

3. The transgenic mouse lines as well as bank voles and conventional RIII mice are suitable models to recognize different TSE types in goats; the findings can be useful to make choices for the best models for strain typing and type identification for BSE, classical scrapie, CH1641 scrapie and atypical scrapie as well as a separate class of classical scrapie, Italian type of classical scrapie. Thus, these models serve as improved animal models for detection and discrimination of goat BSE from other goat TSEs.

4. It has been amply possible to check existing methods for their performance to diagnose goat scrapies and BSE. New biochemical parameters and proper tools (i.e. antibodies) have been defined. The methods are suitable at least for expert laboratories, and reagent composition will be open to the interested parties. GoatBSE has contributed to development of improved and validated (existing) diagnostics for goat BSE and scrapie.

5. Unfortunately, the 171R allele as in ARR-sheep does not occur in goats. But there is also good news. Pre-existing information that had appeared in the years right before the project had started together with new field studies in France from the GoatBSE project have confirmed that the **genetic variant codon 222K of PrP** in heterozygous state (222Q/K) is an outstanding allele for breeding towards TSE resistance in goats. This result was also confirmed in experimental challenges by intracerebral route (until now only for scrapie) and the oral route (for 1st and 2nd passaged BSE). Still, studies on intracerebral scrapie and BSE challenges in homozygous 222K/K goats have to be performed; these have been initiated lately at a small scale. These results contribute to propose improved guidelines for control of goat TSEs by selective breeding-culling strategies.

6. The large study on a set of different natural goat TSE isolates has yielded useful information on the variability of goat scrapie which confirms that several phenotypes can often be present in one host.
Also, BSE (experimental isolates from different goat PrP genotypes, as well as two previously reported goatBSE field isolates from France and UK) and atypical scrapie do behave different from all other isolates. This work can be considered as a goat TSE reference regarding geographical distribution of natural goat TSE strains in Europe.

7. The above points are useful for scientific reasons, policy makers and stakeholders. The data on geographical TSE distribution, the resistance related information, the tissue spread of infectivity and a potential enhanced risk for humans if BSE-infected goat products would be consumed are highly relevant and should keep the society vigilant on any emergence of BSE under goats (and possibly sheep and cattle) to protect end users. This also means that prevention and inactivation tools require attention and potential improvement. In anyway, breeding for resistance appears possible.

8. While the above points described direct practical research output with respect to impact on the small ruminants sector, in more generalized terms this project has contributed to generation of knowledge in small ruminant genetics and pathology research. Dissemination and exploitation of results has been promoted and the objectives of the project have been adapted to general and strategic lines being:

- General measures to promote research, technological development and innovation:
  - actions in Human Resources: staff dedicated to supporting research.
- Strategic lines and priorities by:
  - development of the reginal territories by promoting respect and animal welfare.
  - safety and quality of individual and collective life contributing to food safety, and development of a sustainable, safe and healthy food chain.
  - sustainability of social and economic development through protecting the economy against the impact of animal diseases.
  - its strategic impact by acquiring knowledge about risk factors for human exposure to BSE contaminated goat products (milk and meat and meat products).
DISSEMINATION AND USE OF RESULTS

Publishable results of the Final plan for using and disseminating the knowledge (see format in Appendix 1).

The strategic impact of this project has been to prove sound scientific information for use in quantitative risk assessment of human exposure to BSE via goat products. The scientific insights gained can contribute to promoting food quality and safety through control of goat TSEs within EU Member states and regions. This will benefit EU consumer driven concerns with regard to food safety and animal welfare and it will assist EU milk and meat producers, by reinforcing competitiveness in a global market. Objectives and deliverables of this project have provided essential information allowing quantification of the risk to humans that BSE potentially presents if in goats and their products. In addition, it will facilitate the initiation of direct control of TSEs in goats, since currently limited control of goat TSEs was based solely on our knowledge of TSE in sheep. The cases of caprine BSE found in France and the UK\(^9\) have indicated that, with respect to TSEs, goats might respond differently than sheep, at least after passage in goats BSE is better transmissible to several species PrP’s including human PrP. As has been seen with sheep, improved genetic and diagnostic tools for controlling goat TSEs should lead to significant reductions of scrapie, and perhaps even to control of caprine TSEs in EU regions with high TSE outbreaks. Such an outcome would greatly reduce both food safety concerns and welfare problems in infected animals. Moreover, it would contribute to the sustained development of the dairy and meat sector of EU agri-food business by decreasing and eventually eliminating the load of TSEs entering the food chain. Besides EU interests, this project is serving a world with intentions to reduce prion risks for human consumers and animals.

The GoatBSE consortium’s plan for using and disseminating the knowledge at the end of the project, has been to provide a complete picture of the activities undertaken and most importantly will provide information on the future route to full use (exploitation or use in further research) and dissemination of the knowledge.

Section 1 - Exploitable knowledge and its Use

The contractors have not developed any special exploitable knowledge having a potential for industrial or commercial application in research activities or for developing, creating or marketing a product or process or for creating or providing a service. The project was intended to yield scientific progress in prion field, and information for safety and risk assessment towards humans and animals with respect to BSE in goats.
Section 2 – Dissemination of knowledge.

Final plan for using and disseminating the knowledge

see table: “Final plan for using and disseminating the knowledge”
after page 27.
Section 3 - Publishable results
GoatBSE - being a typical scientific project with eye mark on human and animal risk for BSE transmission – has been publishing mainly in scientific peer review articles, which are displayed in the previous section “Dissemination of knowledge” and “Impact on research and small ruminants sector”. No exploitable results are opportune in this project. The GoatBSE project is rather a scientific contribution with significant practical results for goat farming; the only material contribution are potentially transgenic mouse lines which have been created to express variant goat PrP alleles; the publication of the production and use of these lines can be expected within two years from now by partner 4 via peer reviewed scientific papers(INIA, Madrid). We have produced a stakeholders publication in International Innovation entitled Eradicating BSE in goats (see green pages at the end of this report).

Pilot activities to TSE-resistance breeding have been initiated in a new EU-supported follow-up study in the EMIDA ERA net under the acronym GOAT TSE FREE.
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>BSE</td>
<td>bovine spongiform encephalopathy</td>
</tr>
<tr>
<td>CNS</td>
<td>central nervous system (brain and spinal cord)</td>
</tr>
<tr>
<td>DOW</td>
<td>Description of work document; in fact the official technical description of the whole project.</td>
</tr>
<tr>
<td>dpi</td>
<td>days post infection</td>
</tr>
<tr>
<td>ENS</td>
<td>enteric nervous system (nerve cells in the gut)</td>
</tr>
<tr>
<td>Ggl</td>
<td>ganglion; a junction outside the central nervous system where nervous cells have their connections</td>
</tr>
<tr>
<td>IHC</td>
<td>immunohistochemistry, a microscopic technique that uses PrP\textsuperscript{Sc} detection with PrP specific antibodies</td>
</tr>
<tr>
<td>IPP</td>
<td>ileal Peyer’s patch; a lymph node in the ileum</td>
</tr>
<tr>
<td>LRS</td>
<td>lympho-reticular system (such as lymph nodes and spleen)</td>
</tr>
<tr>
<td>mpc</td>
<td>months post challenge</td>
</tr>
<tr>
<td>mpi</td>
<td>months post infection</td>
</tr>
<tr>
<td>PK</td>
<td>proteinase K, a powerful microbial protease removing all PrP\textsuperscript{C} but leaving PrP\textsuperscript{Sc} partially unaffected</td>
</tr>
<tr>
<td>PRNP</td>
<td>gene coding for PrP</td>
</tr>
<tr>
<td>PrP</td>
<td>prion protein, in normal form a cellular protein (PrP\textsuperscript{C}) considered to be the target and potential agent in TSEs or prion diseases</td>
</tr>
<tr>
<td>PrP\textsuperscript{Dis}</td>
<td>PrP deposits observed when performing IHC on TSE diseased tissue</td>
</tr>
<tr>
<td>PrP\textsuperscript{Pres}</td>
<td>the PK-resistant part of PrP\textsuperscript{Sc} as seen with Wbl and ELISA</td>
</tr>
<tr>
<td>PrP\textsuperscript{Sc}</td>
<td>the disease associated form of PrP originating from a change in structure and aggregation of PrP.</td>
</tr>
<tr>
<td>Tg</td>
<td>transgenic, which means that the PRNP gene has been replaced by a foreign PrP with DNA techniques</td>
</tr>
<tr>
<td>TSE</td>
<td>transmissible spongiform encephalopathy</td>
</tr>
<tr>
<td>Wbl</td>
<td>Western-blotting, a biochemical technique that uses degradation of proteins with proteinase K, subsequent separation of the digest followed by detection of the resistant PrP\textsuperscript{Sc} with PrP-specific antibodies</td>
</tr>
<tr>
<td>wt</td>
<td>wild type for the PrP sequence; in the DOW v41 mentioned as I_{142}R_{211}Q_{222}. On position 154 these animals are also wild type with a R_{154}</td>
</tr>
</tbody>
</table>
ACKNOWLEDGEMENTS

We are grateful to the collaboration in providing goat tissue materials from the United Kingdom through our colleagues Hope, Gonzalez and Spiropoulos (AHVLA) in consultation with DEFRA, from France through our colleague Thierry Baron (ANSES), and from the Central Veterinary Services of Cyprus collaborating with consortium partner 6 FLI.

We want to acknowledge for the opportunities which the EU Network of Excellence NeuroPrion (EC FOOD-CT-2004-506579; coordinator Dr. Jean Philippe Deslys, CEA, Fontenay-aux-Roses, FR) initially has given the GoatBSE consortium partners that enabled to combine their knowledge and national activities into a Goat TSE project group.

FINAL PLAN FOR USING AND DISSEMINATING THE KNOWLEDGE

Results of dissemination plan

See pages 28-32
Dissemination of information to general public

- Peer reviewed publications (35 until now, more to come)
  - Complete project period Dec2006-Nov2012
  - Dissemination of information

<table>
<thead>
<tr>
<th>Planned/actual Dates</th>
<th>Type</th>
<th>Type of audience</th>
<th>Countries addressed</th>
<th>Size audience</th>
<th>Partners involved/responsible</th>
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<td>5-10-2007</td>
<td>Flyer</td>
<td>General project information flyer</td>
<td>Research &amp; Higher education</td>
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<td>EU</td>
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<tr>
<td>6-2008</td>
<td>Publication</td>
<td>GoatBSE: project showcase. In Emerging Epidemics Research (EU funded projects) 2002-2008 by the EC-European Research Area. Pages 74-75.</td>
<td>Policy &amp; Research</td>
<td>EU</td>
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<td>Oct 8, 2008</td>
<td>Information</td>
<td>Animal TSEs workshop in Prion2011 at Madrid</td>
<td>Policy &amp; Research</td>
<td>world</td>
<td>120</td>
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<tr>
<td>Nov-08</td>
<td>Publication</td>
<td>Several contributions (pages 1-2 and 8-9) in the NeuroPrion Prion2008 special issue newsletter.</td>
<td>Policy &amp; Research</td>
<td>world</td>
<td>world</td>
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<tr>
<td>3-2009</td>
<td>Information</td>
<td>EFSA Scientific Opinion Genetic TSE resistance in goats</td>
<td>Policy &amp; Research</td>
<td>EU</td>
<td>world</td>
</tr>
<tr>
<td>23 September, 2009</td>
<td>Information</td>
<td>Animal TSEs workshop in Prion2009 at Porto Carras, Greece</td>
<td>Research, policy makers</td>
<td>100</td>
<td>world</td>
</tr>
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<td>7-2009</td>
<td>Information</td>
<td>State-of-the-art review goat TSEs</td>
<td>Public and Research</td>
<td>world</td>
<td>world</td>
</tr>
<tr>
<td>during 2010-2012</td>
<td>Information</td>
<td>Several items like literature list, workshop announcements, announcements on new papers, stakeholders lists see website: <a href="http://www.goatbse.eu">www.goatbse.eu</a></td>
<td>Public and Research and Stakeholders</td>
<td>world</td>
<td>world</td>
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<td>during 2011</td>
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<td>Research</td>
<td>world</td>
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<td>9 May 2012</td>
<td>Information</td>
<td>Animal TSEs workshop in Prion2012 at Amsterdam; May 9th 2012</td>
<td>Research</td>
<td>world</td>
<td>200</td>
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<tr>
<td>2012</td>
<td>Publication</td>
<td>Eradicating BSE in goats: in international Innovation, EuroFocus Issue February 2012 pages 100-102. (SEE AT END OF THIS PLAN)</td>
<td>Stakeholders: policymakers, goat sector, EU-officers, national agricultural offices.</td>
<td>Europe</td>
<td>Europe</td>
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</tbody>
</table>

Project web-site activities

- dec-2006 Private consortium web-portal operational | Research | consortium + EC | <30 | P1 |
- sep-2007 First version public website operational www.goatbse.eu | Research & Higher education | Worldwide | - | P1 |
- sep-2008 Full public website operational www.goatbse.eu | Research & Higher education | Worldwide | - | P1 |
- oct-nov-2008 Several meeting announcements (www.goatbse.eu) | Research & Higher education | Worldwide | - | P1 |
- 19-11-2008 Executive summary of year 1 posted (www.goatbse.eu) | Research & Higher education | Worldwide | - | P1 |
- dec-08 Post Executive summary year 2 (www.goatbse.eu) | Research & Higher education | Worldwide | - | P1 |
- Apr & Oct 2009 Announcements and report on workshop on Animal TSEs, Porto Carras, Greece (22Sep2009) | Research & Higher education | Worldwide | 100 | P1 |
- 9-nov-09 Workshop: Goat genetics and TSEs | Research specialists | United Kingdom | 10 | P3-P12 |
- cont 2010 Newsflashes on website (www.goatbse.eu) | Research & Higher education | Worldwide | - | P1 |
- cont 2010 Some popular articles (www.goatbse.eu) | General public & Higher education | Worldwide | - | P1 / all |
- 2010 Improve stakeholder section | General public & Higher education | Worldwide | - | P1 / all |
- 2010 Executive summary year 3 (www.goatbse.eu) | Research & Higher education | Worldwide | - | P1 / all |
- 2011 Goat TSEs literature list | Research & Higher education | Worldwide | - | P1 / all |
- 2012 Executive summary year 5 (www.goatbse.eu) | Research & Higher education | Worldwide | - | P1 / all |
- 2013 Executive summary year 6 (www.goatbse.eu) | Research & Higher education | Worldwide | - | P1 / all |
- 2013 Final report GoatBSE project for public | Research & Higher education | Worldwide | - | P1 / all |

Peer reviewed publications (35 until now, more to come)


Workshops, exhibitions, conferences

Workshop: Workshop on TSEs in Goats, Cervids and Sheep. Prior to the international Prion2008 conference. Research & Higher education

World-wide 180

P2

P12

P5

P5

P5, P7, AHVLA

P9

P1

CODA

P4, P2, P1

P5

P5

P6

P9

P1

P12, P1, AHVLA

P1

P2, P1

P12, P2, AHVLA, ANSES, NIAH

P7

P12, P2, AHVLA, ANSES, NIAH

P7

P2a,c

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<th>Date</th>
<th>Event</th>
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<tr>
<td>22-Sep-09</td>
<td>Workshop: Developments in TSEs of domestic and wild animals. Prior to the international Prion2009 conference.</td>
<td>Research &amp; Higher education</td>
<td>World-wide 100 P1</td>
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<tr>
<td>24-25 Sep 2009</td>
<td>Information: International Prion conference Prion 2009; Porto Carras Greece</td>
<td>Research, patients</td>
<td>world 650 P9</td>
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<td>8-Sep-10</td>
<td>Workshop: TSEs in animals and their environment. Prior to the international Prion2010 conference.</td>
<td>Research &amp; Higher education</td>
<td>World-wide 95 P1</td>
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<td>Oct 8-10</td>
<td>Information: International Prion conference Prion 2008; Madrid Spain</td>
<td>Research</td>
<td>world 750 P4</td>
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<td>16-May-2011</td>
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<td>9-12 May 2012</td>
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<td>Research, patients</td>
<td>world 540 P1</td>
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<td>9-May-2012</td>
<td>Information: Animal TSEs workshop in Prion2012 at Amsterdam; May 9th 2012.</td>
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<td>9-2007 Prion2007</td>
<td>Posters: Modeling the within herd prevalence of scrapie in goats in Italy.</td>
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<td>9-2007 Prion2007</td>
<td>Posters: Studies on pathogenesis of Spanish goat scrapie analyzing PrPSc distribution by immunohistochemistry</td>
<td>Research</td>
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<td>10-2008 Prion2008</td>
<td>Posters: Analysis of monoclonal antibodies for detection of the prion protein in goats bearing different PRNP gene polymorphisms</td>
<td>Research</td>
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<td>10-2008 Prion2008</td>
<td>Posters: Genetic susceptibility of goats to Nrdh: AHIQ as risk factor</td>
<td>Research</td>
<td>Europe, America, Asia 800 P7, P1</td>
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<td>10-2008 Prion2008</td>
<td>Posters: Intracerebrally induced BSE in goats</td>
<td>Research</td>
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<td>10-2008 Prion2008</td>
<td>Posters: Genetic variability of the PRNP gene in wild ruminants from Italy and Scotland</td>
<td>Research</td>
<td>Europe, America, Asia 800 P7 P1/2/3 P5</td>
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<td>2008</td>
<td>Conference: Update on BSE and scrapie in Spain</td>
<td>General public &amp; Higher education</td>
<td>Spain, 50 P5</td>
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<td>2008</td>
<td>Conference: Genetic factors associated with TSE. Control methods</td>
<td>General public &amp; Higher education</td>
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<td>2008</td>
<td>Conference: TSEs surveillance and control program</td>
<td>General public &amp; Higher education</td>
<td>Spain, 50 P5</td>
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<td>Sep 2009, Joint funders</td>
<td>Presentation: Genetics of sheep and goat scrapie</td>
<td>Research</td>
<td>United Kingdom 250 P1/2/3</td>
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<td>Nov 2009, Vet. School</td>
<td>Poster: Genetics of sheep and goat scrapie</td>
<td>Research &amp; Higher education</td>
<td>Mexico 200 P12/3</td>
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<tr>
<td>May-2009</td>
<td>Conference: Breeding plans Italy</td>
<td>Research, Stakeholders, Policy Makers</td>
<td>Italy, 50 P7</td>
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<td>Nov-09</td>
<td>Conference: Genetics and biology of goat and sheep TSEs</td>
<td>Research</td>
<td>Italy, 100 P7</td>
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<td>23-9-2009, ± 5 Posters: Ruminant TSEs subjects</td>
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<td>Europe, America, Asia &gt;500 all partners</td>
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<td>9 Sep 2010</td>
<td>Posters: A one step triple immunofluorometric assay enables differential diagnosis of BSE, classical and atypical scrapie;</td>
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<td>9 Sep 2010</td>
<td>Comparative performance of three rapid post mortem tests for active surveillance of TSE in goats.</td>
<td>Research</td>
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<td>9 Sep 2010</td>
<td>Distinct Multiplex Diagnosis of Seven TSE-types from Cattle and Sheep.</td>
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<td>25-Nov-2010</td>
<td>DISTINCT GGT BSE PROPAGATE MORE EFFICIENTLY THAN CATTLE BSE IN HUMAN PRP TRANSGENIC MICE. Congreso Nacional de Priones. 25-26 Nov 2010.</td>
<td>Research</td>
<td>Spain, 100 P4, P2</td>
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<td>15-17 Sep 2010</td>
<td>A Distinct Proteinase K Resistant Prion Protein Fragment Challenges the Diagnosis of prion diseases in goats</td>
<td>Research, R&amp;D</td>
<td>Europe, America, Asia, Africa 250 P1, P9</td>
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<td>8-Sep-10</td>
<td>Developments in goat TSEs</td>
<td>Research</td>
<td>Europe, America, Africa &gt;500 all partners</td>
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<td>15-17 Sep 2010</td>
<td>Antibody and antigen properties in prion detection of ruminants.</td>
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<td>Europe, America, Asia, Africa 250 P1, P1, AHVLA</td>
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<td>28th Meeting ESVE/ECVP</td>
<td>Posters: Lack of association between scrapie genetic risk groups and Visna/maedi antibody titres</td>
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<td>XXII SEA PV 2010</td>
<td>Posters: Relacion entre la neuropatologia y expresion genica de ovinos infectados por scrapie</td>
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<td>XXII SEA PV 2010</td>
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**Expert activities**

- 2009 EFSA expert support for opinion formation on “Scientific and technical assistance on the provisional results of the study on genetic resistance to Classical scrapie in goats in Cyprus”. November 2009
  - Expert activities
  - Europe P1, P2, P6, P7, P8
- 2009 SCIENTIFIC OPINION Genetic TSE resistance in goats Scientific Opinion of the Panel on Biological Hazards, March 2009
  - Expert activities
  - Europe P2a
  - Expert activities
  - Europe P1, P2, P6, P7
- 2012 Request for scientific and technical assistance on the provisional results of the study on genetic resistance to scrapie in goats in Cyprus. July 2012
  - Expert activities
  - Europe P1, P2a, P7, P8
- 2008-2012 Brain typing expert group (STEG) activities, supporting EURL tasks.
  - Expert activities
  - Europe P1, P2, P6, P8, P10
Special issue towards public “Eradicating BSE in goats”

in journal: INTERNATIONAL INNOVATION, Euro-Focus February 2012 issue, pp 100-102
Eradicating BSE in goats

Dr Jan Langeveld, acting coordinator of the GoatBSE consortium, and former coordinator Dr Alex Bossers discuss their plans for breeding goats which are insensitive to the infectious agents responsible for past epidemics of ‘mad cow disease’, namely the bovine spongiform encephalopathy agent and similar prions.

Eradicating BSE crisis, have shown in a very costly way that certain infectious diseases are behaving as new agents, are difficult to pasteurise and have unpredictable epizootic and zoonotic behaviour (properties which mean animal diseases can affect other animal species including humans). BSE infected several hundred thousand cattle, and also a few hundred people suffered from the human variant that causes Creutzfeldt-Jakob disease. The disease in humans and animals is still incurable and untreatable, and involves progressive brain degeneration.

Eradicating TSEs in goats means no possibility of hazardous transmissions through the animal production sector or from animals to society.

Could you outline the primary aims of your research?

Our consortium aims to generate data for human bovine spongiform encephalopathy (BSE) risk estimations related to the consumption of goat products. We will obtain an EU-wide insight into the characteristics and control of transmissible spongiform encephalopathies (TSEs, a generic name for prion disease like BSE and scrapie) in goats. Our field research collects national and regional information on TSE variants and the genetic background of susceptibility in animals from the main goat producing countries in Europe. The laboratory research will estimate the risk of disease transmissibility, especially between goats and humans.

Why is the field of TSEs so significant?

The BSE epidemic that started in the UK in the 1980s and subsequently spread through Europe provides the impetus for our research. The source of the epidemic remains unknown, but the most plausible explanations are either small ruminants such as goats that are known targets of this disease, or infected cattle that went unnoticed because infections were rare or because clinical signs appear late in the animals’ lifespan.

The epidemic developed following the feeding of insufficiently inactivated ruminant derived meat and bone meal rations to cattle. TSEs, and especially the BSE crisis, have shown in a very costly way that certain infectious diseases are behaving as new agents, are difficult to pasteurise and have unpredictable epizootic and zoonotic behaviour (properties which mean animal diseases can affect other animal species including humans). BSE infected several hundred thousand cattle, and also a few hundred people suffered from the human variant that causes Creutzfeldt-Jakob disease. The disease in humans and animals is still incurable and untreatable, and involves progressive brain degeneration.

Eradicating TSEs in goats means no possibility of hazardous transmissions through the animal production sector or from animals to society.

Can you provide a summary of some of the techniques you are using in the project?

This approach combines many techniques: we are testing healthy and infected goats with prion protein (PrP) gene polymorphisms for their genetic susceptibility to infection, producing transgenic mice with goat PrP genes highly susceptible to the disease; in vitro PrP misfolding studies using specially designed PrP variants; and testing goats with different PrP polymorphisms for resistance to BSE and scrapie infection.

We are also analysing caprine TSE strain variations in seven EU countries (Greece, Cyprus, Italy, Spain, France, The Netherlands and the UK), testing the infectivity levels of TSE-infected goat tissues using caprinised and humanised transgenic mice; and testing for the potential survival of TSE infectivity in the cheesemaking process.

Why is it important to study samples from a large geographical range?

The PrP polymorphisms conferring resistance to TSEs are rare, so sampling many animals with a broad range of genetic diversity is important – data from many geographical locations and breeds of production goat are desired. Furthermore, different TSE strains are expected to occur at various geographical locations and these might have different PrP-based genetic resistance profiles.

What are some achievements of the project so far?

A very promising resistance allele has been detected in goats in Cyprus, though in most countries of Europe this is absent. This is already being used in a resistance breeding programme in Cypriot goats as European Food Safety Authority (EFSA) approved emergency action due to the very high scrapie incidence there. However, more experimental work is needed to confirm the effectiveness of this strategy. Another allele found at higher frequencies in the other EU countries has yet to be confirmed as a resistance allele, though we have a better experimental basis to start a breeding programme with this than we do with the allele identified in Cyprus.

Who will benefit from the project?

The European Food Safety Authority (EFSA) advocates our work for the promotion of safe food for consumption in the EU. Also, this work is of particular concern for producers of goat milk: their products are enjoying an increased appreciation throughout Europe.

Goat TSEs are serious issues, since identifying one infected animal means the whole herd must be culled, which is unethical, economically difficult for farmers, and goes against public opinion if there are alternatives that could be developed. Although the BSE problem has subsided in cattle, goat TSEs still have a continuing impact on farmers who raise these animals.
Avoiding the cull

BSE, the prions responsible for epidemics of ‘mad cow disease’, have recently been identified in goats. In response, the GoatBSE consortium is examining the risk posed to humans, and hopes to breed disease resistant animals.

THE BOVINE SPONGIFORM encephalopathy (BSE) outbreaks in the 1980s led to the culling of several million cattle in the UK and Europe, and the infectious agents responsible – proteins called prions – grew in notoriety by crossing the species barrier to infect humans, causing around 200 cases of the fatal neurodegenerative condition Creutzfeldt-Jakob disease.

The tolls of the BSE epidemics helped to spur on the formation of the European Food Safety Agency (EFSA) in 2002, which began actively surveying livestock other than cattle for various forms of BSE related transmissible spongiform encephalopathies (TSEs) in order to safeguard humans from new routes of infection through the consumption of small ruminants (sheep and goats).

In 2005, concerns about ulterior risks of TSEs grew when the EFSA reported the first case of a BSE infection in goats. In response to this, researchers convened to structure and stimulate goat TSE studies; this was initially executed under the EU-funded FP6 Neuroprion network. Subsequently, the GoatBSE project was commissioned to investigate the control of TSEs in goats and to estimate the risk of transmissibility of the agents, both between members of a herd and between goats and humans. Previous efforts made to avoid future mass culls of infected livestock successfully led, in several EU regions, to the breeding of sheep that appear resistant to TSEs, and the European Commission has pushed for a repeat of such a breeding programme in goats.

However, as Dr Jan Langeveld – an acting coordinator of the GoatBSE consortium – explains, TSEs and BSE in goats have had a long history without much scrutiny: “For centuries, TSEs have been known as scrapie in sheep and goats but it only became apparent that the disease was transmissible in the 1930s,” he outlines. “TSEs are commonly recognised to cause three main forms of disease in small ruminants: classical scrapie, BSE, and atypical scrapie. BSE has recently been found in two goats to date (one in France and another in the U.K.), and while no cases have been reported in sheep thus far, both are susceptible to infection when fed brain tissue from infected cattle.”

Comprising of a consortium of 10 institutions in seven EU Member States and funded by the EU’s FP6 Food Programme and national governments, GoatBSE is now addressing the paucity of knowledge surrounding TSE infections in goats. An up-to-date website is available to inform scientists and stakeholders (www.goatTSE.eu).

TISSUE INFECTIVITY

The project has already made headway on several fronts. One of the primary aims of GoatBSE is the clinical investigation of the diseases in goats which are inoculated with – or orally exposed to – prions. The team is specifically looking at the infectivity distribution of BSE and TSEs in cells involved in the central nervous system or peripheral nervous and lymphatic tissues (which prions target), as well as tissues which may be more relevant to human consumption such as muscle, intestines and milk-based products.

Screening tissues for the presence of infection, and for the presence of the malfolded protein PrPSc which is associated with disease status diagnostics, is conducted by testing in highly susceptible transgenic mice.
GOATBSE

PROPOSAL FOR IMPROVEMENT OF GOAT TSE DISCRIMINATIVE DIAGNOSIS AND SUSCEPTIBILITY-BASED ASSESSMENT OF BSE INFECTIVITY IN GOAT MILK AND MEAT

OBJECTIVES

The project’s main goal is to provide sound scientific information which can be used to quantitatively assess the risk of human exposure to BSE via goat milk, meat and products thereof. Such knowledge only can be usefully obtained if scientific insights are gained into the control of TSEs in goats within EU Member states and regions.

PARTNERS

Central Veterinary Institute of Wageningen UR (CVI-WUR), The Netherlands
- Instituto National de la recherche Agronomique (INRA), France
- The Roslin Institute & R(D)SVS, University of Edinburgh (UEDIN), UK
- Instituto Nacional de Investigación y tecnología Agroalimentaria (INIA), Spain
- Research Centre for TSE and Emerging Transmissible Diseases, University of Zaragoza (UNIZAR), Spain
- Institute for Novel and Emerging Infectious Diseases at the Friedrich-Loeffl-Institut (FLI), Germany
- Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d’Aosta (IZSLPV), Italy
- Istituto Superiore di Sanità (ISS), Italy
- Centre for Research and Technology Hellas (CERTH-INA), Greece
- Commissariat à l’Energie Atomique et aux Energies alternatives (CEA), France

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DR JAN P M LANGEVELD, PhD and DR ALEX BOSSERS, PhD MSC BSC are senior scientists at the Central Veterinary Institute of Wageningen University and Research Centre at Lelystad, The Netherlands. Langeveld’s research is focused on protein issues related to understanding, diagnosing and fighting veterinary diseases. Bossers is in charge of TSE research coordination and biomolecular studies. His focus is moving towards pathogenomics.

So far, clinical disease has been observed in brain tissue following the inoculation of goats with varying levels of natural resistance to TSEs offered by different prion protein gene (PrP) polymorphisms (gene characteristics which vary in populations). Some goats have shown BSE infection status in enteric nerve cells within a year of oral passage of the prion.

Further work to quantify the risk of transmissibility of diseases from animal to animal, and to humans via the consumption of infected meat or milk, is underway. “Concerns about milk consumption are warranted because, in sheep, milk from infected ewes can be involved in the transmission of TSEs to their lambs,” Langeveld points out. More understanding of the risks involved in consuming goat products will help to promote food safety in the EU Member States.

Understanding disease status also depends on surveying goat populations for different strains of TSEs across Europe. To do so, the GoatBSE consortium conducted a large study on the geographical distribution of the diseases in major goat-producing regions of seven EU Member States (Greece, Cyprus, Italy, Spain, France, The Netherlands and the UK). Biochemical analysis and determination of biological transmission characteristics in susceptible rodent models is ongoing, with more samples currently being collected from institutions outside of the consortium. Such efforts should serve to characterise the breadth of variation in strains of prions which infect goats locally and internationally.

BREEDING RESISTANCE

Another line of enquiry for the team is to identify and determine the influence of genetic polymorphisms in PrP on the susceptibility of goats to infection by BSE and TSEs. This has been carried out by comparing the genes of scrapie – infected animals with those of their uninfected peers in the same herds. Initially, this involved analysing infected field herds where susceptibility appeared dependent on PrP polymorphisms. Subsequently, the team used various TSE inoculations to infect a series of goats with differing PrP polymorphisms, and also tested the transmissibility of infection between goats with different genetic variants using capriniised transgenic mice or in vitro models. Useful PrP polymorphisms may be rare among goats, and the frequency of different alleles can vary across regional and/or national populations, so the project screened and genotyped goats for variation in the PrP gene in each of the seven countries involved.

The efforts have led to the identification of possible resistance polymorphisms which are present at low frequencies throughout the various European goat populations. Evidence confirms that the 146 mutation in the PrP gene offers a strong degree of resistance to TSE infection, but currently this is a very rare allele in goat populations outside of Cyprus. The 222 mutation is present at higher frequencies than 146 in other EU countries, and there is a stronger basis for it being used in breeding resistance, but work to confirm the level of resistance that it confers is ongoing. Further in vitro studies of prion protein malfolding in animals with the 222 mutation will help with this.

When suitable polymorphisms are found to have meaningful benefits for boosting resistance to prions in the animals, the next stage is to use breeding programmes to increase the frequency of these. Langeveld states the potential of such programmes: “There are very promising results from goat challenge experiments (with scrapie and BSE) that suggest breeding resistance to TSEs is possible. At this stage, little action has been undertaken to use breeding but it is subject for new work and pilot field studies”.

IMPLICATIONS OF GOATBSE

All of these insights will contribute to a safer and more robust market for goat products in the EU. Though concerns about BSE epidemics in cattle have reduced recently, the risks of prion infections posed by the consumption of goat meat and milk have not been quantified. Some regions of the EU (such as Cyprus) currently have a high incidence of scrapie in goat herds, at a time when Europeans are increasingly consuming goat meat, milk and milk-derived products. These factors, along with new concerns about the rise of caprine BSE cases, suggest that assessing the transmissibility of these diseases from goats to humans is rightly deemed a priority for minimising health risks to consumers.

The developments made by GoatBSE will also facilitate the control of TSEs in goats. Scrapie and related infections of single animals currently necessitate the culling of entire herds, the results of which are extremely wasteful, evoke strong public outcry and severely damage the financial prospects of farmers. By reining in the impact of TSEs on goat populations through breeding programmes used to raise prion resistant animals, mass culls can be avoided. Thanks to GoatBSE, both consumers and producers will eventually benefit from healthier herds.