4.3. Alternative disease models

The third method, to find an alternative natural disease model that can be studied in the field, is more problematic. Studies of the natural transmission of the only known naturally-occurring TSE of small ruminants, scrapie, might provide a model for BSE in sheep, should it occur under field conditions. Both scrapie and experimental BSE in sheep have similar clinical signs and they have similar diffuse tissue distributions of PrP^{Sc} [34, 35, 59, 115]. If natural ovine BSE is similar to experimental ovine BSE, then ovine BSE may potentially behave in a similar manner to scrapie as far as routes and mechanisms of transmission are concerned.

4.3.1. Scrapie

This is the most extensively studied TSE model. Several institutions have established, maintained and recorded naturally infected flocks of sheep in order to study various aspects of scrapie, including its transmission. These include the INRA Langlade flock of Romanov sheep, various Institute for Animal Health flocks and the VLA scrapie-affected flock.

Analyses of data collected over more than a decade from the first of these have provided epidemiological evidence for both a maternal and lateral component of transmission [22, 99]. Higher relative risks of clinical scrapie were observed associated with lambing periods. There was also a reduced risk of clinical scrapie in artificially-reared lambs from healthy dams, and an increased risk in maternally-reared lambs from scrapie-affected dams. They proposed that transmission may occur within the first 24 h of life with additional risk for those that then continue to share the maternal environment (all lambs remained on their dams for the first intake of colostrum and then for 24 h).

The Institute for Animal Health flocks have established that, despite earlier contradictory findings [30, 31, 33], true vertical transmission of ovine scrapie (via the germ-line or in utero) is improbable [36, 37]. A scrapie-free flock has been established by embryo-transfer (ET) from one with a long-standing scrapie problem. The ET-derived flock has remained scrapie-free since its establishment in 1996, even though it has a similar *PrP* genetic profile to the original flock. Of interest to mechanisms of horizontal/lateral transmission is the fact that the "clean" flock was established and maintained in a scrapie naïve environment; a parallel ET-derived flock that was maintained in close proximity to, but separate from, the original scrapie-affected flock did experience clinical scrapie cases [37]. Lateral transmission has also been shown to occur in the absence of lambing [38].

In the VLA flock it has been shown that lateral transmission occurs [84] and that exposure to a contaminated environment only is sufficient to produce disease (Dexter, Tongue, Bellworthy, unpublished data).

These flocks are managed in a way that maintains high frequencies of sheep with PrP genotypes at high risk of developing clinical disease. Thus with a high incidence of clinical disease and high infectious load, they also provide controlled environments in which to study the pathogenesis of naturally acquired disease. They effectively counter the difficulties of studying a disease that occurs at a low flock-level incidence, however it must be recognised that whilst they provide evidence for routes and mechanisms of natural transmission and estimates of transmission parameters, they are probably not representative of any but the most heavily affected (worst-case scenario) commercial flocks. They are also limited in the range of breeds present, and (potentially) in the number of different scrapie isolates/strains present. These flocks may mimic natural exposure, but at a level that no commercial flock-owner would be able to tolerate and remain as an economically viable unit. Because of this the relative importance of different components of transmission may vary in commercial field flocks and therefore intervention measures may have different outcomes. These institutionalised research flocks, therefore, act as an important bridge between the artificial exposure – natural route transmission experiments – and the true field situation.

A variety of experimental studies using the approaches outlined above have provided

evidence for possible routes of transmission of scrapie. PrP^{Sc} has been found in tissues that could be involved in the natural dissemination of the infectious agent i.e., routes that could lead to exit of the infectious agent from the animal, and result in either environmental contamination or direct transmission. These tissues include the lympho-reticular system of the gut [40, 103, 115], chronically inflamed mammary tissue associated with lymphocyctic mastitis [73], kidney tissue [90], salivary glands [104], nictitating membrane [77], and placentae [2, 81, 101].

For the majority of these tissues, evidence of infectivity or the presence of PrP^{Sc} in associated secretions and excretions is still elusive for scrapie in small ruminants. The exception is blood [55]. Although experimental blood transfusions have resulted in clinical scrapie [55], just as with BSE, it is unlikely to play a major role: blood transfusions are not regular occurrences in sheep veterinary practice.

On the other hand, not only has PrP^{Sc} and infectivity been demonstrated in placentae [3, 81, 101], but it has also been shown to produce clinical scrapie when administered orally to sheep [78, 79]. This was proposed by the authors as a mechanism for lateral transmission from ewe to ewe at lambing time. Placenta has also been cited as a possible explanation for some of the epidemiological findings thought to be associated with mechanisms of maternal transmission [74], although much of the epidemiological evidence may also be interpreted as a contribution to transmission via the lateral route, especially that of environmental contamination. For example, there are reduced odds of ever becoming a scrapie-affected flock if the flock sometimes lambs in different places, compared to those flocks that always lamb in the same place [74]; there is decreased risk of disease associated with lambing in individual pens [75], and there were increased odds for scrapie-positive status of a flock that was found to be associated with failure to remove placenta from bedding along with its disposal in compost.

Epidemiological cross-sectional [74, 75] and case-control studies [47, 51, 80] have provided supporting evidence for the role of various allied management practices in the transmission of scrapie in the field. So far they lack the consistency and specifics necessary for the development of appropriate intervention measures. The scrapie literature does however illustrate how the different types of investigations into aspects of transmission, and the different disciplines, are complementary. Experimental studies of transmission routes and epidemiological studies of risk factors are intrinsically linked in a positive feedback loop, each informing the other.

4.3.2. Chronic wasting disease

The other naturally occurring TSE, CWD of deer is probably less relevant as a model for BSE in small ruminants, has been recently reviewed elsewhere [123] and is covered by Sigurdson in this special issue [89].

4.3.3. Other disease models

Host-specific experimental studies in large animals are expensive and do take time to produce results. The former means that they are difficult to fund. The latter means that they may have to be run in parallel with other experiments, often with more start-up assumptions than desirable, rather than in a logical step-wise order following on from previous findings. They are, however, of paramount importance. They provide an opportunity to study the disease in the original host species; they can be comparable across studies, if standardised protocols are used, and they eliminate the noise of variability, the difficulties of loss to follow-up and the potential biases that are experienced with epidemiological studies. To counter the time and resource limitations, other models have been sought.

The role of hamsters, mice, the burgeoning range of murine transgenes and other models such as voles is a large subject in its own right, and is covered by Groschup and Buschmann in this special issue [44] and elsewhere [28, 43]. In the past such models have been useful [12, 13], but they also have limitations. For example, laboratory wild-type mice cannot replace the original donor species due to the species-transmission barrier and to their different biology and physiology compared

to ruminants. The former has been addressed with the advent of transgenic mice, the latter is insurmountable. Even these do not replicate reality, and the interpretation and extrapolation of any results back to the donor/host-species needs to be a considered, objective process. For example, data from different transgenic mouse lines are not directly comparable, even between lines which have a common transgene [16, 105].

5. PUBLIC HEALTH

The ultimate question of whether a TSE has implications for public health – i.e. is transmissible to man – is difficult to address in the absence of transmission experiments on people. The most appropriate alternative is to use non-human primates [48, 67, 68, 70] which have indicated that BSE transmits with a end-stage disease indistinguishable from variant Creutzfeldt-Jacob disease (vCJD). However these experiments are limited by ethical constraints. Here the development of transgenic mice has been of prospective value, but at the same time, can be misleading. For example, mice with a single copy of the human *PrP* gene were not susceptible experimentally to BSE [10] while at the same time, epidemiological and strain-typing studies were producing a very strong body of circumstantial evidence that vCJD was a consequence of BSE infection in man. The inevitable limitation of such transgenic mice is that only one human gene is present in the model, and disease susceptibility and incubation period are inevitably multi-factorial. Transgenic mouse models which overexpress human *PrP* are also available, and they are highly susceptible to BSE [7, 15, 65, 106] but these may not be a true indicator of susceptibility in humans. Detailed discussion of these models is outwith the scope of this paper and is covered in detail by Groschup and Buschmann in this special issue [44].

6. REMAINING CHALLENGES

Many challenges remain even when a spongiform encephalopathy has been identified as transmissible, and when routes and mechanisms have been proposed.

What are the effects of repeated low dose exposure? What happens when there is intercurrent disease? How do *PrP* genetics influence the transmission process? Is any apparent reduction in susceptibility actually an effect of incubation period prolongation to beyond the natural lifespan? What is the implication of carrier state/subclinical disease for disease control and health? How can we detect animals in the early stage of disease incubation – a phase "silent" to current investigative tools?

For BSE and scrapie some of these questions have been addressed partially [39, 42, 45, 49, 56, 61]. It is possible that for novel TSE many of these questions will remain unanswered or unpursued, except by the most determined of researchers after the funding, stimulated by the public health and political aspects of BSE and vCJD, has dwindled.

Perhaps the greatest conundrum for researchers faced with a new TSE in a species, or a TSE in a species in which it has not previously been described, is whether it is "new", or merely "newly observed". This is a particular issue for BSE, should it be found in the sheep population. With much speculation over the years that scrapie could be the origin of BSE, it might not be too surprising if a detailed study of scrapie isolates revealed one with BSE-like characteristics. A number of studies in the UK and elsewhere [19, 66, 82] have taken a direct approach to this question by looking at the experimental phenotype in cattle experimentally challenged with scrapie isolates, but the diversity of scrapie isolates precludes this approach being exhaustive.

Given that no one type of study can provide all the details or all the answers required, and because of the constraints implicit in each type of study, it is important that researchers respect and integrate the work from other areas, are rigorous, do not overestimate their findings despite various pressures to do so, and are honest: both in the presentation of their findings and in the value of the outcomes. Some of those interested in pure science may disparage studies that they deem to be of low scientific merit, but which are actually of high value to those involved in policy and decision-making: equally some work of high scientific merit may

be extremely interesting in its own right, but not actually necessary to advance disease control and protect public health.

7. CONCLUSION

The approaches to the investigation of the transmission of BSE and scrapie, outlined above, differ only slightly. Those differences are due to the nature of the two diseases. BSE was a novel spongiform encephalopathy, in a hitherto unaffected species, that had characteristics of a point source epidemic, with an agent that could have been incorporated into a wide variety of feedstuffs and iatrogenically administered to naïve populations, and there was early evidence that it was not restricted to bovines. It was vital to establish, albeit experimentally, which other species might be affected, and whether the epidemic could be maintained by natural transmission, if the source was removed. In contrast, scrapie has been endemic throughout Great Britain for centuries, is maintained naturally (even if we don't know exactly how) and has a known host range. The principles, process and integration of evidence from different types of studies, however, are similar for both of these TSE and can be applied to any emerging or suspected spongiform encephalopathy.

REFERENCES

[1] Aguzzi A., Glatzel M., vCJD tissue distribution and transmission by transfusion – a worst case scenario coming true?, Lancet (2004) 363:411–412.

[2] Andréoletti O., Lacroux C., Chabert A., Monnereau L., Tabouret G., Lantier F., et al., PrP^{Sc} accumulation in placentas of ewes exposed to natural scrapie: influence of foetal PrP genotype and effect on ewe-to-lamb transmission, J. Gen. Virol. (2002) 83:2607–2616.

[3] Anonymous, Encyclopaedic word dictionary, Hanks P. (Ed.), Paul Hamlyn, London, New York, Sydney, Toronto, 1971.

[4] Anonymous, The concise Oxford English dictionary of current English, 9th ed., Thomson D. (Ed.), Oxford university press, 1995.

[5] Arnold M., Wilesmith J.W., Estimation of the age-dependent risk of infection to BSE of dairy cattle in Great Britain, Prev. Vet. Med. (2004) 66:35–47.

[6] Arnold M.E., Ryan J.B.M., Konold T., Simmons M.M., Spencer Y.I., Wear A., et al., Estimating the temporal relationship between PrP^{Sc} detection and incubation period in experimental bovine spongiform encephalopathy (BSE) of cattle, J. Gen. Virol. (2007) 88:3198–3208.

[7] Asante E.A., Linehan J.M., Desbruslais M., Joiner S., Gowland I., Wood A.L., et al., BSE prions propagate as either variant CJD-like or sporadic CJD-like prion strains in transgenic mice expressing human prion protein, EMBO J. (2002) 21:6358–6366.

[8] Bellworthy S.J., Dexter G., Stack M., Chaplin M., Hawkins S.A.C., Simmons M.M., et al., Natural transmission of BSE between sheep within an experimental flock, Vet. Rec. (2005) 157:206.

[9] Bencsik A., Baron T., Bovine spongiform encephalopathy agent in a prion protein $(PrP)^{ARR/ARR}$ genotype sheep after peripheral challenge: complete immunohistochemical analysis of disease-associated PrP and transmission studies to ovine-transgenic mice, J. Infect. Dis. (2007) 195:989–996.

[10] Bishop M.T., Hart P., Aitchison L., Baybutt H.N., Plinston C., Thomson V., et al., Predicting susceptibility and incubation time of human-to-human transmission of vCJD, Lancet Neurol. (2006) 5:393–398.

[11] Blood D.C., Studdert V.P., Baillière's comprehensive veterinary dictionary, Baillière Tindall, London, UK, 1988.

[12] Bruce M.E., Strain typing studies of scrapie and BSE, in: Baker H.F., Ridley R.M. (Eds.), Methods in molecular medicine – Prion diseases, Humana Press, Totowa, USA, 1996, pp. 223–236.

[13] Bruce M.E., Boyle A., Cousens S., McConnell I., Foster J., Goldmann W., Fraser H., Strain characterization of natural sheep scrapie and comparison with BSE, J. Gen. Virol. (2002) 83:695–704.

[14] Bundza A., Charlton K.M., Comparison of spongiform lesions in experimental scrapie and rabies in skunks, Acta Neuropathol. (1988) 76:275–280.

[15] Collinge J., Palmer M.S., Sidle K.C., Hill A.F., Gowland I., Meads J., et al., Unaltered susceptibility to BSE in transgenic mice expressing human prion protein, Nature (1995) 378: 779–783.

[16] Cordier C., Bencsik A., Philippe S., Betemps D., Ronzon F., Calavas D., Crozet C., Baron T., Transmission and characterization of bovine spongiform encephalopathy sources in two ovine transgenic mouse lines (TgOvPrP4 and TgOvPrP59), J. Gen. Virol. (2006) 87:3763–3771.

[17] Cuillé J., Chelle P.L., La maladie dite "tremblante" du mouton ; est-elle inoculable ?, C. R. Acad. Sci. Paris (1936) 203:1552–1554.

[18] Cuillé J., Chelle P.L., La maladie dite "tremblante" du mouton est bien inoculable, C. R. Acad. Sci. Paris (1938) 206:78–79.

[19] Cutlip R.C., Miller J.M., Race R.E., Jenny A.L., Katz J.B., Lehmkuhl H.D., et al., Intracerebral transmission of scrapie to cattle, J. Infect. Dis. (1994) 169:814–820.

[20] Dawson M., Wells G.A., Parker B.N., Preliminary evidence of the experimental transmissibility of bovine

spongiform encephalopathy to cattle, Vet. Rec. (1990) 126:112–113.

[21] Dawson M., Wells G.A., Parker B.N., Scott A.C., Primary parenteral transmission of bovine spongiform encephalopathy to the pig, Vet. Rec. (1990) 127:338.

[22] Diaz C., Vitezica Z.G., Rupp R., Andréoletti O., Elsen J.M., Polygenic variation and transmission factors involved in the resistance/susceptibility to scrapie in a Romanov flock, J. Gen. Virol. (2005) 86:849–857.

[23] Donnelly C.A., Ferguson N.M., Ghani A.C., Anderson R.M., Implications of BSE infection screening data for the scale of the British BSE epidemic and current European infection levels, Proc. Biol. Sci. (2002) 269:2179–2190.

[24] Eloit M., Adjou K., Coulpier M., Fontaine J.J., Hamel R., Lilin T. et al., BSE agent signatures in a goat, Vet. Rec. (2005) 156:523–524.

[25] Elsen J.M., Amigues Y., Schelcher F., Ducrocq V., Andréoletti A., Eychenne F., et al., Genetic susceptibility and transmission factors in scrapie: detailed analysis of an epidemic in a closed flock of Romanov, Arch. Virol. (1999) 144:431–445.

[26] Everest S.J., Thorne L.T., Hawthorn J.A., Jenkins R., Hammersley C., Ramsay A.M., et al., No abnormal prion protein detected in the milk of cattle infected with the bovine spongiform encephalopathy agent, J. Gen. Virol. (2006) 87:2433–2441.

[27] Fernie K., Steele P.J., Taylor D.M., Somerville R.A., Comparative studies on the thermostability of five strains of transmissible-spongiform-encephalopathy agent, Biotechnol. Appl. Biochem. (2007) 47:175–183.

[28] Flechsig E., Hegyi I., Raeber A.J., Cozzio A., Aguzzi A., Weissmann C., Knockouts and transgenic mice in prion research, in: Hörnlimann B., Riesner D., Kretzschmar H. (Eds.), Prions in humans and animals, de Gruyter, Berlin, Germany, 2007, pp. 134–150.

[29] Foley G.L., Zachary J.F., Rabies-induced spongiform change and encephalitis in a heifer, Vet. Pathol. (1995) 32:309–311.

[30] Foote W.C., Clark W., Maciulis A., Call J.W., Hourrigan J., Evans R.C. et al., Prevention of scrapie transmission in sheep using embryo transfer, Am. J. Vet. Res. (1993) 54:1863–1868.

[31] Foster J.D., McKelvey W.A., Mylne M.J., Williams A., Hunter N., Hope J., Fraser H., Studies on maternal transmission of scrapie in sheep by embryo transfer, Vet. Rec. (1992) 130:341–343.

[32] Foster J.D., Hope J., Fraser H., Transmission of bovine spongiform encephalopathy to sheep and goats, Vet. Rec. (1993) 133:339–341.

[33] Foster J.D., Hunter N., Williams A., Mylne M.J., McKelvey W.A., Hope J., Fraser H., Bostock C., Observations on the transmission of scrapie in experiments using embryo transfer, Vet. Rec. (1996) 138:559–562.

[34] Foster J.D., Parnham D.W., Hunter N., Bruce M., Distribution of the prion protein in sheep terminally affected with BSE following experimental oral transmission, J. Gen. Virol. (2001) 82:2319–2326.

[35] Foster J.D., Parnham D., Chong A., Goldmann W., Hunter N., Clinical signs, histopathology and genetics of experimental transmission of BSE and natural scrapie to sheep and goats, Vet. Rec. (2001) 148:165–171.

[36] Foster J.D., Goldmann W., McKenzie C., Smith A., Parnham D.W., Hunter N., Maternal transmission studies of BSE in sheep, J. Gen. Virol. (2004) 85:3159–3163.

[37] Foster J., McKenzie C., Parnham D., Drummond D., Goldmann W., Stevenson E., Hunter N., Derivation of a scrapie-free sheep flock from the progeny of a flock affected by scrapie, Vet. Rec. (2006) 159:42–45.

[38] Foster J., McKenzie C., Parnham D., Drummond D., Chong A., Goldmann W., Hunter N., Lateral transmission of natural scrapie to scrapie-free New Zealand sheep placed in an endemically infected UK flock, Vet. Rec. (2006) 159:633–634.

[39] Goldmann W., Houston F., Stewart P., Perucchini M., Foster J., Hunter N., Ovine prion protein variant A(136)R(154)L(168)Q(171) increases resistance to experimental challenge with bovine spongiform encephalopathy agent, J. Gen. Virol. (2006) 87:3741–3745.

[40] González L., Dagleish M.P., Bellworthy S.J., Sisó S., Stack M.J., Chaplin M.J., et al., Postmortem diagnosis of preclinical and clinical scrapie in sheep by the detection of disease-associated PrP in their rectal mucosa, Vet. Rec. (2006) 158:325–331.

[41] Gordon W.S., Advances in veterinary research. Louping-ill, tick borne fever and scrapie, Vet. Rec. (1946) 58:516–520.

[42] Gravenor M.B., Stallard N., Curnow R., McLean A.R., Repeated challenge with prion disease: the risk of infection and impact on incubation period, Proc. Natl. Acad. Sci. USA (2003) 100:10960–10965.

[43] Groschup M.H., Geissen M., Buschmann A., The experimental transmissibility of prions and infectivity distribution in the body, in: Hörnlimann B., Riesner D., Kretzschmar H. (Eds.), Prions in humans and animals, de Gruyter, Berlin, Germany, 2007, pp. 473–478.

[44] Groschup M.H., Buschmann A., Rodent models for prion diseases, Vet. Res. (2008) 39:32.

[45] Gruner L., Elsen J.M., Vu Tein Khang J., Eychenne F., Caritez J.C., Jacquiet P., et al., Nematode parasites and scrapie: experiments in sheep and mice, Parasitol. Res. (2004) 93:493–498.

[46] Hawkins S.A.C., Wells G.A.H., Simmons M.M., Blamire I.W.H., Meek S.C., Harris P., The topographic distribution pattern of vacuolation in the central nervous system of cattle infected orally with bovine spongiform encephalopathy, Bovine Practitioner (1997) 31.2:73–76.

[47] Healy A.M., Hannon D., Morgan K.L., Weavers E., Collins J.D., Doherty M.L., A paired case-control study of risk factors for scrapie in Irish sheep flocks, Prev. Vet. Med. (2004) 64:73–83.

[48] Herzog C., Salès N., Etchegaray N., Charbonnier A., Freire S., Dormont D., et al., Tissue distribution of bovine spongiform encephalopathy agent in primates after intravenous or oral infection, Lancet (2004) 363:422–428.

[49] Hoffmann C., Ziegler U., Buschmann A., Weber A., Kupfer L., Oelschlegel A., Hammerschmidt B., Groschup M.H., Prions spread via the autonomic nervous system from the gut to the central nervous system in catle incubating bovine spongiform encephalopathy, J. Gen. Virol. (2007) 88:1048–1055.

[50] Hoinville L.J., Decline in the incidence of BSE in cattle born after the introduction of the "feed ban", Vet. Rec. (1994) 134:274–275.

[51] Hopp P., Ulvund M.J., Jarp J., A case-control study on scrapie in Norwegian sheep flocks, Prev. Vet. Med. (2001) 51:183–198.

[52] Hornlimann B., Riesner D., Kretzschmar H. (Eds.), Prions in humans and animals, de Gruyter Berlin, New York, 2007.

[53] Houston F., Foster J.D., Chong A., Hunter N., Bostock C.J., Transmission of BSE by blood transfusion in sheep, Lancet (2000) 356:999–1000.

[54] Houston F., Goldmann W., Chong A., Jeffrey M., González L., Foster J., et al., Prion diseases: BSE in sheep bred for resistance to infection, Nature (2003) 423:498.

[55] Hunter N., Foster J., Chong A., McCutcheon S., Parnham D., Eaton S., et al., Transmission of prion diseases by blood transfusion, J. Gen. Virol. (2002) 83:2897–2905.

[56] Jackman R., Everest D.J., Schmerr M.J., Khawaja M., Keep P., Docherty J., Evaluation of a preclinical blood test for scrapie in sheep using immunocapillary electrophoresis, J. AOAC Int. (2006) 89:720–727.

[57] Jeffrey M., Wells G.A.H., Spongiform encephalopathy in a nyala (*Tragelaphus angasi*), Vet. Pathol. (1988) 25:398–399.

[58] Jeffrey M., Martin S., Gonzalez L., Ryder S.J., Bellworthy S.J., Jackman R., Differential diagnosis of infections with the bovine spongiform encephalopathy (BSE) and scrapie agents in sheep, J. Comp. Pathol. (2001) 125:271–284.

[59] Jeffrey M., Ryder S., Martin S., Hawkins S.A., Terry L.M., Berthelin-Baker C., Bellworthy S.J., Oral inoculation of sheep with the agent of bovine spongiform encephalopathy (BSE). 1. Onset and distribution of disease-specific PrP accumulation in brain and viscera. J. Comp. Pathol. (2001) 124:280–289.

[60] Jeffrey M., Martin S., Gonzalez L., Foster J., Langeveld J.P., van Zijderveld F.G., et al., Immunohistochemical features of PrP(d) accumulation in natural and experimental goat transmissible spongiform encephalopathies, J. Comp. Pathol. (2006) 134:171–181.

[61] Kao R.R., Houston F., Baylis M., Chihota C.M., Goldmann W., Gravenor M.B., et al., Epidemiological implications of the susceptibility to BSE of putatively resistant sheep, J. Gen. Virol. (2003) 84:3503–3512.

[62] Kelly D.F., Gaskell C.J., Spongy degeneration of the central nervous system in kittens, Acta Neuropathol. (1976) 35:151–158.

[63] Kinney H.C., Sidman R.L., Pathology of the spongiform encephalopathy in the gray tremor mutant mouse, J. Neuropathol. Exp. Neurol. (1986) 45:108–126.

[64] Kirkwood J.K., Cunningham A.A., Epidemiological observations on spongiform encephalopathies in captive wild animals in the British Isles, Vet. Rec. (1994) 135:296–303.

[65] Kong Q., Huang S., Zou W., Vanegas D., Wang M., Wu D., et al., Chronic wasting disease of elk: transmissibility to humans examined by transgenic mouse models, J. Neurosci. (2005) 25:7944–7949.

[66] Konold T., Ryder S.J., Lee Y.H., Stack M.J., Horrocks C., Green R., et al., Different prion disease phenotypes result from inoculation of cattle with two temporally separated sources of sheep scrapie from Great Britain, BMC Vet. Res. (2006) 2:31.

[67] Lasmézas C.I., Comoy E., Hawkins S., Herzog C., Mouthon F., Konold T., et al., Risk of oral infection with bovine spongiform encephalopathy agent in primates, Lancet (2005) 365:781–783.

[68] Lasmézas C.I., Deslys J.P., Demaimay R., Adjou K.T., Lamoury F., Dormont D., et al., BSE transmission to macaques, Nature (1996) 381:743–744.

[69] Lasmezas C.I., Deslys J.P., Robain O., Jaegly A., Béringue V., Peyrin J.M., et al., Transmission of the BSE agent to mice in the absence of detectable abnormal prion protein, Science (1997) 275:402–405.

[70] Lasmézas C.I., Fournier J.G., Nouvel V., Boe H., Marcé D., Lamoury F., et al., Adaptation of the bovine spongiform encephalopathy agent to primates and comparison with Creutzfeldt-Jakob disease: implications for human health, Proc. Natl. Acad. Sci. USA (2001) 98:4142–4147.

[71] Last J.M., A Dictionary of Epidemiology, 2nd ed., Oxford University Press, New York, USA, 1988.

[72] Lezmi S., Ronzon F., Bencsik A., Bedin A., Clavas D., Richard Y., et al., PrP^{d} accumulation in organs of ARQ/ARQ sheep experimentally infected with BSE by peripheral routes, Acta Biochim. Pol. (2006) 53:399–405.

[73] Ligios C., Sigurdson C.J., Santucciu C., Carcassola G., Manco G., Basagni M., et al., PrP^{Sc} in mammary glands of sheep affected by scrapie and mastitis, Nat. Med. (2005) 11:1137–1138.

[74] McIntyre M., Gubbins S., Sivam S.K., Baylis M., Flock-level risk factors for scrapie in Great Britain: analysis of a 2002 anonymous postal survey, BMC Vet. Res. (2006) 2:25.

[75] McLean A.R., Hoek A., Hoinville L.J., Gravenor M.B., Scrapie transmission in Britain: a recipe for a mathematical model, Proc. Biol. Sci. (1999) 266:2531–2538.

[76] Office Internationale Epizootie, Bovine spongiform encephalopathy, in: OIE manual of diagnostic

tests and vaccines for terrestrial animals, 5th ed., 2004, pp. 549–569.

[77] O'Rourke K.I., Baszler T.V., Besser T.E., Miller J.M., Cutlip R.C., Wells G.A., et al., Preclinical diagnosis of scrapie by immunohistochemistry of third eyelid lymphoid tissue, J. Clin. Microbiol. (2000) 38:3254–3259.

[78] Pattison I.H., Hoare M.N., Jebbett J.N., Watson W.A., Spread of scrapie to sheep and goats by oral dosing with foetal membranes from scrapie-affected sheep, Vet. Rec. (1972) 90:465–468.

[79] Pattison I.H., Hoare M.N., Jebbett J.N., Watson W.A., Further observations on the production of scrapie in sheep by oral dosing with foetal membranes from scrapie-affected sheep, Br. Vet. J. (1974) 130:65–67.

[80] Philippe S., Ducrot C., Roy P., Remontet L., Jarrige N., Calavas D., Sheep feed and scrapie, France, Emerging Infect. Dis. (2006) 11:1274–1279.

[81] Race R., Jenny A., Sutton D., Scrapie infectivity and proteinase K-resistant prion protein in sheep placenta, brain, spleen, and lymph node: implications for transmission and antemortem diagnosis, J. Infect. Dis. (1998) 178: 949–953.

[82] Robinson M.M., Hadlow W.J., Knowles D.P., Huff T.P., Lacy P.A., Marsh R.F., Gorham J.R., Experimental infection of cattle with the agents of transmissible mink encephalopathy and scrapie, J. Comp. Pathol. (1995) 113:241–251.

[83] Ronzon F., Bencsik A., Lezmi S., Vulin J., Kodjo A., Baron T., BSE inoculation to prion diseases-resistant sheep reveals tricky silent carriers, Biochem. Biophys. Res. Commun. (2006) 350:872–877.

[84] Ryder S., Dexter G., Bellworthy S., Tongue S., Demonstration of lateral transmission of scrapie between sheep kept under natural conditions using lymphoid tissue biopsy, Res. Vet. Sci. (2004) 76:211–217.

[85] Sharpe A.H., Hunter J.J., Chassler P., Jaenisch R., Role of abortive retroviral infection of neurons in spongiform CNS degeneration, Nature (1990) 346:181–183.

[86] Schoon H.A., Brunckhorst D., Pohlenz J., Spongiforme encephalopathie beim Rothalsstrauss (*Struthio camelus*) Eim Kasuistischer Beitrag, Teirartztl. Prax. (1991) 19:263–265.

[87] Schoon H.A., Brunckhorst D., Pohlenz J., Beitrag zur neuropathology beim Rothalsstrauss (*Struthio camelus*) – Spongiforme Encephalopathie, Verh. Ber. Erkrg. Zootiere (Akademischer Verlag) (1991) 33: 309.

[88] Schätzl H.M., The phylogeny of mammalian and nonmammalian prion proteins, in: Hörnlimann B., Riesner D., Kretzschmar H. (Eds.), Prions in humans and animals, de Gruyter, Berlin, Germany, 2007, pp. 119–121.

[89] Sigurdson C.J., A prion disease of cervids: chronic wasting disease, Vet. Res. (2008) 39:41.

[90] Sisó S., Gonzalez L., Jeffrey M., Martin S., Chianini F., Steele P., Prion protein in kidneys of scrapie-infected sheep, Vet. Rec. (2006) 159:327–328.

[91] Somerville R.A., Oberthur R.C., Havekost U., MacDonald F., Taylor D.M., Dickinson A.G., Characterization of thermodynamic diversity between transmissible spongiform encephalopathy agent strains and its theoretical implications, J. Biol. Chem. (2002) 277:11084–11089.

[92] Soto C., Prions: the new biology of proteins, CRC Press, Florida, USA, 2006.

[93] St Rose S.G., Hunter N., Matthews L., Foster J.D., Chase-Topping M.E., Kruuk L.E., et al., Comparative evidence for a link between Peyer's patch development and susceptibility to transmissible spongiform encephalopathies, BMC Infect. Dis. (2006) 6:5.

[94] Taylor D.M., Woodgate S.L., Atkinson M.J., Inactivation of the bovine spongiform encephalopathy agent by rendering procedures, Vet. Rec. (1995) 137:605–610.

[95] Taylor D.M., Woodgate S.L., Fleetwood A.J., Cawthorne R.J., The effect of rendering procedures on scrapie agent, Vet. Rec. (1997) 141:643–649.

[96] Taylor D.M., Woodgate S.L., Rendering practices and inactivation of transmissible spongiform encephalopathy agents, Rev. Sci. Tech. (2003) 22:297–310.

[97] Terry L.A., Marsh S., Ryder S.J., Hawkins S.A., Wells G.A., Spencer Y.I., Detection of disease-specific PrP in the distal ileum of cattle orally exposed to the agent of bovine spongiform encephalopathy, Vet. Rec. (2003) 152:387–392.

[98] Tongue S.C., Pfeiffer D.U., Warner R., Elliott H., del Rio Vilas V., Estimation of the relative risk of developing clinical scrapie: the role of prion protein (PrP) genotype and selection bias, Vet. Rec. (2006) 158:43–50.

[99] Touzeau S., Chase-Topping M.E., Matthews L., Lajous D., Eychenne F., Hunter N., et al., Modelling the spread of scrapie in a sheep flock: evidence for increased transmission during lambing seasons, Arch. Virol. (2006) 151:735–751.

[100] Tranulis M., Influence of the prion protein gene, *Prnp*, on scrapie susceptibility in sheep, APMIS (2002) 110:33–43.

[101] Tuo W., Zhuang D., Knowles D.P., Cheevers W.P., Sy M.S., O'Rourke K.I., PrP^{c} and PrP^{Sc} at the fetal-maternal interface, J. Biol. Chem. (2001) 276:18229–18234.

[102] Van den Ingh T.S., Mandigers P.J., van Nes J.J., A neuronal vacuolar disorder in young rottweiler dogs, Vet. Rec. (1998) 142:245–247.

[103] Van Keulen L.J., Schreuder B.E., Meloen R.H., Mooij-Harkes G., Vromans M.E., Langeveld J., Immunohistochemical detection of prion protein in lymphoid tissues of sheep with natural scrapie, J. Clin. Microbiol. (1996) 34:1228–1231.

[104] Vascellari M., Nonno R., Mutinelli F., Bigolaro M., Di Bari M.A., Melchiotti E., et al., PrP^{Sc} in salivary glands of scrapie-affected sheep. J. Virol. (2007) 81:4872–4876.

[105] Vilotte J.L., Soulier S., Essalmani R., Stinnakre M.G., Vaiman D., Lepourry L., et al., Markedly increased susceptibility to natural sheep scrapie of transgenic mice expressing ovine PrP. J. Virol. (2001) 75:5977–5984.

[106] Wadsworth J.D., Asante E.A., Desbruslais M., Linehan J.M., Joiner S., Gowland I., et al., Human prion protein with valine 129 prevents expression of variant CJD phenotype, Science (2004) 306:1793–1796.

[107] Wells G.A., Scott A.C., Johnson C.T., Gunning R.F., Hancock R.D., Jeffrey M., et al., A novel progressive spongiform encephalopathy in cattle, Vet. Rec. (1987) 121:419–420.

[108] Wells G.A., Wells M., Neuropil vacuolation in brain: a reproducible histological processing artefact, J. Comp. Pathol. (1989) 101:355–362.

[109] Wells G.A., Dawson M., Hawkins S.A., Green R.B., Dexter I., Francis M.E., et al., Infectivity in the ileum of cattle challenged orally with bovine spongiform encephalopathy, Vet. Rec. (1994) 135:40–41.

[110] Wells G.A., Simmons M.M., The essential lesion profile of bovine spongiform encephalopathy (BSE) in cattle is unaffected by breed or route of infection, Neuropathol. Appl. Neurobiol. (1996) 22:453.

[111] Wells G.A., Hawkins S.A., Green R.B., Austin A.R., Dexter I., Spencer Y.I., et al., Preliminary observations on the pathogenesis of experimental bovine spongiform encephalopathy (BSE): an update, Vet. Rec. (1998) 142:103–106.

[112] Wells G.A., Hawkins S.A., Green R.B., Spencer Y.I., Dexter I., Dawson M., Limited detection of sternal bone marrow infectivity in the clinical phase of experimental bovine spongiform encephalopathy (BSE), Vet. Rec. (1999) 144:292–294.

[113] Wells G.A., Spiropoulos J., Hawkins S.A., Ryder S.J., Pathogenesis of experimental bovine spongiform encephalopathy (BSE): preclinical infectivity in tonsil and observations on the distribution of lingual tonsil in slaughtered cattle, Vet. Rec. (2005) 156:401–407.

[114] Wells G.A., Hawkins S.A., Austin A.R., Ryder S.J., Done S.H., Green R.B., et al., Studies of the transmissibility of the agent of bovine spongiform encephalopathy to pigs, J. Gen. Virol. (2003) 84:1021–1031.

[115] Wells G.A., Ryder S.J., Hadlow W.J., The pathology of prion diseases in animals, in: Hörnlimann B., Riesner D., Kretzschmar H. (Eds.), Prions in humans and animals, de Gruyter, Berlin, Germany, 2007, pp. 315–323.

[116] Wells G.A., Pohlenz J., Hawkins S.A., Matthews D., Portrait of a spongiform encephalopathy in birds and the transmissibility of mammalian prion diseases to birds, in: Hörnlimann B., Riesner D., Kretzschmar H. (Eds.), Prions in humans and animals, de Gruyter, Berlin, Germany, 2007, pp. 279–282.

[117] Wells G.A., Konold T., Arnold M.E., Austin A.R., Hawkins S.A., Stack M., et al., Bovine spongiform encephalopathy: the effect of oral exposure dose on attack rate and incubation period in cattle, J. Gen. Virol. (2007) 88:1363–1373.

[118] Wilesmith J.W., Wells G.A., Cranwell M.P., Ryan J.B., Bovine spongiform encephalopathy: epidemiological studies, Vet. Rec. (1988) 123:638–644.

[119] Wilesmith J.W., Ryan J.B., Atkinson M.J., Bovine spongiform encephalopathy: epidemiological studies on the origin, Vet. Rec. (1991) 128:199–203.

[120] Wilesmith J.W., Ryan J.B., Hueston W.D., Bovine spongiform encephalopathy: case-control studies of calf feeding practices and meat and bonemeal inclusion in proprietary concentrates, Res. Vet. Sci. (1992) 52:325–331.

[121] Wilesmith J.W., Ryan J.B., Bovine spongiform encephalopathy: observations on the incidence during 1992, Vet. Rec. (1993) 132:300–301.

[122] Wilesmith J.W., Wells G.A., Ryan J.B., Gavier-Widen D., Simmons M.M., A cohort study to examine maternally-associated risk factors for bovine spongiform encephalopathy, Vet. Rec. (1997) 141:239–243.

[123] Williams E.S., Miller M.W., Portrait of chronic wasting disease in deer species, in: Hörnlimann B., Riesner D., Kretzschmar H. (Eds.), Prions in humans and animals, de Gruyter, Berlin, Germany, 2007, pp. 257–262.

[124] Wrathall A.E., Brown K.F., Sayers A.R., Wells G.A., Simmons M.M., Farrelly S., et al., Studies of embryo transfer from cattle clinically affected by bovine spongiform encephalopathy (BSE), Vet. Rec. (2002) 150:365–378.

[125] Wyatt J.M., Pearson G.R., Smerdon T.N., Gruffydd-Jones T.J., Wells G.A., Wilesmith J.W., Naturally occurring scrapie-like spongiform encephalopathy in five domestic cats, Vet. Rec. (1991) 129:233–236.

[126] Zlotnik I., Rennie J.C., A comparative study of the incidence of vacuolated neurons in the medulla from apparently healthy sheep of various breeds, J. Comp. Pathol. (1958) 68:411–415.

別紙様式第2-1
番号9

医薬品
医薬部外品　　研究報告　調査報告書
化粧品

識別番号・報告回数			報告日	第一報入手日 2008年3月21日	新医薬品等の区分 該当なし	厚生労働省処理欄

一般的名称	乾燥濃縮人アンチトロンビンIII	研究報告の公表状況	56th Annual Meeting of the American-Society-of-Tropical-Medicine-and-Hygiene 1044	公表国 アメリカ
販売名（企業名）	①ノイアート（ベネシス） ②ノイアート静注用1500単位（ベネシス）			

研究報告の概要

ヒト顆粒球アナプラズマ症［Human Granulocytic Anaplasmosis (HGA)］の発生率は、1999年以来2倍になった。原因病原体のAnaplasma phagocytophilumは、ニューイングランドの風土病であり、主にマダニ Ixodes scapularis の流行によってヒトに感染する。A. phagocytophilumによって引き起こされる疾患は、無症候なものから重篤なものまであり、一様ではない。A. phagocytophilumの輸血感染が1例報告されているが、現在HGAのスクリーニングは実施されていない。
この病原体によって引き起こされる血液の安全リスクを調査するため、我々はコネチカット州及びマサチューセッツ州の血液ドナーの陽性率を測定した。血液サンプルを春の後半から冬の初め（2001-2005年）及び2006年の初めから1年間、採取した。参加ドナーからの血清について、間接蛍光分析（IFA）を使ってA. phagocytophilumのヒトIgG抗体の試験を実施した。IFA力価が≧1:64のときに陽性とした。IFAによって検査した15,828名のドナー中、432名（2.7%）がA. phagocytophilum抗体陽性であった。力価の分布は以下の通りであった。1:64が256名（59%）、1:128が115名（27%）、1:256が42名（9.7%）、1:512が14名（3.2%）、≧1:1024が5名（1.2%）であった。マサチューセッツ州ドナーの陽性率は2.2%（30/1,346）、コネチカット州ドナーの陽性率は2.8%（402/14,482）であった。血清陽性率ピークは、次の月に生じた：2月（4.7%）、12月（3.7%）と9月（3.4%）。全体的に、年間陽性率は1.7%（2004年）から4.1%（2001年）まで変化が見られた。年間血清陽性率で観察された変動は、おそらくA. phagocytophilumの複雑なライフサイクルに影響する気候および環境因子よるものであろう。
比較的高い陽性率が持続していることから、A. phagocytophilumの血液安全性に及ぼす影響を調査する必要がある。

報告企業の意見	今後の対応
米国ニューイングランド地方の供血者中のA. phagocytophilumの血清陽性率が比較的高い値を持続しているとの報告である。 アナプラズマ属菌は、ウシ科、シカ科、ラクダ科動物の赤血球内に寄生する直径0.2～1μmのグラム陰性桿菌である。万一、原料血漿にアナプラズマ属菌が混入したとしても、除菌ろ過等の製造工程にて除去されるものと考えている。	本報告は本剤の安全性に影響を与えないと考えるので、特段の措置はとらない。

使用上の注意記載状況・その他参考事項等

代表としてノイアート（献血）の記載を示す。
2. 重要な基本的注意
(1) 本剤の原材料となる献血者の血液については、HBs抗原、抗HCV抗体、抗HIV-1抗体、抗HIV-2抗体、抗HTLV-I抗体陰性で、かつALT(GPT)値でスクリーニングを実施している。更に、プールした試験血漿については、HIV-1、HBV及びHCVについて核酸増幅検査（NAT）を実施し、適合した血漿を本剤の製造に使用しているが、当該NATの検出限界以下のウイルスが混入している可能性が常に存在する。本剤は、以上の検査に適合した血漿を原料として、Cohnの低温エタノール分画で得た画分から人アンチトロンビンIIIを濃縮・精製した製剤であり、ウイルス不活化・除去を目的として、製造工程において60℃、10時間の液状加熱処理及びろ過膜処理（ナノフィルトレーション）を施しているが、投与に際しては、次の点に十分注意すること。