Selenium and GPX activity in blood samples from pregnant and non-pregnant ewes and selenium in hay on scrapie-free, scrapie-prone and scrapie-afflicted farms in Iceland

TORKELL JÓHANNESSON [1]

KRISTÍN BJÖRG GUDMUNDSDÓTTIR [2]

TRYGGVI EIRÍKSSON [3]

JED BARASH [1, 2]

JAKOB KRISTINSSON [1]

AND

SIGURDUR SIGURDARSON [2]

[1]*Department of Pharmacology and Toxicology, Institute of Pharmacy, Pharmacology and Toxicology, University of Iceland, Hofsvallagata 53, 107 Reykjavík*
E-mail: dr.thorkell@simnet.is
E-mail: jedbarash@hotmail.com
E-mail: jakobk@hi.is

[2]*Chief Veterinary Officer, Section for Animal Diseases, at the Institute of Experimental Pathology, University of Iceland, Keldur, 112 Reykjavík*
E-mail: kristigu@hi.is
E-mail: sigsig@hi.is

[3]*The Agricultural Research Institute, Keldnaholt, 112 Reykjavík*
E-mail: tryggvie@rala.is

SUMMARY

The aim of this study was to investigate whether an imbalance of selenium (Se) or the activity of glutathione peroxidase (GPX) in blood of sheep might be connected with the sporadic occurrence of scrapie in Iceland. Hay samples (in total 88) from the harvests in 2002 and/or 2003 and blood samples (in total 125) were collected during the experimental period (autumn 2002 – autumn 2003) from 2–5 year old ewes on 19 farms, 14 of which are located in two adjacent valleys in northern Iceland. Farms were divided in three categories: Scrapie-free (never afflicted by scrapie, or prior to 1960 and then restocked with healthy sheep), scrapie-prone (afflicted by scrapie after 1980 and restocked with healthy sheep) and scrapie-afflicted (scrapie diagnosed in respective herds during the experimental period). The farms are all located in four scrapie affected counties. Selenium concentration was low or very low in almost all hay samples and was statistically the same on farms in all categories. Information gathered from 20 veterinarians all over the country revealed that selenium deficiency in lambs is a ubiquitous phenomenon in Iceland. In ewes on eight scrapie-free and scrapie-prone farms the selenium concentration in whole blood declined sharply from the non-pregnant state in autumn to the pregnant state the following spring. The glutathione peroxidase activity declined concomitantly in whole blood, but to a lesser degree, resulting in a significantly higher GPX/Se ratio in the pregnant state and a far less significant correlation between Se concentration and GPX activity than while the ewes were not pregnant. Ewes on scrapie-free farms had a significantly higher concentration of Se and higher GPX activity in the non-pregnant state than non-pregnant ewes on scrapie-prone farms but this was not true of the pregnant state. Se concentration and GPX activity in non-pregnant ewes on 4 scrapie-afflicted farms did not differ significantly from neither non-pregnant ewes on scrapie-free farms nor scrapie-prone farms. The three main conclusions of this research are: First, the low selenium concentration in hay on farms in all categories, and probably all over the country, is not likely to be directly connected to sporadic occurrence of clinical scrapie. Second, it is controversial whether ewes on scrapie-prone or scrapie-afflicted farms may differ in selenium status from ewes on scrapie-free farms. Third, it is strictly necessary to define the status of the ewes, non-pregnant, pregnant or otherwise, at the time of sampling for determination of selenium concentration or GPX activity in blood. This last point applies equally to studies relevant to other conditions as well as to scrapie.

Key words: blood, GPX activity, hay, scrapie, selenium, sheep

YFIRLIT

Selen og glútatíonperoxídasavirkni (GPX virkni) í blóði úr meðgengnum og ólembdum ám og selen í heysýnum á riðulausum býlum, fjárskiptabýlum og riðusýktum býlum á Íslandi

Tilgangur rannsóknarinnar var að kanna, hvort breytingar á seleni eða GPX virkni í blóði sauðfjár gæti tengst staksettri (sporadic) uppkomu riðu hér á landi. Heysýnum úr rúlluböggum (alls 88 sýnum) frá uppskeru áranna 2002 og/eða 2003 og blóðsýnum (alls 125 sýni) var safnað á rannsóknartímabilinu (frá hausti 2002 – til hausts 2003) úr 2–5 vetra gömlum ám á 19 býlum, en 14 þeirra eru í tveimur samliggjandi dölum norðanlands (Vatnsdalur – Víðidalur). Býlum var skipt í þrjá flokka: riðulaus býli („scrapie-free"; riða annaðhvort aldrei komið upp eða fyrir 1960 og þá skipt um fé), fjárskiptabýli („scrapie-prone"; riða komið upp eftir 1980, en síðar skipt um fé) og riðusýkt býli („scrapie-afflicted"; riða í gangi í hlutaðeigandi hjörðum á tímabilinu). Býli þessi eru öll staðsett í fjórum sýslum, þar sem riðu hefur orðið vart. Þéttni selens (Se) var lítil eða mjög lítil í nær öllum heysýnum og var staðtölulega hin sama í sýnum frá býlum í öllum flokkum. Upplýsingar, sem safnað var frá 20 dýralæknum um allt land, gáfu til kynna, að selenskortur í lömbum væri alls staðar algengur á landinu. Í ám á átta riðulausum býlum og fjárskiptabýlum minnkaði þéttni Se í blóði mjög frá hausti, þegar ærnar voru ólembdar, og til næsta vors, þegar nærri kom burði. GPX virkni í blóðinu minnkaði einnig, en minna. Hlutfallið milli GPX og Se, GPX/Se, var því marktækt hærra að vori, þegar nær dró burði, en að hausti og marktæk fylgni milli þéttni Se og virkni GPX minnkaði stórlega frá hausti til vors. Í ólembdum ám á riðulausum býlum var marktækt meira selen í blóðinu og meiri GPX virkni en í blóði ólembdra áa á fjárskiptabýlum. Þessi munur var ekki til staðar að vori, þegar nálgaðist burð. Í blóði ólembdra áa á fjórum riðusýktum býlum var þéttni selens og virkni GPX staðtölulega hin sama og í blóði ólembdra áa á riðulausum býlum og fjárskiptabýlum. Af þessari rannsókn má einkum draga þrjár ályktanir: Í fyrsta lagi er lítil þéttni Se í heyi á býlum í öllum flokkum, og mjög sennilega um allt land, ekki líkleg til þess beinlínis að tengjast staksettri uppkomu riðu. Í öðru lagi er með öllu óljóst, hvort selenbúskapur fjár á riðusýktum býlum eða fjárskiptabýlum kunni að vera annar en í fé á riðulausum bæjum. Í þriðja lagi er mjög mikilvægt að skilgreina nákvæmlega ástand áa, sem taka á úr blóðsýni til ákvörðunar á selen og/eða virkni glútatíonperoxídasa (þungun, ekki þungun, beit, innistaða o.fl.). Þetta á jafnt við rannsóknir á riðu og aðrar rannsóknir.

INTRODUCTION

Scrapie is found sporadically each year in one or more farms in Iceland, usually in four or five localities in southern and northern Iceland (especially nr. 1-4, Fig. 1). The sporadic occurrence of scrapie thus apparently occurs in a non-random fashion suggesting a link to some environmental factor (or factors) that either may cause or is a risk factor for the development of clinical scrapie. Among such factors might be metals like copper and manganese that are essential components of important enzymes in mammals (e.g. Jóhannesson *et al.* 2004) or selenium. In mammalian tissues selenium functions essentially as an active site in glutathione peroxidases (GPXs) that scavenge hydrogen peroxide and other peroxides and of which at least four have been described (Arthur 2000). GPX activity in erythrocytes is generally taken as an index of the ability to scavenge hydrogen peroxide formed normally *in vivo* by the activity of superoxide dismutase (SOD1) or peroxides resulting from oxidative attack on cell membranes or other tissue constituents (Símonarson 1986, Jóhannesson *et al*. 2003). In the blood of sheep (and some other mammals) more than 80% of the selenium concentration is found in erythrocytes (Nève 1989). Glutathione peroxidase activity in whole blood, expressed as units g Hb^{-1} or otherwise, has accordingly been used in Iceland as indicative of the concentration of selenium in the blood of sheep (Eiríksdóttir *et al*. 1981, Símonarson *et al*. 1984) and also in cows or heifers (Þorkelsson 1997, Arnþórsdóttir 2002). In a preliminary study it was found that GPX activity in blood from ewes on scrapie-afflicted or scrapie-prone farms was significantly lower than in blood from ewes on less scrapie-prone or scrapie-free farms. Selenium was, however, not determined in these experiments (Jóhannesson *et al.* 2003).

This study is essentially based on fourteen scrapie-free, scrapie-prone and scrapie-afflicted farms in two adjacent valleys in northern Iceland where scrapie has occurred sporadically on certain farms throughout recent decades whereas on other farms in that area sheep have remained free of the disease. Included in the study are also three scrapie-afflicted farms diagnosed during the experimental period (autumn 2002 – autumn 2003) and two scrapie-free farms but localised in other parts of the country. On the farms hay samples were collected from the 2002 and/or 2003 harvests and the concentration of selenium determined. Blood samples were collected from a representative number of ewes on most of the farms and GPX activity as well as the concentration of selenium determined in whole blood. When feasible, blood samples were taken from the ewes both not pregnant in the autumn 2002 and again in the pregnant state in the spring 2003. Finally a detailed questionnaire was sent to a number of veterinarians located at a various places around the country. This was done in order to assess the degree to which selenium deficiency might be recognized as a clinical problem of importance in Iceland.

The primary aim of this research was to study the possible role of an imbalance of selenium or the activity of glutathione peroxidase on the occurrence of scrapie in Iceland.

MATERIALS AND METHODS

Categories of farms and collection of hay and blood samples

Fourteen farms located in the Vatnsdalur – Vídidalur area (two adjacent valleys running parallel, north to south in northern Iceland) were divided in three categories. *Scrapie-free*: Farms never afflicted by scrapie, or prior to 1960 and then restocked with healthy sheep. *Scrapie-prone*: Farms afflicted by scrapie after 1980 and subsequently restocked with healthy sheep. *Scrapie-afflicted*: Farms where scrapie had been diagnosed in the herds during the experimental period (autumn 2002 - autumn 2003). Included in the study were also three scrapie-afflicted farms diagnosed during the experimental period, as well as two scrapie-free farms located in other parts of the country. It should be noted that the farms in all categories were located in five areas in four scrapie-affected counties (Figure 1) and that on the scrapie-afflicted farms all sheep were, according to government regulations, killed shortly after the disease had been diagnosed and the farms placed in quarantine.

The forage samples consisted mostly of grass silage from round-bales wrapped in plastic with variable dry matter content (from 30-70%). From each bale, samples were taken at 3-4 sites by an experienced person wearing plastic gloves (about 300-400 g from each bale), and placed directly into labeled plastic bags. In some instances it was not feasible to slit the bales open. In these instances the grass silage samples were collected by an electric

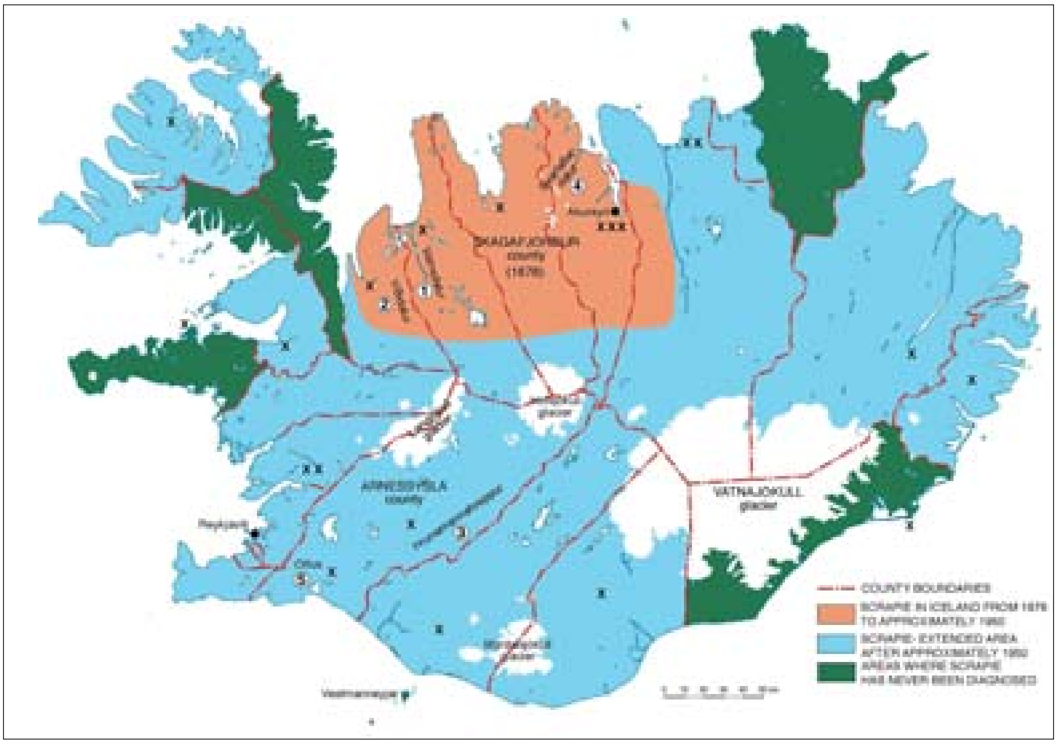

Figure 1. Scrapie in Iceland, localities of farms and sites of veterinarians.
Scrapie arrived in Iceland with an infected, imported ram of foreign stock in Skagafjördur in 1878. It was confined to a part of northern Iceland until ca. 1950 (orange). Scrapie afterwards spread patchily to greater or lesser parts of all counties (blue) except for four (green) until 1978 when successful preventive measures were first systemically enforced (Sigurdarson 1991). The large green area in the north-east indicates one of the main areas in the country that provides scrapie-free, healthy sheep for restocking formerly scrapie-afflicted farms. The numbers 1-5 indicate the five different localities of the 19 farms where hay samples were collected for analysis of selenium and blood samples collected from ewes for analysis of selenium and GPX activity. X indicates the various locations of twenty veterinarians to whom questionnaires were sent.

hay sampler with a 4 cm diameter. Although no strictly controlled comparison has been carried out of the two methods, results of metal analyses in this and our previous study (e.g. on grass silage from farms in Vatnsdalur valley) were the same independent of which one of the two methods was applied (cf. Jóhannesson *et al*. 2004). The sample bags were then tightly closed and kept in a cool place until analysed. About three different grass silage samples (two to five) were collected at a time from the 2002 and/or 2003 harvests on each of the 18 farms, making a total of 88 samples. Samples were taken of grass silage from the cultivated home fields and usually from older and more recently cultivated plots, respectively.

A few farmers still adhere to the old practice of hay making, that is, harvesting the forage as fully dry (> 85% dry). In those few instances samples were collected by hand as described above. For convenience all samples are referred to as *hay* whether fully dry (classical hay) or only partially dry (grass silage).

For each sheep two blood samples were drawn from the jugular vein at one side of the neck by a skilled individual. The first blood sample for the determination of glutathione peroxidase activity in whole blood was drawn into a 9 ml tube containing the lithium salt of heparin. The second blood sample for the determination of selenium in whole blood was collected in a 7.5 ml tube (Sarstedt) specially prepared for metal analysis. Samples used for determination of glutathione peroxidase activity were analysed within 48 hours. The blood samples for selenium determination were kept frozen until analysed. Blood samples were collected from ewes 2-5 year old on 14 farms in all categories, usually from 5-7 sheep on each farm at a time. The total number of blood samples was 125.

On eight farms in the Vatnsdalur – Vídidalur area, characterized as scrapie-free and scrapie-prone, respectively, blood samples were collected in two rounds. In the first round, in October 2002, samples were taken a few weeks after the sheep had been rounded up and driven from the highland pastures and before the animals were admitted to stables for winter feeding. From early December 2002 to January 2003, the ewes were ushered to rams for breeding. A second blood sampling round took place in March – April 2003 because pregnant ewes fed in stables for prolonged periods may exhibit altered selenium levels or enzymic activities. In ewes on the scrapie-afflicted farms (six in number of which three were outside the Vatnsdalur – Vídidalur area) blood samples were collected either while the ewes were not pregnant or in the pregnant state as the herds were, as stated before, culled shortly after diagnosis was made.

A detailed questionnaire was sent to 20 veterinarians around the country. They were asked about how frequently signs of selenium deficiency, especially in newborn lambs, but also in heifers and foals, were observed in their respective areas. Inquiry was also made about what preventive measures, such as injection of lambs with selenite, the use of special feeding salts or fertilizers, were taken in cases of suspected selenium deficiency.

The location of the farms, the scrapie situation in Iceland and the localities where the veterinarians worked to whom questionnaires were sent are shown in Figure 1. A summary of the number of farms in the three categories, their location in the country, the number of samples collected, as well as the survey of blood samples are given in Table 1.

Table 1. Number of farms in each category, their location in the country and total number of hay samples analysed from the 2002 and/or 2003 harvests collected on farms in the resepective categories. - Blood samples were collected on eight scrapie-free and scrapie-prone farms and six scrapie-afflicted farms. On one scrapie afflicted-farm (local. 1), not included in the table, blood samples were collected but, by omission, no hay samples.

Category	No. of farms	Total number of hay samples	Location (see Figure 1)
Scrapie-free	7	34	Localities 1, 2, 5
Scrapie-prone	6	30	Localities 1, 3
Scrapie-afflicted	5	24	Localities 2, 3, 4, 5

Preparation of samples and Se analyses

Hay samples were dried at 65º C in a forced air oven for 48 hours. When stabilized at room temperature samples were milled in a hammer mill to pass through a 1 mm screen. Milled samples were weighed for metal analyses and at the same time weighed to estimate the weight of dry matter. Samples were then digested in duplicate in closed quartz bombs in concentrated HNO_3 (Merck, Suprapur) with temperature programming up to 180° C. After digestion selenium in the samples was reduced from Se (VI) to Se (IV) by 5M HCl (Merck, Suprapur) for at least 16 hours at room temperature. After a suitable dilution the samples were analysed for selenium by hydride generation atomic absorption (Perkin Elmer MHS-20). A typical method limit of detection was 10 ng g^{-1} of the samples.

The results are the means of duplicate measurements and are expressed as µg kg^{-1} of dry matter. External standards, prepared from certified and traceable commercially available stock solution (Merck), were taken through the analytical procedure to confirm accuracy of measurements. Analysis of a certified reference material, sea lettuce (*Ulva lactuca* BCR nr. 279), as a certified grass reference standard was to our knowledge not available, containing a certified amount of 0.593 ± 0.032 µg g^{-1}, gave a recovery of 90 ± 5% (n = 3). A standard addition of 10 ppb selenium gave a recovery of 110 ± 5% (n = 3).

Blood samples were digested in two different ways. First, the samples were digested in duplicate in closed quartz bombs in HNO_3 (Merck, Suprapur). After digestion, the solvent was evaporated to dryness and the residue diluted to an appropriate volume by dilute HNO_3 (Merck, Suprapur). The selenium in the samples was then reduced from Se (VI) to Se (IV) and the amount of selenium determined as described above. Secondly, duplicate samples were solubilized with HNO_3 and HCl followed by dry-ashing with magnesium nitrate (Merck, Suprapur) as an ashing aid. The residue was dissolved in 5M HCl (Merck, Suprapur), and, after reduction of selenium in the samples and dilution to an appropriate volume, subjected to analysis as already described. Accuracy of measurements by use of external standards and recovery of certified Se amounts (also including mussel tissue BCR, CRM 278) were secured in the same way as for the hay analysis, as a sheep blood reference standard was to our knowledge not available.

The results of the selenium determinations were comparable independent of the digestion procedure used. On many occasions both digestion procedures were applied to the samples but on some occasions only one was used. The results are accordingly presented either as the means of duplicate or quadruplicate measurements and are expressed as ng ml^{-1}.

All determinations of selenium were performed at the Fisheries Laboratories, Reykjavík, Iceland.

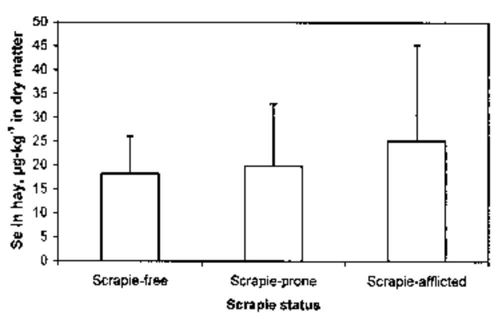

Figure 2. Mean selenium (Se) concentration (µg kg^{-1} in dry matter) in hay samples from 18 scrapie-free, scrapie-prone and scrapie-afflicted farms (see also Table 1). Vertical bars show the standard deviation.

Determination of GPX activity

Glutathione peroxidase activity in whole blood was determined with a spectrophotometric assay originally described by Paglia & Valentine (1967). The reagents used in the analyses were obtained from Randox Laboratories Ltd., UK. Glutathione peroxidase activity was determined within 2 days after sample collection using a UNIFAST 3 analyzer (Sclavo Diagnostics). The results are expressed as units g Hb^{-1}. The coefficient of variation (C.V.) for this method was 8.4%. All sheep had Hb levels in the normal range (90–145 g l^{-1}).

All determinations of GPX activity were performed at the Institute of Experimental Pathology, University of Iceland, Keldur, Reykjavík.

Statistical analysis

A one way analysis of variance (ANOVA) was used for the comparison between categories of selenium concentrations in blood or hay and glutathione peroxidase activity in blood. The Student-Newman-Keuls test was used for all pairwise comparisons where significant differences were found in the ANOVA. Linear regression analysis was used to test the relationship between selenium concentrations and glutathione peroxidase activity in blood.

Table 2. Mean selenium (Se) concentration and mean GPX activity in whole blood ± S.D. of non-pregnant and pregnant ewes (samples from 5-6 animals at a time) on eight scrapie-free and scrapie-prone farms in Vatnsdalur valley (locality 1, Figure 1).

Farm no.	Category	Se (ng ml^{-1}) (non-pregnant)	Se (ng ml^{-1}) (pregnant)	GPX (units g Hb^{-1}) (non-pregnant)	GPX (units g Hb^{-1}) (pregnant)
1	Scrapie-free	191 ± 35	81 ± 23	352 ± 106	179 ± 46
2	“	133 ± 27	89 ± 13	236 ± 81	201 ± 40
3	“	230 ± 66	66 ± 14	314 ± 108	166 ± 38
4	Scrapie-prone	178 ± 32	122 ± 40	287 ± 94	216 ± 74
5	“	127 ± 46	43 ± 11	186 ± 79	132 ± 37
6	“	136 ± 39	74 ± 29	237 ± 82	141 ± 55
7	“	102 ± 49	57 ± 5	184 ± 85	237 ± 49
8	“	171 ± 40	40 ± 13	224 ± 68	123 ± 15

Slopes of two regression lines were compared as described by Glantz (1997).

RESULTS

The mean selenium concentration in hay samples from farms in all three categories was in the range 15-25 µg kg^{-1} and the difference between categories was not statistically significant ($P > 0.05$). Singularly high values (60-90 µg kg^{-1}) were found in a few samples from farms in all categories but especially from scrapie-afflicted farms (Figure 2).

In the blood of ewes on eight scrapie-free and scrapie-prone farms in the Vatnsdalur Valley (local. 1, Figure 1) selenium concentration declined sharply from the non-pregnant state in the autumn 2002 to the pregnant state of the ewes in the spring 2003. A concomitant decrease, but to a lesser degree, in GPX activity in blood was also observed (Table 2). The GPX/Se ratio was thus significantly higher in the pregnant state than in the non-pregnant state. Although the selenium concentration was

Table 3. Mean selenium (Se) concentration and mean GPX activity in whole blood ± S.D. of ewes on six scrapie-afflicted farms at various localities in the country. The ewes were either non-pregnant or pregnant when scrapie was diagnosed (usually 1-2 animals per herd). Samples were collected from 7-10 animals just before killing the respective herds.

Farm no.	Pregnancy status	Se ng ml^{-1}	GPX units g Hb^{-1}	Location (see fig. 1)
9	pregnant	120 ± 85	295 ± 85	2
10	“	121 ± 51	254 ± 51	5
11	non-pregnant	191 ± 47	516 ± 40	3
12	“	87 ± 26	189 ± 39	1
13	“	289 ± 56	468 ± 191	4
14	“	56 ± 16	82 ± 34	2

Figure 3. The relationship between selenium concentration and GPX activity in whole blood in sheep on eight farms in the Vatnsdalur valley. The samples were collected in autumn 2002 (non-pregnant; filled circles) and spring 2003 (pregnant; open circles). Regression lines were fitted to the data points by the method of least squares. The slopes of the two lines were significantly different ($P < 0.01$).

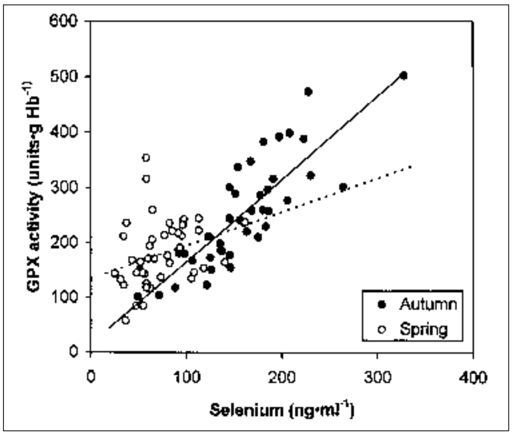

Table 4. Answers of 20 veterinarians to whom questionnaires were sent (see text).

Question no. 1: Do you see signs of selenium deficiency in your area?			
Species:	Lambs	Heifers, calves	Foals*
Number of veterinarians answering yes:	20 (100%)	10 (50%)	4 (20%)
* Suspicions of selenium deficiency in foals			
Question no. 2: Do you apply/recommend prophylactic measures for sheep (lambs)?:			
	Se/VitE injections	Feed suppl.	Se enriched fertilizer
Number of veterinarians answering yes:	20 (100%)	12 (60%)	0

disproportionately lower in the pregnant state than when the ewes were not pregnant the two parameters were positively correlated on both occasions ($P < 0.05$) albeit barely in the pregnant state (R^2=0.7019 and R^2=0.1060, respectiverly) (Figure 3).

In the blood of non-pregnant ewes on the three scrapie-free farms in the Vatnsdalur valley concentration of selenium and GPX activity in the blood were significantly higher ($P < 0.05$), respectively, than in the blood of the non-pregnant ewes on the five scrapie-prone farms (Table 2). In the pregnant state the differences between the respective parameters did not reach the level of significance ($P > 0.05$) (Table 2).

Scrapie was diagnosed on six farms during the experimental period (usually 1-2 animals per herd). When scrapie was diagnosed ewes on four of these farms (two of which were in the Vatnsdalur – Vídidalur area) were in the non-pregnant state whereas the ewes were pregnant on two of the farms (Table 3). The selenium concentration and GPX activity were quite variable in ewes on these four farms, but neither parameter differed significantly ($P > 0.05$) from the corresponding results for the non-pregnant ewes on scrapie-free or scrapie-prone farms (Table 2).

In January 2004 a questionnaire was sent to Icelandic veterinarians by e-mail. Twenty district or practicing veterinarians replied. The locations of these veterinarians are shown in Figure 1. All of them had seen signs of selenium deficiency in lambs recently; however, the incidence was no longer high due to widespread prophylactic use of selenium as selenite injections (mostly of newborn lambs) and/or as feed supplement to the sheep. The use of selenium enriched fertilizer does not seem to be commonly practiced among Icelandic farmers (Table 4).

DISCUSSION

It is generally considered that a selenium concentration in hay lower than 100 µg kg^{-1} may indicate that animals feeding on it can suffer from selenium deficiency (Adriano 2001). The selenium concentration in hay samples collected from the 2002 and 2003 harvests on the 18 scrapie-free, scrapie-prone and scrapie-afflicted farms that were studied was almost uniformly low or on an average 15-25 µg kg^{-1} (Figure 2). Selenium was only in a few instances found in the hay samples in amounts that were near to "normal levels" (above 60 µg kg^{-1}).

Eiríksdóttir *et al*. (1981) and Símonarson *et al*. (1984) found that the concentration of selenium was low or rather low in dry hay and silage at the experimental station at Hvanneyri, Iceland, as well as in grass (except from the highlands, see below) in southwest, western and northern Iceland. Purdey (2000) moreover found selenium concentration to be uniformly low or very low (similar range as our results) in samples of herbage collected in northern Iceland.

The farms on which the hay samples were collected are located in one county in southern Iceland and three counties in northern Iceland, all counties where scrapie has been found sporadically throughout several decades (Figure 1). Inquiries sent to twenty veterinarians located all over the country, i.e. situated in totally scrapie-free counties as well as in the scrapie-free areas, scrapie-prone or scrapie-afflicted areas of other counties, indicate that signs of clinical selenium deficiency are commonly seen in lambs, or even in heifers or foals, unless abated with selenium feed supplements or preventive administration of selenium as medicine (Figure 1, Table 4). Low selenium concentration in hay harvested as fodder for sheep (or other herbivorous animals) therefore seems to be a ubiquitous phenomenon in Iceland and it is accordingly not likely to be directly connected with the sporadic occurrence of clinical scrapie in certain areas of the country. This is in contrast to the possible promotive effect of low manganese concentration in hay on the occurrence of clinical scrapie in Iceland (Jóhannesson *et al*. 2004).

Soil in Iceland is considered rich in selenium (levels about 3000 ng g^{-1} have been found), but it is at the same time rather acid and the availability of selenium can therefore be low (Símonarson *et al*. 1984). These authors emphasized that this may particularly be true for hay harvested on cultivated home fields early in the summer after a short and fast growing period. They found higher amounts of selenium in grass from the highlands, where the grass (or other grazing plants) grows slowly, than in the lowlands with faster growth of the grass. It should therefore be anticipated that the concentration of selenium in ovine blood is highest in the autumn shortly after the sheep have been collected from the highlands or other uncultivated pastures. This was also found to be the case in our study (see below). In this context it is moreover of interest that elder veterinarians and sheep farmers have personally informed us that clinical signs of selenium deficiency were rarely seen prior to 1960. Up to that time it was customary to let the sheep graze in the field outside the sheds, in addition to feeding them inside during winter time. Feeding the herds almost entirely inside the stables during winter, until spring time, has since become the general rule on the majority of sheep farms (> 70%) in Iceland.

Selenium concentration in the blood of ewes on three scrapie-free and five scrapie-prone farms in the Vatnsdalur valley declined sharply from the non-pregnant (autumn 2002) to the pregnant state (spring 2003) (Table 2) in spite of the fact that ewes on most of the farms were given selenium supplement with the hay. The general consensus is that a selenium concentration in blood above 100 ng ml^{-1} is sufficient, levels between 50 ng ml^{-1} and 100 ng ml^{-1} marginally sufficient and levels below 50 ng ml^{-1} deficient (Hamliri *et al*. 1990, Nève 1991). It thus follows that the ewes were in sufficient selenium status when not pregnant but only in marginally sufficient status in the pregnant state. The GPX activity in blood also declined in pregnancy but proportionately less than the selenium concentration (Table 2). This is due to the fact that GPX activity in whole blood, residing mostly in the erythrocytes (Nève 1989), depends essentially on the availability of selenium during formation of the entire erythrocyte population (Hamliri *et al*. 1990). The GPX/Se ratio was thus significantly higher when the ewes were in the pregnant state than when non-pregnant and the correlation between selenium concentration and GPX activity was much less significant in the pregnant state (Figure 3 and RESULTS). From these results it is necessary to define the status of the sheep (pregnant/non-pregnant or otherwise) when using the results of selenium or GPX determinations in order to predict whether or not changes in these parameters have relevance for the occurrence of clinical scrapie. This conclusion is also valid for other studies including determinations of Se concentration and GPX activity in blood.

It should be mentioned that Eiríksdóttir *et al*. (1981) have previously found seasonal changes in Se concentration and GPX activity in the blood of ewes on the experimental station at Hvanneyri, Iceland, similar to that which we found.

There was a significantly higher concentration of selenium in the blood of non-pregnant ewes on scrapie-free farms than in blood of non-pregnant ewes on scrapie-prone farms. The same significant difference was also found for GPX activity. These differences levelled out in the pregnant state (Table 2). At first glance these results might support our previous findings that a low GPX activity was seen in the blood of ewes on scrapie-prone and scrapie-afflicted farms (Jóhannesson *et al*. 2003). Our present results show, however, that the selenium concentration and GPX activity in the blood of non-pregnant ewes on four scrapie-afflicted farms did not differ significantly from these parameters in the blood of non-pregnant ewes on scrapie-free and scrapie-prone farms. The same apparently also applies to the pregnant ewes on scrapie-afflicted farms (cf. Tables 2 and 3 and RESULTS).

In summary: The results indicate conclusively that selenium deficiency in hay is ubiquitous in Iceland, and that it is not likely to be directly connected with the occurrence of sporadic scrapie, but they are inconclusive as to whether the selenium concentration or GPX activity in the blood of ewes on scrapie-prone or scrapie-afflicted farms are significantly different from ewes on scrapie-free farms. This research is hampered by the fact that due to the low prevalence of scrapie in Iceland the number of scrapie-afflicted farms is always likely to be low during any chosen experimental period. The impact of selenium deficiency on the health of domestic animals in Iceland obviously warrants further study.

ACKNOWLEDGEMENTS

This work is a part of the FATEPRIDE PROJECT (Contract No QLK 4-CT-2002-02723 Quality of Life Programme – Framework K5). The experimental work was performed in close co-operation with the Office of the Chief Veterinary Officer. We also want to express our gratitude towards Dr. Guðjón Atli Auðunsson, of the Fisheries Laboratories, Mrs. Steinunn Árnadóttir, of the Keldur Institute, and towards all the farmers and their families for the assistance provided during collection of samples on their respective farms. Finally we wish to thank Mrs. Jóhanna Edwald for assistance in preparing the manuscript. Jed Barash was a Fulbright Fellow in Iceland.

REFERENCES

Adriano AC 2001. *Trace elements in terrestrial environments. Biogeochemistry, bioavailability, and risks of metals*. Chapter 18 (selenium). Springer, 2nd ed. 867 p.

Arnþórsdóttir AL 2002. Selen og júgurbólgusýklar hjá fyrsta kálfs kvígum [Selenium and mastitis in cows at first calving]. *Freyr* 98, 26-29. (In Icelandic).

Arthur JR 2000. The glutathione peroxidases. *Cell. Mol. Life Sci*. 57,1825-1835.

Glantz SA 1997. *Primer of Biostatistics*. McGraw-Hill, 4th ed. pp. 238-245.

Hamliri A, Johnson DW, Kessabi M & Olson WG 1990. The evaluation of selenium status of sheep from the major production areas of Morocco. *Ann. Rech. Vét*. 21, 137-142.

Eiríksdóttir G, Símonarson B, Thorsteinsson T, Gudmundsson B & Jónmundsson JV 1981. Árstíðabundnar breytingar á seleni í blóði sauðfjár. Tilraun á Hvanneyri 1980.

[Seasonal variation of selenium in the blood of sheep: Experiment at Hvanneyri in 1980]. *J. Agr. Res. Icel*. 13, 25-33. (In Icelandic, English summary).

Jóhannesson T, Kristinsson J & Snædal J 2003. Hrörnunarsjúkdómar í heila – oxavarnarensím og kopar. Kynning á rannsóknum [Neurodegenerative diseases, antioxidative enzymes and copper. A review of experimental research]. *Læknablaðið* 89, 659-671. (In Icelandic, English summary).

Jóhannesson T, Gudmundsdóttir KB, Eiríksson T, Kristinsson J & Sigurdarson S 2004. Copper and manganese in hay samples from scrapie-free, scrapie-prone and scrapie-afflicted farms in Iceland. *Icel. Agric. Sci*. (in print).

Nève J 1989. Biological functions of selenium. In: Nève J &. Favier A (eds.). *Selenium in Medicine and Biology.* Proceedings of the Second International Congress on Trace Elements in Medicine and Biology. March 1988, Avoriaz, France, Walter de Gruyter, Berlin and New York. pp. 97-111.

Nève J 1991. Physiological and nutrional importance of selenium. *Experientia* 47, 187-193.

Paglia DE & Valentine WN 1967. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *J. Lab. Clin. Med*. 70, 158-169.

Purdey M 2000. Ecosystems supporting clusters of sporadic TSEs demonstrate excesses of the radical-generating divalent cation manganese and deficiencies of antioxidant cofactors Cu, Se, Fe, Zn. Does foreign cation substitution at prion protein's Cu domain initiate TSE? *Med. Hypotheses* 54, 278-306.

Sigurdarson S 1991. Epidemiology of scrapie in Iceland and experience with control measures. In: Bradley R, Savey M & Marchant B (eds.). *Sub-acute Spongiform Encephalopathies.* Proc. Seminar CFC Agricultural Research Programme. Kluwer Academic Publishers, Brussels and Luxembourg. pp. 233-242.

Símonarson B, Eiríksdóttir G, Sigurdarson S & Thorsteinsson T 1984. Selenskortur og seleneitrun [Selenium deficiency and selenium toxicity]. *Freyr* 80, 910-912. (In Icelandic).

Símonarson B 1986. Selen [Selenium]. *Tímarit um lyfjafræði* 21, 17-24. (In Icelandic, English summary).

Þorkelsson G 1997. Tengsl seleninnihalds i blóði mjólkurkúa og fastra hilda [The relationship between selenium concentration in blood of dairy cows and retained placenta]. *Dýralækaritið* 9, 12-15. (In Icelandic).

Manuscript recieved June 16, 2004
Accepted October 18, 2004