

# Analysis of BSE monitoring system in Lithuania

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## Summary

Lithuania has been strengthening its BSE monitoring program since July 2001. A total of 264,268 cattle were examined during the period of 2001-2006. No BSE positive case was found. A total of 93.1% of healthy slaughtered cattle, 6.3% of fallen stock, 0.4% of emergency slaughtered cattle and 0.2% of cattle with clinical signs were tested at ante mortem inspection. The number of tested adult cattle has increased 4.5 times from 4.17% in 2001 to 18.88% in 2006. 2.41% adult cattle were tested in 2002, 1.97% in 2003, 10.59% in 2004 and 18.92% in 2005. The number of tested healthy slaughtered adult cattle has increased 4 times from 4.09% in 2001 to 17.41% in 2006. 2.11% of healthy slaughtered adult cattle were tested in 2002, 1.5% in 2003, 9.96% in 2004 and 17.66% in 2005. The number of tested risk group adult cattle has increased 18 times from 0.08% in 2001 to 1.47% in 2006. 0.3% of risk group of adult cattle were tested in 2002, 0.47% in 2003, 0.63% in 2004 and 0.96% in 2005. 94.3% of all tested cattle were from 2 to 11 years old, 0.1% of them were less than 2 years old, 3.0% were 12 years old and 2.6% were 13 years old and more. 54.3% of all tested cattle were more than 5 years old. A total of 53.9% of healthy slaughtered cattle, 59.0% of fallen stock, 61.9% emergency slaughtered cattle and 62.6% of cattle with clinical signs at ante mortem inspection were more than 5 years old. Significant correlation was found between the age of healthy slaughtered cattle and emergency slaughtered cattle and cattle with clinical signs at ante mortem inspection ( $P < 0.05$ ). There was no correlation between the age of fallen stock and healthy slaughtered cattle. The mean age of healthy slaughtered cattle was 6.5 years and the mean age of fallen stock was 6.7 years in 2005. Enfer TSE and Bio-Rad rapid tests were applied for BSE monitoring. Inconclusive test results were subjected to histopathological and immunocytochemical examination.

**Keywords:** BSE, monitoring, descriptive analysis

Bovine spongiform encephalopathy (BSE) is a fatal neurodegenerative disease characterized by a long incubation period and a gradual accumulation of an anomalous isoform of prion protein (PrP<sup>Res</sup>) in the central nervous system causing degeneration of neurons and ultimately leading to death in all cases (12, 17). Cattle affected by BSE demonstrate chronic neurological signs consisting of behavioural changes, abnormalities of posture and movement, and hyperaesthesia (15). Diagnostics of BSE in live cattle is impossible since pathologic prion protein (PrP<sup>Res</sup>) does not stimulate an immune or inflammatory response in the host (16). BSE was first described in Great Britain in 1987 (17). Since then, BSE has occurred in many countries involving imported and indigenous cattle (9, 10). BSE has been identified since 1988 in Great Britain (14) and in Lithuania since 1990. In passive monitoring any person who has in their possession or charge a bovine animal suspected or being affected by BSE must report about the case immediately to the relative authorities. Since July 2001, Lithuania, like other European

countries, has begun to actively monitor BSE among specific populations of bovine animals (3).

The aim of this study was to evaluate BSE monitoring system in Lithuania during the period of 2001-2006.

## Material and methods

Since 2000 all cattle in Lithuania have been individually identified by ear tags. Individual data of bovine animals are collected at birth and stored in the Central Data Base of Identification and Registration of bovine animals (therefore – CDB). All movements of cattle are traced and recorded by competent authorities; slaughterhouses, farmers and processing plants of animal by-products have access to CDB in order to check identification, age and movements of bovine animals. All slaughter products of bovine animals are identified and kept at the slaughterhouse before BSE test results are obtained. All animal origin by-products are collected and processed at the processing plant.

The monitoring of cattle for the presence of BSE was divided into target groups described below (tab. 1). Healthy slaughtered bovine animals were cattle subject to normal

Tab. 1. BSE monitoring of bovine animals

Categories of bovine animals	Age of bovine animals
<b>Active monitoring</b>	
Healthy slaughtered	All > 30 months
Emergency slaughtered	All > 24 months
Clinical signs at ante mortem inspection	All > 24 months
Fallen stock	All > 24 months
<b>Passive monitoring</b>	
BSE suspects	All

slaughter for human consumption and cattle without clinical signs of disease slaughtered in the context of a disease eradication campaign other than BSE. Emergency slaughtered bovine animals were cattle subject to special emergency slaughtering. Bovine animals with clinical signs at ante mortem inspection were cattle sent for normal slaughter but suspected of suffering from a disease which is communicable to man and to animals or showing symptoms or being in a general condition such as to indicate that such a disease may occur and cattle showing symptoms of a disease or of a disorder of their general conditions which is likely to make their meat unfit for human consumption. Fallen stock was cattle that had died or been killed on the farm or in transport, but not slaughtered for human consumption nor killed in the framework of an epidemic. BSE suspects were cattle reported as suspects of BSE.

The study data were extrapolated from the BSE monitoring reports in 2001-2006. Brainstem samples from slaughtered cattle were collected at a slaughterhouse and from dead cattle on a farm or at a processing plant of animal origin by-products using the methods and protocols laid down in the Fifth Edition of the Manual Diagnostic Tests and Vaccines for Terrestrial Animals published by the World Organisation

for Animal Health (OIE) (2). The samples were correctly marked as to identify the sampled cattle. A form which accompanies the samples was filled in and sent to National Veterinary Laboratory (thereafter – NVL), which is TSE reference laboratory in Lithuania. Samples from bovine animals were subjected to rapid tests (Bio-Rad and Enfer TSE) and to histopathological and immunocytochemical examination (3). Monthly reports from NVL and regional competent authorities were analyzed. The data were broken-down by categories of cattle tested in the framework of BSE monitoring program and distribution of cattle by year of birth and geographical origin. Records of post mortem examination were examined.

Descriptive epidemiology and time series data analysis were performed to describe the trends of BSE monitoring program. Statistical analysis was done by statistical program Prism 2.01. Correlation coefficient ( $r$ ) and its 95% confidence interval and  $P$  value were calculated.

## Results and discussion

A total of 264,268 cattle were tested in the framework of BSE monitoring program during 2001-2006 in Lithuania. No BSE positive case was found. There was a significant difference between sizes of target groups of cattle tested in 2001-2006 ( $P < 0.05$ ). A total of 246 018 healthy slaughtered cattle, 1206 emergency slaughtered cattle, 447 cattle with clinical signs at ante mortem inspection and 16597 fallen stocks were tested in the framework of BSE monitoring programs in 2001-2006 (fig. 1). 93.1% of all tested cattle were healthy slaughtered cattle, 6.3% – fallen stock, 0.4% – emergency slaughtered cattle and 0.2% – cattle with clinical signs at ante mortem inspection.

The number of tested cattle increased 4.5 times from 4.17% in 2001 to 18.88% in 2006 in Lithuania. 2.41%

adult cattle were tested in 2002, 1.97% in 2003, 10.59% in 2004 and 18.92% in 2005. A total of 19 282 cattle were tested in 2001, 11 136 in 2002, 9746 in 2003, 86 195 in 2005 and 87 406 in 2006 (fig. 1). The number of tested healthy slaughtered adult cattle increased 4 times from 4.09% in 2001 to 17.41% in 2006. 2.11% of healthy slaughtered adult cattle were tested in 2002, 1.5% in 2003, 9.96% in 2004 and 17.66% in 2005. A total of 18 911 healthy slaughtered cattle were tested in the framework of BSE monitoring programs in 2001, 9758 in 2002, 7418 in 2003, 47506 in 2004, 81 769 in

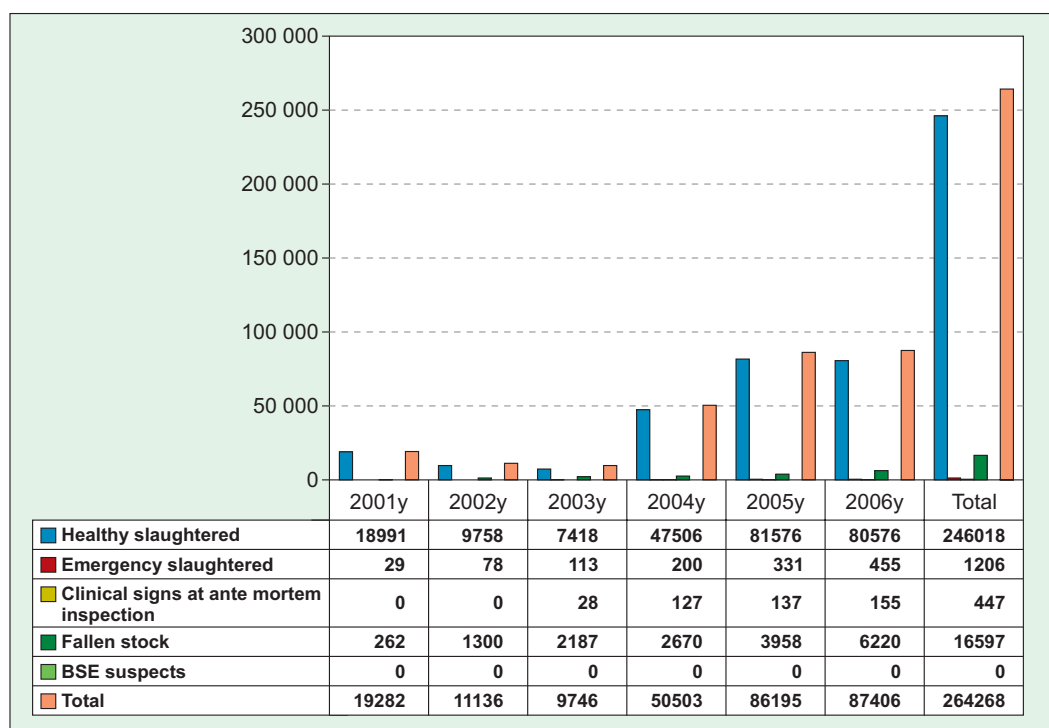


Fig. 1. Number of bovine animals tested in the framework of BSE active and passive monitoring program

2005 and 80576 in 2006. The number of tested risk group adult cattle increased 18 times from 0.08% in 2001 to 1.47% in 2006. 0.3% of risk group of adult cattle were tested in 2002, 0.47% in 2003, 0.63% in 2004 and 0.96% in 2005. A total of 371 risk group cattle were tested in 2001, 1378 in 2002, 2328 in 2003, 2997 in 2004, 4426 in 2005 and 6830 in 2006 (fig. 2). The number of emergency slaughtered cattle increased slightly. A total of 29 emergency cattle animals were tested in 2001, 78 in 2002, 113 in 2003, 200 in 2004, 331 in 2005 and 455 in 2006. The number of cattle with clinical signs at ante mortem inspection was low. No cattle with clinical signs at ante mortem inspection were tested in 2001 and 2002. A total of 28 cattle with clinical signs at ante mortem inspection were tested in 2003, 127 in 2004, 137 in 2005 and 155 in 2006. The number of fallen stock of bovine animals increased significantly from 1.4% in 2001 to 7.1% in 2006. There were no BSE clinical suspects in Lithuania.

94.3% of all tested cattle were from 2 to 11 years old, 0.1% were less than 2 years old, 3.0% were 12 years old and 2.6% were 13 years old and more. 54.3% of all tested cattle were more than 5 years old. 4 year old cattle constituted the largest age group of tested bovine animals (fig. 3). The mean age of tested cattle was 7.1 years in 2001, 7.3 years in 2002, 7.0 years in 2003, 6.8 years in 2004, 6.4 years in 2005 and 5.7 years in 2006. The mean age of healthy slaughtered cattle was 7.1 years in 2001, 6.3 years in 2002, 7.1 years in 2003, 6.9 years in 2004, 6.5 years in 2005 and 5.6 years in 2006. The mean age of tested fallen stock was 5.9 years in 2001, 5.7 years in 2002, 6.7 years in 2003, 6.0 years in 2004, 6.7 years in 2005 and 7.4 years in 2006.

A total of 53.9% of healthy slaughtered cattle, 59.0% of fallen stock, 61.9% emergency slaughtered cattle and 62.6% cattle with clinical signs at ante mortem inspection were more than 5 years old. Significant correlation was found between the age groups of healthy slaughtered cattle and emergency slaughtered cattle and cattle with clinical signs at ante mortem inspection ( $P < 0.05$ ). There was no correlation between the age group of fallen stock and of healthy slaughtered cattle (fig. 4).

A total of 1.2% of all tested cattle were sampled for BSE testing in Alytus county, 14.4% in Kaunas county, 22.9% in Klaipėda county, 5.7% in Marijampolė county, 25.2% in Panevėžys county, 4.5% in Šiauliai county, 8.8% in Tauragė county, 2.3% in Telšiai county, 11.6% in Utena county and 3.4% in Vilnius county (fig. 5).

A total of 116 825 (44.2%) of all samples were tested by Enfer TSE test, 146 412 (55.4%) samples were tested by Bio-Rad test, 1006 samples were subject to

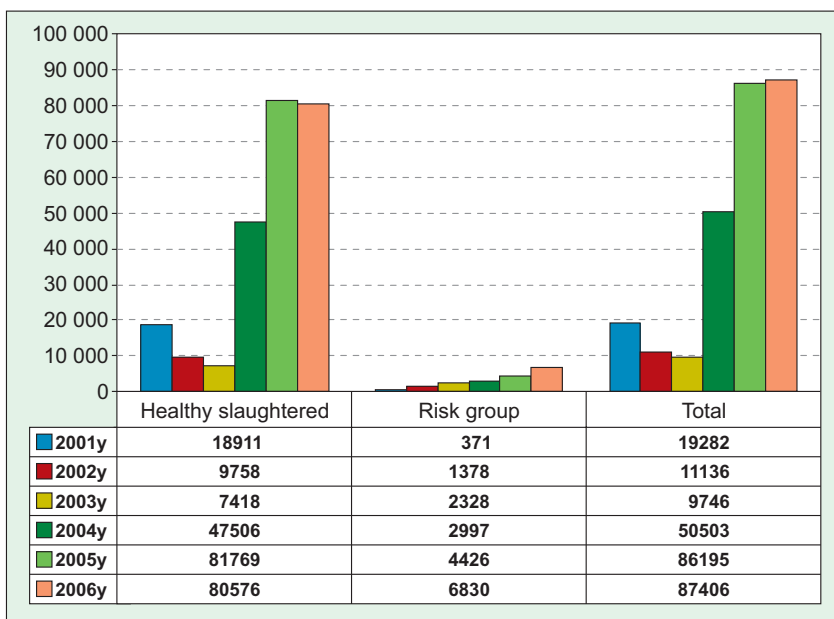


Fig. 2. Number of healthy slaughtered and risk group bovine animals tested in the framework of BSE monitoring program

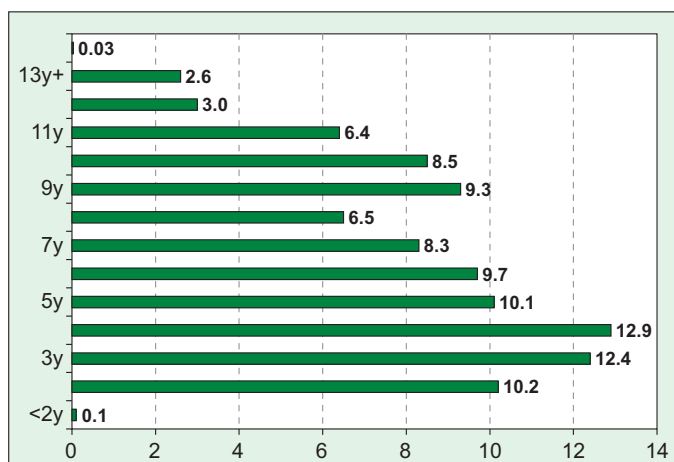


Fig. 3. Extrapolated age distribution of bovine animals tested in the framework of BSE monitoring program in Lithuania, y = year

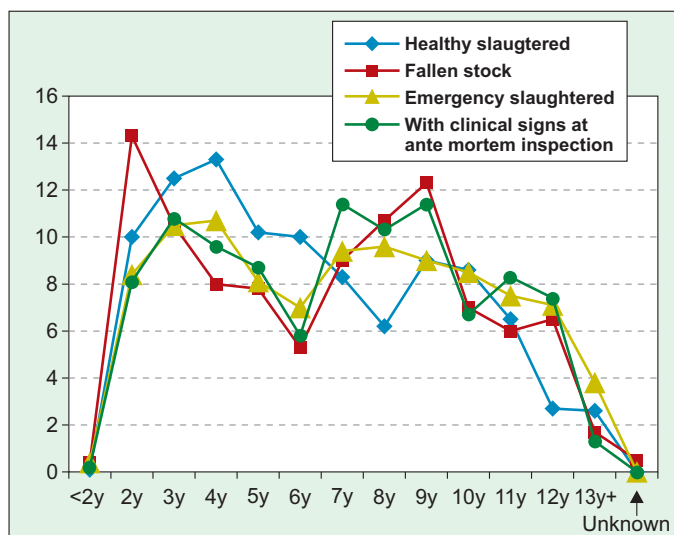


Fig. 4. Extrapolated age distribution of target groups of bovine animals tested in the framework of BSE monitoring program

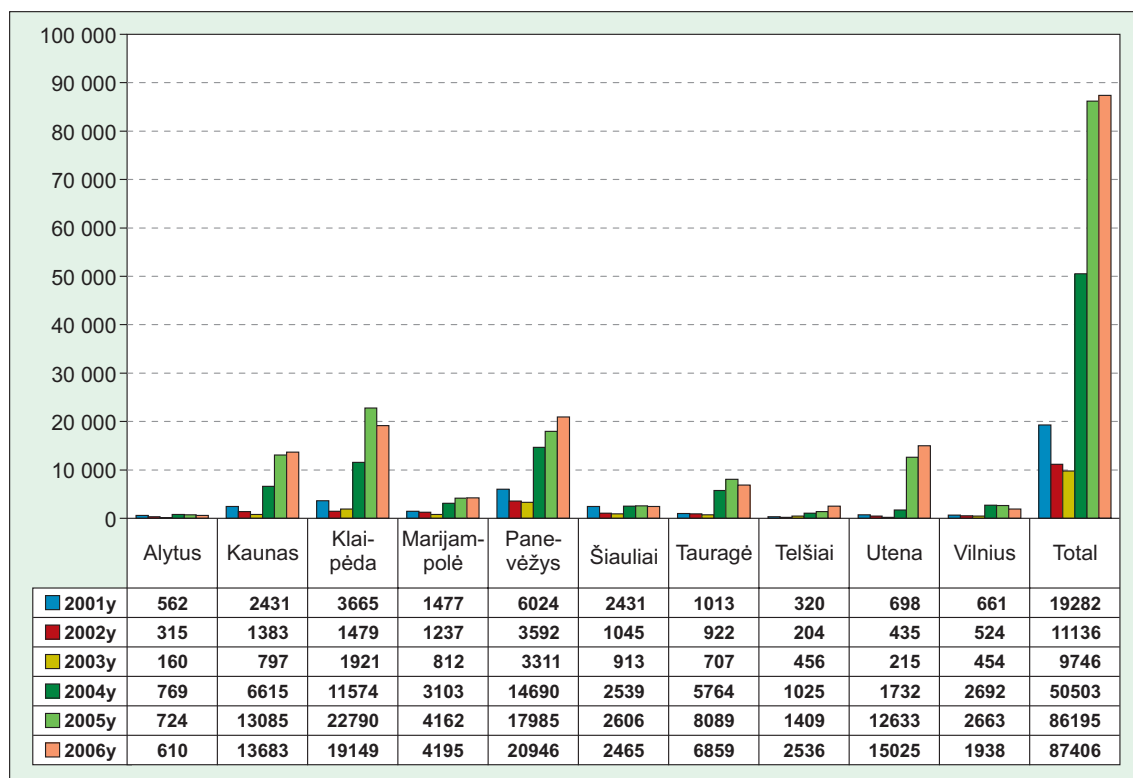


Fig. 5. Number of bovine animals tested in the framework of BSE monitoring program in Counties of Lithuania

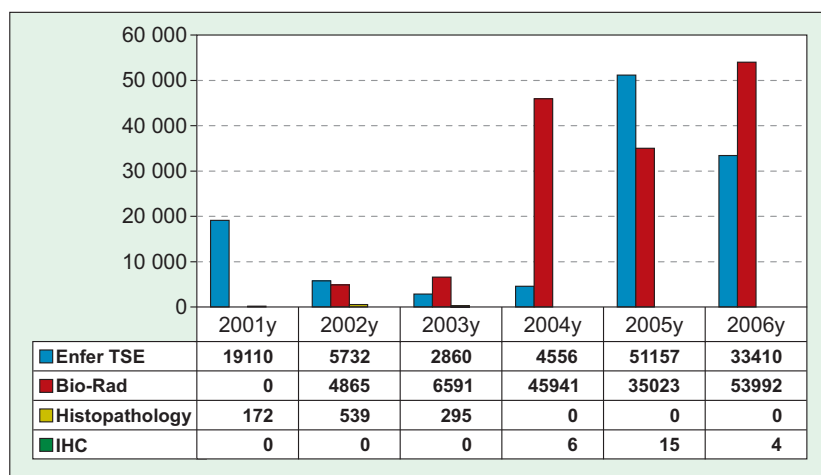


Fig. 6. Laboratory testing for the presence of BSE in bovine animals

histopathological examination and 25 samples were subject to immunocytochemical examination (fig. 6).

There were no significant correlation between the number of healthy slaughtered and fallen stock cattle tested monthly in 2005-2006 ( $P > 0.05$ ). Seasonal differences were noted between the number of sampled healthy slaughtered and fallen stock cattle in 2004-2005 and 2005-2006 (fig. 7).

Analysis of post mortem records revealed that the majority of cattle died from the following diseases: nervous ketoses, ruminal tympany, ruminal acidosis, abomasal dislocation, rabies, parturient paresis, abomasal ulcers, mastitis, traumatic reticulitis, postoperative septicaemia, trauma, poisonings and pathological parturition.

significantly lower than in EU countries. In EU 20.92% of adult cattle were tested in 2001 (EU15), 26.26% in 2002 (EU15), 24.66% in 2003 (EU25), 25.5% in 2004 (EU25) and 23.51% in 2005 (EU25), (4-8). In Lithuania 4.17% of adult cattle were tested in 2001, 2.43% in 2002, 1.97% in 2003, 10.59% in 2004, 18.62% in 2005 and 18.88% in 2006. Therefore the difference in the number of tests in risk cattle was rather significant. In EU 1.92% of adult risk cattle were tested in 2001, 3.13% in 2002, 3.26% in 2003, 3.42% in 2004 and 3.47% in 2005 (4-8). In Lithuania 0.08% of adult risk cattle were tested in 2001, 0.3% in 2002, 0.47% in 2003, 0.63% in 2004, 0.96% in 2005 and 1.47% in 2006. Inadequate financing in 2001-2004 had a negative impact on the BSE monitoring program. 2003 was

Until 1999, epidemiological studies on BSE were based on a mandatory reporting system of clinically suspected bovine animals (11). The new generation of rapid tests for detecting the protein resistant prion protein ( $\text{PrP}^{\text{Res}}$ ) in brain tissue has made it possible to test large numbers of samples, and even poor quality samples, in a short period of time (13). Enfer TSE and Bio-Rad rapid tests were applied for BSE monitoring in Lithuania. All inconclusive test re-

sults were subjected to histopathological and immunocytochemical examination. Since the start of an expanded monitoring program on BSE in 2001, 264 268 cattle have been tested. No BSE case has been diagnosed. The surveillance involves active monitoring of healthy slaughtered cattle, fallen stock, emergency slaughtered cattle and cattle with clinical signs at ante mortem inspection. BSE monitoring programs facilitate comparisons between the same target group over the monitoring years and within the same age group. Risk group cattle appeared to have a significantly higher prevalence of BSE than healthy cattle (12). In Lithuania the percentage of tested healthy slaughtered and risk cattle compared to the adult population was



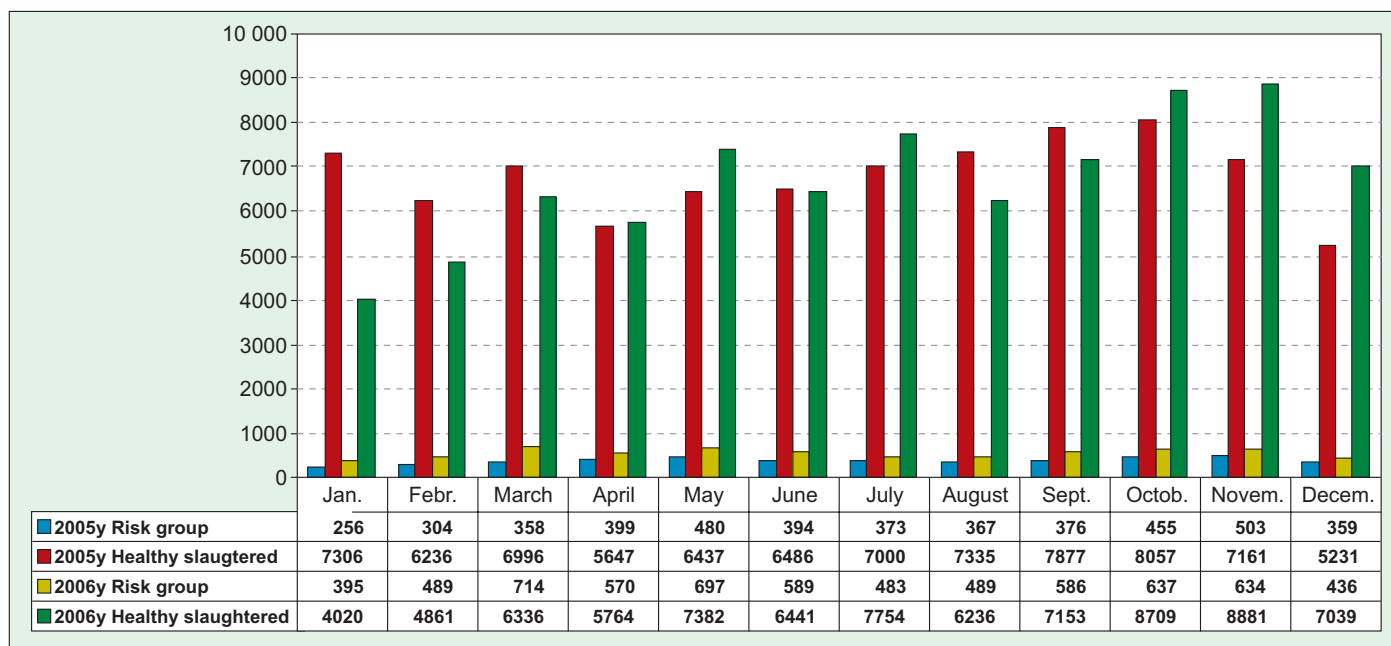


Fig. 7. Number of monthly tested bovine animals in 2005-2006

the worst for the BSE monitoring. The year 2005 was the first year of full BSE monitoring of healthy slaughtered cattle in Lithuania. However, the percentage of tested adult slaughtered cattle in 2005 was lower than EU mean average. The number of adult fallen stock has been increasing from 2004, but still it remains lower than the EU average. The mean incubation period of BSE is estimated to be approximately five years (1). The mean age of healthy slaughtered cattle in Lithuania was 6.5 years, meanwhile in the EU (EU15) it was 7.9 years in 2005 (8). The mean age of fallen stock in Lithuania was 6.7 years while in the EU it was 10.1 years in 2005 (8). It shows that cattle in other EU countries have been used longer than in Lithuania. However, the majority of tested cattle in Lithuania were older than the mean incubation period of BSE. The number of tested cattle in counties was significantly different and it depended on the location of cattle slaughterhouses (fig. 5). The majority of cattle were slaughtered in Kaunas, Klaipėda, Panevėžys and Utena counties. BSE was differentiated mainly from metabolic and postparturient disorders, and rabies.

### Conclusions

The survey of BSE monitoring systems in Lithuania was the first attempt to evaluate the various data provided by the mandatory reporting system. The study gave a precise estimation of the size of targeted groups of cattle monitored for BSE. In recent years BSE monitoring systems have made it possible to check all adult cattle at the time of slaughter or death. Analysis of all these data helps to detect the trends of BSE monitoring system, investigate risk factors and determine the effective control measures for the disease. The cattle population of Lithuania is still free from BSE. Cattle cohorts born at the end of the nineteen-nineties did not carry a risk for human beings.

### References

- Anderson R. M., Donnelly C. A., Ferguson N. M., Woolhouse M. E. J., Watt C. J., Udy H. J., Mawhinney S., Dunstan S. P., Southwood T. R. E., Wilesmith J. W., Ryan J. B. M., Hoinville L. J., Hillerton J. E., Austin A., Wells G. A. H.: Transmission dynamics and epidemiology of BSE in British cattle. *Nature* 1996, 382, 779-788.
- Anon.: Manual of Diagnostic tests and Vaccines for Terrestrial Animals (mammals, birds and bees). World Organization for Animal Health, Paris 2004.
- Anon.: Regulation of the Parliament and of the Council of 22 May 2001 laying down rules for the prevention, control and eradication of certain transmissible spongiform encephalopathies (EC) No 999/2001. OJ L 147, 31.5.01, 1.
- Anon.: Report on the monitoring and testing of bovine animals for the presence of Bovine Spongiform Encephalopathy (BSE) in 2001. European Commission, Brussels 2002, 40 p.
- Anon.: Report on the monitoring and testing of ruminants for the presence of Transmissible Spongiform Encephalopathy (TSE) in 2002. European Communities 2003, 72 p.
- Anon.: Report on the monitoring and testing of ruminants for the presence of Transmissible Spongiform Encephalopathy (TSE) in the EU in 2003, including the results of the survey of prion protein genotypes in sheep breeds. European Communities 2004, 91 p.
- Anon.: Report on the monitoring and testing of ruminants for the presence of Transmissible Spongiform Encephalopathy (TSE) in the EU in 2004. Luxembourg, Official publications of the European Communities 2005 – VIII, 104 p.
- Anon.: Report on the monitoring and testing of ruminants for the presence of Transmissible Spongiform Encephalopathy (TSE) in the EU in 2005. Luxembourg, Official publications of the European Communities 2006 – VIII, 104 p.
- Donnelly C. A.: Likely size of the French BSE epidemic: epidemiological analysis helps in evaluating the potential risk from eating French beef. *Nature* 2000, 408, 787-788.
- Donnelly C. A., Santos R., Ramos M., Galo A., Simas J. P.: BSE in Portugal: anticipating the decline of an epidemic. *J. Epidemiol. Biostat.* 1999, 4, 277-283.
- Ducrot C., Roy P., Morignat E., Baron T., Calavas D.: How the surveillance system may bias the results of analytical epidemiological studies on BSE: prevalence among dairy versus beef suckler cattle breeds in France. *Vet. Rec.* 2003, 34, 185-192.
- Morignat E., Ducrot C., Roy P., Baron T., Vinard J.-L., Biacabe A.-G., Madec J.-Y., Bencsik A., Debeer S., Eliazewicz M., Calavas D.: Targeted surveillance to assess the prevalence of BSE in high risk populations in western France and the associated risk factors. *Vet. Rec.* 2002, 151, 73-77.
- Moynagh J., Schimmel H.: Tests for BSE evaluated. *Nature* 1999, 400, 105.
- Taylor K. C.: The control of bovine spongiform encephalopathy in Great Britain. *Vet. Rec.* 1991, 129, 522-526.
- Van Keulen L. J. M., Langeveld J. P. M., Garssen G. J., Jacobs J. G., Schreuder B. E. C., Smits M. A.: Diagnosis of bovine spongiform encephalopathy: a review. *Vet Quart.* 2000, 22, 197-200.
- Wells G. A. H., Hawkins S. A. C., Green R. B., Austin A. R., Dexter I., Spencer Y. I., Chaplin M. J., Stack M. J., Dawson M.: Preliminary observations on the pathogenesis of experimental bovine spongiform encephalopathy (BSE): an update. *Vet. Rec.* 1998, 142, 103-106.
- Wells G. A. H., Scott A. C., Johnson C. T., Gunning R., Hancock R. D., Jeffrey M., Dawson M., Bradley R.: A novel progressive spongiform encephalopathy in cattle. *Vet. Rec.* 1987, 121, 419-420.

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