

USEFULNESS OF RAPID TESTS FOR DIAGNOSIS OF BSE

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Experience from the application of rapid tests for BSE diagnosis in comparison to reference methods is described. Sensitivity and specificity of all three rapid tests was evaluated. Detection of the first three cases of BSE in Poland was based on rapid tests which gave positive results. Opposite to that histopathology was negative/positive/positive while immunohistochemistry was positive/weak positive/positive in the first, the second and third case of BSE, respectively.

Key words: cattle, bovine spongiform encephalopathy, rapid tests, diagnosis.

When testing cattle for BSE two aspects of successful monitoring should be considered. One is availability of reliable (100% sensitive and specific) and rapid tests and another is testing specific groups of animals where probability of the detection of BSE is the highest - so called risk groups. Preliminary report of the European Commission from July 8, 1999 evaluated four and accepted three rapid tests for BSE monitoring (3). According to David Byrne, EU Commissioner for Health and Consumer Protection, available data shows that rapid tests account for 2 out of 3 of BSE cases detected in the European Union (EU) (1). Definition of risk groups was first introduced in Switzerland in 1999 (2, 5). BSE clinical suspects were tested within passive surveillance while risk animals were included in active monitoring and they comprise: dead-on-farm animals, emergency slaughtered animals and animals sent for normal slaughter but found sick *at ante* mortem inspection (sanitary slaughter). At the moment most cases of BSE (except BSE clinical suspects) are found in risk animals. About one in every thousand animals in this category tests is positive for BSE (0.09%) (1). On the other hand, only one animal per thirty thousand healthy tested, scores positive for BSE (0.003%).

Passive monitoring of all BSE clinical suspects with histopathology was introduced in Poland at the National Veterinary Research Institute (NVRI), Pulawy in 1996. Between March 1996 and August 2002, 1723 samples have been tested - all with negative results. Active monitoring was introduced in January 2001 also at the NVRI, in Department of Virology while additional 4 regional labs in Gdansk, Cracow, Warsaw and Wroclaw were opened in October 2001. At present all healthy animals

above 30 months old slaughtered for human consumption as well as risk animals above 24 months of age are tested for BSE (based on the regulation of Minister of Agriculture & Rural Development of February 1, 2002). According to General Veterinary Inspectorate, 261 281 samples have been tested within active monitoring scheme until the end of October 2002. Most of the samples came from healthy animals, then emergency slaughtered cattle, dead-on-farm animals, sanitary slaughtered cattle and clinical suspects. First case of BSE was detected after 110 000 tests, the second one after 170 000 and the third case after 190 000 tests. Altogether 35 539 samples have been tested in NVRI lab between January 2001 and November 2002.

The aim of the study was to assess three rapid tests available on the market for BSE diagnosis based on our experience with positive samples (sensitivity). We also evaluated specificity of those tests by counting the number of false positive results (specificity). This parameter is very important from economical point of view. Retesting means additional cost for the lab and extended time for the slaughterhouse before the final result is delivered. All rapid tests rely on PrP^{res} (PrP resistant to proteolysis) detection. This protein is regarded as a specific marker of prion diseases. It can be detected before vacuolar changes appear. Practically, it means that rapid tests should detect not only cattle with clinical BSE but also animals incubating the disease (clinically healthy).

Material and Methods

The samples received for testing were collected with special spoons and comprised obex region. All three rapid tests described in the study were used according to manufacturer's instructions on dedicated and recommended equipment (4). Histopathology and immunohistochemistry (IHC) - with rabbit anti-ovine PrP antisera (R524 - kind gift of Dr. Langeveld from ID-DLO Lelystad, the Netherlands) were performed according to standard protocols in Department of Pathology, NVRI, Pulawy.

Results

First case of BSE was detected in an official regional diagnostic laboratory in Cracow with „Biorad Platelia-BSE” test, which gave very high reading (beyond the upper limit of a reader). Two other rapid tests (Enfer-TSE and Prionics-Check) were clearly positive. Luminescence units (LU) are used in Enfer-TSE test and the threshold value for positive cases is 5.5 LU. The reading for the first case was 869.29 LU. Result of Prionics-Check test is presented in Fig. 1. All three criteria to assess the result as positive were fulfilled: presence of PrP after digestion with proteinase K, lower molecular weight of PrP and the presence of three bands corresponding to glycosylation profile of PrP. Immunohistochemistry confirmatory testing done at NVRI was positive while histopathology was negative.

Second BSE case was diagnosed at NVRI during routine monitoring using „Enfer-TSE” rapid test. This time the reading was barely above the threshold value – 24.38 LU, although western-blot (Prionics-Check) was clearly positive. Histopathology confirmatory testing was clearly positive (typical for BSE vacuolar changes have been found mostly in the solitary tract nucleus) and weak positive in IHC.

Third case of BSE was also detected in an official regional diagnostic lab in Cracow with „Biorad Platelia-BSE” test, which again gave very high reading (beyond the upper limit of a reader). Two other rapid tests (Enfer-TSE and Prionics-Check) were also positive. The reading for the third case in Enfer-TSE was 668.89 LU. Both histopathology and immunohistochemistry confirmatory tests done at Department of Pathology, NVRI were positive.

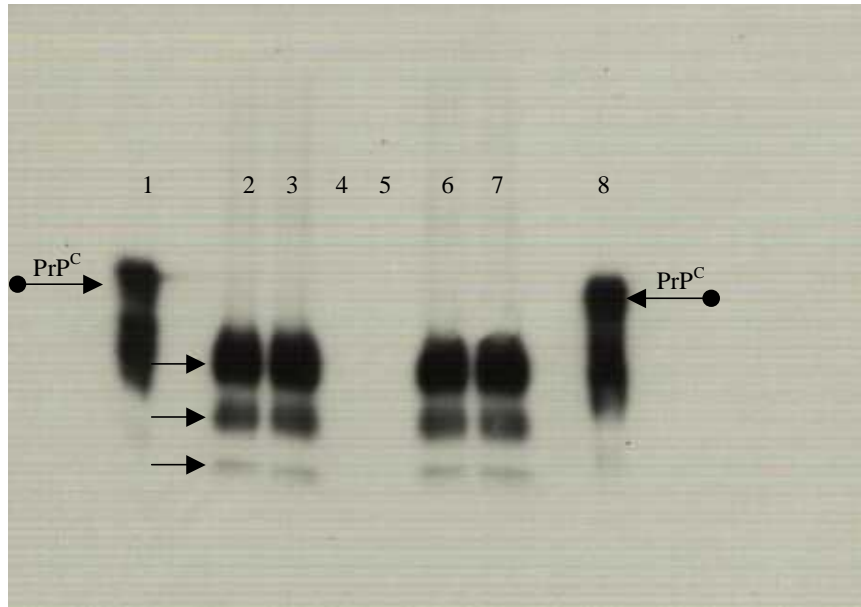


Fig. 1. First Polish case of BSE diagnosed with Prionics-Check.

- 1, 8 - Positive Control of the test – undigested PrP^C (arrow indicates position of PrP^C).
- 2, 3 - Positive sample tested in duplicate. Arrows indicate typical glycosylation pattern of PrP^{res}
- 4, 5 - Negative samples (tested before with Prionics-Check with negative result).
- 6, 7 - Positive sample tested in duplicate.

Specificity of Biorad Platelia-BSE was in a range of 0.1-1% on the basis of our own and regional labs experience (they test 75% of all samples for BSE). In case of Enfer-TSE the range was between 0.8 and 1%. Only Prionics-Check was 100% specific (no retesting was required). All false-positive results in Biorad Platelia-BSE and Enfer-TSE were negative when retested with the same test kits.

Discussion

All three BSE cases have been detected in healthy cattle. These animals were 9, 13 and 6 years old, respectively. First two animals originated from small private farms where other livestock was kept as well, while the third animal came from a big farm with 823 animals. The source of BSE is unknown in the first two cases since both farms have not used meat and bone meals in feedingstuffs. Cows from the farm where the third BSE case was detected were fed concentrates of foreign origin.

When comparing these first three cases of BSE diagnosed in Poland, it is clear that active monitoring with rapid tests is crucial for successful detection of BSE in a country with low incidence of BSE. In all three cases, rapid tests were positive. Comparison of rapid tests shows that Prionics-Check is most reliable test since all cases have given clearly positive results even in the second case which gave much lower readings in Enfer-TSE in comparison to the first case of BSE (26.8 LU and 869.29 LU respectively).

All three cases were diagnosed in healthy cattle where PrP deposits should be present before vacuolar changes in central nervous system (CNS). This statement holds true for the first case, where histopathology was negative. On the other hand, the second and the third case were clearly positive in histopathology with widespread vacuolisation of neuropil grey matter (especially in the solitary tract nucleus) although the animals were healthy before slaughter. Comparing the appearance of vacuolar changes in CNS to the presence of PrP^{res} deposits, no correlation can be drawn between these two diagnostic features. The first case of BSE showed high concentration of PrP^{res} in rapid tests (Biorad Platelia-BSE and Enfer-TSE), while histopathology did not reveal any vacuolar changes in neuropil grey matter. Opposite to that, the second case was weak positive in rapid test (Enfer-TSE), meaning low PrP^{res} concentration in the sample, while histopathology revealed many vacuoles of neuropil grey matter. Summarising, it can be said that the use of rapid tests and testing of all animals over 30 months old slaughtered for human consumption are crucial for detection of BSE. Another point is that any suspicion of BSE should be confirmed or excluded with all available tests. In our study we have not used electron microscopy for SAF detection and O.I.E. immunoblot which are regarded as confirmatory tests for BSE along with histopathology and IHC. SAF detection requires electron microscope and trained personnel and its diagnostic value is questioned (6). O.I.E. immunoblot utilizes the same techniques as Prionics-Check western blot (gives the same results). Because Prionics-Check was positive in all three cases we gave up performing reference immunoblot.

Note: First two Polish cases of BSE have been confirmed in German reference laboratory for BSE, where O.I.E. immunoblot was performed.

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