Bov-tA Short Interspersed Nucleotide Element Sequences in Circulating Nucleic Acids from Sera of Cattle with Bovine Spongiform Encephalopathy (BSE) and Sera of Cattle Exposed to BSE

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Circulating nucleic acids (CNA) are known to be enriched in repetitive DNA sequences in humans. Here, bovine sera CNA were analyzed to determine if cell stress-related short interspersed nucleotide elements (SINEs) could be detected in sera from cattle associated with bovine spongiform encephalopathy (BSE). Nucleic acids were extracted, amplified, cloned, and sequenced from the sera of protease-resistant prion protein (PrP<sup>res</sup>)-positive cattle (n = 2) and sera from BSE-cohort cows (n = 6); 150 out of 163 clones revealed the presence of, on average, an 80-bp sequence from the 3′ region of Bov-tA SINE. A PCR protocol was developed that differentially identified SINE-associated CNA in BSE-exposed versus normal cattle. CNA were extracted from a serum vesicular fraction after controlled blood collection and processing procedures. Sera from four confirmed cases of BSE, 137 BSE-exposed cohort animals associated with eight confirmed BSE cases, and 845 healthy, PrP<sup>res</sup>-negative control cows were tested. All four sera from confirmed BSE cases were repeatedly reactive in the assay. BSE-exposed cohorts had a 100-fold higher occurrence of repeatedly reactive individuals per cohort (average = 63%; range = 33% to 91%), compared to healthy controls (average = 0.6%; P < 0.001). This study shows that BSE-confirmed and cohort animals possess a unique profile of SINE-associated serum CNA that can be utilized as a marker that highly correlates to BSE exposure.

MATERIALS AND METHODS

Study population. Cows of the Holstein breed primarily located in northwest Germany were used for the entire study. BSE-confirmed animals are defined as cattle in which a European Union-approved test for protease-resistant prion
protein (PrP\textsuperscript{res}) in the brain stem showed a positive result and were confirmed by immune histochemistry. Serum of BSE cattle was drawn ante mortem from cows referred to the Institute of Veterinary Medicine as suspicious and confirmed as PrP\textsuperscript{res} positive post mortem. Feeding cohorts are defined according to the official European Union definition as being raised or born on the same farm within 12 months prior to or after the BSE index case. According to the official German BSE statistics (2001, 2002, and 2003) (6), it was calculated that such cows have a 107-fold increased risk of having BSE as defined by PrP\textsuperscript{res} accumulation in the brain stem at the time of BSE diagnosis of the index case (Table 1).

Sera from cohorts were obtained at the day of culling, drawn, and stored under the same standard conditions as BSE and control specimens. All cohorts that could be acquired during the study period were used if the group number exceeded seven for statistical reasons. Healthy control cattle are defined as randomly selected dairy cows from a farm where no BSE-confirmed animal has ever been detected, i.e., no animal slaughtered from the farm has shown positive results with an approved BSE post mortem test. The majority of normal control cattle sera were collected in a slaughterhouse at the time of slaughter. All cohort cattle and cows acquired from the slaughterhouse used as normal controls were tested with a European Union-approved test for PrP\textsuperscript{res} in the brain stem and found to be negative. Blood from these groups was drawn ante mortem. All cows tested were from farms in northern Germany and did not differ in age or husbandry between the groups.

**Serum collection.** Special care was taken in collection, processing, and storage of serum samples. Blood from the tail vein or artery was collected into 18-ml plastic tubes equipped with a coagulation accelerator and kept at room temperature for 2 to 8°C for not longer than 24 h. Centrifugation was done at 2 to 8°C at 2,000 g for 25 min at 4°C in a model 5214 bench top water bath and 250 g at 4°C for 15 min. The serum supernatant was transferred into 1.5-ml microcentrifuge cups in 0.5-ml aliquots and frozen immediately at −20°C or −80°C until use.

**Preparation of serum fractions.** Frozen serum was thawed at 4°C in an ice-water bath and 250 μl was transferred into a 1.5 ml microcentrifuge tube. The tube was centrifuged at 4,000 g for 25 min at 4°C in a model 5214 bench top centrifuge (Eppendorf, Hamburg, Germany) to remove cell debris. The supernatant was transferred into a fresh tube and subjected to 35 min of centrifugation at 20,000 g. The supernatant was carefully removed and the pellet was used for further analyses.

**Nucleic acid extraction.** We used 20,000 × g pellets with a standard silica-based nucleic acid extraction (NucleoSpin kit; catalog number K306a, BD-Clontech, Heidelberg, Germany; field study: NucleoMag kit, Macherey & Nagel, Düren, Germany) according to the manufacturer’s instructions. The resulting nucleic acid solutions were either used immediately or frozen at −80°C until further use.

**Primers.** The primers used in this study were derived from preceding investigations with degenerated differential display primers (data not shown). Briefly, two BSE cattle and four normal controls were used; after PCR differentially abundant bands were extracted and sequenced, specific primers were designed and tested in all possible combinations. The best separating primer combination was designated CHX-1F and CHX-1R. CHX-1F is homologous to a cDNA bovine entry similar to calmodulin (accession number XM_592316), whereas CHX-1R is homologous to the monomer unit of Bov-TA (accession number X64124).

**Sequencing.** Primers CHX-1F and CHX-1R (catalog numbers 42-4103 and 42-91102; respectively; Chronix Biomedical GmbH, Göttingen, Germany) were used on extracted nucleic acids in 20-μl PCR (Advantage-2 PCR kit, BD-Clontech, Heidelberg, Germany), with 30 to 35 cycles at 48 to 55°C annealing (60 seconds), 68°C extension (2 min), and 94°C denaturation (1 min). Samples from confirmed BSE cases and healthy control cows were loaded side-by-side on a polyacrylamide gel electrophoresis (PAGE) gel and analyzed as described. Bands were cut out of gels, eluted, and subjected to reamplification. The products were first blunt-ended with T7 DNA polymerase and phosphorylated with polynucleotide kinase and ATP. This reaction mixture was used for blunt-end ligation into a SmaI-digested, dephosphorylated pGEM-4Z vector.

Ligation was performed overnight at 4°C using 1 U T7 DNA ligase, 1 μg of the vector, and the PCR product prepared as described above. The product was transformed into chemically competent Escherichia coli and plated on TAXI LB-agar containing tetracycline, ampicillin, 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal), and isopropylthiogalactopyranoside (IPTG). After overnight incubation at 37°C, positive (white) clones were picked and cultured in 1.5 ml LB-medium with ampicillin. Bacteria were harvested, and plasmids were isolated according to standard protocols and reconstituted in 50 μl Tris-borate-EDTA buffer. The plasmids were sequenced using either a LICOR model 4200 DNA sequencer with IRD700-labeled M13 forward and M13 reverse primers or with a model 3100 ABI capillary sequencer using unlabeled primers with BigDye-termination.

Raw sequences were processed using Sequencer (MAC OSX). Briefly, after trimming for the unresolved cloning vector and ambiguities, an automatic contig assembly was performed using the default stringency parameters. From the resulting contigs, only those that included clones from at least two different samples were selected. Homologs were then defined as that portion of the contig sequence that was covered by more than 50% of the individual clones. The final homologous sequences were checked for the presence of the used primers. All homologs had one primer sequence; 18 homologs had both primer sequences present.

**Blast analysis.** Genetic analysis was applied to the sequences using the Advance BLAST program (http://www.ncbi.nlm.nih.gov/BLAST/). The following parameters were set using the Megablast subprogram: low complexity filter off, expect 1000, and word size 11. All hits were compared by order and length, and the longest homologies of each region were used for comparisons. Analyses were done using the nr and est databases.

**Diagnostic PCR.** Three μl of the extracted nucleic acids from serum fractions was used without DNase pretreatment in a total PCR volume of 20 μl. Primers CHX-1F and CHX-1R (catalog numbers 42-4103 and 42-91102, Chronix Biomedical GmbH, Göttingen, Germany) were used at 1 μM each using a proof-reading polymerase system (Advantage-2 PCR kit, BD-Clontech, Heidelberg, Germany). After 30 cycles of 95°C for 30 seconds, 55°C for 45 seconds, and 68°C for 1 min, a SybrGreen I-derived melting curve was recorded in an MX4000 PCR system (catalog number 401260, Stratagene, La Jolla, CA). The area under the curve (AUC) of the derived melting function −d(F/T)dT between 87°C and 90°C was used for analysis. This range was used since it was not prone to the influence of nonspecific products, e.g., primer-dimers, which frequently may be present due to the use of SybrGreen I during PCR. The reactivity of each individual sample was calculated on the basis of an AUC above the detection limit, which is defined as the mean ± 5 standard deviations above the baseline of nontemplate controls. All initially reactive samples were retested in duplicate. Samples showing reactivity upon retesting were defined as repeatedly reactive.

**Statistical analysis.** The proportion of repeatedly reactive in the cohort groups and the healthy control groups was calculated. The statistical significance between the cohorts and healthy controls was estimated using the chi-square test. For total group comparisons, a calculation with 9 degrees of freedom has been done, whereas for two-group comparisons, the degrees of freedom was lowered to 1.

**PAGE.** Eight μl of the PCR mixture was mixed with loading buffer and applied to a precast 12 to 20% polyacrylamide gel in Tris-borate-EDTA buffer (45 mM Tris, 45 mM boric acid, 1 mM EDTA) (4 to 20% Tris-borate-EDTA gel; catalog number EC62255, Novex, Karlsruhe, Germany) were used at 1°C during PCR. The reactivity of each individual sample was calculated on the basis of a AUC above the detection limit, which is defined as the mean ± 5 standard deviations above the baseline of nontemplate controls. All initially reactive samples were retested in duplicate. Samples showing reactivity upon retesting were defined as repeatedly reactive.

**Nucleotide sequence accession numbers.** The sequences of the PCR products from BSE and BSE-exposed cows based on primers CHX-1F and CHX-1R were deposited with the EMBL database (accession numbers AJ780924 to AJ780929).

**RESULTS**

A primer derived from a 3’ SINE region (CHX-1R) was evaluated by PCR and differential display for its utility in

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**TABLE 1. Statistics from 2001 to 2003 of the German BSE eradication program**

| Samples                  | No. of animals | % of animals PrP\textsuperscript{res} positive | Odds ratio*       |
|--------------------------|----------------|-----------------------------------------------|------------------|
| **Regular slaughterhouse** |                |                                               |                  |
| Cohort cows              | 103            | 7.70 × 10\textsuperscript{-3}                | 0.0013           |
| Vertical control         | 8              | 5.58 × 10\textsuperscript{-1}                | 0.14             |
| Odds ratio*              | 107            | (48.5–227)                                    |                  |

* The difference in BSE incidence between the population of cows that was regularly slaughtered and cohort cows is highly significant \(P < 0.001; \chi^2\). The lower and upper limits of the 95% confidence interval are in parentheses.
separation of signal from sera of healthy and diseased cattle. Samples from BSE-confirmed cows, BSE-exposed at-risk cohorts, and healthy animals were used. One primer pair (CHX-1F/CHX-1R) showed no reactivity with a control group of healthy animals but gave consistent amplification with BSE-exposed cattle. CHX-1F/CHX-1R was used to amplify CNA from two confirmed BSE samples, PrP\textsuperscript{res}-negative cohort sera, and healthy control cows. Figure 1, upper panel, shows a representative PAGE gel of the PCR products using this primer pair in these groups. Similar multiple banding patterns can be observed for both BSE sera and all four cohort sera, whereas no reactivity is seen in normal control animals. Figure 1, lower panel, shows the results of melting curve analyses (28) of these samples. The melting patterns of both BSE and all four cohort sera show a similar wide-range reaction pattern, whereas a nonreactive pattern was observed in the healthy control samples.

To elucidate the nature of the resulting PCR products, amplicons from two BSE cases and six BSE cohort animals were cloned and sequenced as described. The range of fragment sizes was from 105 to 304 bp, with an average size of 210 bp. A stretch of an average of 80 bp was found in 150 out of 163 CNA-derived clones (Table 2). This 80-bp stretch was identified as part of a SINE referred to as Bov-tA. Three representative CNA fragments are shown in Fig. 2. CNA4 (AJ780927) was identified in both the BSE cases and cohort cows; CNA6

![Image](http://cvl.asm.org/Downloaded from on April 27, 2019 by guest)
(AJ780928) and CNA1 (AJ780924) were CNA fragments from cohort sera with BSE cow 1 (CNA6) and BSE cow 2 (CNA1). The depicted homologies downstream of the SINE are those database entries with the highest homology to the respective CNA sequences. The downstream sequences adjacent to the SINE fragment cannot be found in the genome contiguously; i.e., they are chimeric sequences consisting of truncated genomic DNA. The results in Table 2 show that there were 21 total homologs representing 163 clones; 19 out of the 21 homologs had a detectable Bov-tA SINE fragment. All 19 homologs contained unique contiguous regions 3’ to the Bov-tA SINE fragment. The 19 unique regions represented short sequences ranging from 11-mers to 83-mers homologous with bovine genomic entries in the nonredundant BLAST database.

The data suggest that multiple nucleic acid sequences may be involved in defining the difference between normal and BSE-exposed cattle. The melting curve approach was selected because an entire PCR product profile can be generated. Using the CHX 1F and 1R primers, the melting curve protocol was further applied to a study of four confirmed BSE cases, eight unrelated PrPres-negative cohorts (n = 135) from BSE cases, and 176 healthy control cows (Table 3). To avoid a regional bias, 148 samples from a slaughterhouse processing cattle from the same area where the BSE cases developed were selected randomly and included in the healthy control group together with 28 samples from a BSE-nonexposed healthy control herd.

The parameter used to calculate reactivity in this PCR dif-

| TABLE 2. CNA SINE fragments in BSE-infected and BSE-exposed cohort sera |
|-----------------------------------------------|
| Homolog no. | No. of occurrences of clones | Total no. of clones per homolog | SINE length in homolog (bp) | % Identity with Bov-tA (X63124) | Size of attached non-SINE DNA (bp) |
|-------------------|-----------------------------|---------------------------------|---------------------------|---------------------------------|---------------------------------|
| BSE Cohort        | BSE                          | Cohort                          |                           |                                 |                                 |
| 1                 | 3                            | 6                               | 9                         | 52                              | 94                              | 169                             |
| 2                 | 12                           | 12                              | 12                        | 80                              | 90                              | 19                              |
| 3                 | 12                           | 12                              | 12                        | 79                              | 92                              | 78                              |
| 4                 | 2                            | 6                               | 6                         | 86                              | 90                              | 215                             |
| 5                 | 9                            | 9                               | 9                         | 81                              | 88                              | 166                             |
| 6                 | 3                            | 9                               | 11                        | 82                              | 91                              | 109                             |
| 7                 | 8                            | 8                               | 8                         | 86                              | 90                              | 160                             |
| 8                 | 7                            | 7                               | 7                         | 86                              | 81                              | 135                             |
| 9                 | 7                            | 7                               | 7                         | 0                               | 92                              | 111                             |
| 10                | 7                            | 7                               | 7                         | 80                              | 92                              | 178                             |
| 11                | 4                            | 15                              | 19                        | 82                              | 93                              | 63                              |
| 12                | 6                            | 6                               | 6                         | 87                              | 85                              | 40                              |
| 13                | 6                            | 6                               | 6                         | 85                              | 88                              | 107                             |
| 14                | 1                            | 5                               | 6                         | 83                              | 93                              | 128                             |
| 15                | 1                            | 4                               | 6                         | 80                              | 82                              | 177                             |
| 16                | 6                            | 6                               | 6                         | 80                              | 87                              | 129                             |
| 17                | 6                            | 6                               | 6                         | 0                               | 92                              | 246                             |
| 18                | 5                            | 5                               | 5                         | 87                              | 85                              | 122                             |
| 19                | 4                            | 1                               | 5                         | 65                              | 90                              | 62                              |
| 20                | 2                            | 3                               | 5                         | 80                              | 83                              | 179                             |
| 21                | 1                            | 4                               | 4                         | 80                              | 87                              | 143                             |
| a Overview of CNA sequences derived from the microvesicular fraction of bovine sera. The length and percent identity to the Bov-tA element are given together with the length of the unique fusion sequence. The source (BSE and/or cohort) and occurrence frequency are given. |

| TABLE 3. Study populations |
|-----------------------------|
| Animals | No. in group | Age of BSE indicator case (mo) |
|--------|--------------|-----------------------------|
| BSE cohort 15/03 | 14 | 64 |
| BSE cohort 19/03 | 35 | 55 |
| BSE cohort 02/02 | 18 | 60 |
| BSE cohort 12/03 | 16 | 50 |
| BSE cohort 14/02 | 11 | 71 |
| BSE cohort 42/02 | 17 | 83 |
| BSE cohort 45/02 | 11 | 93 |
| BSE cohort 44/03 | 13 | 65 |
| Healthy control herd | 28 | 27–79 |
| b Random healthy controls* | 148 | 24–90 |
| a Age range of healthy control cows. |
| b Cows randomly selected from the slaughterhouse. |
ferential display used AUCs. The AUCs were calculated according to the trapezoidal rule on the melting curves as exemplified in Fig. 1, lower panel. Figure 3 shows that 33% to 91% of the cohorts demonstrated detectable CNA, an average of 63% for all cohorts tested, while only one of 176 healthy control samples (0.6%) showed repeatedly positive reactivity. In addition, four of four sera from confirmed PrPres-positive BSE cows showed positive results. These results are statistically significant ($P < 0.001$); if calculated separately, every individual cohort group has a significantly ($P < 0.001$) increased reactivity compared to the healthy controls.

In a field study, an additional set of 669 samples from a slaughterhouse were tested. These additional samples originated from 257 different farms and did not differ significantly from the cohort groups in age (60.7 months; range, 30 to 90 months, versus 61.3 months; range, 37 to 91 months; average with 10th to 90th percentile) or regional origin (northern Germany). Samples from known cohorts served as positive controls and yielded 58% repeated reactivity, which was in the middle of the assigned value (50% to 69%). Only four samples from the field study were found to be repeatedly reactive (0.59%), which confirms the results with the previous group of 176 normal control cattle.

**DISCUSSION**

The presence of CNA in the sera of cattle was reported previously (7, 21). The present study was conducted to determine whether BSE exposure correlates with the appearance of serum SINE-associated CNA. BSE was selected for several reasons: BSE is a naturally occurring veterinary chronic illness, clinical samples with sufficient volumes of sera and associated records were available (although the number of confirmed BSE cases available was limited in this study), and there is an associated at-risk population of animals.

BSE-confirmed and at-risk animals from exposed cohorts (see Materials and Methods for definitions) of PrPres-positive BSE index cases were defined as BSE-exposed cases. The rationale for studying at-risk cohorts is clear from Table 1. According to the data provided by the German Ministry of Consumer Safety, Nutrition and Agriculture (6), the likelihood of detecting a PrPres-positive animal among cohorts is more than 100-fold greater than in healthy, noncohort cattle. Such BSE cohort cattle are in fact challenged with the same contaminated chow as the BSE index case. Approximately 1,000 cattle were used in this study to confirm the feasibility of a PCR test to detect SINE-associated CNA as an ante mortem surrogate marker for BSE exposure.

Three major findings arose from the study of SINE-associated CNAs in BSE. First, the 3′ region of the Bov-tA fragments was detected in the PCR products derived from the serum of confirmed BSE cases or BSE-exposed cohorts. The genomes of ruminants contain three related SINE elements: Bov-tA, Bov-A2, and Bov-B. The Bov-tA element, which is present in about 285,000 copies, comprising approximately 1.6% of the bovine genome, is a tRNAGly pseudogene (16). It is frequently present within the 3′ untranslated region of genes. Compared to the other bovine SINEs, the Bov-tA elements are relatively heterogeneous, harboring a 73-bp stretch of the
The third major finding from this study is that repeatedly reactive CNA patterns are found in BSE at-risk, PrP\textsuperscript{res}-negative cows. As mentioned above, cohort animals which are exposed to the infectious agent together with the BSE index case are 100 times more likely to become PrP\textsuperscript{res}-positive than noncohort animals (Table 1). Such BSE-exposed cohorts showed from 33% to 91% repeatedly reactive individuals per cohort in eight out of eight PrP\textsuperscript{res}-negative cohorts. The resulting PCR products generated by repeatedly reactive PrP\textsuperscript{res}-negative cohort animals were similar to the PCR products from four cases of confirmed BSE. In the specificity study, only five samples out of 845 combined healthy controls (0.59%) showed repeated reactivity. These data suggest that there is a low incidence of CHX-IR-specific SINE-associated CNA in the healthy control population.

The presence of a 3’ SINE element-associated CNA supports the notion that SINE detection might be a useful early-stage disease marker of a cell stress-associated clinical disease. The results of this study using primers CHX-1F and CHX-1R show that the general bovine population expresses a low incidence of specific detectable SINE-associated CNAs, while 63% of BSE-exposed cohorts are repeatedly reactive. The CNAs are found in fractions that are enriched for microparticles (exosomes and plasma membrane microvesicles). Fevrier and Raposo have proposed that exosomes/microvesicles are involved in extracellular messaging (10). Further, they note that detection of scrapie prions in exosomes has been reported (11). Although nucleic acids have been reported to be associated with misfolded prions (18), it is not clear if the unique CNAs detected in this study might be involved in such an association.

The results of this study identified genetic sequences associated with BSE and BSE exposure and support the laboratory diagnostic use of circulating nucleic acids for detecting BSE-exposed animals (3, 5, 22, 23, 26). Future studies should be able to determine whether the unique fragments associated with SINE CNA can reveal the cell or cells of origin and consequently define the associated clinical diseases in terms of diagnostic criteria.

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**REFERENCES**

1. Alves, G., M. T. Kawamura, P. Nascimento, C. Maciel, J. A. Oliveira, A. Teixeira, and G. Carvalho Mda. 2000. DNA release by line-1 (L1) retrotransposon. Could it be possible? Ann. N.Y. Acad. Sci. 906:129–133.
2. Anker, P., J. Lyutyey, C. Lederrey, and M. Stroun. 2001. Circulating nucleic acids in plasma or serum. Clin. Chim. Acta 313:143–146.
3. Anker, P., and M. Stroun. 2002. Progress in the knowledge of circulating nucleic acids: plasma RNA is particle-associated. Can it become a general detection marker for a cancer blood test? Clin. Chem. 48:1210–1211.
4. Babcock, M., A. Pavlicek, E. Spiteri, C. D. Kashkhor, I. Ioshikhes, L. G. Shaffer, J. Jurka, and B. E. Morrow. 2003. Shuffling of genes within low-coppy repeats on 22q11 (LCR22) by Alu-mediated recombinations during evolution. Genome Res. 13:2519–2532.
5. Beau-Faller, M., M. P. Gauh, A. Schneider, X. Ducrocq, G. Massiard, B. Gasser, M. P. Chenard, R. Kessler, P. Anker, M. Stroun, E. Weitzenblum, G. Pauli, J. M. Wilm, E. Quoix, and P. Oudet. 2003. Plasma DNA microsatellite panel as sensitive and tumor-specific marker in lung cancer patients. Int. J. Cancer 105:361–370.
6. Bundesministerium für Verbraucherschutz, Ernährung und Landwirtschaft. 2003, posting date. Zahl der durchgeführten BSE-Tests im Berichtszeitraum. [http://www.verbraucherministerium.de/index-4DAFF0905/FB545D436d88BAC235535EC830.html].
7. Brenig, B., E. Schütz, and H. Urnovitz. 2002. Cellular nucleic acids in serum and plasma as new diagnostic tools. Berl. Munch. Tierarztl. Wochenschr. 115:122–124.
8. Bruce, M. E., E. R. Will, J. W. Ironside, I. McConnell, D. Drummond, A. Sutcliffe, L. McCardle, A. Cheve, J. Hope, C. Birkett, S. Couzens, H. Fraser, and C. J. Bostock. 1997. Transmissions to mice indicate that ‘new variant’ CJD is caused by the BSE agent. Nature 389:598–501.
9. Durie, B. G., H. B. Urnovitz, and W. H. Murphy. 2000. RT-PCR amplicons in the plasma of multiple myeloma patients–clinical relevance and molecular pathology. Acta Oncol. 39:789–796.
10. Fevrier, B., and G. Raposo. 2004. Exosomes: endosomal-derived vesicles shipping extracellular messages. Curr. Opin. Cell Biol. 16:415–421.
11. Fevrier, B., D. Vilette, F. Archer, D. Loew, W. Faigle, M. Vidal, H. Laude, and G. Raposo. 2004. Cells release prions in association with exosomes. Proc. Natl. Acad. Sci. USA 101:9683–9688.
12. Gessoni, G., P. Barin, S. Valverde, A. Giacomini, C. Di Natale, E. Orlandini, N. Arreghini, G. De Fusco, A. Frigato, M. Fezzi, F. Antico, and G. Marchiori. 2004. Biological qualification of blood units; considerations about the effects of sample’s handling and storage on stability of nucleic acids. Transfus. Apheresis Sci. 30:197–203.
13. Human BSE Foundation. 2005, posting date. http://www.hbsef.org/statistics.htm.
14. Kalkkila, J. P., F. R. Sharp, I. Karkkainen, M. Reilly, A. Lu, K. Solway, M. Murrel, and J. Honkaniemi. 2004. Cloning and expression of short interspersed elements B1 and B2 in ischemic brain. Eur. J. Neurosci. 19:1199–1206.
15. Lasmezas, C. I., E. Comoy, S. Hawkins, C. Herzog, F. Mouthon, T. Konold, F. Auve, E. Correia, N. Lescoutra-Etchegaray, N. Sales, G. Wells, P. Brown, and J. P. Deslys. 2005. Risk of oral infection with bovine spongiform encephalopathy agent in primates. Lancet 365:781–783.
16. Lenstra, J. A., J. A. van Boxtel, K. A. Zwaagstra, and M. Schwerin. 1993. Short interspersed nuclear element (SINE) sequences of the Bovidae. Anim. Genet. 24:33–39.
17. Liu, W. M., W. M. Chu, P. V. Choudary, and C. W. Schmid. 1995. Cell stress and translational inhibitors transiently increase the abundance of mammalian SINE transcripts. Nucleic Acids Res. 23:1758–1765.
18. Manuelidis, L. 2003. Transmissible encephalopathies: speculations and realities. Viral Immunol. 16:123–139.
19. Sakamoto, K., and N. Okada. 1985. Rodent type 2 Alu family, rat identifier sequence, rabbit C family, and bovine or goat 73-bp repeat may have evolved from tRNA genes. J. Mol Evol. 22:134–140.
20. Schmid, C. W. 1998. Does SINE evolution preclude Alu function? Nucleic Acids Res. 26:4541–4550.
21. Schütz, E., L. Jakoubov, W. Wemheuer, H. B. Urnovitz, W. Schultz-Schaeffer, and B. Brenig. 2004. Serum nucleic acids directly correlate to BSE risk. Presented at the 29th International Conference on Animal Genetics, Tokyo, Japan. 74.4-9902072-1-1.
22. Sisco, K. L. 2001. Is RNA in serum bound to nucleoprotein complexes? Clin. Chem. 47:1744–1745.
23. Stroun, M., J. Lyantye, C. Lederrey, H. E. Mulcahy, and P. Anker. 2001. Alu repeat sequences are present in increased proportions compared to a unique gene in plasma/serum DNA: evidence for a preferential release from viable cells? Ann. N.Y. Acad. Sci. 945:258–264.
24. Taback, B., and D. S. Hoon. 2004. Circulating nucleic acids in plasma and serum: past, present and future. Curr. Opin. Mol. Ther. 6:273–278.
25. Taback, B., S. J. O’Day, P. D. Boasberg, S. Shu, P. Fournier, R. Elashoff, H. J. Wang, and D. S. Hoon. 2004. Circulating DNA microsatellites: molecular determinants of response to biochemotherapy in patients with metastatic melanoma. J. Natl. Cancer Inst. 96:1552–156.
26. Tsui, N. B., E. K. Ng, and Y. M. Lo. 2002. Stability of endogenous and added RNA in blood specimens, serum, and plasma. Clin. Chem. 48:1647–1653.
27. Urnovitz, H. B., J. J. Tuite, J. M. Higashida, and W. H. Murphy. 1999. RNAs in the sera of Persian Gulf War veterans have segments homologous to chromosome 22q11.2. Clin. Diag. Lab. Immunol. 6:330–335.
28. Wittwer, C. T., G. H. Reed, C. N. Gundry, J. G. Vandersteen, and R. J. Pyor. 2003. High-resolution genotyping by amplicon melting analysis using LCGreen. Clin. Chem. 49:853–860.