Isolation and Identification of Enterobacteriaceae from Raw Horsemeat intended for Human Consumption (Basashi)

KATSUNORI FURUHATA¹, NAOTO ISHIZAKI¹, YUTAKA SUGI², AND MASAFUMI FUKUYAMA¹※

¹School of Life and Environmental Science, Azabu University Sagamihara, Kanagawa 252-5201, Japan
²Center for Integrative Medical Sciences, RIKEN Yokohama, Kanagawa 230-0045, Japan

Received 21 February, 2014/Accepted 21 May, 2014

The status of Enterobacteriaceae contamination was investigated in a total of 131 samples of raw horsemeat (Basashi) intended for human consumption purchased from a general meat shop or by mail-order from October 2012 to December 2013. The bacteria were isolated from 105 of the 112 samples (93.8%). Prominent differences in the isolation rate due to the place of manufacture/sale or by the cut of the meat were not observed. Moreover, in a comparison between domestic (92.6%) and imported (100%) samples, the isolation rate was slightly higher in the imported samples.

When Enterobacteriaceae isolated from raw horsemeat was identified, it was highly diverse, with 14 species identified in total. From among these species, Hafnia alvei was the most common, with 33 strains (19.8%), followed by 27 strains (16.2%) of Klebsiella pneumoniae and 26 strains (15.6%) of Enterobacter cloacae, indicating that these three species were dominant. A trend was observed, with the dominant strain differing depending on the place of manufacture/sale or the cut of the meat. H. alvei was isolated at an especially high frequency from imported samples.

An investigation was carried out regarding raw horsemeat intended for human consumption from Yamanashi Prefecture and Canada, regularly purchased from one store in Kanagawa Prefecture. Enterobacteriaceae were isolated during five of nine (55.6%) trials, in which the isolated bacteria were H. alvei, K. pneumoniae, etc. Moreover, they were isolated at a very high isolation rate of seven among 10 trials for the Canadian meat, and H. alvei was the most commonly isolated bacteria. Accordingly, when an investigation was carried out regarding the differences in the strain level in the six isolates of H. alvei periodically isolated from raw horsemeat from Canada by the pulsed-field gel electrophoresis (PFGE) pattern using a restriction enzyme, SfiI, there was a possibility that these were the same H-38 strain (November 2013) and H-64 strain (April 2014) as well as the same H-104 strain (July 2014) and H-131 strain (December 2014).

As mentioned above, it has been demonstrated that a variety of Enterobacteriaceae were isolated from raw horsemeat (Basashi) intended for human consumption, and at a high frequency. Moreover, based on the fact that the same species or strain was chronologically isolated, the possibility of contamination by the same contamination source at different times was suggested.

Key words: Enterobacteriaceae / Raw horsemeat (Basashi) / Isolation / Identification.
resulted. Following this incident, the Ministry of Health, Labour and Welfare determined that regulations with legal force accompanied by penalties were necessary regarding meat intended to be eaten raw, and created standards for raw beef intended for human consumption. However, it was believed that the risk for contamination by intestinal hemorrhagic *Escherichia coli* and genus *Salmonella* was small in horsemeat, thus resulting in its exclusion from these regulations.

However, there are some cases in which cows and horses are raised in the same barn. Moreover, there are some farms in which the slaughtering, animal testing and destruction of cows and horses are carried out in the same facilities, causing concern with regard to cross-contamination during these processes.

The identification of *Enterobacteriaceae* was determined as the index for the new standards, which were much stricter and included a wider range of bacterial groups compared to the conventionally reported standard component target for meat to be eaten raw. When these bacteria are detected in food products, it means that the food product has been contaminated by human or animal feces.

Only beef for raw consumption was subjected to the newly stipulated standards, and as noted above, horsemeat was excluded; therefore, more stores began providing raw horsemeat instead of beef to avoid having to deal with the regulations. Moreover, because horsemeat is lower in calories, fat and cholesterol compared to other animal meat, in addition to being rich in protein, minerals and iron, it has been garnering attention as a nutritious food (Badiani et al., 1997; Lee et al., 2007; Lorezo et al., 2013). In recent years, it has become possible to purchase raw horsemeat anywhere in the country by mail order or by shopping online, and is increasingly being eaten nationwide.

Based on this situation, a standard component test for meat to be eaten raw was used to investigate the status of *Enterobacteriaceae* contamination in commercial raw horsemeat, which is currently not included in the new standards.

**MATERIALS AND METHODS**

**Tested raw horsemeat**

A total of 131 samples of raw horsemeat (Basashi) purchased from a general meat shop or by mail order from October 2012 to December 2013 were used in this study. The breakdown thereof, with regard to the place of processing or sales was: 62 samples from Kumamoto Prefecture, 23 samples from Nagano Prefecture, 17 samples from Yamanashi Prefecture, 13 samples from Fukushima Prefecture, six samples from Tokyo Prefecture, two samples each from Aomori Prefecture, Yamagata Prefecture, Shizuoka Prefecture and Miyazaki Prefecture, and one sample each from Saitama Prefecture and Ehime Prefecture. Moreover, when classified by the cut of the meat, there were 47 thigh samples, 23 sirloin samples, 10 marbled meat, 7 belly (futaego) samples, five filet samples, two flank (obl) samples, and 37 samples of unknown cuts. From among these, 104 samples were from horses raised in Japan and 27 were imported meat (22 samples from Canada, three samples from Italy, and two samples from Argentina).

**Isolation and identification of Enterobacteriaceae**

The test for *Enterobacteriaceae* was carried out according to the method described in ISO 21528-1: 2004 (2004). The samples provided for the experiment were cut into 25 g units, put together with buffered peptone water (225 ml), and cultured at 37°C for 18 h. A total of 1 ml of this culture medium was added to 10 ml of EE broth, and the samples were cultured at 37°C for another 24 h; subsequently, this was smeared in streaks on VRBG agar plates. The samples were then cultured at 37°C for another 24 h. Several model colonies were selected, their oxidase reaction and glucose fermentability were evaluated, and the presence of *Enterobacteriaceae* was investigated. Regarding the identification of the isolates, the API20E system (Sysmex bioMerieux co., Ltd.) was used to carry out a test for characteristics, and when the identification percentage was 85% or greater, the presence of a species was considered to be positive. Moreover, the identification testing was carried out by a genetic method for some strains based on the base sequence of 16S rRNA (approximately 500 bp).

**Pulsed-field gel electrophoresis**

An analysis of the chromosomal DNA restriction profiles in the six test strains was carried out by pulsed-field gel electrophoresis. Agarose gel blocks containing genomic DNA were prepared according to the method described by Rice et al. (1999). The test strains were cultured for 18 h at 37°C on nutrient broth (Oxoid, Hampshire, England). Cells of each strain were suspended in EET buffer (100 mM EDTA, 10 mM EGTA and 10 mM Tris-HCl, pH 8.0), washed twice and resuspended in 300 μl of the same buffer. The cell suspensions were mixed with an equal volume of 1% SeaKem® Gold Agarose (Lonza, Rockland, ME USA), dispensed into a plug mold and allowed to solidify at 4°C.

The agarose gel blocks were treated with EET buffer containing 200 μg/ml lysozyme and 0.05% N-lauroylsarcosine and then were incubated at 37°C. After 3 h of incubation, the gel blocks were placed...
again into EET buffer containing 1 mg/ml Proteinase K and 1% SDS, and were incubated overnight at 50°C. The sample gel blocks were washed three times with TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0). Each gel block was digested for 5 h with 30 U Sfi (New England Biolabs Japan, Tokyo, Japan) at 50°C. The gel blocks were loaded into each well of 1% PFC agarose gels (Bio-Rad Laboratories, CA, USA). The electrophoretic conditions used were based on those reported by Gamage et al. (1998). Electrophoresis was carried out at 6 V/cm for 21 h with the pulse time ranging from 1 to 25 seconds using a CHEF DRIII system (Bio-Rad Laboratories) with 0.5× TBE buffer (44.5 mM Tris, 44.5 mM boric acid and 1 mM EDTA, pH 8.0) at 14°C. The agarose gel was stained with SYBR® Green I (Lonza) and the band patterns were visualized by a UV transilluminator. In addition, the Lambda Ladder PFG Marker (New England Biolabs Japan, Tokyo, Japan) was used as a marker.

RESULTS

Isolation of Enterobacteriaceae from raw horsemeat (Basashi)

The isolation of Enterobacteriaceae from raw horsemeat is shown in TABLE 1. The bacteria were isolated at a very high isolation rate of 105 of the 112 samples (93.8%). Regarding the breakdown by place of manufacture or place of sale, it was isolated from all samples excluding six samples (9.7%) from Kumamoto Prefecture and one sample (12.5%) from Yamanashi Prefecture.

TABLE 2 shows the isolation of Enterobacteriaceae based on the cut of the meat. The bacteria were isolated from all samples of the belly (Futaego) (seven samples) and flank (Obi) (two samples). Moreover, it was isolated from 35 of 38 thigh samples (92.1%), 21 of 23 sirloin samples (91.3%), nine of 10 marbled meat samples (90.0%) and four of five filet samples (80.0%), and was therefore found to be isolated from the horsemeat samples regardless of the cut.

TABLE 3 shows the isolation of Enterobacteriaceae by dividing the samples into domestic samples and imported samples. It was isolated from 88 of 95 domestic samples (92.6%), and all 17 imported samples, with the imported samples therefore having a higher isolation rate.

Species characteristics of Enterobacteriaceae isolated from raw horsemeat

TABLE 4 shows the identification of the 167 Enterobacteriaceae strains isolated from raw horsemeat summarized by species. Shown here are species in which three or more strains were isolated; however, it

| Cut of meat   | No. of samples | No. of positive samples | Positive rate (%) |
|--------------|----------------|------------------------|-------------------|
| Thigh        | 38             | 35                     | 92.1              |
| Sirloin      | 23             | 21                     | 91.3              |
| Marbled meat | 10             | 9                      | 90.0              |
| Belly (Futaego) | 7             | 7                      | 100.0             |
| Filet        | 5              | 4                      | 80.0              |
| Flank (Obi)  | 2              | 2                      | 100.0             |
| Unknown      | 27             | 27                     | 100.0             |
| Total        | 112            | 105                    | 93.8              |

TABLE 3. The isolation of Enterobacteriaceae from raw horsemeat based on the domestic or foreign origin of the meat.

| Origin | No. of samples | No. of positive samples | Positive rate (%) |
|--------|----------------|------------------------|-------------------|
| Domestic | 95             | 88                     | 92.6              |
| Foreign | 17             | 17                     | 100.0             |
| Total   | 112            | 105                    | 93.8              |

TABLE 1. The isolation of Enterobacteriaceae from raw horsemeat (Basashi) based on the place of manufacture or place of sale.

| Places of manufacture/sale | No. of samples | No. of positive samples | Positive rate (%) |
|----------------------------|----------------|------------------------|-------------------|
| Kumamoto                   | 62             | 56                     | 90.3              |
| Fukushima                  | 13             | 13                     | 100.0             |
| Nagano                     | 13             | 13                     | 100.0             |
| Yamanashi                  | 8              | 7                      | 87.5              |
| Tokyo                      | 6              | 6                      | 100.0             |
| Aomori                     | 2              | 2                      | 100.0             |
| Yamagata                   | 2              | 2                      | 100.0             |
| Shizuoka                   | 2              | 2                      | 100.0             |
| Miyazaki                   | 2              | 2                      | 100.0             |
| Saitama                    | 1              | 1                      | 100.0             |
| Ehime                      | 1              | 1                      | 100.0             |
| Total                      | 112            | 105                    | 93.8              |
was still highly diverse, with 14 total species having three or more strains identified. From among these, the species with the highest number of isolated strains was *Hafnia alvei* at 33 strains (19.8%). This was followed by *Klebsiella pneumoniae* at 27 strains (16.2%) and *Enterobacter cloacae* at 26 strains (15.6%), indicating that these three species were dominant. Additionally, there were nine strains (5.4%) each of *Raoultella terrigena* and *Serratia marcescens*, seven strains (4.2%) each of *Citrobacter freundii*, *Serratia liquifaciens*, and *Pantoea* spp., five strains (3.0%) each of *Enterobacter amnigenus*, *Enterobacter gergoviae* and *Klebsiella oxytoxa*, and three strains (1.8%) each of *Citrobacter youngae*, *Escherichia coli* and *Proteus mirabilis*.

FIG.1 shows the results of the identification of the isolated strains by genus. According to this analysis, *Enterobacter* spp. was the most common, at 43 strains (25.7%). This was followed by *Hafnia* sp. at 33 strains (19.8%), *Klebsiella* spp. at 32 strains (19.2%), *Serratia* spp. at 17 strains (10.2%), *Citrobacter* spp. at 12 strains (7.2%), *Raoultella* sp. at nine strains (5.4%), and *Pantoea* sp. at seven strains (4.2%). In this manner, *Enterobacter, Hafnia, Klebsiella, Serratia* and *Citrobacter* were the major genuses isolated from raw horsemeat.

TABLE 5 shows the top ten species isolated from samples by prefecture from the three prefectures with the most samples from which bacteria were isolated. When the species isolated from samples obtained from these prefectures were compared, *K. pneumoniae* (16 strains, 21.1%) and *E. cloacae* (13 strains, 17.1%) were the most commonly isolated from the samples obtained from Kumamoto Prefecture. However, very few of these species were isolated from the samples purchased in Fukushima Prefecture and Nagano Prefecture, where *H. alvei* was the most commonly isolated species. In this manner, a trend was discovered in the isolation frequency of *Enterobacteriaceae*, with the finding differing depending on the place of purchase.
However, H. alvei was dominantly isolated from the imported samples (15 strains), accounting for 42.9%. In this manner, a characteristic trend was observed in the isolation frequency in domestic and imported samples.

Periodic attempts to isolate Enterobacteriaceae from raw horsemeat purchased from a single source

From October 2013 to December 2014, raw horsemeat originating in Yamanashi Prefecture and Canada was regularly purchased from one store in Kanagawa Prefecture, and the isolation of Enterobacteriaceae was attempted using these samples. The results are shown in TABLE 8. The top panel shows the isolation of the different species from samples obtained in Yamanashi Prefecture; wherein, Enterobacteriaceae was isolated in five out of nine (55.6%) trials. The isolated strains were H. alvei (two trials), K. pneumoniae (two trials) and Yersinia manufacture and place of sale of the raw horsemeat.

TABLE 6 shows the top ten species of Enterobacteriaceae isolated and identified from the thigh and sirloin samples, which were most commonly found from among all samples. E. cloacae (11 strains, 19.0%) was the most commonly isolated from the thigh samples, followed by H. alvei in sirloin samples. Meanwhile, H. alvei (five strains, 21.8%) was the most commonly isolated from sirloin samples; however, the isolation rate of this species was third highest in the thigh samples (six strains, 10.3%). In this manner, a trend was discovered of the isolation frequency of Enterobacteriaceae, which differed depending on the cut of the raw horsemeat.

TABLE 7 shows the isolated and identified Enterobacteriaceae by dividing the samples into domestic samples and imported samples. K. pneumoniae (24 strains, 18.2%), E. cloacae (22 strains, 16.7%) and H. alvei (18 strains, 13.6%) were the most frequently isolated from the domestic samples.

TABLE 6. The top ten species of Enterobacteriaceae isolated from thigh and sirloin samples.

| Species                  | Thigh No. of strains (%) | Species No. of strains (%) |
|--------------------------|--------------------------|---------------------------|
| Enterobacter cloacae     | 11 19.0                  | Hafnia alvei              |
| Klebsiella pneumoniae    | 9 15.5                   | Enterobacter cloacae      |
| Hafnia alvei             | 6 10.3                   | Klebsiella pneumoniae     |
| Serratia marcescens      | 4 6.9                    | Citrobacter freundii      |
| Escherichia coli         | 3 5.2                    | Raoultella terrigena      |
| Enterobacter gergoviae   | 3 5.2                    | Serratia liquefaciens     |
| Serratia liquefaciens    | 3 5.2                    | Pantoea sp.               |
| Raoultella terrigena     | 3 5.2                    | Klebsiella oxytoca        |
| Citrobacter braakii      | 2 3.4                    | Rahnella aquatilis        |
| Enterobacter amnigenus   | 2 3.4                    | Serratia marcescens       |
| Others                   | 12 20.7                  | Others                    |
| **Total**                | **58 100.0**             | **Total**                 |

TABLE 7. The top ten species of Enterobacteriaceae isolated from raw horsemeat from domestic and foreign samples.

| Species                  | Domestic No. of strains (%) | Foreign No. of strains (%) |
|--------------------------|-----------------------------|---------------------------|
| Klebsiella pneumoniae    | 24 18.2                     | Hafnia alvei              |
| Enterobacter cloacae     | 22 16.7                     | Enterobacter cloacae      |
| Hafnia alvei             | 18 13.6                     | Klebsiella oxytoca        |
| Raoultella terrigena     | 8 6.1                       | Klebsiella pneumoniae     |
| Serratia marcescens      | 7 5.3                       | Escherichia coli          |
| Pantoea sp.              | 7 5.3                       | Serratia liquefaciens     |
| Citrobacter freundii     | 6 4.5                       | Serratia marcescens       |
| Enterobacter amnigenus   | 5 3.8                       | Citrobacter freundii      |
| Enterobacter gergoviae   | 5 3.8                       | Enterobacter cancerogenus |
| Serratia liquefaciens    | 5 3.8                       | Raoultella terrigena      |
| Rahnella aquatilis       | 4 3.0                       | Others                    |
| Others                   | 21 15.9                     | **Total**                 |

**Total 132 100.0** **Total 35 100.0**
PFGE profiles of the six *H. alvei* strains isolated from periodic examinations

For the six *H. alvei* strains isolated from raw horsemeat that was periodically purchased from the same store, the electrophoretic profiles of the DNA fragments obtained using a restriction enzyme, *Sfi*I, are shown in FIG. 2. The H-104 strain and H-131 strain showed the same band pattern, with a difference in seven or more bands observed in other strains. Moreover, a difference in three bands was observed between the H-38 strain and H-64 strain, along with a difference in seven or more bands with other strains. Furthermore, the H-39 strain and H-129 strain were each observed to have a difference of seven or more bands from other strains.

Based on an investigation carried out about the differences between bacterial strains in terms of the acquired band pattern according to the PFGE pattern criteria published by Tenover et al. (1995; 1997) at the US CDC, there was a strong chance that the H-104 strain and H-131 strain were the same strain. Moreover, there was a possibility that the H-38 strain and H-64 strain may have been the same strain. It was believed that there was a high possibility that the H-39 strain and H-129 strain were different strains. In this manner, the six strains of *H. alvei* isolated at different times from the same raw horsemeat from Canada were divided into four clusters according their PFGE pattern.

**DISCUSSION**

In Japan, there is a tradition of eating raw horsemeat; however, there has never been an incidence of large-scale food poisoning caused by pathogenic bacteria from such horsemeat. That said, parasitic diseases are...
known to be associated with consuming raw horsemeat (Ancelle et al., 1988). In recent years, the Sarcocystis fayeri present in raw horsemeat has been clarified as a cause of diarrhea in Japan (Murata et al., 2012; Saito, 2012).

Due to an outbreak of food poisoning caused by intestinal hemorrhagic Escherichia coli from eating raw beef in Japan, new standards were set for beef intended to be eaten raw. These standards stipulate that such beef must be negative for Enterobacteriaceae. However, raw horsemeat was excluded from this testing although it must still be negative for fecal coliform bacteria and Salmonella, as required the previous standards.

In this study, in the context of increased demand for raw horsemeat, the isolation of Enterobacteriaceae was attempted in store-bought raw horsemeat samples, and it was isolated at a high rate of 93.8%. Raw horsemeat is excluded from the new standards, so there have been no previous reports attempting the isolation of Enterobacteriaceae from such meat. Accordingly, there are no standards for comparatively determining whether or not this isolation rate is constant.

No significant differences in the isolation rate of Enterobacteriaceae were found with respect to the places of manufacture or sale, and there were also no prominent differences according to the cuts of raw horsemeat examined as shown in TABLE 1 and TABLE 2, respectively. In a comparison between domestic and imported samples, the imported samples had a slightly higher isolation rate. Moreover, \( H. \text{alvei} \) was isolated from 42.9% of imported samples, which was higher than the rate in domestic samples.

Raw horsemeat of different lot numbers from Yamanashi Prefecture and Canada were regularly purchased in order to investigate the contamination status of Enterobacteriaceae over time. As a result, Enterobacteriaceae were isolated in five out of nine (55.6\%) trials using samples from Yamanashi Prefecture, and in seven out of 10 (70.0\%) trials with Canadian samples, with the imported samples having a slightly higher isolation rate. Moreover, \( H. \text{alvei} \) was isolated at a high frequency in both kinds of samples, regardless of the isolated strains.

An investigation was then carried out regarding the differences at the strain level within six isolates of \( H. \text{alvei} \) that had been isolated from raw horsemeat obtained at different times from Canada by examining the SfiI PFGE pattern. When an evaluation was carried out using the criteria proposed by Tenover et al. (Tenover et al., 1995; Tenover et al., 1997), there was a high probability that the H-104 strain isolated in July 2014 (lot number: 101203) and the H-131 strain isolated in December (lot number: 103201) were the same strain. Moreover, there was a possibility that the H-38 strain isolated in November 2013 (lot number: 110822) and H-64 strain isolated in April 2014 (lot number: 110215) were the same strain.

The possibility of \( H. \text{alvei} \) contamination at different times from the same contamination source was considered based on the fact that the same strains were identified among \( H. \text{alvei} \) isolated from raw horsemeat from Canada that had different lot numbers and were obtained at different times. In recent years, resistant bacteria have become a concern regarding meat for human consumption (Hiroi et al., 2012). Accordingly, we plan to carry out drug susceptibility testing against various strains isolated from raw horsemeat to understand the presence of associated infections. It is also isolated from acute gastroenteritis and diarrhea patients at times (Westblom and Milligan, 1992).
resistant bacteria. Moreover, thorough sterilization using chlorinated disinfectants is required as a measure for preventing Enterobacteriaceae contamination. In the future, we would like to investigate the chlorine resistance of the strains isolated in this study.

ACKNOWLEDGMENT

This research was supported in part by a research project grant awarded by the Azabu University Research Services Division.

REFERENCES

Ancelle, T., Dupouy-Camet, J., Bougnoux, M.E., Fourestie, V., Petit, H., Mougeot, G., Nozais, J.P., and Lapierre, J. (1988) Two outbreaks of trichinosis caused by horsemeat in France in 1985. Am. J. Epidemiol., 127, 1302-1311.

Badiani, A., Nanni, N., Gatta, P.P., Tolomelli, B., and Manfredini, M. (1997) Nutrient profile of horsemeat. J. Food Compos. Anal., 10, 254-269.

Crandall, C., Abbott, S.L., Zhao, Y.Q., Probert, W., and Janda, J.M. (2006) Isolation of toxigenic Hafnia alvei from a probable case of hemolytic uremic syndrome. Infection, 34, 227-229.

National Research Council. (1997) Nutrient profile of horsemeat. J. Food Compos. Anal., 10, 254-269.

Crandall, C., Abbott, S.L., Zhao, Y.Q., Probert, W., and Janda, J.M. (2006) Isolation of toxigenic Hafnia alvei from a probable case of hemolytic uremic syndrome. Infection, 34, 227-229.

ISO 21528-1:2004, Microbiology of food and animal feeding stuffs—Horizontal methods for the detection and enumeration of Enterobacteriaceae—Part 1: Detection and enumeration by MPN technique with pre-enrichment.

Lee, C.E., Seong, P.N., Oh, W.Y., Ko, M.S., Kim, K.I., and Jeong, J.H. (2007) Nutritional characteristics of horsemeat in comparison with those of beef and pork. Nutr. Res. Pract., 1, 70-73.

Liu, C.H., Lin, W.J., Wang, C.C., Lee, K.L., Tsai, M.C. (2007) Young-infant sepsis combined with urinary tract infection due to Hafnia alvei. J. Formos. Med. Assoc., 106 (3 Suppl), S39-43.

Lorezo, J.M., Sarriés, M.V., Tateo, A., and Plidore, P. (2013) Carcass characteristics, meat quality and nutritional value of horsemeat: A review. Meat Sci., 96, 1478-1488.

Moreno, C., Troñcoso, M., Coria De La, P., Ledermann, W., Del Valle, G., Nunez, C., Araya, P., and Fernández, J. (2010) Report of four clinical cases of Hafnia alvei bacteremia in a pediatric cardiac surgery unit. Rev. Chilena Infectol., 27, 40-44.

Murata, R., Suzuki, J., Sadamasu, K., and Kai, A. (2012) Detection of Sarcocystis fayeri from horsemeat associated with diarrheal diseases in Tokyo (in Japanese). Clin. Parasitol., 23, 96-98.

Rice, D.H., Mcmenamin, K.M., Pritchett, L.C., Hancock, D.D., and Besser, T.E. (1999) Genetic subtyping of Eschericha coli O157 isolates from 41 Pacific Northwest USA cattle farms. Epidemiol. Infect., 122, 479-484.

Saito, M. (2012) Food poisoning caused by Sarcocystis fayeri associated with ingestion of raw horse meat (in Japanese). J. Vet. Epidemiol., 16, 114-125.

Tenover, F.C., Arbeit, R.D., Goering, R.V., Mickelsen, P.A., Murray, B.E., Persing, D.H., and Swarninathan, B. (1995) Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. J. Clin. Microbiol., 33, 2233-2239.

Tenover, F.C., Arbeit, R.D., and Goering, R.V. (1997) How to select and interpret molecular strain typing methods for epidemiological studies of bacterial infection: a review for healthcare epidemiologists. Infect. Control. Hosp. Epidemiol., 18, 426-439.

Westblom, T.U., and Milligan, T.W. (1992) Acute bacterial gastroenteritis caused by Hafnia alvei. Clin. Infect. Dis., 14, 1271-1272.