Microbiological Quality and Safety of Zoo Food†

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Two types of commercial products for feeding zoo animals (a frozen meat product, referred to as zoo food, and a dry product, referred to as dry food) were microbiologically examined for spoilage organisms (aerobic, psychrotrophic, coliform, Escherichia coli, mold, and yeasts) and pathogens (Salmonella spp., Listeria monocytogenes, and Campylobacter jejuni). Levels of microorganisms in frozen ground zoo food were compared with those in frozen ground beef and frozen ground turkey meat. The level of microbial contaminants in frozen ground zoo meat was found to be similar to that in frozen ground beef and higher than that in frozen ground turkey meat. Sixty percent of the frozen zoo meat samples were Salmonella positive, and all of the samples were L. monocytogenes positive. Dry zoo food was documented to have microbial levels lower than those in frozen zoo meat; the pathogen levels were <10,000 g of food. Defrosting zoo meat at 10, 25, and 37°C for 24 h showed that 10°C is the best temperature for defrosting frozen ground zoo meat (length, 9 in. [22.8 cm]; radius, 2 in. [5.1 cm]) without affecting the microbiological quality or safety of the product.

Establishing a new zoological garden is a large financial commitment. At a zoo, providing nondomesticated animals the same food as they are accustomed to in nature is difficult. Some companies specialize in producing and distributing frozen raw ground meat used especially for zoo animals and race dogs. Raw meat can be a potential risk to the health of zoo animals. For human food, raw meat is obtained from healthy animals and cooked before eating, destroying all contaminants as well as pathogenic microorganisms. However, meat used in zoo food is normally obtained from aging dairy cows which are purchased by tanners for their hides. The carcass is not inspected by any government agency. It is shipped to a food processor, where it is ground and the meat is packed and frozen. This frozen ground meat is usually thawed at room temperature before being served to the animals.

Although the microbiological quality and safety of human food have been studied and reported in the literature, little to nothing has been published on the safety and quality of zoo food, even though the most common infectious disease of zoo animals is gastroenteritis (6, 7). It is well documented that raw meats (beef and poultry) harbor such pathogens as Campylobacter, Salmonella, Staphylococcus, Clostridium, and Aeromonas spp. and Escherichia coli (3, 4, 9). Closed environments and poor sanitary draining systems in zoological gardens may lead to depletion of the immune systems of the animals. Animals under stress which consume food containing pathogens may be more susceptible to disease than healthy animals (6).

The objectives of this investigation were (i) to determine the microbiological quality of frozen ground zoo food (ii) to document the presence or absence of bacterial pathogens in zoo food, and (iii) to determine the proper defrosting temperature of the zoo food which would be practical and would not affect the microbiological quality of the product.

MATERIALS AND METHODS

Sample collection. Frozen 5-lb (2.25-kg) loaves (radius, 2 in. [5.1 cm]; length, 9 in. [22.8 cm]) of ground zoo food (Wisconsin Brand, Inc., Iola, Wis.) were obtained from the Columbus Zoo, Columbus, Ohio. Composite samples of dry zoo food containing 16% protein and dry zoo food containing 10% protein (Buckeye Feed Mills, Inc., Dalton, Ohio) were also obtained from the Columbus Zoo. Frozen ground beef and frozen ground turkey meat samples were collected from local retail stores and used as reference samples.

Sample preparation. Five 5-lb loaves of frozen ground zoo food were cut into 0.25-in. (0.64-cm) slices by using a steam-sterilized electric saw. Two slices from each sample were selected randomly and analyzed in the following manner. A 25-g portion of each slice was taken for quality analysis and thawed at room temperature for approximately 1 h. In the same manner, 25 g of each sample was obtained for each of the pathogen tests. For the time and temperature study, duplicate loaves of frozen ground zoo food were thawed at 10, 25, and 37°C for 24 h and microbiologically examined, with 25 g of each loaf as the primary homogenate. Five samples of frozen ground beef and five samples of frozen ground turkey were analyzed in a similar manner.

Defrosting of zoo food. Complete 5-lb loaves of frozen zoo food were fit with two copper-constantan (type T) thermocouples. One thermocouple was placed at the center of the loaf after a 0.25-in. hole had been drilled into the loaf. After the thermocouple junction had been placed at the center of the loaf, the hole was packed with meat and the loaf was refrozen. The other thermocouple was placed between the loaf and the protective plastic wrap. A third thermocouple was placed in the incubator. All thermocouples were connected to a time-temperature recording instrument (DigestriP III; Kaye Instruments, Bedford, Mass.). The temperature of the meat (surface and center) and the incubation temperature were monitored during 24 h of defrosting. Incubator temperature data were subjected to regression analysis for presentation graphically.

Culture and sample inoculation. Salmonella typhimurium was obtained from the Biological Science Culture Collection, The Ohio State University, Columbus, and stored at

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4°C on tryptic soy agar (Difco Laboratories, Detroit, Mich.) slants. *Listeria monocytogenes* ATCC 11915 was obtained from the American Type Culture Collection, Rockville, Md., and maintained at 4°C in tryptic soy agar plus 0.6% yeast extract slants. *Campylobacter jejuni* 29428 was purchased from University Micro Reference Laboratory, Inc., Linthicum, Md., and kept at 4°C until needed.

Frozen meat and dry food were experimentally inoculated with each type of pathogenic organism to verify the detection and enumeration protocol.

Microbiological quality analyses. Standard plate count (SPC), psychrotrophic bacteria count (PBC), coliform count (VRB, MPN), and mold and yeast counts (fungi) were conducted by the standard method outlined in the *Bacteriological Analytical Manual* (5a). *E. coli* was enumerated by using the most-probable-number (MPN) technique, in which lauryl tryptose broth with 4-methylumbelliferyl-β-D-glucuronide (Difco) was used as the primary medium.

**Microbiological safety analyses: *Salmonella* isolation and enumeration.** Meat and dry food samples (25 g) were analyzed for *Salmonella* spp. by methods outlined in the *Bacteriological Analytical Manual*. All isolates were serotyped by the National Veterinary Services Laboratory, Ames, Iowa.

The MPN procedure was used to enumerate the organism in all types of samples. Appropriately diluted subsamples of the meat and dry food were inoculated in tubes of 1% peptone water. The analyses were completed by the procedure described above.

As a control method, isolation of the organism from pure culture was carried out simultaneously with sample analysis.

**L. monocytogenes isolation and enumeration.** Twenty-five grams of food sample was enriched in 225 ml of *Listeria* enrichment broth (10) and incubated at 30°C for 24 h. Isolated colonies were identified as *L. monocytogenes* by the method of Lee and McClain (8).

A three-tube MPN analysis was performed by diluting triplicate 25-g samples in 1.0% peptone water and using the method outlined above to determine the level of *L. monocytogenes* in the samples. *L. monocytogenes* ATCC 19115 was used as a positive control.

**C. jejuni isolation and enumeration.** The procedure of Doyle and Roman (5) was used to detect *C. jejuni* organisms.

**RESULTS AND DISCUSSION**

It is known that freezing is used as a method of preserving a wide variety of food, including meat. Although this method does not destroy all the organisms present in the food, it has been estimated that at least a 10 to 90% reduction in the microbial population can occur after freezing (2).

The results of the microbiological quality analyses conducted with frozen zoo meat are presented in Table 1. As detailed, the SPC count was >10⁶/g. This level is relatively high, particularly for frozen products, and indicates that if the meat is subjected to temperature abuse, spoilage may occur in short time. The results also reveal that the level of coliform bacteria exceeded 10⁶/g. This large number may result from temperature abuse of the product prior to freezing or from poor sanitation in handling or processing the product. Although some of the enteropathogenic strains of *E. coli* are MUG negative, the results show that the possi-

![FIG. 1. Microbiological quality of frozen ground meat.](http://aem.asm.org/)

**TABLE 1. Microbiological analyses of defrosted zoo food**

<table>
<thead>
<tr>
<th>Analysis</th>
<th>Level of contamination</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Time 0 (frozen) (n = 5)</td>
</tr>
<tr>
<td></td>
<td>10°C (n = 2)</td>
</tr>
<tr>
<td>SPC⁺</td>
<td>6.2 ± 0.07</td>
</tr>
<tr>
<td>PBC⁺</td>
<td>6.6 ± 0.12</td>
</tr>
<tr>
<td>Coliforms (VRB)⁺</td>
<td>4.7 ± 0.10</td>
</tr>
<tr>
<td>Coliforms (MPN)⁺</td>
<td>4.9 ± 0.11</td>
</tr>
<tr>
<td><em>E. coli</em>⁺</td>
<td>4.7 ± 0.15</td>
</tr>
<tr>
<td>Yeasts and molds⁺</td>
<td>3.9 ± 0.12</td>
</tr>
<tr>
<td>Salmonella spp.⁺</td>
<td>5.3 ± 1.1</td>
</tr>
<tr>
<td><em>L. monocytogenes</em>⁺</td>
<td>6.6 ± 1.1</td>
</tr>
<tr>
<td><em>C. jejuni</em>⁺</td>
<td>&lt;0.3</td>
</tr>
</tbody>
</table>

⁺ PBC, Psychrotrophic bacteria count; VRB, violet red bile agar.

 Mean log₁₀ CFU/g ± standard error.

 Mean MPN/g ± standard error.
ability that enteropathogenic *E. coli* are present in zoo food is high, since these organisms may come from the same source as nonpathogenic *E. coli*. The frozen zoo food also exhibited a level of 10^6* fungi per g. The results of the microbiological safety analysis of frozen zoo food are also presented in Table 1. The data indicate that 60% of the samples were positive for *Salmonella* spp. and that 100% of the samples were positive for *L. monocytogenes*. No *C. jejuni* organisms were detected in any sample of frozen zoo food investigated. *Salmonella agona* was the most frequently isolated serotype (35%), followed by *S. hadar* (20%), *S. typhimurium* subsp. *copenhagen* (20%), *S. typhimurium* (12.5%), *S. anatum* (10%), and *S. istanbul* (2.5%).

To compare the microbiological quality of frozen meat used to feed nondomesticated animals with that used to feed humans, we microbiologically analyzed frozen ground beef and frozen ground turkey meat. The microbiological quality results demonstrated that the frozen zoo food is similar to frozen ground beef in SPC (10^6/g) and fungi (10^6/g), but that considerably more *E. coli* are present in zoo food (10^6/g) than in ground beef (10^6/g). Zoo food contained higher levels of all organisms tested than did frozen ground turkey meat (Fig. 1).

Only one sample of ground beef tested positive for *L. monocytogenes*. *Salmonella* spp., *L. monocytogenes*, and *C. jejuni* were not detected in any sample of frozen ground turkey.

Since the zoo food must be thawed before being served to the animals, another series of experiments were conducted on zoo food thawed at 10, 25, and 37°C for 24 h to determine the most suitable temperature for defrosting zoo food without affecting its microbiological quality. The times required for complete defrosting of a loaf of zoo food (length, 9 in., radius, 2 in.) at various temperatures are illustrated in Fig. 2. In each case the temperature of the meat rose rapidly until the freezing point of the meat (ca. –3.0°C) was reached. At this point the temperature change was slowed until the meat was defrosted, at which time the temperature began to rise rapidly.

The results in Table 1 indicate that defrosting zoo food at 37°C resulted in an SPC count of >10^6/g, which is considered to be the level of putrefaction and slime production (1). Defrosting at 25°C permits the microbial population to increase, but to lower levels than that thawed at 37°C. When the meat was defrosted at 10°C, there was little change in the level of the organisms, which is expected since only the psychrotrophic organisms can grow rapidly at this temperature.

The level of *Salmonella* organisms increased by approximately 2 log cycles when the meat was defrosted at 37°C, whereas *L. monocytogenes* levels were maximal at 25°C. *C. jejuni* was not detected in zoo food at any defrosting temperature (Table 1). Since the 25 and 37°C defrosting temperatures permitted spoilage as well as growth of the pathogenic organisms to unacceptable levels, they should not be used as defrosting temperature for frozen ground meat loaves of this type. The ideal defrosting temperature of frozen zoo food of this size seems to be 10°C, because it does not allow rapid growth of either spoilage or pathogenic bacteria.

In fact, as mentioned above, the zoo animals are fed with raw meat, and cooking of this type of food is not practical, especially for wild animals. The presence of *Salmonella* spp. and *L. monocytogenes* even at low levels in zoo food, with the contribution of other factors such as large amounts of meat consumed daily, contaminated food other than meat, and poor microbiological quality of water, may compromise the health of zoo animals. For these reasons, control of pathogens in processing plants as well as in the preparation of food must be considered.

The results of the microbiological quality analyses of the dry zoo foods revealed SPC counts of 4.7, psychrotrophic bacteria counts of 4.5, coliform (VRB) counts of 3.5, coliform (MPN) counts of 1.5, *E. coli* counts of 1.1, and fungal counts of 3.2 (mean log_{10} CFU/g) for dry food with 16% protein. Data collected with 10% protein feed were similar. The results of microbial safety tests show that this type of food was of no potential microbiological health risk to zoo animals, since no pathogens tested in this investigation were detected. Unfortunately, the dried food is not used extensively in zoos, especially for feeding wild animals. This may
be because the animals, particularly meat-eating animals, are accustomed to eating raw meat in their native environments. Therefore, changing the eating habits of these animals is thought to be a step toward domestication, which is not consistent with the zoo’s purpose.

This investigation has provided a microbiological evaluation of two different types of zoo food. Data presented indicate that zoo meat does contain pathogenic organisms (Salmonella spp. and L. monocytogenes) which may threaten the health of zoo animals. After defrosting zoo food at different temperatures, 10°C for 24 h was documented to be the ideal defrosting temperature of frozen zoo meat loaves (length, 9 in., radius, 2 in.) without affecting the microbiological quality or safety of the product. The dry zoo food was not documented to contain a microbiological potential health risk to zoo animals. In light of information presented in this investigation, food which is to be consumed raw by nondomesticated animals should be tested for microbiological quality and safety to protect the health of the animal.

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