Evaluation of bacterial and protozoal contamination of commercially available raw meat diets for dogs

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Objective—To evaluate bacterial and protozoal contamination of commercially available raw meat diets for dogs.

Design—Prospective longitudinal study.

Sample Population—240 samples from 20 raw meat diets for dogs (containing beef, lamb, chicken, or turkey), 24 samples from 2 dry dog foods, and 24 samples from 2 canned dog foods.

Procedure—Each product was purchased commercially on 4 dates approximately 2 months apart. Three samples from each product at each sampling period were evaluated via bacterial culture for non–type-specific Escherichia coli (NTSEC), Salmonella enterica, and Campylobacter spp. Antimicrobial susceptibility testing was performed on selected isolates. Polymerase chain reaction assays were used to detect DNA from Cryptosporidium spp, Neospora spp, and Toxoplasma spp in samples obtained in the third and fourth sampling periods.

Results—One hundred fifty-three of 288 (53%) samples were contaminated with NTSEC. Both raw and prepared foods contained NTSEC during at least 1 culture period. Salmonella enterica was recovered from 17 (5.9%) samples, all of which were raw meat products. Campylobacter spp was not isolated from any samples. In 91 of 288 (31.8%) samples, there was no gram-negative bacterial growth before enrichment and in 48 of 288 (16.7%) samples, there was no aerobic bacterial growth before enrichment. Susceptibility phenotypes were variable. Cryptosporidium spp DNA was detected in 3 samples.

Conclusions and Clinical Relevance—Bacterial contamination is common in commercially available raw meat diets, suggesting that there is a risk of foodborne illness in dogs fed these diets as well possible risk for humans associated with the dogs or their environments. (J Am Vet Med Assoc 2006;228:537–542)

Feeding raw meat diets to high-performance dogs such as racing Greyhounds and sled dogs has been a common practice for some time. In recent years, many companion-animal owners have also begun feeding pets a raw meat diet. Proponents of BARF diets claim that such diets support a state of ‘supreme wellness’ in dogs.1 Feeding dogs a BARF diet purportedly increases energy and lean body mass, may resolve certain health problems (eg, dental, skin, anal sac, arthritis, and ear problems), and increases resistance to internal and external parasites.1 Claims regarding the health advantages associated with feeding raw meat have not been objectively supported with scientifically valid data.1,6

A number of public and animal health concerns may be raised in association with feeding raw meat to pets. Although proponents of BARF diets maintain that bacterial contamination of raw meat is of no consequence for >99% of dogs,1 disease outbreaks resulting in morbidity and death in companion animals fed raw meat have been reported.5–10 All raw meat products, whether intended for consumption by humans or pets, may potentially be contaminated with Salmonella spp, Escherichia coli, Campylobacter spp, Yersinia spp, Giardia spp, Toxoplasma spp, Neospora spp, Cryptosporidium spp, Echinococcus spp, Clostridium spp, Staphylococcus aureus, and others.1,2 Not only does feeding these diets pose a risk to the animals consuming them, but there is also risk to humans from preparation and handling of the raw meat and from contact with pathogens if the companion animal becomes infected, clinically or subclinically, with a pathogen from the diet. In 1 study,9 30% of fecal samples from dogs fed a homemade BARF diet contained Salmonella serovars. Salmonellosis in humans may result from direct contact with infected animals.11 The purpose of the study reported here was to evaluate commercially available raw meat and processed canine diets for contamination with NTSEC, Salmonella spp, and Campylobacter spp via bacterial culture and with Neospora spp, Toxoplasma spp, and Cryptosporidium spp via PCR assay for DNA.

Materials and Methods

Study overview—Commercial dog food products were purchased, and samples were evaluated for microbial contamination on 4 occasions during a 1-year period. Diets evaluated included raw meat products sold as dog food, commercial dry dog foods, and commercial canned dog foods. Samples were evaluated by means of microbial culture for detection of NTSEC.

BARF Biologically appropriate raw food
NTSEC Non–type-specific Escherichia coli
ATCC American Type Culture Collection
CFU Colony-forming unit
MIC Minimum inhibitory concentration
bp Base pairs

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Salmonella enterica, and Campylobacter spp and to determine the magnitude of bacterial contamination. Polymerase chain reaction assays were used to determine whether the products contained DNA from Neospora spp, Toxoplasma spp, or Cryptosporidium spp.

Diet—Products were arbitrarily selected from an advertised selection of foods available from a large number of retailers with the assumption that products that were most commonly advertised were those most commonly used by consumers. Twenty-one raw meat products sold as diets for dogs were selected for purchase, and 3 retail sources were selected to provide the product from among those identified in an internet search. The raw meat diets were composed of beef, lamb, chicken, or turkey meat and were produced by 7 companies. To minimize the chance of obtaining multiple samples from the same production lots, products were purchased and tested on 4 dates (March 2002, May 2002, August 2002, and October 2002). All products were obtained during each of the 4 purchase periods, with the exception that 1 lamb meat product could only be obtained during the first 2 sampling periods, and a turkey meat product was therefore purchased as a substitute for that diet in sampling periods 3 and 4. As a result of that substitution, 20 of the raw meat products were purchased as a substitute for that diet in sampling periods 3 and 4. In addition, 2 canned and 2 extruded dry dog foods were also arbitrarily selected to serve as controls; those products were major brands that were sold nationally and purchased at local retail outlets. All products were ordered and purchased without informing the suppliers of the intended use for the products. Raw meat products were received frozen and stored at –20°C until evaluated.

Processing for microbial culture—None of the raw meat products were accompanied by instructions for thawing or preparation. Frozen products were thawed at room temperature (22°C) in the original packaging for 8 to 10 hours before sampling. Three 25-g samples of each diet were obtained from different sections of the packaging lot, mixed with 225 mL of sterile saline (0.9% NaCl) solution in a sealed plastic bag, and placed in a paddle mixer for 30 seconds. Sterile swabs were used to transfer samples of the processed mixture for bacteriologic analyses, and 1 mL of each product was placed in a microcentrifuge tube for PCR assay. Processing 3 samples from each of the 4 purchased lots for each diet resulted in 240 samples processed for analysis from the raw meat diets, 24 samples from the dry foods, and 24 samples from the canned foods.

Direct microbial culture—Samples were transferred to tryptic soy agar plates with 5% sheep blood to assess aerobic bacterial contamination and to MacConkey agar to assess gram-negative bacterial contamination. Plates were incubated at 37°C for 18 to 24 hours, and bacterial growth was assessed semiquantitatively by use of a scale (values from 0 to 3) to evaluate bacterial growth. Briefly, this scoring system was developed by use of a reference strain of E coli (ATCC strain 25922) inoculated into tryptic soy broth and incubated for 18 hours at 37°C. Ten-fold dilutions of broth were inoculated onto blood agar plates and incubated at 37°C for 18 to 24 hours to estimate the concentration of bacterial CFUs in the broth. Aliquots of the 10-fold dilutions were transferred into a sterilized organic matrix (finely chopped straw), and samples of the contaminated organic matrix were transferred with sterile swabs to blood agar plates and MacConkey agar (for raw food samples) and incubated at 37°C for 18 hours. Bacterial growth from samples of the contaminated organic matrix was visually scored on agar plates by use of a semiquantitative scale (values from 0 to 3) for scoring numbers of CFUs. The semiquantitative scores were compared with the estimated number of CFUs used to contaminate the matrix. Results suggested that plates with a score of 1 had 5.85 × 10^2 to 5.85 × 10^3 CFUs/g of sample, those with a score of 2 had 5.85 × 10^3 to 5.85 × 10^4 CFUs/g of sample, and those with a score of 3 had >5.85 × 10^4 CFUs/g of sample. Plates with a score of 0 had no visible bacterial growth.

Enriched NTSEC cultures—One milliliter of each processed food sample was added to 9 mL of tryptic soy broth and incubated at 37°C for 18 to 24 hours. Samples were transferred to MacConkey agar with a sterile swab and incubated for 18 to 24 hours at 37°C. A single lactose-fermenting colony, if present, was transferred to a blood agar plate and incubated at 37°C for 18 to 24 hours. Lactose-fermenting colonies that contained indole were assumed to be NTSEC.

Salmonella enterica cultures—One milliliter of each processed food sample was mixed with 9 mL of tetrathionate broth (containing brilliant green and iodoine) and incubated for 18 to 24 hours at 42°C. After incubation, samples were vortexed and 100 µL was added to 10 mL of Rappaport-Vassiliadis R10 broth and incubated at 37°C for 18 to 24 hours. After incubation, sterile swabs were used to transfer samples to xylose-lysine-tergitol 4 agar, plates were incubated at 37°C. Plates were examined at 24 and 48 hours for growth of hydrogen sulfide—producing colonies. If colonies were present, 1 colony from each plate was transferred to a blood agar plate and incubated at 37°C for 18 to 24 hours. Isolates were tested with poly-O grouping antisera, and isolates with positive results were assumed to be S enterica. Salmonella serogroup-specific antisera were used to characterize each isolate, and isolates were sent to the USDA National Veterinary Services Laboratories for determination of serotype.

Campylobacter spp cultures—One milliliter of each processed food sample was mixed with 8 mL of Campylobacter enrichment broth and incubated at 42°C for 48 hours. After incubation, samples were transferred with a swab to Campylobacter agar, incubated in a microaerophilic environment to promote Campylobacter growth, and incubated for 72 hours at 42°C.

Storage of isolates—An individual colony of each NTSEC and S enterica isolate was incubated in tryptic soy broth for 12 hours at 37°C. After incubation, 750 µL was mixed with 750 µL of sterile glycerol. The solution was vortexed and stored at –70°C until further testing.

Antimicrobial susceptibility testing—Isolates were assessed for susceptibility to 16 antimicrobials. Minimum inhibitory concentrations of isolates were determined by use of a semiautomated antimicrobial susceptibility system and interpreted according to the Clinical and Laboratory Standards Institute (CLSI; formerly NCCLS) guidelines for broth microdilution methods. The group of antimicrobial drugs was chosen to be analogous with those used in the United States for the National Antimicrobial Resistance Monitoring System for enteric bacteria. Antimicrobial susceptibility testing was performed according to the manufacturer’s instructions. The following antimicrobials were tested: amikacin, amoxicillin-clavulanate, ampicillin, apramycin, cefotaxime, chloramphenicol, ciprofloxacin, gentamicin, kanamycin, streptomycin, sulfamethoxazole, tetracycline, and trimethoprim–sulfamethoxazole. Escherichia coli ATCC 25922, Enterococcus faecalis ATCC 29212, S aureus ATCC 29213, and Pseudomonas aeruginosa ATCC 27853 were used as controls in antimicrobial MIC determinations.

PCR assays—Polymerase chain reaction assay was performed on all samples purchased in the third and fourth sampling periods. A 1-mL portion of each paddle-mixed sample was placed in a microcentrifuge tube after processing and tested via PCR assay in a single reaction. Samples with posi-
Polymerase chain reaction assays were performed for detection of DNA from Cryptosporidium spp, Neospora spp, and Toxoplasma spp, according to published protocols. For all assays, DNA was extracted by use of a commercially available kit. After PCR analysis, 10 μL of each sample was analyzed on 2% agarose gels in tris-borate–EDTA buffer, stained with ethidium bromide (concentration, 40 μg/mL), and viewed by use of a UV transilluminator. Amplifications were conducted in a thermocycler. A positive and a negative control were included for each run of the PCR assays.

Cryptosporidium spp PCR assay—The assay for detection of Cryptosporidium spp included primers that recognized genomic material from Cryptosporidium parvum, certain strains of Cryptosporidium canis, and certain strains of Cryptosporidium felis. Amplification was performed via a published protocol. Briefly, 2 μL of each sample was added to a thin-walled tube containing the PCR mixture (5 μL of 10X piperazine-EDTA buffer, 3.5 μL of 25mM MgCl₂, 1 μL of 10mM dNTPs, 1 μL of each primer [10μM], 0.4 μL of goldTaq [1 U/reaction], and 34.1 μL of distilled water) for a final volume of 50 μL. The primers used were awA955=5’ CTTCCACCAACTAAGAACGGCC-3’ and awR1206=5’ CTTCCACCAACTAAGAACGGCC-3’, which detect a product of 256 bp. Initial denaturation of DNA proceeded at 96°C for 10 minutes and was followed by a cycle of 30 seconds at 96°C (denature), 30 seconds at 58°C (anneal), and 30 seconds at 72°C (extend). That cycle was repeated 41 times and was followed by 3 minutes of incubation at 72°C.

Neospora spp PCR assay—Amplification was performed according to a published protocol. Briefly, 5 μL of each sample was added to a thin-walled tube containing the PCR mixture (5 μL of 10X piperazine-EDTA buffer, 2 μL of 25mM MgCl₂, 1 μL of 10mM dNTPs, 1 μL of each primer [100μM], 0.5 μL of goldTaq [1 U/reaction], and 95.5 μL of distilled water) for a final volume of 50 μL. The primers used were Np21=5’ GTGCGTCCAATCCTGTAAC-3’ and Np6=5’ CAGTCAACCTAGTCTCTCT-3’, which detect a product of 256 bp. Initial denaturation of DNA proceeded at 96°C for 3 minutes and was followed by a cycle of 60 seconds at 94°C (denature), 60 seconds at 50°C (anneal), and 2 minutes at 72°C (extend). That cycle was repeated 40 times and was followed by 5 minutes of incubation at 72°C.

Toxoplasma spp PCR assay—Amplification was performed according to a published protocol. Briefly, 5 μL of each sample was added to a thin-walled tube containing the PCR mixture (5 μL of 10X piperazine-EDTA buffer, 5 μL of 25mM MgCl₂, 1 μL of 10mM dNTPs, 0.25 μL of each primer [100μM], 0.5 μL of goldTaq [1 U/reaction], and 33 μL of distilled water) for a final volume of 50 μL. The primers used were 5’ CGCTGAGGGAGAAGCAGAAATG3’ and 5’ CGCTGCAGGACACACGAGCTGATGATT3’, which detect a product of 529 bp. Initial denaturation of DNA proceeded at 95°C for 7 minutes and was followed by a cycle of 60 seconds at 94°C (denature), 60 seconds at 55°C (anneal), and 60 seconds at 72°C (extend). That cycle was repeated 35 times and was followed by 10 minutes of incubation at 72°C.

**Results**

Fifty-three percent (153/288) of samples examined via culture in enrichment broth (including raw, dry, and canned products) were contaminated with NTSEC (Table 1). By use of enriched culture methods, NTSEC were recovered at least once from raw meat products derived from all species of source animals tested and 90.5% (19/21) of the specific raw meat products evaluated contained NTSEC during at least 2 of the sampling periods. Ten of the 21 (47.6%) raw meat products contained NTSEC at each of the 4 sampling periods. Among all samples of raw meat products, NTSEC were recovered from 90.6% (143/240); NTSEC were isolated from raw meat products from all vendors and all manufacturers. Those bacteria were also recovered from all 4 of the canned and dry diets during the first sampling period and 1 of the dry-food products during the second sampling period. Salmonella enterica was isolated from 17 samples, all of which were obtained from raw-meat products (7.1% [17/240] of raw meat samples; 5.9% [17/288] of all samples; Table 1). Those 17 samples were derived from 10 raw meat products. Overall, S enterica was isolated at least once from 47.6% (10/21) of raw meat products.

![Table 1](https://example.com/table1.png)

<table>
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<th>Product</th>
<th>No. of samples in each period</th>
<th>Positive results in period 1</th>
<th>Positive results in period 2</th>
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<th>Positive results in period 4</th>
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*Only 9 samples of turkey products were tested in periods 3 and 4. Only 6 samples of lamb products were tested in periods 1 and 2.
Salmonella enterica was recovered from multiple samples in the same sampling period for 4 of those 10 products, and 2 of the 10 products had positive culture results in 2 separate sampling periods. Most (11/17) isolates were obtained during the third sampling period (ie, samples ordered in August of 2002). Six of the S enterica isolates could not be regrown after primary identification and storage and thus were not serotyped. Serotypes among the remaining 11 isolates were S Reading (n = 3 isolates), S Muenster (3), S Cerro (1), S Dublin (1), S Montevideo (1), S Newport, (1), and S Saint Paul (1). No common serotype predominated. Ten of the 17 samples that contained S enterica isolates also contained NTSEC. Campylobacter spp was not isolated from any samples during the study.

Mean quantification scores for aerobic bacterial growth and for gram-negative bacterial growth obtained with unenriched cultures varied among product types (Figure 1). Of the 288 samples, 197 (68.4%) had gram-negative bacterial growth without enrichment; 79 of those 197 (40%) had only 1 type of bacterial colony, whereas the remainder had more than 1 type colony. Excluding the dry and canned foods, 197 of 240 (82.1%) samples had gram-negative bacterial growth without enrichment and 79 (40%) of those had 1 type of bacterial colony: In comparison, 239 of 240 (99%) samples of raw-meat products had aerobic bacterial growth without enrichment and 28 of those 239 (12%) had 1 type of bacterial colony. Overall, 240 of 288 (83.3%) samples had aerobic bacterial growth without enrichment and 29 of 240 (12.1%) had 1 type of bacterial colony.

Polymerase chain reaction assays revealed Cryptosporidium spp DNA in 2.1% (3/144) of samples. Sequencing data confirmed that the DNA in those 3 samples was that of Cryptosporidium spp. One of those isolates was obtained from a raw-beef product, 1 was obtained from a raw-turkey product, and 1 was obtained from a canned turkey product. Neospora spp and Toxoplasma spp were not detected in any samples.

**Antimicrobial susceptibility patterns**—Susceptibility patterns among NTSEC isolates varied; 75 resistance phenotypes were detected. Five common phenotypes accounted for 38.4% (58/151) of isolates (2 of the 153 NTSEC isolates could not be resuscitated after freezing). These included isolates that were susceptible to all drugs evaluated (29/151); those that were resistant to amoxicillin-clavulanic acid, cefoxitin, and cephalothin (9/151); those resistant to streptomycin only (9/151); those resistant to tetracycline (5/151). The remaining 93 isolates were grouped into 70 resistance phenotypes. Resistance to cephalothin was most common (56.3%) among isolates, and no isolates were resistant to amikacin, ciprofloxacin, or ceftiraxone (Figure 2). In general, resistance patterns
among *Salmonella* spp isolates were similar to those in NTSEC. Resistance to sulfamethoxazole was most common among *S. enterica* isolates (71.4%), and no resistance was detected to amikacin, apramycin, ciprofloxacin, ceftriaxone, or trimethoprim-sulfamethoxazole (Figure 3).

**Discussion**

It is well recognized that raw meat may be contaminated with a variety of microbes. Contamination is generally associated with the methods used for harvesting and processing. Although many interventions are required by law to minimize microbial contamination of meat products sold for human consumption, those laws do not apply to meat products sold for consumption by pets. Results of this study indicate that raw meat products sold as dog food are commonly contaminated by various microbial agents. Even with direct (unenriched) culture methods, 99% of raw meat samples had some type of bacterial contamination, and more than 1 type of bacteria was recovered from most of those samples. Without enrichment, nearly 80% of raw meat samples were contaminated with gram-negative bacteria and there is a strong likelihood that at least a small proportion of the gram-negative bacteria were enteric pathogens that could cause infections in humans or animals. Results from bacterial culture of commercial dry and canned diets suggest that those products had less bacterial contamination, compared with raw meat diets. However, a limited number of samples of the dry and canned diets were included as controls, and this did not allow for statistical analyses or comparisons. Further investigation is warranted to make quantitative comparisons of the degree of bacterial contamination among those types of commercial diets. Many of the products contained other raw ingredients (eg, eggs and vegetables) besides meat. Addition of those ingredients, especially raw eggs, could also contribute to the risk of contamination with important bacterial pathogens.  

The USDA’s Food Safety Inspection Service is presently responsible for ensuring that the domestic meat supply is safe and that contamination of meat products with bacterial pathogens is minimal, whereas the FDA’s Centers for Food Safety and Applied Nutrition are responsible for overseeing the safety of eggs and milk. Unlike food intended for human consumption, no regulatory agency is responsible for monitoring bacterial contamination in dog food products made from raw meat, milk, or eggs. The FDA’s Center for Veterinary Medicine has published a guidance document for such products, but no regulatory authority is responsible for assuring that those products meet guidelines for bacterial contamination.

More than 60% of all samples had growth of NTSEC after enrichment. Because the heat and pressure applied in the manufacturing process for dry and canned pet foods are adequate to destroy most bacteria, contamination of the products was evaluated likely occurred after processing. Further testing to detect specific pathogenic strains of *E. coli*, such as O157:H7, was not performed, but isolation of NTSEC is commonly used by the Food Safety Inspection Service and other agencies as a marker for contamination by potentially pathogenic enteric microorganisms. There was great variation in the susceptibility phenotypes among NTSEC isolates; considering that only 1 colony was chosen for analysis per agar plate, it is likely that more phenotypes were included but not detected.

*Salmonella enterica* was recovered from 5.9% of all samples. Excluding the dry and canned products (which all had negative results for growth of *Salmonella* spp), 7.1% of raw-meat diets were contaminated with *S. enterica*. Previous investigators have reported that 20% to 35% of poultry carcasses intended for human consumption have positive results of tests for *Salmonella* spp, an estimate that is in contrast to our findings, in which *Salmonella* spp were isolated from only 2.1% of poultry samples.

It is commonly presumed that half of the raw chicken sold for human consumption in the United States is contaminated with *Campylobacter* spp. In 1 study, *Campylobacter jejuni* was isolated from 98% of samples collected from chicken carcasses intended for human consumption. We expected that some samples from poultry products in our study would contain *Campylobacter* spp, but *Campylobacter* spp was not detected. The most likely explanation for this is that *Campylobacter* spp do not tolerate drying, the organism can be killed by exposure to oxygen, and freezing is known to reduce the number of *Campylobacter* bacteria. Use of PCR assay to detect *Campylobacter* spp DNA may enhance detection of the bacterium from samples in future studies.

Because of reported contamination levels in raw meat samples, we expected that *Toxoplasma* spp, *Cryptosporidium* spp, and *Neospora* spp would be detected in numerous samples in the present study. However, only 3 samples had positive results for *Cryptosporidium* spp DNA, and DNA from neither *Toxoplasma* spp nor *Neospora* spp was detected. The single canned product that was contaminated with *Cryptosporidium* spp contained fishmeal, with or without liver or intestinal tissues that could have served as a source of contamination. The low detection rate for those organisms may have been a consequence of the primers chosen for the PCR assay, but sensitivity and specificity of those primers have been described as high in earlier reports. Samples from the present study may have contained inhibitors of the reaction, or may have been contaminated with the organisms in numbers below the detection threshold for the PCR assay. Detection of DNA via PCR assay does not necessarily correlate with detection of live organisms or risk of infection.

The perceived frequency with which BARF diets are fed to dogs suggests that further investigation of such diets is warranted. Determination of the rate of infection of dogs from raw meat diets in combination with follow-up in dogs fed those diets as well as recovery of pathogens from dogs’ home environments would be useful in assessing more precisely the risks associated with these diets. Our study was not designed to detect infection in dogs fed raw meat diets. However, given the frequency with which microorganisms of fecal origin were detected and the rate of isolation of animal and human pathogens, there may be potential for animal and human infections to occur as a result of feeding raw meat diets to pets.
References

New Veterinary Biologic Products

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<th>Product name</th>
<th>Species and indications for use</th>
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<td>Canine Heartworm Antigen, Anaplasma phagocytophilum, Borrelia burgdorferi, Ehrlichia canis Antibody Test Kit (IDEXX Laboratories Inc, Westbrook, ME, US Vet Lic No. 313)</td>
<td>In vitro diagnostic test for the detection of Dirofilaria immitis antigen, antibody to Anaplasma phagocytophilum, antibody to Borrelia burgdorferi, and antibody to Ehrlichia canis in canine serum, plasma, or whole blood</td>
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<td>Porcine Circovirus Vaccine, Type 2, Killed Baculovirus Vector (Intervet Inc, Millsboro, DE, US Vet Lic No. 286)</td>
<td>For use in healthy swine, 3 weeks of age and older against disease caused by porcine circovirus. Efficacy and potency studies are in progress. Reasonable expectation of efficacy demonstrated in 3-week-old pigs that received 2 doses of vaccine and were challenged exposed 14 days after the second vaccination</td>
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<td>In vitro diagnostic test for the detection of antibody to pseudorabies virus (PRV) in swine serum, plasma, and meat exudates</td>
<td>NA</td>
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