

## MICROBIAL PATHOGEN SURVIVAL STUDY IN A HIGH PLAINS FEEDYARD PLAYA

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**Abstract.**—Sixteen microbes and one enteric protozoal parasite were secured in screw-cap vials (CV) and dialysis tubes (DT) and placed in a feedyard shallow lake (playa) in the West Texas High Plains, USA. They were removed weekly or monthly depending on their susceptibility to the water environment. There were two overlapping studies; one started in September 1996 and was terminated 390 days later. The second study started in May 1997 and was terminated 188 days later. These controlled studies were used to determine the decrease in titers of 10 bacteria (*Pasteurella haemolytica* A1, *Pasteurella multocida* A:3, *Staphylococcus aureus*, *Escherichia coli*, *Enterococcus faecalis*, *Actinomyces pyogenes*, *Salmonella enterica* serovar *dublin*, *Bacillus thuringiensis*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*); two fungi (*Aspergillus fumigatus* and *Aspergillus niger*); four viruses (Infectious Bovine Rhinotracheitis (IBR), Bovine Virus Diarrhea Virus (BVD), Bovine Respiratory Syncytial Virus (BRSV), Bovine Parvovirus (BPV) and one protozoal parasite (*Cryptosporidium parvum*), over time. The *Pasteurella* isolates died in both studies within seven to 35 days. *Actinomyces pyogenes* died within 84 days in the 1996 study and survived for 188 days in the 1997 study. The remaining bacterial isolates in 1996 survived for 390 days with low titers, except for *P. aeruginosa*. Both fungal isolates died by 390 days in the 1996 study. All bacteria and fungi survived the 188 day study in 1997, except for the *Pasteurella* isolates. The titers of the viruses decreased rapidly over 42 days, except for BPV in the 1996 study, and all viruses were inactivated by day 42 in the 1997 study. *Cryptosporidium parvum* survived the 1996 winter but lost its ability to infect infant mice during the month of May, 1997. Microbial survival decreased more rapidly in DT samples compared to CV samples.

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Feedyards with large numbers of calves (25,000 to 100,000 head) are becoming more highly regulated by state and federal governments than in the past regarding their suspected contribution to contamination of the air (Elbers et al. 1996), soil (Faust 1982) and water (Evans & Owens 1972; Miner et al. 1967; Miner et al. 1996). There are many studies which relate to soil and water contamination by manure (Crane et al. 1983; Diesch 1975). More than 20 diseases have been identified as possibly transferred from animal manure (Azevado & Stout 1974).

Manure contamination from dairy (Jansen et al. 1974; Rankin & Taylor 1969) and swine operations are frequently called point source pollution. This pollution often consists of nitrate contamination of storm water runoff which can severely impact animal life and vegetation in streams and rivers (McLead & Hegg 1984; Power & Schepers 1989). The effects of this contamination are compounded by rapid algal-blooms (Alexander 1974). One paper has examined feedyard nitrate contamination in the Texas High Plains of the United States (Stewart et al. 1967).

Water quality is also frequently decreased by contamination from fecal coliforms, *Enterococcus faecalis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Salmonella* due to point source pollution (Geldreich 1969; Geldreich & Kenner 1969; Yanko et al. 1995) and non-point source pollution (Beyer & Perry 1987; Doran & Linn 1979; Doran et al. 1981; Green & Crawford 1990; Pasquarell & Boyer 1995; Thelin & Gifford 1983). In arid areas, waste water becomes a precious commodity and it is frequently used for irrigating crop vegetation and in the production of compost. It is of interest to know how long the pathogens in the waste water will survive on vegetation (Bell 1976; Findlay 1972; Tannock & Smith 1971).

Several studies have addressed the issue of pathogen contamination and survival in waste water (Clark et al. 1981), treatment plants and their products (Rudolfs et al. 1950a; Rudolfs et al. 1950b; Stewart et al. 1967). Some have examined microbial contamination of the feedyard and its immediate environment (Miner et al. 1966; Miner et al. 1967). There is, however, a paucity of literature concerning feedyard microbial contamination of feedyard lakes. Also, little specific information is available on the survival of microbial pathogens located in or near feedyards of the High Plains (Corrier et al. 1990). Two studies determined aerobic microbial counts from manure in a small midwestern feedyard of 5,000 to 10,000 head (Hrubant et al. 1978; Rhodes & Hrubant 1972).

Microbial loading of the feedyard, air, water and soil in the Texas High Plains of the United States is unknown. It is also unknown how long pathogens survive in nearby shallow lakes (playas) of this area. These playas play an important role in containing runoff from large feedyards, since by their nature they have no effective surface drainage systems. Playas provide a valuable source of water for resident wildlife and for migratory birds passing through the semi-arid southwest Texas High Plains. It has been suggested that water contamination from feedyard runoff is less of a problem in arid than in semi-arid areas (Viets 1971) because of fewer rains. Nevertheless, there is a need to determine

how long pathogens might survive in specific environments in order to evaluate the potential risks in using playas as a source of drinking water for wild animals and as a source of water for irrigation purposes of food crops.

This report will focus on the survival of 10 bacteria, two fungi, four viruses and one protozoal parasite over time in a feedyard playa. The study was done in two parts, which overlapped in time. The first was started on 23 September 1996 and concluded 390 days later, and the second was started on 14 May 1997 and concluded 188 days later.

#### METHODS

*Overall design and collection of microbes.*—The design was to place a known titer of 17 different agents in multiple sets of two types of tubes in a typical feedyard playa. Randomly placed duplicate tubes containing the specimens were retrieved at predetermined times and the agents were re-titered to determine decreases over time.

The first tube type was a screw-cap cryo-vial (CV) and the other was a dialysis tube (DT). Multiple tubes of both types were filled with the identified agents and placed in a bucket with numerous holes drilled into the sides and bottom. A lid was attached snugly. The bucket was allowed to fill with water and was submerged to the bottom of the lake (approximately 3 foot depth) with the aid of weights. A line was attached and anchored at the shore line for easy retrieval.

When CV sample tubes were collected from the playa lake they were placed on ice. When collected, the DT were first placed in cold sterile water, then on ice. Sampling during the first study was determined to be more frequent than necessary, therefore, sampling frequency in the second study was reduced. There were two overlapping studies. One started September 1996 (winter), and one started in May 1997 (summer).

At the indicated times, samples in CV and DT were removed from the playa, protected from sunlight, placed on ice and immediately taken to the United States Department of Agriculture laboratory. Exceptions included the viral agents, which were immediately frozen and stored at  $-85^{\circ}\text{C}$ . After the last virus samples were collected, they were all placed on dry ice and delivered to the virus laboratory where they were thawed and titered along with frozen controls which the virus laboratory had

retained. There was also a refrigerated control for the parasite sample. After each collection, the parasite samples were placed on ice packs and immediately mailed overnight to the laboratory, where the samples were inoculated by mouth into newborn mice to determine viability and infectivity of the parasites.

*Sampling intervals.*—In general, all bacteria and fungi were sampled monthly, except for *P. haemolytica* and *P. multocida* which were sampled each week. The four viruses were sampled weekly. The parasite *Cryptosporidium parvum* was sampled monthly.

*Agents.*—The microorganisms used were: (1) bacteria: *Pasteurella haemolytica* A1, *Pasteurella multocida* A:3, *Staphylococcus aureus*, *Escherichia coli*, *Enterococcus faecalis* (old nomenclature *Streptococcus faecalis*), *Actinomyces pyogenes*, *Salmonella dublin*, *Bacillus thuringiensis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*. Viruses: Infectious Bovine Rhinotracheitis virus (IBR), Bovine Virus Diarrhea (BVD), Bovine Respiratory Syncytial Virus (BRSV), Bovine Parvovirus (BPV); (2) fungi: *Aspergillus fumigatus*, *Aspergillus niger*; (3) Parasite: *Cryptosporidium parvum*. The source of most of the bacterial agents was the Texas Veterinary Diagnostic Laboratory, Amarillo, Texas. *Bacillus thuringiensis* and the two fungal agents were supplied by the Department of Microbiology and Immunology, Texas Tech University Health Science Center, Lubbock, Texas. The viruses were supplied by the Texas Veterinary Diagnostic Laboratory. *C. parvum* was supplied by the USDA-ARS, National Animal Disease Center, Ames, Iowa. *Klebsiella pneumoniae* was not included in the Winter 1996 study.

*Bacterial and fungal storage, culturing, titrations, formation of working pools and identification.*—The microbiological media were from Difco (Detroit, Michigan) and the diluent for serial plating was from GIBCO (New York). The 10 bacterial agents were stored at (-85°C) in a double strength skim milk medium (powdered skim milk diluted with 1/2 of the water recommended). The double strength milk medium was prepared by autoclaving the skim milk for 15 minutes. Four milliliters of the sterile milk were poured over a cultured lawn of bacteria or fungi and approximately 100, 2 mm by 2 mm pieces of sterile filter paper were placed in the microbial suspension. This absorption process was facilitated by using a sterile cotton swab and the paper strips containing the microorganisms were frozen at -85°C.

Pools of each bacterial agent were used to inoculate 30 tryptose agar plates containing 5% bovine blood. These plates were then incubated in air with 5% CO<sub>2</sub> at 37°C for 24 hours. Each of the 30 plates was flooded with 4 mL of sterile physiological phosphate buffered saline (PBS), pH 7.4, and the growth was washed off with sterile cotton tipped swabs. All ten bacterial suspensions were each pooled separately. A duplicate 10-fold serial dilution of the pool was prepared in PBS and 0.1 mL from each dilution was spread on the surface of tryptose agar plates containing 5% bovine blood. The bacterial pools were checked for purity by culturing on tryptose agar plates. Each type of bacteria was identified by colony morphology, cell morphology and Gram stain, and by standard bacteriologic identification procedures (Lennette et al. 1980).

The two fungal agents were cultured, pooled and titered similarly to the bacterial agents, except that malt agar medium was used, and they were incubated for 5 days at 28°C. The fungal pools were checked for purity by culturing on malt agar medium. The fungi were identified by colony morphology, and histology of the fruiting bodies (Larone 1987).

*Viruses and virus titers.*—The four virus pools (IBR virus, BVD, BRSV and BPV) were supplied fresh, on ice, and placed in vials. Specific virus titers were determined at the start of the study and at predetermined intervals by end point dilution on Madin Darby Bovine Kidney (MDBK) cells. Virus samples were tested by 10-fold dilutions in eight rows (eight replications) of a 96-well microtiter plate. Virus samples were diluted in minimal essential medium (MEM) starting at 10<sup>-1</sup> through 10<sup>-8</sup>. Twenty-five μL of each virus dilution was added to eight wells on a microtiter plate containing 25 μL of MEM. After adding the virus dilutions, 150 μL of MDBK cells in MEM with 10% bovine fetal serum were added to each well. The added cell suspensions produced a confluent monolayer after 24 hours incubation. The plates were incubated at 37°C in 5% CO<sub>2</sub> and characteristic virus cytopathic effect was observed and recorded for six days. The end point virus titer was calculated by described methods (Reed & Muench 1938).

*Cryptosporidium parvum.*—The parasite was supplied in PBS, on ice and was placed in vials. The concentration of the oocysts was 4.00x10<sup>7</sup>/mL (Sept., 1996) and 1.10x10<sup>7</sup>/mL (May, 1997). Three types of containers were used for the parasite: the playa vials (CV), the playa dialysis tubes (DT), and refrigerator control vials (RCV) were maintained at 4°C.

*Preparation of oocysts for seeding tubes.*—Feces collected from calves experimentally inoculated with *C. parvum* (Iowa isolate) were suspended in two volumes of 2.5% potassium dichromate solution. This suspension was passed through a graded series of sieves to remove large particles. Twelve milliliters of suspension were overlaid onto 15 mL of 1:2 Sheather's sucrose solution with 0.025 M PBS in 50 mL centrifuge tubes. Tubes were centrifuged for 15 min at 600 x g. The interface layer, containing oocysts, was harvested with a Pasteur pipette, and diluted with PBS and centrifuged for 15 min at 750 x g. The pellet was re-suspended in PBS and centrifuged for 15 min at 350 x g. The final pellet was resuspended in PBS and oocysts were enumerated by direct count with a hemocytometer (Arrowood & Sterling 1987).

*Re-isolation of seeded oocysts from tubes.*—Tubes recovered from the playa and refrigerated control tubes, were sent to the National Animal Disease Center (NADC) Ames, Iowa, laboratory at monthly intervals. *Cryptosporidium parvum* oocysts were recovered from the tubes by rinsing with PBS and centrifuging the resultant suspension for 15 min at 1500 x g. The pellet was resuspended in PBS, and centrifuged for 15 min at 750 x g and again resuspended and centrifuged for 15 min at 350 x g. The final pellet, resuspended in PBS, was adjusted to  $10^6$  oocysts per mL for inoculation into infant mice.

*Assessment of oocyst viability by ability to infect infant mice.*—BALB/c mouse pups were obtained from a breeding colony maintained at the NADC. One-week-old pups were inoculated orally with 0.1 mL of *C. parvum* oocysts suspended in PBS at a concentration of  $10^6$  per mL ( $10^5$  oocysts per pup). Ten pup replications were performed. Pups were returned to their dams following inoculation. Seven days post-inoculation, pups were euthanized, and the contents of the distal colon removed. Smears of contents were made on microscope slides, stained with carbol-fuchsin, and examined for the presence of oocysts. Infection was quantitated by counting the numbers of oocysts in each of ten random high power (500 x) microscope fields on smears from each pup. Fields with more than 10 oocysts were recorded as "10". The average number of oocysts per field was then calculated for each group by totaling the number of oocysts counted for all pups in that group, and dividing by the total number of fields examined.

*Vials.*—Sterile polypropylene vials, with screw-tops (CV) (VWR, Scientific Products, McGraw Park, Illinois) were labeled with the agent number. They were used in both the September, 1996 and May, 1997 studies. A refrigerator control vial (RCV) was also used for the *C.*

*parvum* parasite only. The dialysis tubing (DT) used in the September, 1996 study was a screw cap, dispos-dialyzers, 10 mL size, with a molecular cut off of 25,000 (J.J. Nita Burgoon, Co., Forth Worth, Texas), made of regenerated cellulose. The dialysis tubing used in the May, 1997 study was Spectrum, type F-PVDF a teflon type material with a cut off of 80,000 molecular weight (Spectrum/Microgon, Laguna Hills, California).

*Playa water.*—Four liters of playa water were purified by passing through four layers of cheese cloth and one layer of cotton, then centrifuging at 4000 x g for 20 minutes. The amber supernatant fluid was aspirated from the sediment material and processed through a Whatman #3 filter, then through a Whatman #42 filter. It was then filtered through a large volume Millipore 0.22  $\mu\text{m}$  pore size filter. The sterilized playa water was checked repeatedly for bacteria by culturing on tryptose agar plates. The sterile playa water was added to an equal volume of each agent (bacterial and fungal) pool, prior to placing in the vials or filling the dialysis tube bubbles. There were two exceptions: playa water was not added to any of the vials that contained viruses or parasites.

*Other water quality assay tests.*—Playa water was periodically assayed for electrical conductivity with two hand held TDS meters models WD-35661-30 and WD-35661-40 (Whatman Co., Hillsboro, Oregon). The pH of the playa water was measured with an Orion pH meter model 811, equipped with an Orion general purpose probe (9102BN, Cambridge, Massachusetts). The turbidity of the playa water was measured at wavelength 650 nm by a LKB Biochrome spectrophotometer, model 4050 (Cambridge, England). The biological oxygen demand (BOD), chemical oxygen demand (COD), and nitrogen concentrations (nitrate nitrogen and ammonia nitrogen) were determined at the beginning and end of the two studies by the City of Amarillo waste water treatment facility.

*Statistical Methods.*—Pathogen survival was analyzed by first computing the cumulative proportion of dead microorganisms at each observation. PROC GLM (SAS Institute Inc., 1989) was used to do separate analyses of variance (ANOVAs) for each period and pathogen. The ANOVA model tested the following effects: container type (CT), replication within container type (R-CT), days since start (D) and the CT x D interaction. Effect of container type was tested with R-CT as error with the residual used as error for D and CT x D. Using data from days common to both periods, Winter 1996 and Summer 1997 were

Table 1. Feedyard playa microbial means and standard errors. Proportions of dead microorganisms are shown by container type and day, in the 1996 winter study.

Container type	Day	Microorganism	Mean <sup>a</sup>	SE
CV	4	<i>P. haemolytica</i>	0.99	0.00
DT	1	<i>P. haemolytica</i>	0.95	0.01
CV	1	<i>P. multocida</i>	0.95	0.01
DT	1	<i>P. multocida</i>	0.99	0.00
CV	21	<i>S. aureus</i>	0.98	0.01
DT	7	<i>S. aureus</i>	0.98	0.01
CV	84	<i>E. coli</i>	0.96	0.01
DT	1	<i>E. coli</i>	0.86	0.10
CV	207	<i>E. faecalis</i>	0.98	0.01
DT	21	<i>E. faecalis</i>	0.98	0.01
CV	28	<i>A. pyogenes</i>	0.99	0.00
DT	7	<i>A. pyogenes</i>	0.99	0.00
CV	4	<i>B. thuringiensis</i>	0.96	0.00
DT	7	<i>B. thuringiensis</i>	0.99	0.01
CV	84	<i>S. dublin</i>	0.99	0.00
DT	7	<i>S. dublin</i>	0.99	0.01
CV	84	<i>P. aeruginosa</i>	0.99	0.00
DT <sup>a</sup>	28	<i>P. aeruginosa</i>	0.97	NA <sup>b</sup>
CV	84	<i>A. fumigatus</i>	0.94	0.00
DT	10	<i>A. fumigatus</i>	0.95	0.01
CV	295	<i>A. niger</i>	0.92	0.00
DT	14	<i>A. niger</i>	0.85	0.01

<sup>a</sup> CT X D interaction is not significant.

<sup>b</sup> NA, not applicable.

compared for *P. haemolytica* and *P. multocida*. The water quality assays, pH, electrical conductivity, turbidity, BOD, COD, nitrate nitrogen and ammonium nitrogen, were used in comparing locations with ANOVAs done with PROC GLM. An effect was considered to be significant when  $P \leq 0.05$ . For each pathogen, period and container type, an attempt was made to fit a 2-parameter Weibull function to the proportion of dead microorganisms over number of days using the ordinary least squares option of PROC MODEL (SAS Institute Inc., 1993).

## RESULTS

*Analyses.*—ANOVAs of the winter 1996 bacterial survival data (Table 1) indicated that container type (CT) x days (D) since start interaction was significant ( $P \leq 0.05$ ) for all bacteria except *P. aeruginosa* (DT, day 28). In the summer of 1997 (Table 2) the CT x D interaction was significant for all organisms except *B. thuringiensis* (DT, day 28) and *A. pyogenes* (DT, day 28). For the above three cases only D was significant. In Tables 1 and 2, the means and standard errors of the

Table 2. Feedyard playa microbial means and standard errors. Proportions of dead microorganisms are shown by container type and day, in the 1997 summer study.

Container Type	Day	Microorganism	Mean	SE
CV	28	<i>P. haemolytica</i>	0.98	0.01
DT	21	<i>P. haemolytica</i>	0.99	0.00
CV	7	<i>P. multocida</i>	0.95	0.00
DT	7	<i>P. multocida</i>	1.00	0.00
CV	28	<i>S. aureus</i>	0.99	0.00
DT	28	<i>S. aureus</i>	1.00	0.00
CV	90	<i>E. coli</i>	0.99	0.01
DT	56	<i>E. coli</i>	1.00	0.00
CV	56	<i>E. faecalis</i>	0.99	0.00
DT	28	<i>E. faecalis</i>	1.00	0.00
CV	356	<i>A. pyogenes</i>	0.98	0.01
DT <sup>a</sup>	28	<i>A. pyogenes</i>	0.99	NA <sup>b</sup>
CV	28	<i>K. pneumoniae</i>	1.00	0.00
DT	10	<i>K. pneumoniae</i>	0.87	0.03
CV	120	<i>B. thuringiensis</i>	0.99	0.00
DT <sup>a</sup>	28	<i>B. thuringiensis</i>	0.99	NA
CV	35	<i>S. dublin</i>	0.55	0.09
DT	28	<i>S. dublin</i>	1.00	0.00
CV	35	<i>P. aeruginosa</i>	0.82	0.09
DT	28	<i>P. aeruginosa</i>	0.98	0.02
CV	90	<i>A. fumigatus</i>	0.99	0.01
DT	56	<i>A. fumigatus</i>	0.95	0.05
CV	180	<i>A. niger</i>	0.84	0.00
DT	56	<i>A. niger</i>	0.95	0.00

<sup>a</sup> CT X D interaction is not significant.

<sup>b</sup> NA, means not applicable.

proportions of dead microorganisms are shown for container type and days for both periods, except for the three cases where CT x D was not significant. Only the means are given for these three cases. The ANOVAs which included the effect of period, using the proportions of dead *P. haemolytica* and *P. multocida*, showed the P x CT x D interaction to be significant for both bacterial species. The fitting of the Weibull function to the proportions of dead microorganisms over the appropriate number of days was unsuccessful for most bacteria in summer 1997. The non-linear least-squares procedure would not converge with parameter estimates. The ANOVAs, with means and standard errors, taken together with the curves indicated that for *A. fumigatus*, *A. niger*, *K. pneumoniae*, *S. enterica* serovar *dublin*, *S. aureus* and *P. aeruginosa*, survival extended beyond ten days in small numbers.

*Microbial survival, winter 1996.*—The following microbes in CV containers survived for 390 days with the individual colony forming units/mL shown in Table 3: *S. aureus*, *E. coli*, *E. faecalis*, *B. thuringiensis*,

Table 3. Feedyard playa microbial survival data, Winter 1996 study.

Identification and microbial titers (colony forming units/mL) of screw-cap sample vials (CV)										
Day Sampled	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>	<i>Enterococcus faecalis</i> <sup>a</sup>	<i>Actinomyces pyogenes</i>	<i>Bacillus thuringiensis</i>	<i>Salmonella dublin</i>	<i>Pseudomonas aeruginosa</i>	<i>Aspergillus fumigatus</i>	<i>Aspergillus niger</i>	
0	2.95X10 <sup>11</sup>	3.75X10 <sup>11</sup>	7.50X10 <sup>8</sup>	3.60X10 <sup>10</sup>	1.10X10 <sup>10</sup>	3.70X10 <sup>11</sup>	4.50X10 <sup>11</sup>	4.40X10 <sup>5</sup>	2.65X10 <sup>8</sup>	
7	4.30X10 <sup>10</sup>	3.80X10 <sup>10</sup>	1.90X10 <sup>9</sup>	4.65X10 <sup>9</sup>	1.90X10 <sup>7</sup>	2.50X10 <sup>10</sup>	1.60X10 <sup>10</sup>	1.76X10 <sup>5</sup>	7.00X10 <sup>7</sup>	
28	3.85X10 <sup>9</sup>	3.50X10 <sup>10</sup>	2.45X10 <sup>9</sup>	2.80X10 <sup>8</sup>	5.50X10 <sup>6</sup>	1.75X10 <sup>10</sup>	1.10X10 <sup>10</sup>	2.95X10 <sup>5</sup>	4.90X10 <sup>7</sup>	
56	2.15X10 <sup>8</sup>	2.70X10 <sup>10</sup>	2.90X10 <sup>9</sup>	2.85X10 <sup>7</sup>	2.65X10 <sup>6</sup>	6.55X10 <sup>9</sup>	5.30X10 <sup>9</sup>	5.00X10 <sup>4</sup>	4.85X10 <sup>7</sup>	
84	1.14X10 <sup>8</sup>	1.50X10 <sup>10</sup>	1.90X10 <sup>9</sup>	0	5.50X10 <sup>6</sup>	2.45X10 <sup>9</sup>	2.15X10 <sup>9</sup>	2.95X10 <sup>4</sup>	6.00X10 <sup>7</sup>	
174	5.50X10 <sup>6</sup>	6.70X10 <sup>7</sup>	2.08X10 <sup>8</sup>	0	5.70X10 <sup>6</sup>	3.60X10 <sup>7</sup>	1.65X10 <sup>8</sup>	3.10X10 <sup>2</sup>	1.30X10 <sup>8</sup>	
294	5.00X10 <sup>2</sup>	1.02X10 <sup>7</sup>	1.00X10 <sup>6</sup>	0	9.00X10 <sup>4</sup>	1.15X10 <sup>7</sup>	6.05X10 <sup>7</sup>	2.70X10 <sup>2</sup>	2.50X10 <sup>7</sup>	
324	3.95X10 <sup>4</sup>	1.76X10 <sup>6</sup>	1.03X10 <sup>7</sup>	0	2.45X10 <sup>6</sup>	6.30X10 <sup>5</sup>	1.71X10 <sup>7</sup>	0	1.95X10 <sup>4</sup>	
390	1.82X10 <sup>4</sup>	9.30X10 <sup>4</sup>	2.80X10 <sup>3</sup>	0	6.40X10 <sup>5</sup>	1.44X10 <sup>6</sup>	2.35X10 <sup>7</sup>	0	0	
Identification and microbial titers (colony forming units/mL) of dialysis sample tubes (DT)										
0	2.95X10 <sup>11</sup>	3.75X10 <sup>11</sup>	7.50X10 <sup>8</sup>	3.60X10 <sup>10</sup>	1.10X10 <sup>10</sup>	3.70X10 <sup>11</sup>	4.50X10 <sup>11</sup>	4.40X10 <sup>5</sup>	2.65X10 <sup>8</sup>	
7	7.00X10 <sup>9</sup>	2.25X10 <sup>7</sup>	9.45X10 <sup>7</sup>	2.35X10 <sup>8</sup>	1.12X10 <sup>7</sup>	4.30X10 <sup>9</sup>	7.60X10 <sup>8</sup>	2.80X10 <sup>4</sup>	2.65X10 <sup>7</sup>	
21	6.25X10 <sup>6</sup>	2.20X10 <sup>7</sup>	1.65X10 <sup>7</sup>	3.00X10 <sup>5</sup>	1.90X10 <sup>6</sup>	3.25X10 <sup>7</sup>	4.00X10 <sup>4</sup>	1.00X10 <sup>1</sup>	6.50X10 <sup>7</sup>	
28	4.40X10 <sup>6</sup>	5.25X10 <sup>7</sup>	1.50X10 <sup>6</sup>	ND <sup>b</sup>	1.30X10 <sup>6</sup>	1.35X10 <sup>7</sup>	ND	ND	2.65X10 <sup>6</sup>	

<sup>a</sup> Old nomenclature is *Streptococcus faecalis*.<sup>b</sup> ND, not done.

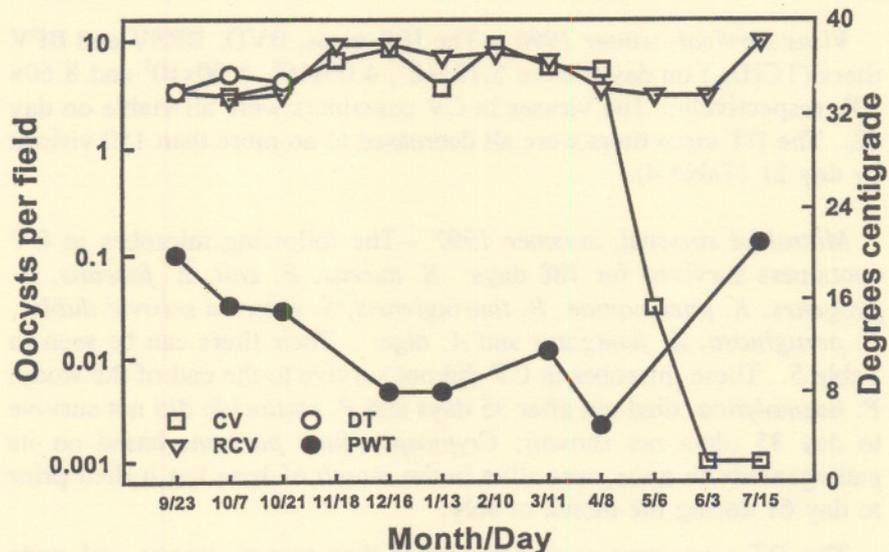


Figure 1. Survival of *Cryptosporidium parvum* (first study) in playa, 1996-97. Viability of recovered *C. parvum* oocysts as assessed by ability to infect infant mice. Left Y axis shows the average number of oocysts per microscopic field in smears from infant mice inoculated with oocysts recovered at the indicated dates. Right Y axis shows temperature of water in playa. CV represents the viability of oocysts recovered from sealed vials immersed in the playa. RCV represents viability of oocysts recovered from vials refrigerated at 4°C. DT represents viability of oocysts recovered from dialysis tubes immersed in the playa. PWT represents playa water temperature at indicated dates.

*S. dublin* and *P. aeruginosa*. The following microbes in CV did not survive to the end of the study: *A. pyogenes*, *A. fumigatus* and *A. niger*. *Pasteurella haemolytica* and *P. multocida* did not survive to day 28 (data not shown). *Cryptosporidium parvum*, based on its pathogenicity in mice (Fig. 1), was able to survive through the winter in the playa and survived through the month of May (day 235) but not June (day 267).

The DT were removed after 28 days. The microbes that were still viable at that time were: *S. aureus*, *E. coli*, *E. faecalis*, *A. pyogenes* (removed on day 21), *B. thuringiensis*, *S. enterica* serovar *dublin*, *P. aeruginosa* (removed on day 21), *A. fumigatus* (removed on day 21), and *A. niger*; their colony forming units/mL can be seen in Table 3. *Pasteurella haemolytica* and *P. multocida* in the DT died out between days seven and 14. The DT containing *C. parvum* was removed at 28 days and still had viable oocysts based on the mouse infectivity assay (Fig. 1).

*Virus survival, winter 1996.*—The IBR virus, BVD, BRSV and BPV titers ( $TCID_{50}$ ) on day 0 were  $5.12 \times 10^7$ ,  $4.00 \times 10^6$ ,  $4.00 \times 10^5$  and  $8.60 \times 10^6$ , respectively. The viruses in CV containers were all viable on day 42. The DT virus titers were all decreased to no more than 150 virions by day 21 (Table 4).

*Microbial survival, summer 1997.*—The following microbes in CV containers survived for 188 days: *S. aureus*, *E. coli*, *E. faecalis*, *A. pyogenes*, *K. pneumoniae*, *B. thuringiensis*, *S. enterica* serovar *dublin*, *P. aeruginosa*, *A. fumigatus* and *A. niger*. Their titers can be seen in Table 5. These microbes in CV did not survive to the end of the study: *P. haemolytica*, died out after 35 days and *P. multocida* did not survive to day 35 (data not shown); *Cryptosporidium parvum*, based on its pathogenicity in mice, was alive in the month of June but it died prior to day 61 during the month of July.

The DT containers were removed if they turned opaque and were found to be dry on the inside of the dialysis bag. None of the DT containers lasted to the end of the 188 day study. The last days that the DT tubes contained fluid for the following microbes in the summer 1997 study were: *S. aureus* 28, *E. coli* 56, *E. faecalis* 84, *A. pyogenes* 56, *K. pneumoniae* 28, *B. thuringiensis* 28, *S. enterica* serovar *dublin* 56, *P. aeruginosa* 28, *A. fumigatus* 84 and *A. niger* 56. The titers (colony forming units/mL) for these dates and microorganisms can be seen in Table 5. The DT containers of *P. haemolytica* and *P. multocida* were removed on day 35 and their respective titers were  $1.8 \times 10^4$  and  $1.2 \times 10^3$ . The DT containers of *C. parvum* were removed on day 28 and were shown to still contain viable organisms based on the mouse infectivity assay.

*Virus survival, winter 1997.*—The IBR virus, BVD, BRSV and BPV titers ( $TCID_{50}$ ) on day 0 were  $1.00 \times 10^7$ ,  $2.20 \times 10^6$ ,  $4.00 \times 10^4$  and  $1.04 \times 10^4$ , respectively. The viruses in CV containers were still viable by day 7. The viruses became inactivated over time in the following order: RSV, day 21; IBR, day 28; BPV, day 35 and BVD, day 42. The DT virus samples were all inactive on day 21 (Table 4).

*Playa electrical conductivity, pH and turbidity.*—The electrical conductivity (reported in  $\mu\text{S}/\text{cm}$ ) of the playa water was variable over time and among samples collected. The electrical conductivity ( $n=17$ ) of the playa water over time ranged from 350 to 3900  $\mu\text{S}/\text{cm}$ : The mean was 1281  $\mu\text{S}/\text{cm}$  with a  $SD \pm 889$ . No discernible patterns were observed.

Table 4. Feedyard playa virus survival data.

Day Sampled	Infectious bovine rhinotracheitis		Bovine virus diarrhoea		Bovine respiratory syncytial virus		Bovine parvovirus <sup>b</sup>	
	Screw-cap vial	Dialysis tube	Screw-cap vial	Dialysis tube	Screw-cap vial	Dialysis tube	Screw-cap vial	Dialysis tube
0	5.12X10 <sup>7</sup>	5.12X10 <sup>7</sup>	4.00X10 <sup>6</sup>	4.00X10 <sup>6</sup>	4.00X10 <sup>5</sup>	4.00X10 <sup>5</sup>	8.60X10 <sup>6</sup>	8.60X10 <sup>6</sup>
7	1.30X10 <sup>7</sup>	8.60X10 <sup>3</sup>	1.00X10 <sup>6</sup>	9.20X10 <sup>3</sup>	6.00X10 <sup>3</sup>	1.30X10 <sup>2</sup>	2.00X10 <sup>7</sup>	1.00X10 <sup>5</sup>
14	1.00X10 <sup>6</sup>	6.40X10 <sup>2</sup>	4.00X10 <sup>5</sup>	1.26X10 <sup>2</sup>	1.80X10 <sup>2</sup>	1.30X10 <sup>2</sup>	1.26X10 <sup>7</sup>	1.26X10 <sup>2</sup>
21	9.20X10 <sup>6</sup>	1.50X10 <sup>2</sup>	1.64X10 <sup>2</sup>	ND	1.30X10 <sup>2</sup>	1.30X10 <sup>2</sup>	5.20X10 <sup>6</sup>	1.26X10 <sup>2</sup>
28	6.40X10 <sup>4</sup>	ND <sup>a</sup>	1.00X10 <sup>5</sup>	ND	1.30X10 <sup>2</sup>	1.30X10 <sup>2</sup>	4.00X10 <sup>6</sup>	1.26X10 <sup>2</sup>
42	6.00X10 <sup>4</sup>	ND	6.00X10 <sup>4</sup>	ND	1.30X10 <sup>2</sup>	ND	7.84X10 <sup>6</sup>	ND
Summer 1997 identification and virus titer (tissue infective dose 50 percent endpoint/mL)								
0	1.00X10 <sup>7</sup>	1.00X10 <sup>7</sup>	2.20X10 <sup>6</sup>	2.20X10 <sup>6</sup>	4.00X10 <sup>4</sup>	4.00X10 <sup>4</sup>	1.04X10 <sup>4</sup>	1.04X10 <sup>4</sup>
7	0	0	4.00X10 <sup>5</sup>	1.25X10 <sup>4</sup>	4.00X10 <sup>2</sup>	1.84X10 <sup>2</sup>	8.40X10 <sup>2</sup>	0
14	ND	ND	ND	0	ND	ND	ND	ND
21	2.52X10 <sup>4</sup>	0	1.00X10 <sup>4</sup>	0	0	0	2.52X10 <sup>3</sup>	0
28	0	ND	1.26X10 <sup>2</sup>	ND	ND	ND	1.48X10 <sup>3</sup>	ND
35	0	ND	4.00X10 <sup>2</sup>	ND	ND	ND	0	ND
42	0	ND	0	ND	ND	ND	0	ND

<sup>a</sup> ND, not done.<sup>b</sup> Retrospectively, it was determined that the Parvovirus inoculum in 1996 was contaminated with an adventitious virus.

Table 5. Feedyard playa microbial survival data, summer 1997 study.

Identification and microbial titers (colony forming units/mL) of screw-cap sample vials (CV)										
Day Sampled	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>	<i>Enterococcus faecalis</i> <sup>a</sup>	<i>Actinomyces pyogenes</i>	<i>Klebsiella pneumoniae</i>	<i>Bacillus thuringiensis</i>	<i>Salmonella dublin</i>	<i>Pseudomonas aeruginosa</i>	<i>Aspergillus fumigatus</i>	<i>Aspergillus niger</i>
0	1.62X10 <sup>10</sup>	2.66X10 <sup>10</sup>	8.10X10 <sup>9</sup>	8.85X10 <sup>9</sup>	5.40X10 <sup>10</sup>	1.90X10 <sup>9</sup>	5.10X10 <sup>10</sup>	4.41X10 <sup>10</sup>	1.25X10 <sup>5</sup>	4.20X10 <sup>7</sup>
28	9.60X10 <sup>7</sup>	3.50X10 <sup>8</sup>	1.52X10 <sup>10</sup>	3.25X10 <sup>9</sup>	1.14X10 <sup>8</sup>	5.25X10 <sup>6</sup>	1.60X10 <sup>10</sup>	1.60X10 <sup>10</sup>	8.15X10 <sup>3</sup>	8.00X10 <sup>7</sup>
56	4.25X10 <sup>5</sup>	7.05X10 <sup>7</sup>	1.05X10 <sup>8</sup>	4.40X10 <sup>7</sup>	3.70X10 <sup>6</sup>	2.60X10 <sup>7</sup>	2.35X10 <sup>7</sup>	1.55X10 <sup>8</sup>	1.10X10 <sup>3</sup>	5.45X10 <sup>7</sup>
84	7.90X10 <sup>5</sup>	1.65X10 <sup>8</sup>	5.75X10 <sup>7</sup>	2.50X10 <sup>7</sup>	1.06X10 <sup>6</sup>	1.40X10 <sup>7</sup>	3.40X10 <sup>6</sup>	4.70X10 <sup>5</sup>	6.55X10 <sup>2</sup>	2.90X10 <sup>7</sup>
188	2.75X10 <sup>6</sup>	1.85X10 <sup>6</sup>	1.23X10 <sup>7</sup>	3.25X10 <sup>6</sup>	1.15X10 <sup>6</sup>	4.05X10 <sup>6</sup>	1.60X10 <sup>6</sup>	6.15X10 <sup>6</sup>	4.30X10 <sup>2</sup>	4.95X10 <sup>6</sup>
Identification and microbial titers (colony forming units/mL) of dialysis sample tubes (DT)										
0	1.62X10 <sup>10</sup>	2.66X10 <sup>10</sup>	8.10X10 <sup>9</sup>	8.85X10 <sup>9</sup>	5.40X10 <sup>10</sup>	1.90X10 <sup>9</sup>	5.10X10 <sup>10</sup>	4.41X10 <sup>10</sup>	1.25X10 <sup>5</sup>	4.20X10 <sup>7</sup>
28	8.00X10 <sup>2</sup>	4.00X10 <sup>6</sup>	3.20X10 <sup>6</sup>	1.70X10 <sup>5</sup>	7.20X10 <sup>9</sup>	1.35X10 <sup>7</sup>	1.30X10 <sup>6</sup>	5.05X10 <sup>8</sup>	5.05X10 <sup>2</sup>	6.00X10 <sup>6</sup>
56	ND <sup>b</sup>	1.10X10 <sup>3</sup>	4.50X10 <sup>5</sup>	1.00X10 <sup>2</sup>	ND	ND	4.25X10 <sup>4</sup>	ND	7.17X10 <sup>3</sup>	1.85X10 <sup>5</sup>
84	ND	ND	2.01X10 <sup>5</sup>	ND	ND	ND	ND	ND	5.00X10 <sup>1</sup>	ND

<sup>a</sup> Old nomenclature is *Streptococcus faecalis*.<sup>b</sup> ND, not done

The pH ( $n=17$ ) activity (reported in units) of the playa water was measured from September 1996 through November 1997. The pH values of the samples were quite similar over extended periods of time, as were pH values on samples taken within an hour of each other. The pH ranged from 7.0 to 8.6 units, with a tri-mode of 7.67 units, a mean of 7.58 units and a SD  $\pm$  0.36.

Turbidity ( $n=9$ ) of the playa water was measured from May through October, 1997. The water was disturbed as little as possible prior to taking the sample. The turbidity was measured at 650 nm wavelength. Turbidity in May was 13.5. This increased to 35.1 in June and decreased to 8.0 in October. The turbidity of the water in November was at its highest, 77.6, when the wind was mixing the water and sediment at the time of sample collection.

*Playa BOD, COD and nitrogen.*—At the beginning of the studies, the following concentrations were found: BOD, 1006mg/L; COD, 813 mg/L; nitrate nitrogen, 0 mg/L; and ammonia nitrogen, 28.5 mg/L. At the end of the studies the following concentrations were found: BOD, 520 mg/L; COD, 1380 mg/L; nitrate nitrogen, 0.5 mg/L and ammonia nitrogen, 39 mg/L.

#### DISCUSSION

It appears probable that most of the microbes that wash into a playa from a feedyard die rapidly over a 120 day period, however a residual population of gram negative pathogens (Tannock & Smith 1971) and some gram positive pathogens are capable of remaining viable for at least a year in feedyard playas. Of course, organisms in screw-capped vials or dialysis tubing were not exposed to the playa environment in the same way as bacteria, viruses and parasites that were freely deposited by animals, birds or reptiles. Therefore, it is difficult to know if the results reported in this study are accurate reflections of what would happen in the wild. However, if they are, the survival of these pathogens in low numbers could potentially pose some danger of infecting other susceptible animals that might come in contact with or drink the water. Therefore, any resultant disease would likely be due to a combination of factors such as the susceptibility of the host, dose of the infecting agent and stressful environmental conditions. A significant additional source of gram negative pathogens in these playas are the large migrating flocks of thousands of geese, ducks and cranes.

Consistently lower titers of bacteria and fungi were found in the

dialysis tubes when compared to screw-cap vials. These were initially filled with 1/2 volume of sterile playa water and 1/2 by volume of the agent in physiological phosphate buffered saline. The dialysis tubes were included in the study to determine if there were natural inhibitors of bacteria and fungi in the playa water. Conversely the investigators wanted to know if the playa water entering the dialysis tubes could act as a growth medium. The latter was not observed to any extent. The possibility of a free exchange of playa water inhibitors in the dialysis tubes exists, whereas it would not exist in the screw-cap tubes. This may account for lower titers of the agents and a more rapid die off in the dialysis tubes, compared to the screw-cap tubes. Also, the lower titers may have been due to the "leakiness" of the cellulose acetate dialysis membranes caused by bacterial degradation.

If the decreased titers of the agents in the dialysis tubes were due to inhibitors in the playa water, then it might be expected that the membrane filtered playa water added to the screw-capped tubes might eventually kill all the microorganisms present. It might also be argued that the filtration technique might take out all or part of the potential inhibitors. Conversely, the filtered playa water might serve as a growth medium.

*Pasteurella haemolytica* and *P. multocida* had the poorest survival of all the bacteria tested. This was not unexpected, since both are rather fastidious agents in the laboratory and will die out after a few days in the incubator at 37°C on Tryptose blood agar. These organisms will not survive in culture plates for a week or longer on a lab bench at ambient temperature and will not survive for more than a few weeks in the refrigerator.

The poor ability of bovine *P. multocida* to survive in playas during the winter or summer months is an important finding for feedyard owners. It has been suggested that perhaps bovine *P. multocida* is involved in avian pasteurellosis (fowl cholera) in feedyard playas; however, bovine isolates were avirulent when injected into mallard ducks. Avian isolates recovered from wild mallards, wild turkeys and a bald eagle were virulent after injections were administered to mallard ducks (Windingstad et al. 1988). Avian strains of *P. multocida* are significant pathogens which induce avian cholera in migratory water fowl, especially ducks and geese inhabiting playas of the High Plains. The avian strains have killed millions of birds since they were first reported. An avian cholera outbreak in 1944 was first seen at the

Muleshoe National Wildlife Refuge in the duck population (Steiert 1995). Avian cholera was also reported in Castro County, Texas in the 1940s. The source was thought to have been chicken carcasses that were dumped in playa drainage (Quortrup et al. 1946).

The CV with *A. niger* maintained a higher titer than those that contained *A. fumigatus*. The main difference between the two fungi was that *A. niger* had a higher initial titer due to its excellent spore production in two days. The *A. fumigatus* culture had fewer spores at seven days. More colonies probably originated from its hyphae, thus a lower original titer occurred compared to *A. niger*. It is probable that hyphal elements are more susceptible to environmental stress than the spores.

All four viruses (IBR, BVD, RSV and BPV) in the DT samples were decreased in titer by day 21 in the September 1996 study and the same decrease was observed by day seven in the 1997 study (Table 4). The 1996 CV samples of the BVD and IBR virus had decreased to a virus titer of 60,000 tissue culture infective doses (TCID<sub>50</sub>)/mL by day 42. The BPV virus titer on day 42 remained high, 7,840,000 TCID<sub>50</sub>/mL. Retrospectively, it was determined that the initial BPV inoculum in 1996 only, was contaminated with an adventitious virus and the RSV titer was practically non-existent. The CV samples of the four viruses decreased more rapidly during the May 1997 study, probably due to the hot ambient temperature. The DT samples of IBR and BPV were inactivated by day seven and BVD and RSV were inactivated by day 14. The CV samples were inactivated in the following order: RSV, day 14; IBR virus, day 28; BVD and BPV, day 42.

The oocysts of *C. parvum* are quite resistant to environmental conditions, however they were susceptible to freezing and apparently to the hot summer weather of the Texas Panhandle. However, the depth of water probably had a bearing on their survival through the summer and winter months. *C. parvum* probably survived the winter because the playa never froze to the bottom where the tubes were located. The shallow water and the hot temperature of the 1997 summer appeared to destroy the oocysts viability.

The pH of the playa was more constant in the neutral and slightly basic range than expected. It was also unexpected to find the electrical conductivity so variable within a playa. Turbidity was quite variable and increased with high winds and also when large flocks of migratory

fowl used the playa as a stopover in their migration.

This study indicates that there was a rapid microbial decline in pathogen viability in the DT samples over a one month period. However, the data also show that pathogens in tubes are capable of surviving in the playa environment at a low population density for long periods of time (a year or longer). It should be pointed out that survival of the test organisms in the sterile playa water in this study could be quite different from survival in nonsterile playa water where inhibition and/or competition by naturally occurring microorganisms could have a negative impact on pathogen survival.

Feedyard playas should be fenced off to prevent stray calves from wandering into the potentially contaminated water. It appears that the practice of placing grass filter strips (Young et al. 1980; Yanko et al. 1995) between the feedyard and playa should reduce the total solids, BOD, COD, ammonia nitrogen, viral, bacterial and endotoxin load of playas and would be a good idea. Finally, individuals responsible for feedyard playa water resources should be cautious in the application of these waters to fields and crops (Bell 1976), and for dust abatement, because the water may be contaminated with pathogens. Particular attention should be taken in irrigating crops with feedyard water. This would include allowing for sufficient time to expire prior to the consumption of the crop. This will allow for a further decrease in potential pathogen viability.

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