RESEARCH REPORT

ENVIRONMENT

Title: Evaluation of dust and pathogens in air exhausted from swine facilities
NPB #00-088

Investigator: Gary L. Bowman, DVM

Institution: The Ohio State University

Co-investigators: Teresa Y. Morishita, DVM, PhD
John C. Gordon, DVM, MPH

Date Received: 8/31/2001

I. Abstract: Five swine finishing buildings were identified in which *Salmonella* organisms were recovered from pen fecal samples. On a weekly basis for 5 weeks, fecal samples were collected from the pens, dust levels were recorded for a 24-hour period both inside and outside the building, and air was sampled both inside and outside the building by drawing it through filters for a 24-hour period. The filter papers were then cultured for salmonellae and *Escherichia coli*. *E. coli* organisms were recovered from some of the indoor and outdoor filters. No *Salmonella* spp were isolated from either the indoor or outdoor filters at any of the five *Salmonella* fecal-positive facilities. Dust levels varied markedly between facilities but were unrelated to the finding of *E. coli* organisms on the filter papers.

II. Introduction: Confinement livestock rearing is being confronted on several issues - odor, waste disposal, pollution of surface water, and use of chemicals in production – to name a few. Many states are contemplating more restrictive regulation of concentrated animal feeding operations (CAFO) based on the public perception that a CAFO poses a health threat to people living and working nearby. Numerous studies have investigated the risk of water contamination by swine pathogens as a result of waste handling methods. Employee health and safety hazards have been identified for those working in confinement swine building. Studies have found these workers to have more respiratory problems than the general population. There is increasing concern about the health risk of aerosolized dust, endotoxins, volatile compounds, and pathogens to those people living and/or working in the areas near confinement facilities. A study found that neighbors of large-scale swine operations reported significantly higher rates of symptom known to represent toxic or inflammatory effects on their respiratory systems. In an attempt to answer questions about the health risk to neighbors from airborne pathogens emanating from swine facilities, we concurrently sampled the air both within and immediately outside the building housing growing swine known to be

shedding salmonellae in their feces. As dust particles can serve as a airborne vehicles for odor molecules, toxins, and bacteria, dust levels were measured both inside and outside the facility concurrently with the air samplings for bacteria to see if dust levels were associated with the recovery of bacteria.

III. Objectives: To better assess the health risk posed by a typical confinement swine feeding facility to people living and working nearby, we wanted 1) to determine if we could recover salmonellae and *E. coli* bacteria from air exhausted from a building where swine are shedding salmonellae in their feces, 2) to determine if airborne dust levels were associated with the recovery of airborne bacteria, and 3) In the event we found the same species of bacteria both on the inside and outside filters, we wanted to determine if they were genetically related through the use of genetic finger-printing techniques.

IV. Procedures: Producers participating in the study were recruited by Ohio veterinarians engaged in swine practice. We estimated we would need to survey approximately 25 units in our search for 5 units where we could find salmonellae in the feces. As we had proposed to conduct the study in mechanically-ventilated finishing units, we found production systems having multiple contract-finishing units to be most suitable because they could provided access to many units typical of the swine industry in the Midwest. To comply with farm biosecurity protocols, contract finishers also afforded us the option of being able to collect samples and data at more than one site on a given day when the same contractor operated all the units visited that day.

On the initial visit to the prospective study unit, multiple small fecal samples were collected from the floor of each pen filling 6 four-ounce sterile containers per building. The fecal samples were frozen at minus-80^{o} C. until culturing procedures could be started. When salmonellae were recovered from the initial fecal samples, we arranged with the contractor to collect dust level data and air samples for a 24-hour period once a week for five weeks.

Two total suspended particulate high volume samplers loaded with 8 x 10 inch high purity glass microfibre filters were used for the air sampling. One air sampler was placed within the building in the central aisle way and the other was set-up outside the building approximately 10 feet downwind of the building or 10 feet from the primary exhaust fan. A sterile filter paper was placed in each air sampler. Both the inside and outside samplers operated concurrently for a 24-hour period. At the end of the 24-hour period, the filter paper was aseptically removed from each sampler and placed in a sterile pouch for transport to the laboratory for immediate culturing.

A light-scattering photometer was mounted on each of the air sampler apparatuses to record the airborne dust levels in the period the air was being drawn through the sampler. The airborne particulate levels were recorded once every 60 seconds during each 24-hour monitoring period; this data was later downloaded to a personal computer for analysis.

Pen fecal samples were collected throughout the unit for culturing coinciding with each of the five 24-hour sampling periods in that unit. Fecal samples were chilled for transport to the laboratory and then maintained at –80^{o} C. until the laboratory could process them.

Isolation of bacteria. For *E. coli* isolation, the specimen (5 gm of the composite fecal sample in each of the six 4-ounce cups, or half the filter paper from both the inside and outside air samplers) was mixed with 100 ml of 1:20 dilution brain-heart infusion broth. This suspension was incubated in air at 37°C for 18 hours and then plated on MacConkey's agar. *E. coli*-suspect colonies were confirmed by observing growth characteristics and biochemical reactions on MIO, TSI, citrate, and urea media. For salmonellae isolation, the specimen (5 gm of the composite fecal sample, or the other one-half of each filter paper) was placed in 50 ml of a pre-enrichment broth to revive injured salmonellae that may be present in the samples. This suspension was incubated for 16-18 hours in air at 37°C. After 24 hours' incubation, 0.1 ml of this culture was transferred to selective enrichment broth. This broth was subcultured after 24 hours of air incubation at 37°C on Brilliant green and XLT_4 agar plates. Suspect *Salmonella* spp. colonies were confirmed by observing growth characteristics and biochemical reactions on LIA, TSI, citrate, and urea media.

Genetic Fingerprinting. To determine if bacterial isolates from the inside and outside air filter elements were related by having similar restriction endonuclease analysis patterns, we used the genetic fingerprinting procedures. Note: While we planned to use the procedure for both *E. coli* and salmonellae isolates, no salmonellae were recovered from any of the air filter elements. Therefore the procedures were used for the *E. coli* isolates only.

V. Results:

All the swine units participating in this study were operated by one of 3 contractors. Each unit had a capacity of approximately 1,100 head and was double-curtained with tunnel ventilation capability. The floors in all participating units were totally slatted with deep manure storage pits below.

As the first objective of our project was to see if we could find airborne salmonellae and *E. coli* organisms in the air exhausted from five buildings housing swine shedding salmonellae in their feces, we began by identifying *Salmonella*-positive units. To find five *Salmonella*-positive units, we collected the initial fecal samples from a total of 24 finishing units (Table 1). We found salmonellae in one or more fecal samples from 7 farms but two units were later excluded. One of the *Salmonella*-positive units (#6) was converted from a finisher to a replacement gilt rearing facility before the weekly surveillance could begin. This unit was dropped from further study at the request of the contractor because the increased traffic associated with our investigation might compromise the higher level of biosecurity afforded replacement breeding stock. The hogs in another unit (#3), found to be *Salmonella*-positive on the initial sampling, were marketed before we could begin the weekly sampling. We were unable to find salmonellae in the feces of the next group in this facility even though we did the fecal testing, air sampling, and dust measurements for five weeks. Hence this unit was also excluded from the study. The dust measurements and the culture results from the five study farms are shown in Table 2.

Outside dust readings and air sample collections were not were obtained on the last 4 of the 5 weekly samples at one unit (#16) because of electrical damage to the dust monitor and air sampler that occurred during the second weekly monitoring period.

No salmonellae were recovered from any of the 25 air filter elements collected inside the buildings housing hogs shedding salmonellae in their feces. Likewise, no

salmonella organisms were found on any of the 21 air filters elements collected 10 feet outside the same five buildings

We selected *E. coli* as a marker organism to see if it could be recovered from the air inside and outside the unit. *E. coli* was recovered from a total of 7 of 25 inside air filters and 5 of 21 outside filters. In only 2 instances did we find *E. coli* on both an inside and outside filter during the same sampling period.

Our second objective was to study the association between airborne dust levels and the recovery of airborne *Salmonella* organisms from the filter elements. The dust levels both inside and immediately outside the buildings (approximately 10 feet) were measured during the weekly 24-hour air sampling periods. Since no *Salmonella* organisms were recovered from any of the filters by bacteriological culturing techniques, no correlation can be made. As expected, the average dust levels were higher inside the building than in the stream of the air exhausted outside the building (2.63 versus 1.4 milligrams/cubic meter of air average). We also found that the dust levels were highly variable over time, both within a given building as well as between buildings. Automatic curtains and fans probably added to the variability in the measured dust levels because some units changed back and forth between mechanical and natural ventilation modes in the course of the sampling period, especially when there was warm days with cool nights.

The third objective was to determine if the bacteria found on the filters inside the building and outside the building were related to each other by the use of genetic finger-printing techniques. *E.coli,* our marker organism, was recovered from a total of 12 filters (7 inside filters and 5 outside filters). On two occasions the finding of *E. coli* on the outside filter coincided with its recovery from the inside filter. By using genetic fingerprinting techniques, we determined on one farm that the *E. coli* found on the outside filter was not related to the *E. coli* isolated from the inside filter during the same 24-hour period. On the other farm, the fingerprinting results were inclusive because of laboratory procedural problems. The culture will be reprocessed and the results reported in September, 2001.

Based on the findings in this preliminary study, there is no evidence that salmonella organisms excreted in the feces of infected hogs pose a health risk to neighbors by airborne dissemination from the swine facility. Even within the building where salmonella-shedding hogs are housed, we were unable to recover salmonella from the air filters attached to high volume air samplers. We were able to find *E. coli* on the air filters collected from both inside and outside the building about one quarter of the time. On two occasions, once at each of two units, the finding of *E. coli* on the inside and outside filters coincided, but we have no evidence to this point that the isolates from the outside filters were genetically related to the isolates from the inside filter. Determining if any of the *E. coli* organisms captured on the filters were related to those excreted by the hogs in the respective units was beyond the scope of this project., however it may be an area for future investigation as the role of CAFOs on the airborne dissemination of pathogens is evaluated.

Table 1, NPPC Project #00-088

Project Farm #	Initial Fecal Survey Date	Initial fecal salmonellae survey results (number of 4-ounce cups in which *Salmonella* spp were found
1	27-Jun-00	2+ of 6
2	27-Jun-00	0+ of 6
3	27-Jun-00	2+ of 6 in one of 2 barns
4	27-Jun-00	0+ of 6
5	12-Jul-00	0+ of 6
6	12-Jul-00	2+ of 6
7	17-Aug-00	0+ of 6
8	17-Aug-00	2+ of 6 in one of 2 barns
9	17-Aug-00	0+ of 6 in each of 2 barns
10	8-Sep-00	1+ of 6
11	8-Sep-00	0+ of 6
12	22-Mar-01	2+ of 6 in one of 2 barns
13	10-Jan-01	0+ of 6 in each of 2 barns
14	5-Mar-01	0+ of 6 in each of 2 barns
15	5-Mar-01	0+ of 6
16	18-Apr-01	5+ of 6
9 (second time)	15 Feb 01	0+ of 6 in each of 2 barns
Total		*Salmonella* spp were found in the feces of 7 of the 24 units sampled.

Table 2, NPPC Project #00-088

		Salmonella cultures			*E. coli* cultures		Dust measurements					
Farm #	Sampling date	Fecal culture results	Inside air filter culture	Outside air filter culture	Inside air filter culture	Outside air filter culture	Inside air highest mg/m^3	Inside air lowest mg/m^3	Inside air average mg/m^3	Outside air highest mg/m^3	Outside air lowest mg/m^3	Outside air average mg/m^3
1	8-Jan-01	0 of 6	0	0	Neg	Neg	none*	none*	none*	0.154	0.001	0.025
1	15-Jan-01	0 of 6	0	0	Neg	**Positive**	1.343	0.215	0.638	0.056	0	0.025
1	23-Jan-01	1 of 6	0	0	**Positive**	Neg	2.248	0.714	1.093	2.861	1.864	2.039
1	29-Jan-01	0 of 6	0	0	**Positive**	Neg	3.744	0.839	1.196	2.289	0	1.317
1	5-Feb-01	1 of 6	0	0	Neg	Neg	3.958	1.24	1.714	1.592	1.352	1.412
8	17-Jan-01	1 of 6	0	0	**Positive**	**Positive**	40.53	0	2.05	11.196	0	0.115
8	25-Jan-01	0 of 6	0	0	Neg	Neg	6.219	0.836	1.212	2.317	0.615	1.31
8	2-Feb-01	1 of 6	0	0	Neg	Neg	6.411	1.301	1.658	30.532	0.665	1.17
8	7-Feb-01	0 of 6	0	0	Neg	**Positive**	5.222	1.424	1.729	2.295	1.377	1.637
8	12-Feb-01	0 of 6	0	0	Neg	**Positive**	46.95	1.433	1.843	3.887	1.736	2.719
10	18-Oct-00	2 of 6	0	0	**Positive**	**Positive**	2.146	0.084	0.28	0.124	0	0
10	24-Oct-00	1 of 6	0	0	Neg	Neg	3.896	0.136	0.326	3.446	0	0.263
10	30-Oct-00	5 of 6	0	0	Neg	Neg	4.478	0.179	0.568	1.041	0	0.011
10	6-Nov-00	5 of 6	0	0	**Positive**	Neg	3.137	0.144	0.515	0.14	0	0.022
10	14-Nov-00	4 of 6	0	0	Neg	Neg	4.21	0.53	1.031	0.129	0.001	0.026
12	27-Mar-01	3 of 6	0	0	Neg	Neg	1.88	0.2	0.324	5.726	3.942	4.118
12	2-Apr-01	4 of 6	0	0	Neg	Neg	5.365	4.11	4.354	3.01	0.864	1.838
12	9-Apr-01	4 of 6	0	0	Neg	Neg	6.089	4.38	4.498	3.893	2.124	2.617
12	16-Apr-01	3 of 6	0	0	Neg	Neg	6.268	4.136	4.56	4.551	2.242	2.557
12	23-Apr-01	4 of 6	0	0	Neg	Neg	6.678	4.53	4.699	3.955	2.788	2.98
16	30-Apr-01	5 of 6	0	0	**Positive**	Neg	6.315	4.67	5.052	3.934	2.687	3.098
16	7-May-01	3 of 6	0	Not done	**Positive**	Not done	6.101	5.23	5.299	Not done	Not done	Not done
16	14-May-01	2 of 6	0	Not done	Neg	Not done	6.169	5.45	5.704	Not done	Not done	Not done
16	21-May-01	3 of 6	0	Not done	Neg	Not done	8.575	5.86	6.219	Not done	Not done	Not done
16	29-May-01	3 of 6	0	Not done	Neg	Not done	8.694	6.32	6.631	Not done	Not done	Not done
Averages and totals		55 of 150	0 of 25	0 of 21	7+ of 25	5+ of 21	8.193	2.248	2.633	4.149	1.06	1.395

* No readings because of electrical power interuption

Not done = equipment damaged on 7 May 01 by electrical surge or lightning