



Potential Cause of Diarrhea in Piglets at Weaning in Hawai'i

L. Woody, J. Ishimoto, Yong Li, and C.N. Lee¹

Department of Human Nutrition, Food and Animal Sciences

¹contact

Abstract

It has been observed on a particular farm that the zebra dove (*Geopelia striata*), also known as the “barred ground dove,” often frequents the feeding troughs in the weanling piglets pens. It was hypothesized that the birds contributed to incidences of diarrhea in the piglets. A simple study was initiated to examine the microbial population present in feed that had been exposed to the zebra doves. The treatments tested (n= 3 samples for each) were a) commercial piglet feed out of a sealed bag (control, C); b) commercial piglet feed exposed to air at the feed trough level with a net screen (air exposure, AE); and c) commercial piglet feed exposed to zebra doves (birds ate from the trough, BE). To simulate eating and digestion, 10g samples of feed were mixed with 90 ml of 1% peptone water, placed in a sterile bag, and subjected to 1 minute of blending in a Stomacher food processor at 260 rpm. The pH of the mixture was then lowered to 3.0–3.5 with hydrochloric acid to mimic the environment of the stomach. A sample of the mixture (the supernatant) was then plated on plate count agar and incubated for 24h at 35°C. Cultures grown were then subjected to laboratory procedures leading to DNA sequencing. The results showed the presence of *Bacillus pumilus*, *Bacillus licheniformis*, and *Acetobacter indonesiensis* in all BE samples and *Leuconostoc mesenteroides* in one BE sample. This paper discusses the potential role of these bacteria in piglet health and their impact on public health.

Keywords: piglets, diarrhea, bacteria, birds, zebra dove

Introduction

Weaning is one of the most stressful times for any mammal, and piglets are no different (Mardec et al. 1988). The young piglets are faced with changes in environment and diet, varying feed intake, dehydration, and new pathogens and social interactions. It is not uncommon for them to develop diarrhea under these circumstances, due to their exposure to enteric pathogens during a susceptible phase of growth (Vondruskova et al. 2010). Attempts to modify diets to help alleviate diarrhea and improve performance have yielded mixed results (Sorensen et al. 2009, Bhandari et al. 2009). One of the possible factors identified as contributing to diarrhea is restricted feeding (Laine et al. 2008).

In Hawai'i's sub-tropical environment it is common to find open housing systems for pig production, as opposed to the enclosed housing systems in the colder regions of the world. Such housing systems provide birds with the opportunity to scavenge for food in the pig pens. Birds are known to be carriers or transmitters of diseases (thepigsite.com). In a new piggery, it was observed that visits by the zebra dove (*Geopelia striata*) were often followed by incidences of diarrhea in weaned piglets. These birds enter into the pens directly by flying in and landing on the floor and feed troughs or just by walking through chain-link fence barrier between pens. Bird netting was effective in preventing the intrusion of zebra doves. However, there was no clear evidence that these birds were transmitting any microorganisms. They are ubiquitous in Hawai'i, being found in parks, on sidewalks, in open dining areas and parking lots, etc.

Objectives

The objectives of this study were to determine if a) if zebra doves contaminate the piglets' feed and b) identify the bacterial types found in the feed following exposure/visitation by the birds. The study was self funded; hence, it has a limited scope. It is a study conducted at a single farm, using feed samples from that farm.

Materials and Methods

Commercial feed for piglets was obtained from bags that were sealed until time of sampling. Samples were then subjected to the following treatment: a) the control (C) treatment was put directly into a zip lock bag and sealed (n= 6); b) the "air exposure" (AE) treatment was placed in a container with bird netting over it and the container was placed on a chair next to the pig pen for air exposure for 30 minutes (n=6); and c) the "bird exposure" (BE) treatment was placed in a trough, where birds were allowed to feed on it for a minimum of 30 minutes (n=3). From each treatment, a minimum of 3 samples were taken for microbial studies in the labora-

tory. [The microbial studies were limited to 3 samples from each treatment because of cost limitations.]

In the laboratory, 10g of samples were weighed from each treatment and diluted with 90ml of 1% sterile peptone water. This mixture was then subjected to 1 minute of blending in a Stomacher food processor at 260 rpm. The pH of the mixture was then lowered to 3.0–3.5 with hydrochloric acid to mimic the environment of the stomach. A sample of the treated mixture, the supernatant, was further diluted prior to plating on plate count agar and incubated for 24h at 35°C. The cultures grown were then subjected to laboratory procedures leading to DNA sequencing.

Bacterial Isolation: Based on morphological appearance in the culture plates, six colonies were selected and streaked for further isolation of bacteria. These colonies were then placed into a tryptic soy broth and incubated for 24h at 35°C. A small amount of this culture was then prepared for polymerase chain reaction (PCR) technique for amplification of 16S rDNA fragments (Vliegen et al. 2006). The samples were then subjected to



Figure 1. The feed for piglets was subjected to air exposure (A) and bird exposure (B) for a minimum of 30 minutes.

gel electrophoresis. Samples were also sent to a DNA lab for nucleic acid sequencing, and the results were matched to the BLAST system for bacterial determination.

Results and Discussion

The exposure of the feed to air and to birds is illustrated in Figures 1A and 1B. In the AE treatment, the feed was protected by bird netting to prevent any direct contact. No birds came close to the feed samples. In the BE treatment, the birds were allowed to consume the feed and walk over it freely. Each exposure was for a minimum of 30 minutes.

The concentration of bacteria in the various feed samples is presented in Figure 2. Samples from the sealed bags showed low bacteria count (3%) when compared to AE samples (29%). Samples from BE had the highest bacteria number in culture, contributing to 68% of total bacteria counts.

The gel electrophoresis for the 16S rDNA amplicons from the isolated bacteria is presented in Figure 3. The far left column is the standard and the other bands showed the presence of bacteria for each column. DNA sequencing showed BE samples had 3 major groups of bacteria species: *Acetobacter indonesiensis*, *Bacillus pumilus*, and *Bacillus licheniformis*. *Acetobacter indonesiensis* is one of the 34 sub-species of *Acetobacter* that have been

identified (Cleenwerck et al. 2002). Some of this group has been reported to cause pneumonia in lung transplant patients (Bittar et al. 2008).

The Gram-positive spore-forming *Bacillus pumilus* resides in soils, which explains why zebra doves, which spend a huge amount of time walking on the ground searching for food, are carriers of this microbe. This bacterium has a wide range of symbiotic relationships with plants and has been known to promote rhizobacteria among the roots of peppers and wheat (Joo et al. 2004, Sari et al. 2007). In general, this bacteria is harmless, although there are reports of its being resistant to hydrogen peroxide. It can also be opportunistic, as in a reported case involving an immunocompetent child (Bentur et al. 2007).

Bacillus licheniformis is a ubiquitous soil bacteria often associated with plant material. This bacteria is commonly found on bird feathers and has known to be involved in molting in birds. This spore-forming bacillus has been reported to cause abortion in pigs (Kirkride et al. 1986) and sheep (Mason and Munday 1968), especially in livestock that already have compromised immune states. This bacterium has also been demonstrated in food spoilage (Pepe et al. 2003). The endospores formed from *Bacillus licheniformis* are heat resistant and cannot be destroyed in the baking process. Toxins from these bacteria can result in stomach pains, diarrhea, and sometimes vomiting. The food-borne gastro-enteritis can lead to septicaemia (Salkinoja-Salonen et al. 1999). Interestingly, the sub-strain of this bacillus (SB3086) has fungicidal properties in plants (Hutton et al. 2001).

The total bacteria count in various types of samples is depicted in Figure 4. The data showed that feed samples from the piglet trough had the highest bacteria count. Trough samples (leftover feed) had 433,333 colony-forming units (cfu) per gram of feed compared to 44,167 cfu/gm for BE samples, under 2,700 cfu/gm for AE, and 1,100 cfu/gm for feed from the bag. Hence, it is very possible that over time, the bacteria transmitted by the zebra doves to the feed increased in numbers. The high concentration of bacteria in the leftover feed could be the cause of diarrhea when ingested by the piglets. Piglets stressed from weaning would be vulnerable to such contaminated feed because they have lowered immune defense mechanisms as they are transitioning from passive immunity to active immunity (Coffery et al. 1995).

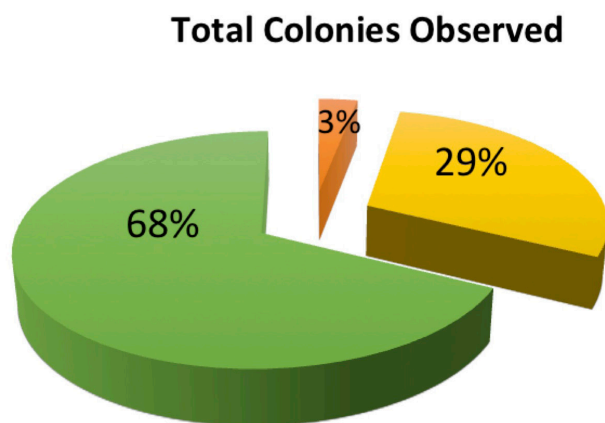


Figure 2. A pie chart representing the distribution of the number of bacteria colonies observed in feed: Control – feed from the sealed bag; Air – feed following 30 minutes of air exposure in proximity to the pigpen, and Bird – feed following 30 minutes of bird exposure (free choice).

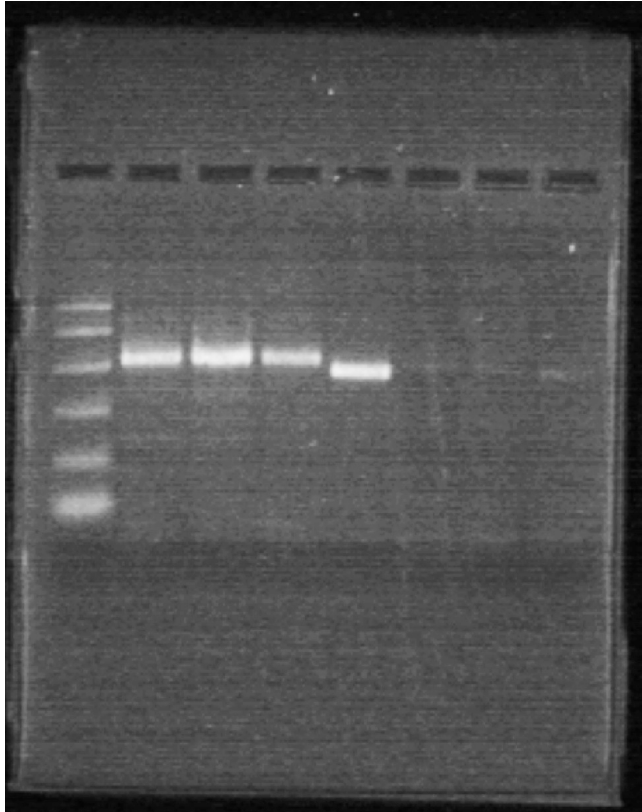


Figure 3. Gel electrophoresis showing the presence of bacteria for the 16S rDNA amplicons.

Summary and Impact

The data from this study (limited and constrained by funding) suggest that the zebra dove is capable of carrying and transmitting potentially harmful bacteria to mammals. The zebra doves are found everywhere in Hawai'i, often in close proximity to human facilities, especially dining areas. This suggests the potential risks to human health, especially to infants, children, and individuals with compromised immune systems. An expanded study would shed more information to the public health risks and animal well-being issues associated with the zebra dove.

Acknowledgements

The study was a voluntary project of an undergraduate student in the Animal Sciences program. The authors would like to thank Ali'i Farms for the feeds samples and collaboration.

References

- Bentur, H.N., Dalzell, A.M., and Riordan, F.A.I. 2007. Central venous catheter infection with *Bacillus pumilus* in an immunocompetent child: a case report. *Ann. Clin. Microbiol Antimicrob.* 6: 12. <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2169249/>

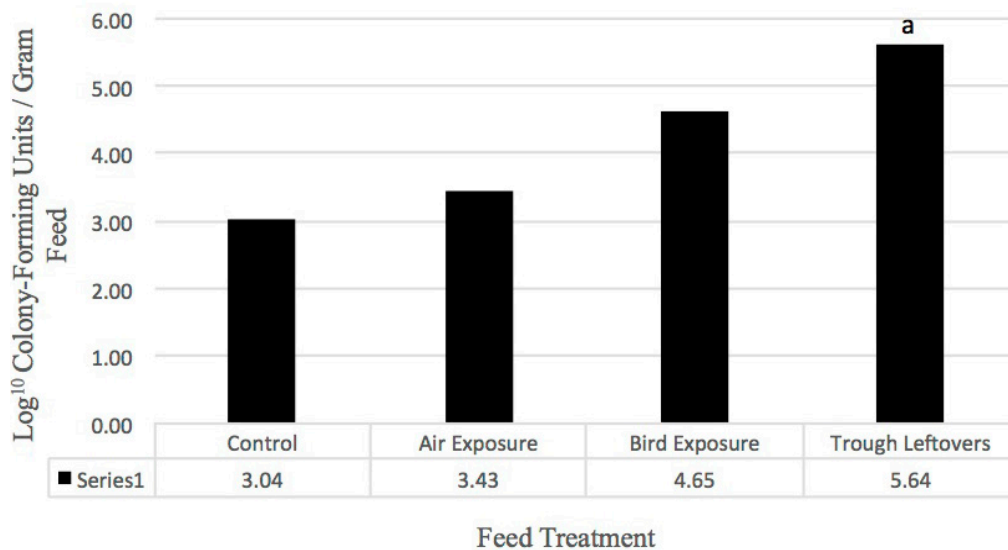


Figure 4. Average number of bacterial colonies per gram of feed: control, air exposure,* bird exposure,* and leftover feed from trough. * Exposure to air or birds was for 30 minutes. ^a $P \leq 0.001$.

- Bhandari, S.K., Nyachoti, C.M., and Krause, D.O. 2009. Raw potato starch in weaned pig diets and its influence on postweaning scours and the molecular microbial ecology of the digestive tract. *J. Anim. Sci.* 87: 984–993.
- Bittar, F., Reynaud-Gaubert, M., Thomas, P., Boniface, S., Raoult, D., and Rolain, J.M. 2008. *Acetobacter indonesiensis* pneumonia after lung transplant. *Emerging Infectious Diseases* 14(6): 997–998.
- Cleenwerck, I., Vandemeulebroecke, K., Janssen, D., and Swings, J. 2002. Re-examination of the genus *Acetobacter*, with descriptions of *Acetobacter cerevisiae* sp. nov. and *Acetobacter malorum* sp. nov. *Int'l. J. of Systematic and Evol. Microbiology* 52: 1551–1558.
- Coffery, R.D., Parker, G.R. and Laurent, K.M. 1995. Feeding and managing of the weanling piglet. Univ. of Kentucky Coop. Ext. Service. ASC-149: 1–8. <https://www.uky.edu/Ag/AnimalSciences/pubs/asc149.pdf>, accessed Aug. 29, 2014.
- Hutton, P., Kough, J., Etsitty, C., Vaituzis, Z., and Cerrelli, S. 2001. Use of *Bacillus licheniformis* strain sb3086 as a biofungicide. http://www.epa.gov/opp00001/chem_search/reg_actions/registration/decision_PC-006492_1-Feb-01.pdf; accessed on Aug. 28, 2014.
- Joo, G., Kim, Y., Lee, I., Song, K. and Rhee, I. 2004. Growth promotion of red peppers plug seedlings and the production of gibberellins by *Bacillus cereus*, *Bacillus macrolides*, and *Bacillus pumilus*. *Biotech. Letters*. 26: 487–491.
- Kirkbride, C.A., J.E. Collins, and C.E. Gates. 1986. Porcine abortion caused by *Bacillus* sp. *J. Amer. Vet. Med. Assoc.* 188: 1060–1061.
- Laine, T.M., Lyytikäinen, T., Yliaho, M., and Anttila, M. 2008. Risk factors for post-weaning diarrhoea on piglet producing farms in Finland. *Acta Vet. Scand.* 50(1): 21
- Madec, F., Bridoux, N., Bounaix, S., Jestin, A. 1998. Measurement of digestive disorders in the piglet at weaning and related risk factors. *Prev. Vet. Med.* 35, 53–72.
- Mason, R.W. and B.L. Munday. 1968. Abortion in sheep and cattle associated with *Bacillus* spp. *Australian Veterinary J.* 44: 297–298.
- Pepe O., Blaiotta G., Moschetti G., Greco T., Villani F. 2003. Rope-producing strains of *Bacillus* spp. from wheat bread and strategy for their control by lactic acid bacteria. *Appl. Environ Microbiol.* 69(4): 2321–2329.
- Sari, E., Etebarian, R., and Aminian H. 2007. The effects of *Bacillus pumilus*, isolated from wheat rhizosphere, on resistance in wheat seedling roots against the Take-all fungus, *Gaeumannomyces graminis* var. tritici. *Journal of Phytopathology*. 155: 720–727.
- Salkinoja-Salonen. S., Vuorio R., Andersson M.A., Kämpfer P., Andersson M.C., Honkanen-Buzalski T., and Scoging A.C. 1999. Toxigenic Strains of *Bacillus licheniformis* related to food poisoning. *Appl Environ. Microbiol.* 65(10): 4637–4645.
- Sorensen, M.T., Vestergaard, E.M., Jensen, S.K., Lauridsen, C. and Hojsgaard, S. 2009. Performance and diarrhea in piglets following weaning at seven weeks of age: Challenge with *E. coli* O 149 and effect of dietary factors. *Livestock Sci.* 123: 314–32.
- The Pig Site. 2014. Airborne transmission and other methods. Accessed on Aug. 25, 2014. <http://www.thepigsite.com/pighealth/article/629/airborne-transmission-and-other-methods>
- Vliegen, I., Jacobs, J.A., Beuken, E., Bruggeman, C.A., and Vink, C. 2006. Rapid identification of bacteria by real-time amplification and sequencing of the 16S rRNA gene. *J. Microbiol. Meth.* 66: 156–164.
- Vondruskova, H., Slamova, R., Trckova, M., Zraly Z. and Pavlik, I. 2010. Alternatives to antibiotic growth promoters in prevention of diarrhoea in weaned piglets. *Vet. Medicina* 55: 199–224.