

Antibiotic Residues in Dairy Manure

Part 2: Sampling dairy manure for antibiotic detection

Standard methods for sampling manure

While well-established standards for water and wastewater examination exist^[1], standard for manure are lacking. In lieu of standards, and given the inherent heterogeneity of dairy manures, care must be taken to insure that samples collected for analysis are representative of the average daily flow. While the most desirable approach would be to amass and homogenize a 24-hour flow of manure, then collect composite samples; this approach is generally impractical, as large volumes of manure would need to be handled. The Eastern Research Group, Inc. in cooperation with U.S. Environmental Protection Agency has been developed to assess the performance of manure-based anaerobic digestion^[1] and suggests that: 1) a series of at least six grab samples should be collected over a period of no less than one hour and combined into a single composite sample; 2) composite samples should be no less than 5 gal. and subsamples withdrawn for analysis should no less than 1 qt.; 3) triplicate subsamples should be collected; 4) sampling should be conducted for at least a 1 year period, ideally on a monthly basis, though it is important that the scope of the work is reasonable and that sample numbers are not cost prohibitive; and 5) there should be an ongoing review of analytical results to determine if the degree of variability is reasonable or if a modification of the sample collecting protocol is necessary.

Standard methods have not yet been developed specifically for sampling manure for antibiotic residues. In lieu of specific sampling standards, the methods of the Eastern Research Group, Inc. were adopted for testing different manure handling systems for antibiotic residues as follows:

Liquid samples

If the sample location is a *manure tank with agitation*, every 10 min. for 1 h a 3.5 qt. sample of 'representative material' will be collected. If *agitation is not available*, a 3.5 qt. sample will be collected from six grab samples taken within the top 3 ft. of the manure tank. If the sample location is the *effluent of an anaerobic digester*, a 3.5 qt., sample will be collected every 10 min. for 1 h when effluent is flowing over the effluent weir. If effluent is not flowing, six 3.5 qt. samples will be collected from within the top 3 ft. of the digester effluent weir chamber. If the sample location is *continuously flowing* (e.g. liquids post separation), a 3.5 qt. sample will be collected every 10 min. for 1 h. If the sample location is a *lift station*, a 3.5 qt. sample will be collected from six different depths. For each location, the 6 samples will be combined in a clean and sterilized 5 gal. bucket, homogenized using a drill and paint-mixing paddle, then after 2 min. of mixing, composite subsamples will be collected using a sterile sampling scoop and transferred into sample containers. If the sample location is a *long-term storage*, several 5 gal. daily collections will be made throughout each the spring and fall agitation and pump out from pumping equipment or tanker trucks. If practical, each daily sample will be the composite of several 3.5 qt. samples collected at different times during the pumping operations that day.

Solid samples

If the sample location is a *static pile* of manure solids, six unique 3.5 qt. samples will be collected and combined into a 5 gal. bucket and after 2 min. of hand-mixing, a composite subsample will be collected and loaded into labeled sample containers.

Sample container preparation

Antibiotics can strongly adsorb to solids, including the plastics and glass of sampling containers. To minimize this adsorption, carryover, and contamination, all sample containers must first be acid washed in a 2% nitric acid bath for a eight hours to strip and minimize adsorptions of antibiotics.

Sample preservation

Filled sample containers are transported on ice to the laboratory where they are immediately frozen. Frozen samples are then dried using a specialized machine (called a freeze-drier) that removes all water from the frozen samples. Once freeze-dried, samples are moved into a specialized -112°F freezer where they are stored until analysis. All these measures help minimize degradation of antibiotic residues in the samples and ensure accurate detection.

Limitations

Currently, sample heterogeneity and variability are not well characterized, but are

likely greater for trace-level antibiotics than for the manure characteristic measurements (e.g. total and volatile solids) that the Eastern Research Group, Inc. protocol was developed for. It is also unpractical to extract antibiotics from a 1 qt. subsample of manure due to the extraction costs, capacities of purification equipment, and limits on sample throughput. Large samples also impact the noise:signal ratio as impurities in the manure will interfere with the antibiotic residue signal. Instead, a small (0.1g) subsample is ideal. Consequently, there is the potential that the sampling methods employed do not adequately capture the heterogeneity of antibiotic residues in the manure samples. At this time, the knowledge to characterize this heterogeneity and improve sampling methodologies does not exist. Our current research efforts are aimed in part at resolving this variability, and despite limitations, the data acquired using the methods outlined above will still provide useful knowledge about antibiotic residues in dairy manure.

FACT SHEET SERIES Antibiotic Residues in Dairy Manure

- Part 1: Critically important antimicrobials labeled for dairy use
- Part 2: Sampling dairy manure for antibiotic detection
- Part 3: Laboratory methods for extracting antibiotic residues from dairy manure
- Part 4: Laboratory methods for analyzing antibiotic residues extracted from dairy manure
- Part 5: What is known about antibiotic residues in dairy manure?

AUTHORS

Jason P. Oliver, PhD
Curt Gooch, PE

jpo53@cornell.edu
cag26@cornell.edu

(607) 227-7943
(607) 225-2088

REFERENCES

- [1] APHA, American Public Health Association. 2012. Standard methods for the examination of water and wastewater. American Water Works Association.
- [2] Eastern Research Group, Inc. March 2011. Protocol for quantifying and reporting the performance of anaerobic digestion systems for livestock manures. U.S. Environmental Protection Agency AgSTAR Program. <http://www.epa.gov/sites/production/files/2014-12/documents/protocol.pdf>



This material is based upon work that is supported by the National Institute of Food and Agriculture, U.S. Department of Agriculture, under award number 2016-68003-24601. Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the authors and do not necessarily reflect the view of the U.S. Department of Agriculture.