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Clinical Significance of the Persistence of Pathogens in Pig (Sus Scrofa) Manure Used as Biofertilizer and Food

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ABSTRACT

Pig manure is a by-product of the swine industry; it can be used as biofertilizer or as ruminant feed. However, its use is limited because of the possibility of transmitting pathogens. The persistence of: bacterias, molds, yeasts and parasites has been found after processing fresh pig manure by storage tanks, biodigesters, silage, sun drying, earthworm compost and preparation of earthworm flour. In this study, the clinical significance of such persistence in case of using the manure for feeding cattle or biofertilization, was performed. It was found that persistent agents not always represent a risk to the human and cattle health, because several are specific to the pig. Other agents require specific measures because they are zoonotic (Escherichia coli, Salmonella spp., Listeria monocytogenes and Balantidium coli). The use of processed pig manure represents a lower risk than the fresh one and therefore can be used more safely.

Keywords: Pig manure, feeding, biofertilization, pathogens, Bioremediation.

INTRODUCTION

Pork is the most consumed meat around the world, and is expected that this trend continue because the human population could exceed nine billion people in 2050 [3] which consequently increases demand; besides, scientific advances in pork industry are making animal protein production more profitable and of better quality. This will allow the pork industry to continue climbing in terms of competitiveness. Nevertheless, this favorable outlook is challenged by the large amount of manure produced, which can reach up to 10 kg of pig manure per day. Although it is possible to use this product as a source of nutrients and renewable energy, its strategic use as raw material for animal feed or as bio-fertilizer, is limited by the Colombian Health Authorities

due to the possible risk of contamination [4]. The main barrier is that part of the pathogenic load present in the pig manure persists, even after undergoing different treatment processes [1, 2]. If this obstacle can be overcome, there is no doubt of the benefits of this by-product in its different possibilities of use. On one hand, the solid fraction of pig manure is a resource with high potential for use as an ingredient in animal feed, especially in ruminants, due to its low cost, high organic matter content and good levels of crude protein, with the additional advantage of being a raw material that is available throughout the year [5]. Studies such as that of Estrada-Álvarez et al. [6], showed that the use of pig manure silage with ground integral sugarcane (Saccharum officinarum L.) maintains production curves in dual-purpose cattle, without affecting their sanitary status.

On the other hand, the liquid fraction can be used as an element that improves soil structure and fertility, since it is a source rich in organic and inorganic nutrients such as nitrogen, phosphorus. potassium. sulfur. calcium. magnesium, chlorine, copper, iron, boron, manganese, molybdenum and zinc [7]. In this sense, González [8] found that biofertilization with liquid pig manure in pastures of Cynodon plectostachyus allowed to increase not only the production of forage but also the crude protein, with a very low possibility of environmental contamination by infiltration of nitrates towards the subsoil and groundwater. In countries like Brazil, as in other parts of the world, the storage of liquid pig manure and subsequent application to the land is the predominant practice management for this by-product due to its simplicity, low cost and possible reduction of costs in agricultural production through the replacement of chemical fertilizers with manure nutrients [9]. Another form of use is the production of renewable energy, which is possible thanks to the implementation of anaerobic digestion systems that allows to recover energy in the biogas form [10].

In Colombia, liquid pig manure is processed in biodigesters and manure tanks before final disposal, while the solid fraction is used in silage, dehydration in the sun, earthworm compost and earthworm flour. Recent studies have shown that different pathogens persist in these treatment ways, such as Lawsonia intracellularis, aerobic mesophilic bacteria, Staphylococcus aureus. molds. Clostridium sulfite reducers, total coliforms, Escherichia coli.Listeria monocytogenes, Salmonella Balantidium spp., Strongyloides spp. and coccidia [1, 2]; however, the clinical implications of this persistence have not been specified, and some of them require special attention because of their zoonotic potential such as: Escherichia coli, Salmonella spp., Listeria monocytogenes, and Balantidium coli. The present work had the objective of opening a deep discussion of the true clinical importance of the persistence of pathogens in processed pig manure for biofertilization and animal feeding purposes.

MATERIALS AND METHODS

This study was done as result from previous investigations of Betancur et al. [1, 2] who evaluated the persistence of 26 pathogens in processed pig manure by different

methodologies (manure tanks, biodigesters, silage, dehydration in the sun, earthworm compost and earthworm flour), carried out in central-western Colombia, considering viruses, bacteria, molds, veasts and parasites of higher importance for the pork production in the area. For this evaluation the following diagnostic techniques were used: Porcine Parvovirus (PPV), Porcine Circovirus type 1 (PCV-1), Porcine Circovirus type 2 (PCV-2), Porcine Herpesvirus type 1 (PHV-1), Actinobacillus pleuropneumoniae and Lawsonia intracellularis the conventional PCR technique; Porcine Reproductive and Respiratory Syndrome virus (PRRSV), nested RT-PCR; Clostridium sulfite reducers, aerobic mesophilic bacteria, Staphylococcus aureus, total coliforms, Escherichia coli, molds and yeasts culture, and agar counts specific for each species: Salmonella spp., sequential incubation, Gram staining, oxidase testing and agglutination with "O"; polyvalent antiserum Listeria monocytogenes incubation and ELFA (Enzyme Linked Fluorescent Assay) in the miniVIDAS kit (BioMérieux, Craponne, Fr); Leptospira spp., culture in EMJH medium (Ellinghausen and McCullough as modified by Johnson and Harris) and evaluation by darkfield microscopy; Ascaris suum, Trichuris suis, Balantidium coli, Metastrongylus Strongyloides spp., coccidia and strongylids, using McMaster and Sloss; Giardia intestinalis with Ritchie; and Cryptosporidium parvum with Zielh-Neelsen cold stain. The findings recorded in these studies are detailed in Table 1, which was constructed strictly according to the persistence of each pathogen in each of the six routes of use of the pig manure considered; It is pertinent to highlight that the starting point was always the pig manure freshly produced on the farm, either in its liquid or solid phase.

The clinical importance of the persistent pathogens was established by comparing the results of the afore mentioned investigations with the findings reported in research publications in the clinical, epidemiological and porcine production areas.

RESULTS AND DISCUSSION

Of the 26 pathogens considered, *Lawsonia* intracellularis, aerobic mesophilic bacteria, *Staphylococcus aureus*, molds, yeasts, *Clostridium* sulfite reducers, total coliforms, *Escherichia coli*, *Listeria monocytogenes*, *Salmonella* spp., *Balantidium coli*,

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Strongyloides spp. and coccidia persisted in the different routes (Table 1). It is known that chemical (pH, enzymes), biological (microbiological activity) and physical factors (temperature, desiccation) may influence the persistence of bacteria, viruses and parasites in the different animal manure treatment systems [11]. The rates of decline of pathogens in manure depend on their management and storage conditions [12]. The clinical implications of persistent agents will depend on the destination of processed pig manure. In terms of usefulness, pig manure that comes from manure tanks, biodigesters and earthworm compost is usually used as a biofertilizer for soils, while that from sun dehydrated, silage and earthworm flour sometimes is used as animal feed. Biodigesters and manure tanks use the liquid fraction of pig manure, while silage, sun dehydration, earthworm compost and earthworm flour use the solid fraction [1, 2].

Lawsonia intracellularis is the causal agent of porcine proliferative enteropathy, a disease of high economic impact in this industry. Apparently, the ruminants are not affected, while in the equines can produce an illness [13]. Persistence was found in liquid pig manure, as well as in the sun dehydrated manure and in silage. Their viability could not be specified, since the use of the PCR technique also detects DNA from dead cells [14]. Supported by these facts, the provision of silage and pig manure dehydrated in the sun to the ruminants should not be a problem because they are not specific hosts of the agent. On the other hand, biofertilization of grasslands with pig manure for ruminant feeding purposes would not be risky, as it is known that this pathogen only survives outside its host for one to two weeks [15], and the rotation cycles of grasslands and of grasses biofertilization regularly exceeds this time. It is recommended that in situations where horses will graze on biofertilized meadows with pig manure, they do it after a minimum of two weeks post application.

Balantidium coli is a parasite that has the pig as its main host; It can affect several species including humans and to a lesser extent cattle [16]. These parasites can be observed in two stages: one of mobile trophozoite that is sensitive to desiccation, and another cystic, which can be kept viable for more than two weeks in the environment [17]. As can be seen in Table 1, the parasite did not persist in silage, pig manure dehydrated in the sun and transformed into earthworm flour, which are the

routes used in cattle feed, and therefore they can be considered appropriate processes for the elimination of the agent. In the case of liquid pig manure coming from manure tanks, it did not persist and therefore can also be used for biofertilization of soils. However, in the case of earthworm compost, persistence was observed, but no analysis was performed to verify its viability. Based on this, it could be noticed that the earthworm compost that has this parasite in viable states, should be carefully managed in the biofertilization of soils that are later grazed by cattle.

Table1. Persistence of Pathogens in the Different Routes of Pig Manure Used in Central Western Colombia.

| PATHOGEN | R1 | R2 | R3 | R4 | R5 | R6 |
|----------------------|----|----|----|----|----|----|
| PPV | ** | ** | ** | ** | ** | ** |
| PCV-1 | ** | ** | ** | ** | ** | ** |
| PCV-2 | * | * | ** | ** | ** | ** |
| PRRSV | ** | ** | ** | ** | ** | ** |
| PHV-1 | ** | ** | ** | ** | ** | ** |
| Lawsonia | X | X | X | X | * | * |
| intracellularis | | | | | | |
| Actinobacillus | ** | ** | ** | ** | ** | ** |
| pleuropneumoniae | | | | | | |
| Aerobic mesophilic | X | X | X | X | X | X |
| bacteria | | | | | | |
| Staphylococcus | X | X | * | * | X | * |
| aureus | | | | | | |
| Molds | ** | * | * | X | X | * |
| Yeast | * | * | * | X | X | * |
| Clostridium sulfite | X | X | X | X | X | X |
| reducers | | | | | | |
| Total coliforms | X | X | X | X | X | X |
| Escherichia coli | X | X | X | X | X | X |
| Listeria | ** | X | X | * | * | * |
| monocytogenes | | | | | | |
| Salmonella spp. | X | * | * | * | * | * |
| Leptospira spp. | ** | ** | ** | ** | ** | ** |
| Ascaris suum | ** | ** | ** | ** | ** | ** |
| Cryptosporidium | ** | ** | ** | ** | ** | ** |
| parvum | | | | | | |
| Trichuris suis | ** | ** | ** | ** | ** | ** |
| Balantidium coli | ** | * | * | * | X | * |
| Metastrongylus spp | ** | ** | ** | ** | ** | ** |
| Giardia intestinalis | ** | ** | ** | ** | ** | ** |
| Strongylids | * | * | ** | * | ** | ** |
| Strongyloides spp. | * | X | * | * | X | * |
| Coccidias | X | X | * | * | * | * |

R = Route. R 1 = biodigester; R 2 = manure tank; R 3 = dehydration in the sun; R 4 = silage; R 5 = earthworm compost; R 6 = earthworm flour. X = persistence; * the pathogen was found but did not persist; ** the pathogen was not found.

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The eggs of Strongyloides (quite possibly Strongyloides ransomi), did not persist in the routes that involve direct use of the pig manure for the feeding of cattle (silage, dehydrated by sun, earthworm flour) being therefore suitable routes for this purpose. Strongyloides ransomi also did not survive in the biodigesters and, therefore, the material obtained by that route, can be used in biofertilization processes. Although Strongyloides ransomi seems to have persisted in manure tanks and earthworm composting, the products obtained by these routes can be used in biofertilization of grassland to be grazed by cattle, horses, goats or sheep, because, unlike Strongyloides estercoralis, which can infect dog, cat, fox, man, coati, chimpanzee, gibbon and orangutan, Strongyloides ransomi is a very specific parasite of pigs [18, 19].

Coccidia persisted in biodigesters and manure tanks, whose materials are not used for animal or human consumption. If the coccidia persisted in other routes linked to biofertilization of pastures, which could eventually end in animal consumption, the risk of infecting ruminants or equines would be minimal, since the different species of Eimeria are very host specific. The species found in cattle are not even reported in other ruminants, and there is no cross infection among small ruminants such as sheep and goats [19]. However, the potential for transmission of diseases to animals through the application of pig manure to the grasslands depends on variables such as the dose, number and species of pathogens present in them, frequency and the seasonality of the pathogen, the effectiveness of any treatment on the persistence of the agents, the ability of the pathogens to survive in the environment, and the susceptibility of the host [20].

Aerobic mesophilic bacteria, Staphylococcus aureus, molds, yeasts, Clostridium sulfite reducers, total coliforms, Escherichia coli, Listeria monocytogenes and Salmonella spp., are used as indicators of the sanitary quality of food [21]. All these microorganisms persisted in one or more treatment routes used; aerobic Clostridium mesophilic bacteria, sulfite reducers, total coliforms and Escherichia coli persisted on all routes; molds and yeasts persisted in silage and earthworm compost, while Staphylococcus aureus persisted in manure tanks, biodigesters and earthworm compost. Although the persistence of these microorganisms would in some way limit the use of pig manure for biofertilization or feeding of cattle, it is worth underlining that there is evidence suggesting that pathogenic bacteria disappear along the digestive tract of ruminants fed with fresh pig manure contaminated with Salmonella spp., Escherichia coli and Yersinia spp, because the ruminants have developed a natural mechanism for the digestion of the food, based on volatile fatty acids, anaerobiosis, temperature, osmotic pressure and saturated fatty acids of the rumen, in addition to proteolytic enzymes and abomasal pH, which probably allows the elimination of pathogenic bacteria [22].

The persistence of molds and yeasts in silage is an undesirable condition of the process; its clinical implications in case of being supplied as cattle feed would depend on the species found; It is known that the presence of molds in food for animal consumption can be considered normal, however the absence of toxigenic molds should be verified [23]. In the rumen it is normal to find different species of molds and yeasts that enter with the food, most of the yeasts are destroyed during their transit through the alimentary tract, whereas large amount of molds can be excreted even in viable state [24]. The presence of molds and yeasts in the earthworm compost is considered normal due to the biological interaction of the worms with these microorganisms [25], so its presence should not be a limitation for its use as a biofertilizer.

Special attention has been given to Escherichia coli; this microorganism is of great importance for public health, especially strain 0157: H7, of which ruminants are important reservoirs [26]. In general, the serotypes associated with disease in pigs (0138, 0139, 0141) are not found in humans, in which, in addition, the prevalence of human pathogenic Escherichia coli is very low [27]. It is important to consider that due to the specificity of fimbrial adhesins of Escherichia coli (F4 and F18 for pigs, as well as F5, F17 and F41 in cattle) [28, 29], it is not possible to consider the true risk involved in the use of pig manure in cattle; in another related study, it is mentioned that dry bird excreta are safe for ruminant feeding even when the amount of Escherichia coli is sometimes greater than 100 CFU / g [30]; this was the limit reference value considered in our studies [1, 2]; this fact added to the cattle feeding reports with fresh pig manure without harmful effects on their health, evidences that the risk of feeding cattle directly

with pig manure processed by silage, dehydrated by the sun or transformed into earthworm flour is almost nil, as well as the consumption of pastures that have been biofertilized with liquid pig manure or coming from earthworm compost should represent a lower risk. It is known that *Escherichia coli* does not survive in the soil for a long time, although in soils saturated by the application of pig manure can live up to 68 days [31].

Listeria monocytogenes, is a bacterium capable of infecting humans by eating of contaminated food; it is found in the intestines of animals and people who acting as carriers and, it is also widely distributed in natural environments such as soil, water, effluent, fodder and silage, where they survive for extended periods [32]. As mentioned in Goberna et al. [31], Listeria spp. is able to survive up to 128 days on biofertilized soils with pig manure. Although porcine and bovine are susceptible to develop listeriosis. they frequently act as asymptomatic carriers [33]. There was no persistence of the pathogen in silage of pig manure, earthworm compost, or transformed in earthworm flour, therefore are considered safe routes for implementation in integrated bovine-porcine production systems. In cattle, cases of listeriosis have been mainly associated with the consumption of poorly elaborated or poorly preserved silages that allow the development and multiplication of Listeria monocytogenes, and reach levels capable of causing disease [34]. It is important to clarify that when the process is elaborated with technical rigor, there is no doubt of its effectiveness in the elimination of pathogens [35]; however, persistence in manure tanks and the dehydration in the sun, makes it necessary to take all biosecurity measures to prevent it reaching humans indirectly. The infective dose in cattle should be considered before discarding these routes as alternatives in the use of pig manure; in this respect it is known that this bacterium is able to traverse the entire digestive tract without causing infection [36].

Finally, one of the major public health concerns is salmonellosis; it is one of the world's leading food-borne zoonoses, *Salmonella* spp. can survive for months on pig manure [37]. In pigs, salmonellosis presents both health and economic importance; the main serotype involved in the septicemic form is *Salmonella choleraesuis*, while the enteric form is mainly caused by *Salmonella typhimurium* [38]. Due to their zoonotic potential, all biosecurity measures

should be taken to avoid infection in humans. This bacterium did not persist in the silage, dehydrated by the sun and earthworm flour routes, therefore pig manure from these processes could be used to feed cattle. In the case of the biodigester, there was persistence, but a reduced risk could be expected in its use in biofertilization due to the possibility that the strains found are not pathogenic [39], some studies conducted under in vitro conditions have shown that volatile fatty acids, molasses and the ruminal and abomasal environment, affect the growth of Salmonella typhimurium [40]. The process implies that the bacterium once comes into contact with the soil, competes with native flora and is exposed to environmental conditions that could affect its survival [12].

The practice of feeding ruminants with pig manure is widespread in Latin America, because its processing eliminates many of the potential health risks, improves palatability and reduces odors [41]. In addition, the recycling of excreta in ruminant feed promotes economically the pork production [5]; in this regard, there are different reports in which mention is made of the use of pig manure as an ingredient in ruminant feed: Gutiérrez-Vázquez and Preston [22] tried a mixed diet composed of fresh pig manure, molasses and stubble of cereals, for the fattening of steers and obtained weight gains of up to 1 kg per day; Alvarez and Gutiérrez-Vázquez [42], fed steers with fresh pig manure with 30% of dry matter, sugarcane molasses and sorghum or maize, with daily weight gains of 0.92-0.94 kg, being cheaper in terms of production costs the use of maize with respect to sorghum; the same authors determined in their study that the manure of 10 pigs is required to feed one bovine daily. On the other hand, studies have demonstrated the beneficial effect of using a pig-pasture-milk system, in which the biofertilization of the Kikuyo (Pennisetum clandestinum) pastures with pig manure allowed to increase the production in 1.8 liters of milk per day [43]. According to Rojas and Ojeda [44], pig manure can be incorporated into cattle fed under confinement conditions, replacing up 30% of by-products of agro-industrial processing of cereals, such as wheat bran, without altering the animal response. It is economically viable to incorporate pig manure in the diet of ruminants without representing a sanitary risk for the animals, and to achieve weight gains equivalent to other commercial diets [45].

When young animals eat pastures biofertilized with excreta there is a risk of developing some disease induced by persistent pathogens, because young cattle are more susceptible than adult cattle; however, the risk is greatly reduced when biofertilization of the soil is done early and long before the grassland is ready to graze again; additionally, it is possible that even when some pathogens persist, they are in an unviable state [46]. Goberna et al. [31] mentioned that the endogenous soil microbiota competes with the reduces manure pathogens and populations.

CONCLUSIONS

Although some sectors of the scientific community reject the use of excreta in biofertilization systems because of the possibility of transmission of pathogens through contaminated water, it is also clear that the use of pig manure should be structured into a plan of biofertilization that guarantees a safe application without affecting these sources.

Although all the routes of pig manure use analyzed in this study showed variability in the persistence of pathogens, the specificity of some of them allows pig manure to be used in bovine feed or in biofertilization systems in a safe way. However, it is a requirement to adopt personal safety measures that prevent or limit the possibility of infection of humans handling fresh pig manure, due to the presence of some agents with zoonotic potential. It is advisable to carry out a microbiological and parasitological analysis of the pig manure prior to their use, in which the specificity of the agents is determined and their damage capacity is established.

It would be advisable to evaluate the persistence and viability of some of the pathogens, after applying liquid pig manure to the soil for biofertilization coming from manure tanks or biodigesters, and in that same way that solid pig manure from the earthworm compost.

It is necessary to establish the minimum level of *Listeria monocytogenes* capable of causing infection in ruminants, in order to be able to make a more precise judgment on the transcendence of using pig manure in bovine feeding or biofertilization of soils.

Studies confirming the viability of the parasites after the different treatments are required, as well as complementary tests that specify persistent species of *Salmonella* spp., molds and yeasts that may be pathogenic.

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Clinical Significance of the Persistence of Pathogens in Pig (Sus Scrofa) Manure Used As Biofertilizer and Food



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