Animal Health

Review

Four Elements Contributing to the Persistence of Colibacillosis in Camagüey

Four elements contribute to the persistence of porcine colibacillosis in Camagüey

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ABSTRACT

Background: Colibacillosis is one of the catastrophic diseases in swine production. Despite several research aimed to diagnose, prevent, and control the disease for many years, it has not been eradicated. **Aim.** To analyze four elements that favor the persistence of the disease in Camagüey. **Development**: Overall, 115 related sources were reviewed. A total of 50 references were selected according to the editorial guidelines of the Journal of Animal Production with the goal of establishing the discrimination element. Based on their current relevance, the references were divided into three groups: last five-year period (19), 2000-2010 (11), and before 2000 (20). In the 1985-2005 period, more attention was given to colibacillosis in the province of Camaguey, as demonstrated with the development of a vaccine (VACOLI), a diagnostic kit (AuBIODOT-EtEC), and sustainable antidiarrheal treatments from plants (Eucabev). At the end of that period, the disease was increasingly underestimated; the etiological agent was unknown; the diagnostic methods put aside. On the other hand, preventive and control measures tackled the effect, not the cause of the disease. **Conclusions**: four elements contributed to the persistence of swine colibacillosis in Camagüey, with ensuing economic losses due to underestimation of the disease; no knowledge about the *E. coli* patotypes involved; absence of accurate and rapid diagnostic kits; and the application of inappropriate preventive and control measures. The utilization of probiotics or just multipurpose autochthonous micro-organisms could help reduce the negative impact of this disease.

Key words pigs diagnostic *Escherichia coli*, prevention (*Source MeSH)*

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INTRODUCTION

As stated by the *Acquisition Services Directorate And Risk Management Agency* (2015), colibacillosis is among the catastrophic diseases that affect pigs worldwide. Hence, *Escherichia coli* is among the 40 pathogens affecting this species, according to a study that comprised 57 471 papers published between 1966 and 2016. They show that this gastrointestinal syndrome targets post-weaned litters and piglets (Vander Waal and Deen, 2018).

The considerable economic losses mostly due to colibacillosis cause mortality, decreased animal weight gain, and higher treatment costs (García-Meniño *et al.*, 2018; Barreto, Rodríguez, Vázquez, and Junco, 2020). Antibiotherapy poses new challenges as conflictive as the disease. In that sense, the accumulation of antibioresistant strains, and occasional irreversible damage to the environment due to the discharge of these products in the feces and urine, etc., are significant. (Vander Waal and Deen, 2018).

Swine production in the province of Camagüey, Cuba, is among the most promising directions to produce animal food, both in the commercial and non-commercial sectors. This effort is permeated by constant outbreaks of colibacillosis (Barreto *et al.*, 2020). The aim of this proposal is to analyze four elements that contribute to the persistence of the disease in the province.

DEVELOPMENT

A total of 115 related sources were reviewed. Accordingly, 50 references were selected, in keeping with the editorial guidelines of the Journal of Animal Production. The goal was to establish the discriminating element. The sources were classified according to the current relevance, as follows: 19 (last five-year period), 11 (2000-2010), and 20 (before 2000). Then, information was summarized and presented in order to develop the topics for discussion.

Underestimation of the disease

Underestimating a disease may respond to multiple conditions, both subjective and objective, which overlap to offer a mistaken idea of its extent and consequences. It is frequent in diseases with an everyday effect, like swine colibacillosis, instead of those presented sporadically. When they appear, though, their lethality is unquestionable as to the pathogenicity of their etiological agents, and the need to emphasize on diagnostic and prevention (*Acquisition Services Directorate And Risk Management Agency*, 2015). All are harmful, and affect human and animal health. Coincidentally, the pathogenic enteric patotypes of *E. coli* that affect pigs are included in most published articles during the last fifty years (Vander Waal and Deen, 2018). Unfortunately, the underestimation given to this enteropathy extends to humans (Barreto, 1996; Barreto *et al.*, 1998, 2000, 2001, 2020; Barreto and Benítez, 2000).

Outdated knowledge of the etiological agent

The etiological agent of colibacillosis, was first named *Bacterium coli commune* when it was isolated (1885), but thirty-four years later, it was named after its discoverer, Theodor Escherich, as *Escherichia coli*. This denomination drew the attention of researchers in different areas of knowledge to the extent of becoming the most widely studied living species so far. However, such dedication throughout 135 years has been insufficient to elucidate some doubts in relation to its adaptability and behavior in diverse environments (Barreto, 2007; Barreto, Rodríguez, and Barreto, 2016a; Vila *et al.,* 2016; García-Meniño *et al.,* 2018).

Although *E. coli* is the most abundant commensal facultative anaerobic species in the intestinal microbiota of hot-blooded animals, and it contributes to the normal functioning of this organ, which is essential in this topic, there are strains with sufficient virulence traits to be grouped in six enteric patotypes. These groups are, classic enteropathogenic (EPEC), enterotoxigenic (ETEC), enteroinvasive (EIEC), enteroaggregative (EAEC), diffuse enteroadherent (EAEC-D), and Shiga Toxin producers (STEC) (Kaper, Nataro, and Mobley, 2004; Bai *et al.*, 2019). All are pathogenic to humans; hence many of the studies done and papers published so far refer to it as a target species. Some, however, can cause colibacillosis in newborn and post-weaned pigs (Barreto, 2007; Barreto *et al.*, 2020); particularly ETEC and STEC (García-Meniño *et al.*, 2018).

The ETEC pathotype comprises strains codifying for heat-stable (STa and/or STb) and heat-labile (LT) enterotoxins. Meanwhile, STEC groups the ones capable of producing the Shiga-like 2e (Stx2e) toxin, responsible for oedema in post-weaned pigs. Due to the high genetic transference, especially by conjugation, the frequency at which hybrid strains containing information for Stx2e and enterotoxins (TSEC/ETEC) are isolated, is increasingly higher (García-Meniño *et al.*, 2018; Barreto *et al.*, 2020).

Strains of ETEC pathotype cause most cases and outbreaks of colibacillosis in intensive swine systems worldwide. These strains bind to receptors in the epithelium of the small intestine of the host through diverse fimbriae (Barreto, 2007; Luppi, 2017; Sun, and Kim, 2017). The most commonly occurring are F4, F5, F6, previously known as K88, K99, and 987p, respectively), F41 and F18. The last two are prevalent, as the receptors of the others are reduced with animal age. F4 is presented in three different antigenic forms (F4ab, F4ac, F4 ad), and F18 in two (F18ab and F18ac). Multiple fimbriae (F4, F5, F6, and F41) enable the adhesion of ETEC to the receptors of enterocytes in the litters, whereas only two (F4 and F18ac) favor this type of binding in pre-fattening animals (Sun y Kim, 2017; Luppi, 2017). In turn, the F18ab ones cause edema in pigs (García-Meniño *et al.*, 2018).

Although (ETEC) and STEC are the most deeply involved pathotypes in swine colibacillosis, others common to humans should not be excluded. In that respect, strains considered as classic enteropathogenic (EPEC) stand out. They adhere to the intestinal epithelium by means of adhesion or "intimate" proteins, through a complex mechanism that leads to adhesion and structural and functional distortion at the apex of microvilli (Kaper *et al.*, 2004). The EIEC pathotype uses an invasive mechanism that causes similar diarrheal manifestations to the ones

caused by *Shigella* spp. in humans. Additionally, it affects pigs, and has been previously reported in isolates from litters with colibacillosis in Camagüey (Barreto, Clavería, and Ortiz, 1987; Barreto and Guevara, 1987).

The growing participation of hybrid strains, which are a significant cause of this enteric syndrome, as virulence factors, especially in post-weaning diarrhea, has encouraged researchers to introduce the term *Intestinal pathogenic Escherichia coli* -InPEC (Yang *et al.,* 2019).

The confirmation of each of these pathotypes involves more than a simple identification of *E. coli,* by means of routine biochemical tests (Ewing, 1996). Excluding EIEC (Barreto *et al.,* 1987), both commensal strains and pathogenic strains share the same biotype (Barreto, 2007). Accordingly, the value of bacteriological isolates could be low if the resources to identify the patotype are scarce, which is exclusive to specific laboratories.

Last, but not least, the majority of studies of *E. coli* as the etiological agent of colibacillosis only consider its planktonic phenotype, thus undermining the biofilm phase, regardless of its hegemonic performance in every natural environment, and its undeniable virulence factor (Barreto *et al.,* 2016a, b).

Diagnostic shortage

Often, bacteriological diagnostic of *E. coli* is performed from isolated colonies in selective culture media (Agar MacConkey, Shining green Agar, etc.), which undergo biochemical tests for identification (Ewing, 1996). The only information is to use this method to demonstrate it is a species of *E. coli* which does not homologate its pathogenic potential. To elucidate this question, Kauffmann (1947), suggested serological tests based on parietal O antigens (somatic), K (capsular), and H (flagellar), just as was done for the typification of *Salmonella.* This variant, which was improved eventually, showed a valuable serotype-host relation (Ørskov and Ørskov, 1983; Blanco, Blanco, Garabal, and González, 1991; Blanco *et al.,* 2006). Despite its specificity, this variant is painstaking, and it was applied in Cuba until the 1980s (Barreto, 2007).

Other variants to elucidate the commensal-pathogen relationship were intended to determine, from *E. coli* phenotype isolates, the production of enterotoxins (Smith and Halls, 1967), colicines (Fredericq, 1965), and fimbriae (Brinton, 1965; Blanco *et al.,* 1991; Harvey, Anderson, Genovese, Callaway and Nisbet, 2005; Campal *et al.,* 2007, 2008; Campal, 2009). The molecular characterization of the last ones enabled the production of polyclonal (PAb), and monoclonal (MAb) antibodies, both to design diagnostic kits and to manufacture vaccines against swine colibacillosis (Harvey *et al.,* 2005; Campal, 2009; Nadeau *et al.,* 2017).

AuBIODOT-ECET, a panel of monoclonal antibodies against F4, F5, F6, and F41, which is capable of detecting ETEC from rectal swabs on the farms in 45 minutes, was the first of its kind, and made the best example of the above mentioned. Regardless of hours, resources, and talent

invested at the Center of Genetic Engineering and Biotechnology for development, start up, and implementation, this kit has not been applied since mid-2000 (Campal, 2009; Barreto *et al.*, 2020).

There are other effective variants for rapid and accurate identification of enteropathogenic *E. coli.* One of them is using the samples collected from fresh feces deposited on the floor of the pen or house. Then, Polymerase Chain Reaction is used to amplify the virulence genes of the enteropathogen (Weber *et al.,* 2017; García-Meniño *et al.,* 2018; Yang *et al.,* 2019).

A reputed international health institution that published a manual on the diagnostic of diarrheal diseases added this words on the cover: "diagnostic, at least, must be on top of therapy" (*World Health Organization* –WHO, 1983). This assertion is the promoter of this study, and has been assumed by the authors of the present article. Therefore, orior to the analysis of the next topic, if diagnostic could be matched to therapy, the coming events would be obviously predicted.

Inappropriate methods for prevention and control of the disease

In the above topics, the interest observed in Camaguey, particularly between 1985 and 2005, was apparent. So it was backed by research and published papers during that period by the current authors (Campal, 2009; Barreto *et al.*, 2020). Just in the middle of that period, the CIGB in Camaguey offered MINAG a recombinant vaccine against swine colibacillosis caused by ETEC, containing fimbriae F4, F5, F6, and F41 (VACOLI), which had a positive effect on the control of the disease (Wong *et al.*, 1995, 1996; Campal, 2009). However, AuBIODOT-ECET was not applied any further as soon as all the free tests finished on several local swine farms, and other provinces (Campal, 2009).

Some might think that such decision was made because that preventive choice was outdated. In fact, before and after VACOLI, the rest of the world has continued to manufacture vaccines, as evidenced in Spain and China, the top two swine producers (García-Meniño *et al.*, 2018; Yang *et al.*, 2019).

In the same way the best preventive alternative was rejected before the end of the twentieth century, other non-environmentally invading variants to treat colibacillosis had similar outcome. Eucabev, made from *Eucalyptus saligna* crust, which, in addition to its proven antidiarrheal action, had a vital re-hydrating effect on animal recovery (Velázquez, Barreto, Izquierdo, and Palacios, 1991). Its effectiveness against (ETEC), among others, depended on blocking the expression and/or adhesion of fimbriae (F4, F5, F41) to receptors of the host (Barreto *et al.*, 1993a, b, c, 1995a, b, 1996). Although it met the requirements for medications of this kind in animals, and demonstrated its effectiveness to control colibacillosis in newborn pigs and calves (Velázquez *et al.*, 1991; Barreto *et al.*, 1993b, c), it ended up like the other treatments.

It has persisted along with the gradual neglect that continues to exist, which is characterized by underestimation of the disease, poor knowledge of the pathotypes involved, and deficient diagnostic. Regardless of the pathological one, when the animal has no cure. The rest is made of the prevention and control methods in place. Both goals are based on the use of antibiotics in sub lethal concentrations added to feeds, as prophylactic treatment following weaning, for prevention, in the dose recommended by the manufacturer to control colibacillosis outbreaks (Barreto *et al.*, 2015, 2016a, b, 2020). The first choice was internationally banned since the beginning of the twentieth century (Barreto *et al.*, 2016b). The related literature is so abundant that insisting on this preventive strategy would be absurd.

The question all swine farmers and involved professionals should ask themselves is why colibacillosis is affecting litters and pre-fattening animals like this. Solutions are given by dealing with the causes, not the effect. In the two moments, there is a stable and functional lack of intestinal microbiota. It occurs in newborn animals, where it begins to settle, just like in animals with an immature immune system. After weaning, the stress of the moment is increased by changes in the diet, usually with the inclusion of dry feeds that further lacerate the already harmed microvilli (Barreto *et al.*, 2015), compromising around 60% of it (Missotten, Michiels, Degroote, and de Smet, 2015).

In both categories, an ideal breach is created for the establishment of enteropathogens from the environment. The absence of specific vaccines, and the inclusion of antibiotics in the feed, which cause more depression of the existing microbiota, lead to enormous losses produced by this disease (Dou *et al.*, 2017; Lépine *et al.*, 2019; Mukhopadhya, O'Doherty, and Sweeney, 2019; Barreto *et al.* 2020).

Hence, the proper solution consists in supplying the adequate microbiota in the diet during the growing stage. Then help re-establish it at weaning through liquid feed formulations that favor speedy re-establishment of microvilli (Barreto *et al.*, 2015; Missotten *et al.*, 2015).

These choices are easily accessible by any farm engaged in growing and pre-fattening pigs. One of the most broadly known is the utilization of prebiotics and probiotics. The former are non-degradable compounds that enable the development of probiotic microorganisms present in the intestinal microbiota. The latter are supplied in capsules or in the feed (Mukhopadhya *et al.*, 2019). In other provinces of Cuba, these microorganisms have been successfully used in swine production (Vega-Cañizares *et al.*, 2018).

Another simpler and affordable solution, also including probiotics and prebiotics, consists in the utilization of multipurpose autochthonous microorganisms (MAM). They are microbial mixes made with decomposing dry leaves in areas free from chemicals. They are the Camagueyan version of the well-known efficient microorganisms, originally from Japan (Rodríguez *et al.*, 2013). They are used to treat water for the consumption of these animals, and can be previously mixed with feed to produce fermented feed rich in microbial protein, which can be easily

assimilated, and can contribute to intestinal microbiota recovery (Rodríguez *et al.*, 2013; Barreto *et al.*, 2015).

CONCLUSIONS

Colibacillosis is a major and frequent cause of mortality in litters and pre-fattening animals in Camagüey. The contributing elements are underestimation of this disease, the lack of knowledge about the *E. coli* pathotypes involved, the absence of rapid and accurate diagnostic kits, and inappropriate application of preventive and control measures. The utilization of prebiotics, probiotics or simply, multipurpose autochthonous microorganisms, could help reduce the negative impact of this enteric disease.

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AUTHOR CONTRIBUTION

Research design and idea: GBA, HRT, ACE; data analysis and interpretation: GBA, HRT, HBR; redaction of the manuscript: GBA, HRT, ACE.

CONFLICT OF INTERESTS

The authors declare no conflict of interests.