Review

Diarrheagenic and Commensal *E. coli* in Cattle, Implications for Health and Current Antibiotherapy

Guillermo Barreto Argilagos* , Herlinda de la Caridad Rodríguez Torrens*

*Faculty of Agricultural Sciences, Ignacio Agramonte Loynaz University of Camaguey, Camaguey, Cuba. Correspondence: guillermo.barreto@reduc.edu.cu

ABSTRACT

Background: Diarrheagenic *E. coli* poses health and economic problems to cattle raising. They also participate in the expansion of antibioresistance genes, hindering current antibiotherapies. **Aim.** To summarize updated information on *E. coli*-cattle interaction, with emphasis on newborn calf diarrhea, and its implications on current antibiotherapies. **Development:** Bovines are an excellent reservoir of *E. coli.* The six diarrheagenic patotypes affect newborn calves directly, with a lesser or greater intensity. Patotypes ETEC and STEC stand out among them; the former causes the largest colibacillosis outbreaks, the latter is also a growing global zoonotic agent. Every patotype causes major economic losses due to morbidity and mortality of these forms of animal rearing. Both commensal and diarrheagenic strains are sources of antibioresistance, which is stimulated using antibiotics as growth promoters in cattle, making current therapies difficult. Probiotics and efficient microorganisms are sustainable alternatives. **Conclusions**: all the Diarrheagenic patotypes of *E. coli* cause intense or less intense diarrhea in calves, with major economic losses. The STEC strains are zoonotic. Both diarrheagenic and commensal strains participate in the spreading of antibioresistance. This phenomenon is stimulated by inadequate antibiotic use, both for prophylaxis and as growth promoters. Other, more efficient alternatives do not cause side risks to health and the environment, thus not compromising the effectiveness of current antibiotherapies.

Key words: antibioresistance, bovines, *Escherichia coli*, prevention, zoonosis (*Source*: *MeSH*)

INTRODUCTION

Few microorganisms have caught the attention of researchers and scientific institutions, as *Escherichia coli.* This has occurred over the course of 136 years with a completely antagonist,

still puzzling, protagonism. On one extreme end of the scale, it stands out as a commensal in the microintestinal biota, a prominent member of facultative anaerobic representatives of all hot-blooded animals. On the other end, it acts as an intestinal pathogen (*intestinal pathogenic E .coli* -IPEC), and extraintestinal pathogen (*extraintestinal pathogenic E. coli* -ExPEC) (Ramos *et al.,* 2020; Barreto, Rodríguez, and Campal, 2020a).

This dual feature is valid, though it should never be interpreted as absolute. The non-diarrheagenic strains present in the microintestinal biota of all animal species used for food production have an increasing participation in the spreading of antibiotic resistance genes to humans (Ahmed *et al.,* 2019). Although it has been particularly analyzed in birds, bovines also have an outstanding position in this respect (Hang *et al.,* 2019). Underestimating diarrheagenic variants (DEC), many of them zoonotic, has been a regrettable error (Ryu *et al.,* 2020; Barreto *et al.,* 2020a; Barreto *et al.,* 2020b). Cattle are reservoirs of quite a few extraintestinal human pathogenic *E. coli* strains responsible for infections of the urinary tract (UTI) through newborn meningitis (Nielsen *et al.,* 2020).

For about 10 000 years, bovines have been the main source of meat and milk for human consumption. This long period has turned them into an important reservoir of pathogens causing food-transmitted diseases (FTD), among which are the Shiga toxin-producing *E. coli* patotype (STEC) (Sapountzis *et al.,* 2020). At the same time, almost all the IPEC variants stand out among newborn calf diarrhea-producing agents. Their negative impact is more intense during the first weeks of life. A stage characterized by high morbidity and mortality, growth retardation, and increased economic losses due to treatments and others (Awad *et al.,* 2020).

Consequently, the above-mentioned scale is tilted. *A priori*, it is tilted to the negative side, leaving the opposite dish full of uncertainties. Is there something positive to add to this story? Certainly, but it depends on those who organize and run production systems.

Accordingly, the aim of this review is to summarize updated information on *E. coli*-cattle interaction, with emphasis on newborn calf diarrhea, and its implications on current antibiotherapy.

DEVELOPMENT

A previous clarification

The information available in relation to the taxonomy of *E. coli* is very abundant. It has resulted from the negative impact on human and animal health, in addition to becoming a relevant part of their intestinal microbiota (Ramos *et al.,* 2020). For that reason, it was chosen as an appropriate indicator to determine possible water and food contamination from feces. However, despite years past, the proposal of Nataro and Kaper (1998) has remained as a referent of any approximation to this enterobacterium and its pathogeny. This review only focuses on some critical details for

scholars conducting research on the links between bovines, this prolific bacterial species, and the consequences to humans and calves.

E. coli, *morphological and biochemical aspects*

Escherichia coli is the type species from the genus that comprises other four (*Escherichia blattae, Escherichia fergusonnii, Escherichia hermannii*, and *Escherichia vulneris*), without their epidemiological transcendence (Barreto, 2007). It is presented in the form of small, gram negative, anaerobic, facultative, oxidase negative colibacilli (Edwards and Ewing, 1972), motile (swimming, individual motility by peritrichous flagella; or swarming, collective motility regulated by *quorum-sensing*), or nonmotile (Swiecicki, Sliusarenko, and Weibel, 2013). Although the capacity for lactose fermentation is a predominant character, which is used in biochemical schemes for presumptive identification, there are exceptions. The most commonly known is the one that characterizes the enteroinvasive *E. coli* (EIEC) patotype that comprises strains with a phenotype like *Shigella* spp. with a variable pathogenicity in calves, and high virulence in humans (Barreto, 2007; Awad *et al.,* 2020).

The fermentation (or lack of fermentation) of certain carbohydrates has been a way used by several small laboratories as an effort to approach diarrheagenic *E. coli* variants, which are pathogenic to humans and animals. One example is the fermentation of sorbitol, raffinose, and dulcitol, along with decarboxylation of lysine, arginine, and ornithine, to determine the vero toxigenicity character, in strains isolated from cattle (or derived foods), or humans. Generally, rhamnose-non-fermenting *E. coli* O26 strains correspond to the STEC patotype (shigatoxigenic, also named *Shiga* like Toxin *E. coli*) (Gebregiorgis and Tessema, 2016). The addition of adonitol (0.2%) to Simmon's Citrate Agar medium (Pohl *et al.,* 1984) turned it into a simple tool to detect ETEC K99$^+$ (now ETEC F5$^+$), by forming typical yellow mucoid colonies. This variant appeared in the first reports of strains with that phenotype, which caused newborn calf diarrhea in Camaguey (del Risco and Barreto, 1988).

These techniques were painstaking and inaccurate, and required serological confirmation of *E. coli biotype* isolates. It was another complex variant to deal with, whose determination required experienced staff. They relied on the determination of somatic (O), flagellar (H), and capsular (K) antigens. Overtime, it was limited to the detection of fimbrial antigens (F5, F17, and F41), from diarrheagenic ECET in calves (Barreto, 2007). Simultaneously, biological tests were introduced to confirm the presence of highly aggressive enterotoxins to the laboratory animals used. These issues led to the fact that since the end of the 1990s, researchers have suggested using molecular assays to conduct this diagnostic (Mutkar *et al.,* 2015). Unfortunately, there are laboratories where the biochemical variant is their only choice.

Enteropathogenic E. coli patotypes

The diarrheagenic variants of *E. coli* are distributed in six patotypes, according to the virulence factor of the agent, and the harm level caused in the host. They are enteroxigenic (ETEC), enteropathogenic (EPEC), shigatoxigenic (STEC), enteroinvasive (EIEC), enteroaggregative (EAEC), and enteroadherent (EAdEC or DAEC _diffusely adherent *E. coli*]) (Awad *et al.,* 2020).

Within this classification, the STEC patotype incorporates the EHEC subgroup (enterohemorrhagic *E. coli*), which had been an independent patotype in previous classifications due to its relevance (Barreto, 2007; Andrade *et al.,* 2012; Awad *et al.,* 2020). Then, as a EHEC patotype, it included vero toxin-producing strains (vero toxigenic *E. coli* –VTEC) (Karmali *et al.,* 1985), which now belongs to the STEC patotype. Both EHEC and VTEC play an outstanding role in the pathogeny of humans and bovines, so they will be revisited in this review, though its current subordination to STEC will be excluded.

Consequently, it is important to say that ETEC is the predominant patotype in newborn calf diarrhea, particularly in the first four days of life (Andrade *et al.,* 2012). The success of these strains in infecting and further developing the diarrheal syndrome is associated to its broad arsenal of virulence attributes. First, the presence of diverse fimbria that make adherence to small intestinal receptors of the host possible. Although F5 is prevalent, the participation of F17 and F41 cannot be disregarded. The completion of this step facilitates colonization and, when the necessary *quorum* is reached, the release of toxins that cause diarrhea. They can be of two types: heat labile (HL) and heat stable (HSa and Hsb) (Ramos *et al.,* 2020).

The involvement of the other patotypes in the syndrome is controversial. Some researchers report them in isolates from healthy and diarrheal animals (Awad *et al.,* 2020). However, though STEC can be present in the feces of healthy animals, it often acts through Shiga toxins (Stx1 and Stx2). Meanwhile, EPEC and EHEC, with similar pathogenicity mechanisms, affect 2-8-week-old calves, especially during the fourth week (Awad *et al.,* 2020). Protein (intimin) mediated adherence leads to the destruction of the apex of intestinal microvilli (attaching and effacing AE), a damage that causes diarrhea (Nataro and Kaper, 1998; Andrade *et al.,* 2012).

Since the 1980s, *E. coli* O157:H7, a EHEC serotype, has called global attention. Its zoonotic character has been demonstrated, appearing in the United States and Canada simultaneously (Barreto *et al.,* 2007). Then, it spread across Europe, and has been listed among the major enteropathogens found in food quality control analyses, particularly ground beef used to make hamburgers (Ramos *et al.,* 2020). Just like other EHECs, it is present in the microbiota of healthy cattle, accumulating in their feces. It can contaminate milk, water, vegetables, and so on, using different ways. (Nobili *et al.,* 2017).

STEC strains can survive and persist in the gastrointestinal tract of cattle, thus evading its immunological mechanisms. The interaction with the intestinal microbiota may favor or hinder

its persistence in the host, an area worth investigating more thoroughly (Sapountzis *et al.,* 2020). Because of its validity for the control of enteropathogens like the previous, this topic will be analyzed further on.

Due to the elevated rate of pathogenic gene transfer in *E. coli*, STEC strains are often isolated with plasmids, to which information related to intimin (eae) and a potent hemolysin (*hylA*) is added to that of Stx1 and Stx2. These hybrids are termed AESTEC (attaching and effacing STEC) (Thiry *et al.,* 2017). It can occur in healthy and sick calves, as well as in beef and dairy cattle. All act as reservoirs and sources of transmission to humans, causing hemorrhagic colitis through hemolytic uremic syndrome (Awad *et al.,* 2020).

The capture of foreign plasmids in *E. coli* has produced diverse hybrids like the above-mentioned. This phenomenon complicates the epidemiological study, as well as the taxonomic classification into patotypes. In addition to the one described, ETEC/STEC and ETEC/EPEC associations have been reported in 14.7% and 2.7% of cases, respectively. These atypical combinations are more frequent in buffaloes (Awad *et al.,* 2020).

The high gene transference in *E. coli,* besides the one already mentioned, has encouraged this species to play an outstanding role in the transmission of antibioresistance, a phenomenon that set the alarms last century, to the point of being identified as the silent epidemic of the twentieth century (Oliva and Baez, 2019). Bovines are reservoirs of these enteropathogenic entities that play an outstanding role in their persistence and spreading.

E. coli–cattle interrelation and the expansion of antibioresistance

The presence of antibioresistance in commensal bacteria of the intestinal microbiota is frequent and rising. This increment is dual: quantity of bacteria and extension of polyresistance. This phenomenon is like a snowball going down a mountain, causing havoc. This simile is no exaggeration, particularly when the analysis focuses on animal production systems that contribute to human food consumption. The species involved (cattle, pigs, birds), rather than being the exception, are an example of the problem, being the food chain the main route of transmission to consumers (Ramos *et al.,* 2020).

This reality derived from continuous exposure of animals to antibiotics for over half a century, both in sublethal concentrations to promote growth, and for prophylaxis (Barreto, Rodríguez, and Barreto, 2016a, b). The former is under international restrictions, which are not always met. The latter is excessively applied in the prevention of diseases that would not occur if their causes were prevented. To achieve either purpose there are multiple sustainable choices free from the adverse effects of these antimicrobials (Belookov *et al.,* 2019; Barreto *et al.,* 2017; Barreto *et al.,* 2020a; Barreto *et al.,* 2020b).

Because of the amounts of *E. coli* in the intestines, its remarkable adaptability to the most diverse environment, and the capacity to accept foreign genes, it stands out among antibioresistance commensals, and during the transmission and spreading of such resistance (Ramos *et al.,* 2020). The above aspects are favored by the presence of motile genetic elements in their genome (integrons, plasmids, and transposons) (Schrijver *et al.,* 2018; Wyrsch *et al.,* 2019). In that sense, broad spectrum strains of β-lactamase (BLEE) are capable of hydrolyzing third generation cephalosporins and aminoglycosides (*European Centre for Disease Prevention and Control -* ECDC, 2018). The presence of BLEE[+] *E. coli* has been reported in Cuba, in bird isolates (Baez *et al.,* 2021). This practice should be implemented in bovines, since they are reservoirs of patotypes of this enterobacterium, which are pathogenic to humans, and carriers of critical antimicrobial resistance genes during therapy (Coppola *et al.,* 2020).

In Germany, the circulation of *E. coli* resistant to third generation cephalosporins was reported in 70% of cattle farms, and 85% of dairy farms (Hille *et al.,* 2017). This problem is present in all Europe. More than half of the isolates analyzed in 2018 were resistant to at least one type of antibiotic, ranking from top to bottom: aminopenicilline, fluoroquinolons, third generation cephalosporins, and aminoglycosides. The same report points out that *E. coli* stood out both for its tolerance to antimicrobials and the number of deaths caused (ECDC, 2018).

Meanwhile, the European Food Safety Authority (EFSA) made a report about the growing concerns regarding the high proportions of *Salmonella, Campylobacter,* and *E. coli* isolates with a diminished sensitivity to fluoroquinolones (EFSA, 2019).

In the Americas, the United States has reported an increase of strains with these features for several years (Schrijver *et al.,* 2018). In Latin America, two South American beef production giants have also reported a similar context. In 2017, a first report was published on *E. coli* as carriers of genes resistant to antibiotics used in humans in Uruguay (Umpiérrez *et al.,* 2017). Three years later, this topic was reiterated, though more strikingly, since the problem already included major antibiotics for humans (Coppola *et al.,* 2020). In Argentina, scholars referred to a similar situation (González *et al.,* 2019).

In Cuba, during a study to evaluate antibioresistance in isolates from foods, 62.1% of it was observed to come from at least one antibiotic. *E. coli* and *V. cholerae* showed over 50% resistance to tetracycline and ampicillin. The greatest percentages of antimicrobial insensitive strains were obtained in isolates from meat and meat-by products, including beef (Puig-Peña *et al.,* 2020). It was the only national report on these observations, though cattle derivatives were not analyzed by separate throughout the article.

In conclusion, the common denominator of quinolones, cholistine, and third-generation cephalosporins is their priority use in human antimicrobial therapy. Hence, any form of resistance observed in the target bacteria becomes a global concern that calls for permanent surveillance and immediate solutions.

Barreto Argilagos, G., Rodríguez Torrens, H.C.

Are growth promoters strictly necessary for bovine production?

Growth promoters are the substances that, without being nutrients, can increase the efficiency of food conversion, mean daily gain, and carcass quality, or increase in milk production. The most commonly used in calves are classified into five groups: nutritional additives, hormonal implants, growth hormones (BTS-*Bovine Somatotropine*), β-agonists agents, and probiotics. Detailed information has been published about each of these variants, their advantages, and limitations, since the last century (Herago and Agonafir, 2017). According to the aim of this proposal, only the nutritional additives will be explained thoroughly, particularly the case of antibiotics and probiotics.

In a broader sense, a nutritional additive is the substance incorporated to the food to a) meet certain animal needs, and b) increase resistance to diseases (Abd-Elhakeem *et al.,* 1998). There are multiple candidates; since the first half of the last century, antibiotics (sublethal concentrations), organic acids, and certain enzymes are among the most important (Barreto *et al.,* 2017; Herago and Agonafir, 2017). Below some key aspects have been summarized.

<u>Antibiotics as growth promoters</u>

Although since the mid-1940s the stimulating properties of some antibiotics for animal growth were known, their introduction in the diet of calves as nutritional additives was not fully established until the 1950s. This decision came from a hypothesis: by eliminating undesirable microorganisms (and toxins) in the intestinal tract, antibiotics create a favorable environment in the intestinal mucosa for a more efficient absorption of nutrients. Since then, this practice was assumed as an essential component of bovine productions (Kertz *et al.,* 2017).

Between January 1957 and December 1976, a number of 150 papers were published in the United States and Canada, dealing with the impact of antibiotics on the production of calves, and dairy cattle in general, from physiological, genetic, and immunological standpoints (Kertz *et al.,* 2017). Unfortunately, the long chain of successes was based on three major biases: their effect on the generation of antibioresistance strains (commensal and/or transit pathogenic), and their consequences on consumers and the general surrounding (Barreto *et al.,* 2016a). This was confirmed later.

Throughout more than half a century, such successful practice contributed to a reduction of the efficacy of antimicrobials used in humans and animals, due to the generation of resistance bacteria. The first signs of alarm were observed in the mid-1960s when *Salmonella* strains causing outbreaks of food-transmitted diseases in The United Kingdom showed unusual polyresistance. The severity of this led to the creation of a commission to analyze the case (*The Swann Committee*). Among others, it recommended to restrict the use of antibiotics as additives in the diet of animals, except those outside (or with very little application) human and animal

therapies. It also stressed that some antibiotics, like Tylosin, could only be used if officially prescribed (Edqvist and Pedersen, 2002).

Ironically, as Edqvist and Pedersen (2002) noted that *subsequent action turned into inaction, and conclusions into dilutions*, regrettably. The European Union approved the use of macrolides (Tylosin and Spiramycin) as growth promoters. This decision was influenced by the pressures of the pharmaceutical industry, and the community of animal breeders on one side; and on the other, thanks to a mistaken scientific conception (very opportunistic, by the way). According to Walton (1988), antibiotic use in sublethal or inhibiting concentrations did not exert enough pressure to generate a resistance response in bacteria. The broad resistance to macrolides observed in macrobacteria, in subsequent years (Edqvist and Pedersen, 2002; Tang *et al.,* 2019) would have been enough to rebut such theory, but it was otherwise.

In the late Twentieth Century, the World Health Organization called for a meeting to evaluate the consequences generated by this variant. In a general sense, a spine-chilling conclusion was drawn: the extent of the impact of antimicrobial use in animal production on medicine and public health was unknown. Such uncertainty forced a new recommendation: replacing them by safer growth promoters (WHO, 1997).

More years had to pass, until stricter decisions were made. In 1999, the European Union (EU) issued a decree banning this practice, partially. Then, in January 2006, it was established in absolute terms (*US Government Accountability Office*, 2011; Maron, Smith, and Nachman, 2013). Despite the justified decision, breeders in developing countries fail to abide by this rule, though they are not the only ones (Maron *et al.,* 2013; Van *et al.,* 2020).

It is a quite real approximation to world developments after the EU regulation in 2006, as expressed in the proposal of Maron *et al.* (2013), including the justifications to turn a blind eye, based on legal proceedings. This had been anticipated in another famous irony of Edqvist and Pedersen (2002): *antimicrobials as growth promoters*: *resistance to common sense.*

Antibiotic alternatives for growth promotion

Current technologies for animal production not always consider critical aspects of animal physiology. By not adapting to nutritional and environmental changes undergone, they often become targets of pathogenic entities, both enteric and respiratory. The several, already mentioned, patotypes of *E. coli*, are a proof of it. Besides making them ill, their productivity declines, and the economic losses are enormous. This phenomenon can be reversed. Though not relying on antibiotics for therapy or prophylaxis; much less, as growth and health promoters. (Belookov *et al.,* 2019; Tang *et al.,* 2019).

At birth, the immune system of calves is immature, making them dependent on the passive protection they receive in the colostrum. As their only means of defense, they must face the

change from a sterile environment in their mother's uterus, to an environment loaded with microorganisms, many of them pathogenic. With these microorganisms, the ones acquired through lactation from their mothers and other feedstuffs, calves begin conforming their intestinal microbiota. It is a pivotal moment: a) the ideal time for the establishment of enteropathogens; b) the appropriate moment to help with the formation of suitable and stable microbiota, by means of probiotics (Al-Shawi *et al.,* 2020), or compatible microbial mixes (efficient microorganisms _EM) (Belookov *et al.,* 2019; Rodríguez *et al.,* 2021). The result depends on the breeder.

Additionally, newborn calves are far from being functional ruminants. They must be fed as monogastric animals. A liquid diet ensures most of their nutrition until weaning, when they start consuming a dry diet in sufficient amounts to contribute to rumen development. A liquid alternative is the best way to introduce probiotics or EM mixed with nutrients before consumption, or fermented before supplying them to the animals (Missotten *et al.,* 2015). The other variant, in addition to being more economical, can increase the quality and protein levels of nutrients, their digestibility, while maintaining a balanced intestinal microbiota, stimulating protecting immune response at that level, limiting the adherence of enteropathogens, and improving animal health parameters (Rodríguez *et al.,* 2021).

Weaning poses a drastic challenge to animals, only comparable to after birth. The radical change to a dry diet may lead to damage in microintestinal microvilli. These injuries could be irreversible or not, depending on the zootechnical measures adopted. Some of these challenges include the aboe-mentioned suggestions, which are positive in terms of stabilizing altered microbiota (Alayande, Aiyegoro, and Ateba, 2020).

An illustration of the above is the following: The purpose was to evaluate the probiotic effect of *Saccharomyces cerevisiae* on post-weaned Siboney de Cuba (5/8 Holstein, 3/8 Zebu) calves, with an average age of 180 days. Chopped sugar cane was supplied *ad libitum*, together with 100 mL of liquid *S. cerevisiae* culture, C-40 (1.3×10^8 cfu/g)/Norgold/animal. The introduction of this yeast in the conventional diet contributed to weight gains of 10 kg (p<0.05) higher than the control animals. Mean daily gain was higher as well (over 100 g/animal/day) (Delgado, Barreto, and Rodríguez, 2019). Perhaps, the fermentation of nutrients with *S. cerevisiae* for 24-48 hours before feeding the calves could provide even better results.

CONCLUSIONS

Bovines are an excellent reservoir of *E. coli.* All the diarrheagenic patotypes of *E. coli* cause intense or less intense diarrhea in calves, producing major economic losses; STEC is an ascending world zoonotic agent. Both commensal and diarrheagenic strains are sources of antibioresistance, which is stimulated with the use of antibiotics as growth promoters, making current therapies in calves and humans difficult.

REFERENCES

Abd El-Hakeam, A. A., Abd El-Moty, A. K. I., El-Feel, F. M. R., & Abu-Elawa, M. (1998). Reproductive performance of buffalo and Friesian calves as affected by flavomycin growth promoter. *Egyptian Journal of Animal Production*, *35*(1), 55. https://doi.org/10.21608/ejap.1998.112568

Alayande, K. A., Aiyegoro, O. A., & Ateba, C. N. (2020). Probiotics in animal husbandry: applicability and associated risk factors. *Sustainability*, *12*(3), 1087. https://doi.org/10.3390/su12031087

Al-Shawi, S. G., Dang, D. S., Yousif, A. Y., Al-Younis, Z. K., Najm, T. A., & Matarneh, S. K. (2020). The potential use of probiotics to improve animal health, efficiency, and meat quality: A Review. *Agriculture*, *10*(10), 452. https://doi.org/10.3390/agriculture10100452

El-Sayed Ahmed, M. A. E. G., Zhong, L. L., Shen, C., Yang, Y., Doi, Y., & Tian, G. B. (2020). Colistin and its role in the Era of antibiotic resistance: an extended review (2000–2019). *Emerging microbes & infections*, *9*(1), 868-885. https://doi.org/10.1080/22221751.2020.1754133

Andrade, G. I., Coura, F. M., Santos, E. L., Ferreira, M. G., Galinari, G. C., Facury Filho, E. J., de Carvalho, A. U., Lage, A. P., & Heinemann, M. B. (2012). Identification of virulence factors by multiplex PCR in Escherichia coli isolated from calves in Minas Gerais, Brazil. *Tropical animal health and production*, *44*(7), 1783-1790. https://doi.org/10.1007/s11250-012-0139-8

Awad, W. S., El-Sayed, A. A., Mohammed, F. F., Bakry, N. M., Abdou, N. E. M., & Kamel, M. S. (2020). Molecular characterization of pathogenic Escherichia coli isolated from diarrheic and in-contact cattle and buffalo calves. *Tropical Animal Health and Production*, *52*(6), 3173-3185. https://doi.org/10.1007/s11250-020-02343-1

Baez Arias, M., Espinosa, I., Collaud, A., Miranda, I., Montano Valle, D. D. L. N., Feria, A. L., Hernández-Fillor, R. E., Obregón, D., Alfonso, P., & Perreten, V. (2021). Genetic Features of Extended-Spectrum β-Lactamase-Producing Escherichia coli from Poultry in Mayabeque Province, Cuba. *Antibiotics*, *10*(2), 107. https://doi.org/10.3390/antibiotics10020107

Barreto Argilagos, G. (2007). Escherichia coli, últimos 122 años. Revista de Producción Animal, 19(2 Número Especial), 55-67.

Barreto Argilagos, G., Rodríguez Torrens, H., & Barreto Rodríguez, H. (2016a). Comportamiento in vitro de Escherichia coli enterotoxigénica ante concentraciones

crecientes de cobre. *Revista de Producción Animal*, *28*(1), 39-43. https://revistas.reduc.edu.cu/index.php/rpa/article/view/14

Barreto Argilagos, G., Rodríguez Torrens, H., & Barreto Rodríguez, H. (2016b). Antibiorresistencia en Escherichia coli enterotoxigénica inducida in vitro con cobre. *Revista de Producción Animal*, *28*(1), 34-38. https://revistas.reduc.edu.cu/index.php/rpa/article/view/15

Barreto Argilagos, G., Bidot Fernández, A. I., Rodríguez Torrens, H. D. L. C., & Delgado Fernández, R. (2017). Microorganismos autóctonos multipropósito en las producciones caprinas. http://rediuc.reduc.edu.cu/jspui/bitstream/123456789/1097/1/Microorganismos_autoctonos_multiproposito.pdf

Barreto Argilagos, G., Rodríguez Torrens, H., & Campal Espinosa, A. (2020). Cuatro elementos contribuyen a que la colibacilosis porcina persista en Camagüey. *Revista de Producción Animal*, *32*(3). https://revistas.reduc.edu.cu/index.php/rpa/article/view/e3550

Barreto Argilagos, G., Rodríguez Torrens, H., Vázquez Montes de Oca, R., & Junco Pichardo, Y. (2020). Mortalidad por colibacilosis y salmonelosis en crías y precebas porcinas en una unidad especializada. *Revista de Producción Animal*, *32*(1). https://revistas.reduc.edu.cu/index.php/rpa/article/view/e3408

Belookov, A., Belookova, O., Zhuravel, V., Gritsenko, S., Bobyleva, I., Ermolova, E., Ermolov, S., Matrosova, Y. V., Rebezov, M., & Ponomarev, E. (2019). Using of EM-technology (effective microorganism) for increasing the productivity of calves. *International Journal of Engineering and Advanced Technology*, *8*(4), 1058-1061. https://www.elibrary.ru/item.asp?id=39695019

Coppola, N., Freire, B., Umpiérrez, A., Cordeiro, N. F., Ávila, P., Trenchi, G., Castro, G., Casaux, M. L., Fraga, M., Zunino, P., Bado, I., & Vignoli, R. (2020). Transferable resistance to highest priority critically important antibiotics for human health in Escherichia coli strains obtained from livestock feces in Uruguay. *Frontiers in veterinary science*, *7*, 940. https://doi.org/10.3389/fvets.2020.588919

Fernández, R. D., Argilagos, G. B., & Torrens, H. R. (2019). Empleo de Saccharomyces cerevisiae como tecnología para incrementar la ganancia de peso de terneros. *Avances*, *21*(1), 117-128. https://dialnet.unirioja.es/servlet/articulo?codigo=6789900

Del Risco, Y. & Barreto, G. (1988). Estudio preliminar de cepas de E. coli asociadas a síndrome diarreico en terneros, mediante seroaglutinación y siembra en agar citrato de Simmons suplementado con adonitol (0,1%). Rev. Prod. Anim. 4(2), 129-133.

Edwards, P. R., & Ewing, W. H. (1972). Identification of Enterobacteriaceae 3rd edition Minneaplis: Burgess. Publishing Company.

ECDC. Annual Report of the European Antimicrobial Resistance Surveillance Network (EARS-Net) 2017; ECDC: Stockholm, Sweden, 2018.

Edqvist, L. E., & Pedersen, K. B. (2002). Antibiotics as growth promotors: resistance to common sense. The precautionary principle in the 20th century: late lessons from early warnings, 100-110.

Authority, E. F. S. (2019). The European Union summary report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2017. *Efsa Journal*, *17*(2). https://doi.org/10.2903/j.efsa.2019.5598

Gebregiorgis, A., & Tessema, T. S. (2016). Characterization of Escherichia coli isolated from calf diarrhea in and around Kombolcha, South Wollo, Amhara Region, Ethiopia. *Tropical animal health and production*, *48*(2), 273-281. https://doi.org/10.1007/s11250-015-0946-9

Pasayo, R. A. G., Sanz, M. E., Padola, N. L., & Moreira, A. R. (2019). Phenotypic and genotypic characterization of enterotoxigenic Escherichia coli isolated from diarrheic calves in Argentina. *Open veterinary journal*, *9*(1), 65-73. https://doi.org/10.4314/ovj.v9i1.12

Hang, B. P. T., Wredle, E., Börjesson, S., Sjaunja, K. S., Dicksved, J., & Duse, A. (2019). High level of multidrug-resistant Escherichia coli in young dairy calves in southern Vietnam. *Tropical animal health and production*, *51*(6), 1405-1411. https://doi.org/10.1007/s11250-019-01820-6

Herago, T., & Agonafir, A. (2017). Growth promoters in cattle. *Advances in Biological Research*, *11*(1), 24-34. https://doi.org/10.5829/idosi.abr.2017.24.34

Hille, K., Ruddat, I., Schmid, A., Hering, J., Hartmann, M., von Münchhausen, C., Schneider, B., Messelhäusser, U., Friese, A., Mansfeld, R., Käsbohrer, A., Hörmansdorfer, S., Roesler, U., & Kreienbrock, L. (2017). Cefotaxime-resistant E. coli in dairy and beef cattle farms—Joint analyses of two cross-sectional investigations in Germany. *Preventive veterinary medicine*, *142*, 39-45. https://doi.org/10.1016/j.prevetmed.2017.05.003

Karmali, M. A., Petric, M., Lim, C., Fleming, P. C., Arbus, G. S., & Lior, H. (1985). The association between idiopathic hemolytic uremic syndrome and infection by verotoxin-producing Escherichia coli. *Journal of Infectious Diseases*, *151*(5), 775-782. https://doi.org/10.1093/infdis.151.5.775

.Kertz, A. F., Hill, T. M., Quigley III, J. D., Heinrichs, A. J., Linn, J. G., & Drackley, J. K. (2017). A 100-Year Review: Calf nutrition and management. *Journal of dairy science*, *100*(12), 10151-10172. https://doi.org/10.3168/jds.2017-13062

Maron, D. F., Smith, T. J., & Nachman, K. E. (2013). Restrictions on antimicrobial use in food animal production: an international regulatory and economic survey. *Globalization and health*, *9*(1), 1-11. https://doi.org/10.1186/1744-8603-9-48

Missotten, J. A., Michiels, J., Degroote, J., & De Smet, S. (2015). Fermented liquid feed for pigs: an ancient technique for the future. *Journal of animal science and biotechnology*, *6*(1), 1-9. https://doi.org/10.1186/2049-1891-6-4

Muktar, Y., Mamo, G., Tesfaye, B., & Belina, D. (2015). A review on major bacterial causes of calf diarrhea and its diagnostic method. *Journal of Veterinary Medicine and Animal Health*, *7*(5), 173-185. https://doi.org/10.5897/JVMAH2014. 0351

Nataro, J. P., & Kaper, J. B. (1998). Diarrheagenic escherichia coli. *Clinical microbiology reviews*, *11*(1), 142-201. https://doi.org/10.1128/CMR.11.1.142

Nielsen, D. W., Ricker, N., Barbieri, N. L., Allen, H. K., Nolan, L. K., & Logue, C. M. (2020). Outer membrane protein A (OmpA) of extraintestinal pathogenic Escherichia coli. *BMC research notes*, *13*(1), 1-7. https://doi.org/10.1186/s13104-020-4917-5

Nobili, G., Franconieri, I., La Bella, G., Basanisi, M. G., & La Salandra, G. (2017). Prevalence of Verocytotoxigenic Escherichia coli strains isolated from raw beef in southern Italy. *International journal of food microbiology*, *257*, 201-205. https://doi.org/10.1016/j.ijfoodmicro.2017.06.022

Martínez, M. M. O., & Gómez, A. L. B. (2019). Epidemia silente del siglo XXI. Resistencia microbiana a los antibióticos. *Medimay*, *26*(2), 233-247. http://www.medimay.sld.cu/index.php/rcmh/article/view/1419

Pohl, P., Lintermans, P., Vandergheynst, M. C., Van Muylem, K., Schlicker, C., & Van Robaeys, G. (1984). Milieu sélectif citrate-adonitol pour l'isolement de certaines souches de Escherichia coli K99. *Annales de l'Institut Pasteur/Microbiologie 135*(1), 29-33. https://doi.org/10.1016/S0769-2609(84)80040-X

Puig-Peña, Y., Leyva-Castillo, V., Tejedor-Arias, R., Illnait-Zaragozí, M. T., Aportela-López, N., Camejo-Jardines, A., & Ramírez-Areces, J. (2020). Antimicrobial Resistance in Bacteria Isolated from Foods in Cuba. *MEDICC review*, *22*(3). https://doi.org/10.37757/MR2020.V22.N3.9

Ramos, S., Silva, V., Dapkevicius, M. D. L. E., Caniça, M., Tejedor-Junco, M. T., Igrejas, G., & Poeta, P. (2020). Escherichia coli as commensal and pathogenic bacteria among food-producing animals: Health implications of extended spectrum β-lactamase (ESBL) production. *Animals*, *10*(12), 2239. https://doi.org/10.3390/ani10122239

Torrens, H. C. R., Argilagos, G. B., Cabrera, A. L., Sierra, I. L. M., Rodríguez, P. J. B., Esquijerosa, Y. C., & de Oca, R. V. M. (2021). Behavior of Hematologic Indicators in Pre-Fattening Pigs Fed with Multipurpose Autochthonous Microorganisms' Fermented Concentrates. *EC Veterinary Science*, *6*(4), 17-23. https://www.ecronicon.com/ecve/ECVE-06-00375.php

Ryu, J. H., Kim, S., Park, J., & Choi, K. S. (2020). Characterization of virulence genes in Escherichia coli strains isolated from pre-weaned calves in the Republic of Korea. *Acta Veterinaria Scandinavica*, *62*(1), 1-7. https://doi.org/10.1186/s13028-020-00543-1

Sapountzis, P., Segura, A., Desvaux, M., & Forano, E. (2020). An overview of the elusive passenger in the gastrointestinal tract of cattle: The Shiga toxin producing Escherichia coli. *Microorganisms*, *8*(6), 877. https://doi.org/10.3390/microorganisms8060877

Schrijver, R., Stijntjes, M., Rodríguez-Baño, J., Tacconelli, E., Rajendran, N. B., & Voss, A. (2018). Review of antimicrobial resistance surveillance programmes in livestock and meat in EU with focus on humans. *Clinical microbiology and infection*, *24*(6), 577-590. https://doi.org/10.1016/j.cmi.2017.09.013

Swiecicki, J. M., Sliusarenko, O., & Weibel, D. B. (2013). From swimming to swarming: Escherichia coli cell motility in two-dimensions. *Integrative biology*, *5*(12), 1490-1494. https://doi.org/10.1039/c3ib40130h

Tang, K. L., Caffrey, N. P., Nóbrega, D. B., Cork, S. C., Ronksley, P. E., Barkema, H. W., Polachek, A. J., Ganshorn, H., Sharma, N., Kellner, J. D., Checkley, S. L., & Ghali, W. A. (2019). Comparison of different approaches to antibiotic restriction in food-producing animals: stratified results from a systematic review and meta-analysis. *BMJ global health*, *4*(4). http://dx.doi.org/10.1136/bmjgh-2019-001710

Thiry, D., Saulmont, M., Takaki, S., De Rauw, K., Duprez, J. N., Iguchi, A., Piérard, D., & Mainil, J. G. (2017). Enteropathogenic Escherichia coli O80: H2 in young calves with diarrhea, Belgium. *Emerging infectious diseases*, *23*(12), 2093. https://doi.org/10.3201/eid2312.170450

US Government Accountability Office. (2011). Antibiotic resistance: Agencies have made limited progress addressing antibiotic use in animals. *Report no. GAO-11-801*.

Umpiérrez, A., Bado, I., Oliver, M., Acquistapace, S., Etcheverría, A., Padola, N. L., Vignoli, R., & Zunino, P. (2017). Zoonotic potential and antibiotic resistance of Escherichia coli in neonatal calves in Uruguay. *Microbes and environments*, ME17046. https://doi.org/10.1264/jsme2.ME17046

Van, T. T. H., Yidana, Z., Smooker, P. M., & Coloe, P. J. (2020). Antibiotic use in food animals worldwide, with a focus on Africa: Pluses and minuses. *Journal of global antimicrobial resistance*, *20*, 170-177. https://doi.org/10.1016/j.jgar.2019.07.031

Walton, J. R. (1988). Antibiotic resistance: an overview. The Veterinary Record, 122(11), 249-251.

World Health Organization. (1997). *The Medical impact of the use of antimicrobials in food animals: report of a WHO meeting, Berlin, Germany, 13-17 October 1997* (No. WHO/EMC/ZOO/97.4). World Health Organization. https://apps.who.int/iris/bitstream/handle/10665/64439/WHO_EMC_ZOO_97.4.pdf

Wyrsch, E. R., Reid, C. J., DeMaere, M. Z., Liu, M. Y., Chapman, T. A., Roy Chowdhury, P., & Djordjevic, S. P. (2019). Complete sequences of multiple-drug resistant IncHI2 ST3 plasmids in Escherichia coli of porcine origin in Australia. *Frontiers in Sustainable Food Systems*, *3*, 18. https://doi.org/10.3389/fsufs.2019.00018

AUTHOR CONTRIBUTION

CONFLICT OF INTERESTS