



## AVANT: developing alternatives to veterinary antimicrobials in the European Union regulatory context

R. Brunner <sup>(1)</sup>, J. Straube <sup>(1)</sup>, S. Richter <sup>(1)</sup>, C. Huettinger <sup>(1)</sup>, M. Gambino <sup>(2, 3)</sup>, T. Thymann <sup>(3)</sup>, L. Good <sup>(4)</sup>, A. Middelkoop <sup>(5)</sup>, L. Guardabassi <sup>(3)</sup> & K. Hellmann\* <sup>(1)</sup>

(1) KLIFOVET GmbH (doing business as Argenta), Geyerspergerstrasse 27, D-80689 Munich, Germany

(2) Royal Danish Academy – Architecture, Design, Conservation, Philip de Langes Allé 10, 1435 Copenhagen, Denmark

(3) Department of Veterinary and Animal Sciences, University of Copenhagen, Stigbøjlen 4, 1870 Frederiksberg, Denmark

(4) Royal Veterinary College, Royal College Street, London NW1 0TU, United Kingdom

(5) Schothorst Feed Research B.V., Meerkoetenweg 26, 8218 NA Lelystad, The Netherlands

\*Corresponding author: [klaus.hellmann@argentaglobal.com](mailto:klaus.hellmann@argentaglobal.com)

### Summary

The aim of the European Union Innovation Action ‘Alternatives to Veterinary Antimicrobials’, known as AVANT, was to develop new, targeted, non-antibiotic alternatives for the control of post-weaning diarrhoea in pigs. The project focused on four types of alternatives: gut microbiota modulators to maintain a healthy microbiome, therapies targeting the pathogen (enterotoxigenic *Escherichia coli*), immunostimulants and feeding strategies (dietary formulations). Up to now, limited regulatory guidance and support for the development and authorisation of alternatives to antimicrobials have been established. The lack of guidance leads to uncertainties in how to progress towards a marketing authorisation in the European Union, either as a veterinary medicinal product or as a feed additive.

This article reviews the regulatory framework for seven specific, mostly orally administered alternatives to antibiotics: synbiotics (combinations of probiotics and prebiotics), faecal microbiota transplantation (FMT)/faecal filtrate transplantation (FFT), bacteriophages, polymers, immunostimulants (oral and injectable) and feeding strategies. The potential legal classification of these interventions is evaluated, together with the data requirements for authorisation in the European Union. A particular focus is placed on FMT/FFT, which have shown promising results in reducing diarrhoea prevalence. Several hurdles need to be addressed to facilitate applications for marketing authorisations/registrations, as applicable. The development of innovative, non-antibiotic interventions in livestock production is a societal priority that requires legislative support. The article concludes with a set of pragmatic strategies to facilitate the registration and use of such interventions.

## Keywords

Alternatives – Antimicrobials – Authorisation – Feed additives – Innovative interventions – Pigs – Post-weaning diarrhoea – Regulatory – Veterinary medicinal product.

## Required citation

Brunner R, Straube J, Richter S, Huettinger C, Gambino M, Thyman T, *et al.* AVANT: developing alternatives to veterinary antimicrobials in the European Union regulatory context. *Rev. Sci. Tech.* 2025;44:3736. <https://doi.org/10.20506/rst.44.3736>

## Copyright

© 2025 R. Brunner, J. Straube, S. Richter, C. Huettinger, M. Gambino, T. Thyman, L. Good, A. Middelkoop, L. Guardabassi & K. Hellmann; licensee the World Organisation for Animal Health. This is an open access article distributed under the terms of the Creative Commons Attribution IGO Licence (<https://creativecommons.org/licenses/by/3.0/igo/legalcode>), which permits unrestricted use, distribution and reproduction in any medium, provided the original work is properly cited. In any reproduction of this article there should not be any suggestion that WOAHA or this article endorses any specific organisation, product or service. The use of the WOAHA logo is not permitted. This notice should be preserved along with the article's original URL.

## Introduction

Evidence that antimicrobial use in livestock contributes to multidrug-resistant bacterial infections in animals and humans has led to increased consumer awareness and governmental pressure to reduce antimicrobial use in both the human and veterinary sectors [1]. Although sales of antimicrobials in food-producing animals in the European Union (EU) decreased by more than 40% between 2011 and 2022, maintaining an annual decrease of sales of approximately 5% is required to achieve the reduction target set by the European Commission for 2030 in its Farm to Fork Strategy [2].

The aim of the EU Innovation Action project entitled Alternatives to Veterinary Antimicrobials (AVANT) was to develop a variety of new, targeted, non-antibiotic alternatives for the control of post-weaning diarrhoea (PWD). This was achieved through a multi-actor One Health approach, involving stakeholders and professionals from various sectors. The focus on PWD stems from its significant contribution to antimicrobial use in pig production [3]. PWD is one of the most significant threats to the swine industry worldwide, with mortality rates reaching, in extreme cases, up to 20–30% within just two weeks after weaning [4,5]. Treatment options for this disease are increasingly limited in the EU due to restrictions on use of colistin [6] and the recent phase-out of zinc oxide [7], which were previously the two most broadly used antimicrobial/antidiarrheal substances for control of PWD. The situation has further worsened due to rising resistance to alternative antimicrobials, e.g. aminoglycosides, in the main causative pathogen, enterotoxigenic *Escherichia coli* (ETEC), posing significant animal health concerns [8]. Currently, only one live oral vaccine is available on the market (Coliprotec F4/F18), but its effectiveness appears to vary across herds and studies [9-11].

To reduce antimicrobial use and economic losses associated with PWD, AVANT developed and tested seven interventions based on synbiotics, faecal microbiota transplantation (FMT)/faecal filtrate transplantation (FFT), bacteriophages (phages), polymers, immunostimulants (oral and injectable) and feeding strategies. Throughout the project, several challenges emerged regarding the testing and implementation of some of these interventions, particularly in demonstrating safety and efficacy, legal classification and meeting current EU regulatory requirements for feed, feed additives or veterinary medicines, as applicable. Such challenges should be considered by regulatory bodies seeking to support the development of alternatives. This article reviews the EU regulatory framework for each intervention, focusing on classification, data requirements for authorisation, and potential strategies to overcome existing regulatory barriers.

Special emphasis is placed on interventions that are difficult to classify and lack clear regulatory pathways – particularly FMT, which showed the most promising results in reducing PWD in challenge and field trials but is currently prohibited for oral feeding under EU legislation.

## Background

### The interventions

Within AVANT, four types of interventions were studied and developed as potential alternatives to antimicrobials for controlling PWD ([Table I](#)):

- Gut microbiota modulators, including synbiotic feed additive candidates composed of prebiotics and probiotics and an on-farm FMT protocol using faecal filtrate from healthy animals;
- Products targeting the main PWD causative agent, ETEC: a cocktail of phages, and a natural biopolymer derived from chitin (chitosan);
- Immunostimulants targeting the pig immune response, consisting of both injectable and oral immunostimulants extracted from *Ochrobactrum intermedium*;
- Feeding strategies (diets) based on fibrous feeds targeting either the sow or the piglets in transition periods.

The interventions were optimised and tested both *in vitro* and *in vivo* through extensive research. FFT and fibre-rich feed were shown to reduce the prevalence of PWD and/or improve the faecal consistency of weaned piglets on experimental farms [unpublished data] and were chosen from the range of interventions for larger-scale farm trials in Denmark, France and the Netherlands to assess their effect on antimicrobial usage. However, due to EU Feed Regulation (EC) No. 767/2009 [12], which prohibits the placing on the market or use for animal nutritional purposes of any faecal derivatives, FFT was authorised by the respective national competent authorities for use in a trial in the Netherlands on an experimental farm (by the Medicines Evaluation Board) and for use in Denmark on commercial farms only under the condition that all pigs had to be euthanised at the end of the study (by the Danish National Committee on Animal Experimentation). Consequently, only a limited assessment of the efficacy of FFT on antimicrobial use and pig morbidity and mortality in the field across commercial European pig farms facing PWD problems was possible. Nevertheless, FFT was shown to halve pre- and post-weaning mortality and PWD on the Danish study farm [13], in which no antibiotics were allowed due to experimental standardisation.

While the observed parameters did not demonstrate a clear clinical effect of FFT on piglet health on the Dutch farm, the total antibiotic use was reduced [14].

## **Classification of the interventions into regulatory categories**

Classifying the AVANT interventions with respect to the applicable legal framework was essential to determine their marketing authorisation requirements, if any, and development strategy. Products used in animals can be classified as feed, feed additives or veterinary medicinal products (VMPs), with significant differences between these categories regarding quality, safety and efficacy requirements. In the EU, three regulations define these requirements for each category.

- Regulation (EC) No. 767/2009 on the placing on the market and use of feed [12]. Feed materials are generally considered safe, and claims usually refer to specific characteristics of the feed; consequently, no marketing authorisation application is required. Their status as feed materials (*versus* feed additives) is confirmed by an entry in the Catalogue of Feed Materials [15] or Feed Materials Register [16]. Compound feed is a mixture of feed materials that may also contain (registered) feed additives.
- Regulation (EC) No. 1831/2003 on additives for use in animal nutrition [17]. According to Article 2 and Article 5(f), ‘feed additive’ refers to substances, microorganisms or preparations that are intentionally added to feed or water in order to favourably affect animal production, performance or welfare, particularly by affecting the gastro-intestinal flora or digestibility of feeding stuff. A feed additive may not claim the treatment or prevention of a disease. Performance-related claims are possible for ‘zotechnical feed additives’, which may favourably affect the performance of animals in good health.
- Regulation (EU) 2019/6 on VMPs [18]. This regulation defines a product that claims to treat or prevent a disease as a VMP. In addition, Regulation (EU) 2019/6 strictly limits the preventive use of antibiotic substances. It defines an antibiotic substance as ‘any substance with a direct action on bacteria used for treatment or prevention of infections or infectious disease’. Preventive (prophylactic) use is limited to exceptional cases and to administration to individual animals or a restricted number of animals only when the risk of an infection or of an infectious disease is very high and the consequences are likely to be severe. The regulation also limits

metaphylactic use in animals at risk of developing an infectious disease, which is another reason why alternative approaches are needed.

Based on existing categories, the synbiotic product was classified as a feed additive, the phage cocktail and chitosan could be classified as either feed additives or VMPs, the fibre-rich diets were classified as feed, and FMT and immunostimulants as VMPs ([Table II](#)). As the classifications of some AVANT interventions are borderline, there is regulatory uncertainty about how to develop them to obtain registration, which represents a major obstacle to innovation in this field.

The definition of ‘antibiotics’ (as opposed to ‘alternatives to antibiotics’) is not fully clear in the legal context and also differs between VMP and feed regulations. In the context of feed additives, Regulation (EC) No. 1831/2003, Article 2, defines antibiotics as ‘antimicrobials produced by, or derived from, a micro-organism, which destroy or inhibit the growth of other micro-organisms’ [17]. Consequently, substances that would be antibiotics in the VMP context may not be antibiotics in the feed context and vice versa.

Polymers (chitosan) and phages act directly on bacteria by lysis and mechanical disruption of the cell membrane, respectively. Polymers may consequently be considered antibiotic substances in VMP contexts, and thus their preventive use may be restricted by Article 107(3) of the regulation [18]. According to Annex II, point V.1.5.4.1. of the regulation, phage therapy is considered an alternative to antibiotics, and prophylactic claims are explicitly permitted. However, if also considered antimicrobial due to their action on microorganisms, their actual prophylactic use may be limited by the provisions of Article 107(3) to exceptional cases: for administration to an individual animal or a restricted number of animals when the risk of an infection or of an infectious disease is very high and the consequences are likely to be severe.

Any antibiotic, except coccidiostats or histomonostats targeting protozoan parasites, is generally prohibited as feed additive (Regulation (EC) No. 1831/2003 [17], Article 5(4)) and may not be registered, but by definition this applies only for antibiotics produced by or derived from microorganisms. Phages, as well as chitosan, are not produced by or derived from microorganisms and therefore do not fall under the definition of an antibiotic in a feed additive context. Recently, a phage product was assessed by the European Food Safety Authority (EFSA) for feed additive authorisation and was approved (see below).

From a marketing perspective, it also must be considered that claims can be made for treatment or prevention of ETEC-induced diarrhoea only for VMPs, while for feed additives claims can only be made related to performance of healthy animals during the post-weaning phase.

## Regulatory perspectives

The following paragraphs outline the regulatory perspective for each type of intervention developed in AVANT.

### Gut microbiome modulators

#### Synbiotics

Synbiotic products (combining pre- and probiotics) for in-feed use aim to stabilise the intestinal flora of piglets, especially in the stressful post-weaning phase, by enhancing the presence of favourable bacteria in the gut and thereby suppressing the unfavourable ones. The candidate interventions were combinations of well-characterised and freeze-dried probiotic strains of *Enterococcus faecium*, *Pediococcus acidilactici*, *Bifidobacterium adolescentis* and *Lactobacillus reuteri*, isolated from intestinal content of healthy pigs, and the oligofructose inulin. Single bacterial strains were previously evaluated *in vitro* for probiotic properties such as inhibition of pathogenic *E. coli* strains and ability to adhere to pig intestinal epithelial cells. The product was intended for in-feed use, and it was eligible for an authorisation as feed additive. Specifically, such a product can be developed as a zootechnical feed additive under the functional group 'gut flora stabilisers' – microorganisms or other chemically defined substances that have a positive effect on the gut microbiota when fed to animals. Depending on the availability of adequate clinical efficacy data, it may also qualify as a 'physiological condition stabiliser', which supports the animal's health and resilience to stress, or fall under 'other zootechnical additives'. Notably, a specific claim for the treatment or prevention of PWD cannot be made for a feed additive.

#### Faecal microbiota transplantation

FMT involves the transfer of (processed) faeces from healthy donor pigs to recipient pigs at risk of PWD. Prior to AVANT, a study showed promising effects not only of FMT but also of transplant of a derivative of faeces in which all bacteria, fungi and parasites are removed in a filtering process, leaving only virus particles including phages in the filtrate (FFT). Such filtrates have shown efficacy in humans [19] and in neonatal piglets [20,21].

To ensure the safety of the FMT, the FMT protocol used in the AVANT framework was performed within one farm, with recipients receiving the faecal filtrate from sows located at the same farm. The donor faeces were screened for a wide range of swine pathogens, and only pathogen-free faeces were used for further processing.

While faecal transplants/faecal extracts or filtrates may meet the definition of feed [22] with regards to their oral administration, Regulation (EC) No. 767/2009 [12] does not allow the placing on the market or use of faeces, urine and separated digestive tract content resulting from the emptying or removal of digestive tract, irrespective of any form of treatment or admixture. Consequently, the registration and placing on the market of FMT as feed additive is legally not possible. However, studies show that pigs naturally ingest faeces at higher levels than those used in the FMT protocols developed in the AVANT framework (~1 g/d *versus* 20 g/d [23]) and that this exposure to maternal faeces may positively influence piglet performance [24]. Despite the prohibition to market or use faeces as feed, well-defined, isolated faecal extracts or separated substances or microorganisms originating from faeces could potentially be registered as feed additives, provided they are thoroughly characterised and their safety and efficacy are demonstrated according to Regulation (EC) No. 429/2008 [25], as amended. However, this option was not readily available for the FMT protocol developed in AVANT.

FMT/FFT may generally also be considered for authorisation as a biological VMP (other than immunological VMPs). However, certain difficulties are expected when it comes to the requirements as outlined in Regulation (EU) 2021/805 (replacing Annex II of Regulation (EU) 2019/6) for the quality of such products. It is expected that the high level of standardisation required for both the active substance and the final product of a VMP to be manufactured to the rigid requirements of EudraLex Volume 4's good manufacturing practice (GMP) guidelines [26], to be replaced by Regulation (EU) 2025/2091 and Regulation (EU) 2025/2154 as of 16 July 2026, cannot be met with a product that naturally differs significantly depending on the individual donor pig and external factors such as its nutrition and health status. Nevertheless, if these hurdles can be overcome with a well-characterised active ingredient and demonstrated product quality, safety and efficacy, it may be possible to register a filtrate or extract as a VMP, allowing claims for prevention and/or treatment of PWD. This is further discussed below.

## Anti-enterotoxigenic *Escherichia coli* products

### Phages

Lytic phages are viruses that infect and kill bacteria with high specificity, displaying antimicrobial effects via a combination of enzymatic and mechanical mechanisms. To infect a bacterial cell, each phage recognises a specific receptor on the bacterial surface. Because of the extreme diversity of ETEC surface and available receptors, a cocktail of different phages is desirable to ensure efficacy against a range of PWD-inducing ETEC [27]. In AVANT, a product previously designed within the BioPIGLET project (Innovation Fund Denmark grant 7076-00038B) based on the phage ETEP21B was further developed. *In vitro* specificity and efficacy of this phage encouraged testing of the phage product *in vivo*, using a previously developed model for ETEC-induced PWD [28]. Available preliminary results suggest that the addition of the phage product to the feed from weaning might reduce disease severity, but a further optimisation of the phage product will be necessary to obtain a measurable effect on clinical signs [N. Ács *et al.*, article under review]. Phages can be considered for authorisation as a novel therapy VMP, being a biological VMP according to Regulation (EU) 2019/6 [18]. Some guidance is provided in Annex II, V.1.5.4., for phage therapy and in the guideline on quality, safety and efficacy of VMPs specifically designed for phage therapy [29].

Up to now, no phage-based VMP has been authorised in the EU owing to various challenges. Phage therapy VMPs may consist of monophage or multiphage preparations. Their composition may require regular updating/reconditioning due to the narrow bacterial host ranges, the possible development of resistance in the targeted bacteria and the immune response of the treated animal against the phages [30], which may lead to reduced efficacy. Therefore, permanent updating or targeted adaptation (training) of the phages used to treat the same condition is expected to be necessary.

As authorised VMPs, phages will need to be manufactured from characterised and quality-controlled seed lots, and the finished product is subjected to quality control tests in accordance with European Pharmacopoeia 5.31. on phage therapy medicinal products [31]. Currently, safety tests should address target animal safety, user safety and environmental risks. Special considerations need to be addressed for biological products, here for their potential immunogenicity and immunotoxicity, although lytic phages are generally considered safe. A comprehensive efficacy dossier must be submitted, including a pivotal field trial using the final formulation in the target animal species and indication showing efficacy under commercial conditions.

While the European Medicines Agency (EMA) has published comprehensive yet high-level guidance on data requirements for phage products, and a corresponding monograph exists in the European Pharmacopoeia, data requirements must be tailored individually for each product.

An alternative approach for using phages under the current legislation is through the prescription of an 'official formula' (*formula officinale*). European Pharmacopoeia 5.31. also defines the requirements for on-demand, extemporaneous phage preparations. These requirements specify that specialised pharmacies must conduct the preparation. The conditions for using extemporaneous preparations in piglets are discussed below.

Phages may also qualify as feed additives; however, a targeted claim for the treatment or prevention of PWD would not be permitted. As a feed additive, they would likely be classified under zootechnical additives, either as 'gut flora stabilisers' or 'other zootechnical additives'.

Recently, the first phage product (Bafasal) was approved by the European Commission as 'other zootechnical feed additive'. EFSA's Panel on Additives and Products or Substances Used in Animal Feed, known as FEEDAP, did not agree on an effect of the product on animal performance; the claim was finally granted for the reduction of environmental contamination of *Salmonella* Enteritidis strain when used in drinking water in all poultry species. However, the benefit of the product is for food hygiene, and the EFSA Panel expressed doubts on the clinical relevance of the proven effect of reducing *Salmonella* by one log while targeting the worker/consumer [32].

## Polymers

Polymers are substances with a molecular structure built up mainly or completely from many similar units bonded together. The polymers under development are based on chitosan (smaller chito-oligosaccharides and larger molecular weight chitosan) and exhibit an antimicrobial effect via mechanical mechanisms on bacteria that lead to the partial or full damage of the cell membranes. Anti-inflammatory effects have been reported for chitosan and could contribute to clinical benefit; however, it is not clear whether such effects are direct or an indirect effect of antimicrobial action. Preliminary results indicate some *in vivo* efficacy of some chitosan-based polymers against PWD in pigs [unpublished data].

Chitosan-based polymers can be considered for authorisation as a VMP. Due to the manufacturing process, it is expected that the requirements as outlined in Regulation (EU) 2021/805 for VMPs other than biologicals will need to be taken into consideration despite the biological origin of chitosan (crustaceans). Production following the EU-GMP requirements for VMPs [26] will be required, and safety and efficacy must be proven. Analytical characterisation and measurement are challenging due to variabilities in polymer length and degree of deacetylation.

Chitosan-based polymers may also meet the definition of a feed additive; however, a targeted claim for the prevention or treatment of diarrhoea caused by ETEC will not be possible. Within the category of zootechnical feed additives, polymers could fall under the category 'gut flora stabilisers', 'physiological condition stabilisers' (which may favourably affect resilience to stress factors) or 'other zootechnical additives', depending on the effect that can be demonstrated.

### **Immunostimulants**

Immunostimulants based on lipopolysaccharides (LPS) can bind to receptors recognising bacteria in host immune cells and subsequently activate the innate immune system to produce immunoglobulin G. The LPS-based immunostimulants proposed in AVANT are derived from *Ochrobactrum intermedium* and are intended for oral or injectable administration, with oral administration being the preferred route for routine preventive use in piglets due to practicability.

An LPS-based product may be considered for authorisation as a biological, non-immunological VMP. LPS are known to be strong activators of immune cells including B cells, monocytes, macrophages and other LPS reactive cells. However, due to their unspecific effect, they would not be classified as an immunological VMP. For their development, the requirements outlined in Annex II of Regulation (EU) 2019/6 [18] for biological non-immunologicals must be followed. The technology has already been registered as a VMP in Spain for use in dairy cows with mastitis. In a feeding study including sows and their piglets, a positive effect of the oral product on the sows and weaned piglets could be seen. Both the number of piglets weaned and the piglets' weight at weaning were higher [unpublished data]. As LPS are an unspecific immunostimulant, it may be challenging to prove their efficacy against PWD. This will depend on the selected claim to be evaluated.

An oral LPS-based product can also meet the definition of a zootechnical feed additive; however, no claim concerning immunostimulant activity could be made. As described above for polymers, LPS would fall under the category 'gut flora stabilisers', 'physiological condition stabilisers' or 'other zootechnical additives', depending on the demonstrated effects. Injectable immunostimulants are, due to their route of administration, excluded from registration as feed additives.

### **Diets based on fibrous feeds**

Diets varying in fibre level and type, whether inert or fermentable, adjusted to the animal's physiological stage, have been evaluated in sows around farrowing and in piglets around weaning for prevention of PWD. Fibres have been specifically included in the transition diet of sows around farrowing and in the transition diet of piglets around weaning to reduce PWD and are considered to have a positive effect on gut health beyond meeting the pigs' nutritional requirements [33,34].

Animal feed materials and compound feed do not need an authorisation in the EU. To be placed on the market, feed needs to be compliant with relevant EU regulations for feed and feed hygiene. All feed materials used must be listed in the Catalogue of Feed Materials or entered in the EU Feed Materials Register. If feed additives are included, they need to be registered. No claims on the treatment or prevention of disease can be made. If scientifically substantiated and verifiable, claims for compound feed may refer to the protection or maintenance of (healthy) physiological conditions.

Feed for particular nutritional purposes (PARNUT) may be placed on the market with certain disease-related claims. However, in this case, it must fulfil the specific requirements listed in Regulation (EU) 2020/354 [35]. Currently, no intended use is listed in this regulation that would cover any (post-weaning) diarrhoea-related nutritional purposes in pigs. Reduction of stress reactions in pigs is a particular nutritional purpose listed in Regulation (EU) 2020/345, but the essential nutritional characteristics do not fit with the intended composition of the diets proposed in the AVANT project. An application for the addition of a PARNUT claim to the respective list is possible: the applicant needs to submit a dossier to EFSA demonstrating that the specific composition of the feed fulfils the particular intended nutritional purpose and that it has no adverse effects on animal health, human health, the environment or animal welfare. A guideline on the data requirements is currently drafted. After addition to the list, the PARNUT claim may be used by every feed business operator that can show that its own product fulfils the respective requirements.

## Faecal microbiota transplantation: challenges and regulatory perspectives

The following section specifically addressed the regulatory challenges for FMT/FFT. In human medicine, FMT usually refers to the transfer of biological material incorporating faecal microorganisms from screened, healthy human donors to the gastro-intestinal tracts of patients, but in some cases, there may also be autologous use. The main use is currently the treatment of recurrent *Clostridium difficile* infections, but other indications have also been investigated, such as inflammatory bowel disease, hepatic encephalopathy and metabolic syndrome [33].

The EU Innovation Network [36] highlights in a report on the topic, however, that standardised and screened FMT products for use in humans must be readily available to make this a practical treatment option. To promote standards for stool banks, international expert groups have formed and provided detailed descriptions of all processes that relate to the collection, handling and clinical application of human donor stool (e.g. [37]).

FMT preparations in human medicine can be extemporaneously or industrially manufactured. An extemporaneous preparation would usually be collected material that is administered freshly or after freezing and thawing (with minimal processing) prepared on-site in healthcare facilities. The extent of screening of the faecal donations for pathogenic bacterial species is variable. In contrast, a manufactured FMT is further processed, e.g. by removal of undigested faecal matter, and may undergo a more standardised screening process. In human medicine, clinical studies for the registration of such manufactured FMT products are ongoing, and the first registrations are expected towards the end of the 2020s [36]. Experience from the United States of America has shown the importance of robust screening and traceability, as cases of hospitalisation and even death have occurred that are probably connected to FMT containing multidrug-resistant organisms [38].

As summarised by the EU Innovation Network [36], there is currently no agreed EU position on the regulation of FMT, and there is no specific regulatory guidance for FMT. As a consequence of the lack of an EU position, EU Member States have been free to regulate such products on a national level, by either creating a specific framework or applying an existing framework, such as the tissues and cells quality and safety requirements. This has led to a lack of consistency across the EU Member States.

In veterinary medicine, the way to register or use FMT is even less clear. FMT application in animals has been investigated, and it is known that it can treat diarrhoea, infections and dysbiosis (microbial imbalance) both in farm animals (also see above) and in companion animals (e.g. [39-41]). However, results are inconclusive and may be donor-dependent [42,43]. There is an interest in using FMT and putting FMT products on the market, especially in light of the necessary limitation of antimicrobial use and increasing antimicrobial resistance. Notably, clinical guidelines are available for FMT as an adjunct microbial therapy for canine parvovirus enteritis, acute diarrhoea and chronic enteropathy in both dogs and cats, providing recommendations on formulation, dosing and frequency of administration based on current veterinary evidence [44].

Regulation (EU) 2019/6, Article 113, specifies 'the cascade' for the allowed use of medicinal products outside the terms of their marketing authorisation in food-producing terrestrial animal species in exceptional cases, including the use of extemporaneously prepared products under certain circumstances. The use of an extemporaneously prepared FMT product under the cascade (Article 113, 1d) would be permitted only under certain very limited conditions by the responsible veterinarian under their direct responsibility: i) to avoid causing unacceptable suffering; and ii) under the prerequisite that no suitable authorised product is available:

- in the relevant or another EU Member State for the same or another food-producing terrestrial animal species for the same or another indication; and
- in the relevant EU Member State for use in a non-food producing animal species for the same indication; and
- in the EU for human use.

As the application of the cascade is allowed only for the exceptional treatment of animals to avoid unacceptable suffering, routine preventive use of FMT would essentially be excluded. Consequently, the use of an extemporaneously prepared FMT via the cascade is limited to the treatment of severely diseased animals, e.g. those for which there are no other, registered treatment options left. As there are currently no products, including antimicrobials, specifically authorised for the treatment of PWD, there may be opportunities for the use of extemporaneously prepared FMTs via the cascade, which could also contribute to reducing antimicrobial use.

The experiences documented in humans and companion animals demonstrate that to date FMT has been investigated and used as a personalised therapy for individual patients. This approach is feasible mainly when a standardised procedure is in place,

including a donor bank and an 'in-house' pharmacy equipped for the production and storage of faecal transplants. However, scaling this up to herd level may be impractical if donor material needs to be screened for swine pathogens by an external lab, which is costly. Production and storage of faecal matter subjected to a standardised process requires appropriate lab facilities and equipment such as (micro-)filtration equipment. Administering the product, e.g. via an oral drencher to each individual piglet, likely over a period of several days, is highly labour intensive, so that the application of FMT/FFT as standard procedure for piglets under these conditions seems rather unrealistic for commercial farms. Alternative approaches, such as administration with feed, or fewer repetitions of the administration, have not been studied yet.

The alternative would be the development of authorised FMT/FFT products produced in an industrialised manufacturing process. A product with demonstrated stability would also facilitate use on farms. For example, administration via supplemental milk might be possible, and farms might consider contemporaneous administration with ear tagging and iron injections, resulting in more convenient administration and reduced workload.

Unfortunately, it appears nearly impossible to define such a product correctly within the current regulatory framework. In human medicine, complete faeces can only be transferred between individual patients applying a high standard of testing as indicated in the EU Innovation Network report [36]. Similar requirements would need to be considered for treating individual animal patients.

To obtain a VMP marketing authorisation for such a product, a thorough characterisation of the active substance(s) would be required, most likely involving further processing of the faecal material leading to an extract or filtrate. Overall, it is considered rather challenging to fulfil the requirements for authorisation as a VMP in accordance with Annex II of Regulation (EU) 2019/6 [18] for biological products other than immunologicals. With regards to quality, in particular the exact characterisation of the active substance(s) is considered a major challenge as it will be a broad mixture of cells, cell fragments, enzymes, metabolites, toxins, bacteria, viruses and various other substances.

In human medicine, FMT has rapidly developed from a procedure involving different routes of administration of raw stool from identified donors to the use of purified and cryopreserved standardised preparations of FMT from highly selected donors [45].

It is still not fully understood which of the numerous and highly variable components contained in faecal matter are responsible for the beneficial effect of FMT. Thus, their separation from the rest of the faecal matter and their use as individual active substances is currently not possible. Likewise, the characterisation of the donor animals and the production and control of the starting material are associated with major difficulties. The manufacturing process, purification and, if applicable, inactivation procedures, with their validation and all in-process control procedures, will need to be adequately evaluated and described to ensure the quality, safety and batch-to-batch consistency of the finished product. Measures used to ensure freedom from extraneous agents will need to be established as well. Finally, manufacturing will need to be in compliance with the EU-GMP requirements for VMPs [26]. As another hurdle, the establishment and implementation of specifications for the finished product and appropriate control tests, including identification of the active substances and potency testing, must be mentioned. Finally, it is questionable how batch-to-batch consistency can be ensured and what could be expected with regards to the stability of such a product. A product produced by an external manufacturer needs to be stable at least long enough to allow for its distribution and administration.

In the case that a limited number of components (bacterial strains, their metabolites, or other) can be identified as being responsible for the beneficial effects of FMT, and these components can be produced under standardised conditions (culture, synthesis, etc.), they may be placed on the market as feed additives or be used as active substances in a VMP.

With regards to the pharmacology of the FMT product, the mode of action of the active substance(s) needs to be described along with information on its absorption, distribution, metabolism and excretion. This is associated with difficulties as many different substances/components are involved. If the components of the FMT are not absorbed and/or consumer safety can be justified by product tests, for a chemical-unlike product such as this, a maximum residue limit and the definition of a withdrawal period may be obsolete. However, the topic of maximum residue limits needs to be addressed given that pigs are a food-producing species. No conventional toxicology studies are deemed necessary for this type of product; however, investigations into side effects such as immunogenicity or immunotoxicity may be needed.

No clear dose–response relationship is expected for this type of product, and therefore, the dose finding based on conventional dose determination studies is considered irrelevant. Nevertheless, the selected dose will need to be adequately justified.

The safety and clinical efficacy of the product will need to be demonstrated in preclinical and clinical studies in controlled field studies in pigs. Finally, the commercial viability remains questionable due to the above-described circumstances.

## Future perspectives

The introduction of innovative interventions to reduce antimicrobial use, disease incidence and production losses in pig farming is expected to have a great commercial and economic impact. However, when it comes to individual innovative strategies, there is often a lack of specific regulatory guidance. This leads to uncertainties for applicants as to how to progress towards a marketing authorisation in the EU, either as a VMP or as a feed additive.

This lack has been recognised by the competent authorities, and a joint scientific opinion by EMA and EFSA [3] proposed as early as 2017 that a framework should be developed at the EU level for the regulation of substances that reduce the need for or use of antimicrobials and do not sit within the definition of a VMP or feed additive, considering the possible use of specific claims.

Established measures supporting developers of new VMPs and veterinary therapeutic protocols are available for applicants developing alternatives to antibiotics, where the relevant prerequisites are fulfilled, such as scientific advice, limited market applications, incentives for micro-, small- and medium sized companies, Innovation Task Force meetings and Novel Therapies and Technologies Working Party meetings with EMA's Committee for Veterinary Medicinal Products (CVMP; as summarised by [46]). However, up to now limited guidance or specific support for the development and authorisation of alternatives to antimicrobials has been established.

In EMA's strategic reflection on regulatory science to 2025 [47], some proposals are made to support research and incentivise the development of new antimicrobial agents and their alternatives, such as to:

- develop a regulatory approach/framework to promote alternatives to conventional antimicrobials and novel therapies;
- explore the possibility of new funding models to generate data to support existing authorised products and to incentivise new product development;
- provide scientific and regulatory support to encourage development of veterinary antimicrobials and alternatives in order to fill therapeutic gaps without adversely impacting public health.

EMA's 'Reflection paper on promoting the authorisation of alternatives to antimicrobial veterinary medicinal products in the EU' [46] provides a gap analysis that clearly shows that 'making progress on this topic will require a long-term approach and a set of coordinated actions with engagement of resources across the regulatory network, by industry and other relevant stakeholders'. Applicants would benefit from an EU-wide, harmonised approach that streamlines development and registration for all EU markets, reducing costs and timelines and thereby facilitating innovation.

Below are a set of pragmatic strategies that may facilitate and support the registration and use of alternatives to veterinary antimicrobials.

*Establish a framework for on-farm use of alternative non-antibiotic treatments, similar to the existing guidance for autogenous vaccines.*

Autogenous vaccines may be derived from the treated animal itself, or from animals in an epidemiological unit or from a unit having a confirmed epidemiological link (Regulation (EU) 2019/6 [18], Article 2(3)) and are therefore individualised therapies for which a lower degree of standardisation is required (and possible) than for industrially manufactured vaccines. A similar approach may be a way forward, e.g. for FMT or phages. However, unlike autogenous vaccines, which must be inactivated to ensure the absence of adventitious agents, FMT material contains viable microorganisms, including potential pathogens as well as phages, which may be critical for its efficacy. Therefore, any use of FMT would require stringent safeguards to ensure the absence of harmful pathogens while preserving the components critical to its intended effect. In the AVANT project, this was addressed by selecting donor faecal material free from known pig pathogens and applying several (micro-)filtration steps to remove most viable microorganisms (FFT).

Autogenous vaccines must comply with certain articles of Regulation (EU) 2019/6 [18] with regards to the obligation for GMPs (with specific GMP provisions proposed to be developed for these products) and veterinary prescriptions, record-keeping by farmers of food-producing animals, collection and disposal of waste, prohibition of advertisement, the control of manufacturers and importers, and conditions for prohibiting the supply of inactivated autogenous vaccines. It has already been proposed in Regulation (EU) 2019/6 [18], recital no. (70) of the preamble, that detailed GMP guidelines should specifically be prepared for those products given that they are manufactured in a way that is different from industrially prepared products to preserve their quality without hindering their manufacturing and availability.

Regulation (EU) 2019/6 [18], as amended, specifies in Article 159 that the obligations for certificates of GMP for autogenous vaccines will apply only when specific GMP requirements have been laid down in implementing acts. EMA has advised on the implementation of such guidelines, providing some basic obligations for GMP production of autologous vaccines [48]. A similar strategy could be applied to FMT and phages, balancing their potential benefit with effective risk management to ensure safe use of these interventions.

*Due to the lack of treatment alternatives, the use of extemporaneous preparations under 'the cascade' may be justified.*

The manufacturing of these preparations is based on pharmacopoeial monographs (national or European, which in many cases need to be developed first) that can serve as a basis for 'officinal formula' preparations safeguarding a certain level of quality, safety and efficacy. However, such extemporaneous preparations are currently permitted only for prescribed treatments, provided the conditions of the cascade are met, and not for disease prevention.

*Establish a clearer definition and differentiation of antibacterial 'alternatives to antimicrobials' as opposed to 'antibiotics' within the regulatory context. This should include provisions to justify and potentially permit preventive use.*

While alternatives to antimicrobials are defined by the CVMP [46] as 'the use of a VMP which provides an alternative treatment approach to the use of antimicrobials in animals or that reduces the need for the use of antimicrobials by preventing or controlling infectious disease', under the current legislation, alternatives to antibiotics such as polymers (e.g. chitosan) could be classified as antibiotics themselves as they meet the definition of antibiotics as 'substances with a direct action on bacteria'. There is a need for regulatory certainty that despite antibacterial action, they would not be considered to fall under the definition of an antibiotic. Otherwise, their development would become economically unattractive. Based on a risk assessment on their specific impact on public health and the potential of bacteria to become resistant to such treatments, it may be necessary to consider excluding them from the definition of 'antibiotic substance', thus omitting certain limitations.

*Establish incentives and an approach allowing for a reduced set of data for authorisation of alternatives to antibiotics.*

Based on the interpretation of Article 23 of Regulation (EU) 2019/6 [18] by the CVMP/EMA, (classical) antibiotics are not deemed eligible for registration under Article 23 ('Limited Market'). As, per definition, certain alternatives to antimicrobials would qualify as antimicrobials themselves, regulatory requirements and required data are perceived as relevant hurdles in motivating industry to develop innovative alternatives to antimicrobials. A facilitated route to authorisation, similar to the 'limited market' approach, could certainly stimulate investment in such technologies. At EMA, the dialogue between applicants and authorities for obtaining early scientific, regulatory and procedural advice for the development of alternatives is available but may be improved to help de-risk such investments in new technologies.

*Facilitate the registration of feed additives that target the reduction of antimicrobial use by:*

- *providing authority advice/dialogue to developers of such feed additives;*
- *providing the option to make certain claims for feed additives, such as reduction of use of antimicrobials, e.g. as a secondary claim; and*
- *shorten review times for feed additive applications for these specific feed additives to make their development more attractive.*

Overall, the development of innovative interventions that provide alternatives to classical antimicrobials – aimed at reducing antimicrobial use in pigs and other animal species – is a critical strategy to preserve human and animal health that requires continued support and further promotion.

This can be accomplished in the short term by:

- facilitating the dialogue between authorities evaluating VMPs and feed additives and applicants to support clarity and alignment, for example by offering meetings with specialised innovation offices for alternatives to antibiotics providing legally binding regulatory advice;
- offering targeted incentives to encourage the development and registration of non-antibiotic alternatives;
- providing specific guidance for the registration of non-antibiotic alternatives.

In the long term, this may be accomplished by:

- adjusting the legal framework by developing specific guidance to help de-risk investments in the development of alternatives to antimicrobials for animal use;
- defining streamlined data requirements based on a favourable benefit–risk assessment for such interventions.

### Acknowledgements

This project has received funding from the European Union’s Horizon 2020 Research and Innovation Programme under Grant Agreement No. 862829.

The authors thank all AVANT members for their input and discussions during the course of the project.

## **AVANT : le développement d'alternatives aux antimicrobiens vétérinaires dans le contexte réglementaire de l'Union européenne**

R. Brunner, J. Straube, S. Richter, C. Huettinger, M. Gambino, T. Thymann, L. Good, A. Middelkoop, L. Guardabassi & K. Hellmann

### **Résumé**

L'action de recherche et d'innovation de l'Union européenne « Alternatives aux antimicrobiens vétérinaires », dénommé AVANT, a pour objet de mettre au point des alternatives ciblées et non antibiotiques pour lutter contre la diarrhée post-sevrage des porcelets. Le projet s'est concentré sur quatre types d'alternatives : des modulateurs du microbiote intestinal visant à préserver un microbiome sain, des thérapies ciblant l'agent pathogène (*Escherichia coli* entérotoxigène), des immunostimulants, et des stratégies nutritionnelles (composition du régime alimentaire). À ce jour, les orientations réglementaires en faveur du développement de solutions alternatives aux antimicrobiens sont rares, de même que le soutien à leur autorisation. L'absence de directives claires donne lieu à des incertitudes quant aux voies à suivre pour obtenir une autorisation de mise sur le marché au sein de l'Union européenne, soit en tant que médicament vétérinaire, soit en tant qu'additif alimentaire.

Les auteurs passent en revue le cadre réglementaire applicable à sept alternatives spécifiques, principalement administrées par voie orale : les synbiotiques (combinaisons de probiotiques et prébiotiques), la transplantation de microbiote fécal/transplantation de filtrat fécal, les bactériophages, les polymères, les immunostimulants (oraux et injectables) et les stratégies nutritionnelles. Les classifications juridiques dont pourraient relever ces différentes interventions est examinée, ainsi que les données requises pour obtenir leur autorisation dans l'Union européenne. Une attention particulière est portée à la transplantation de microbiote fécal/transplantation de filtrat fécal, qui a donné des résultats prometteurs en termes de réduction de la fréquence des diarrhées. Plusieurs obstacles doivent être levés pour faciliter les demandes d'autorisation, ou d'enregistrement selon le cas. La mise au point d'interventions innovantes non antibiotiques dans les élevages constitue une priorité sociétale qui doit être soutenue par la législation. En conclusion, les auteurs présentent un ensemble de stratégies pragmatiques destinées à faciliter l'enregistrement et le déploiement de ces interventions.

### **Mots-clés**

Additifs alimentaires – Alternatives – Antimicrobiens – Autorisation – Diarrhée post-sevrage des porcelets – Interventions innovantes – Porcins – Produit médico-vétérinaire – Réglementation.

## **AVANT: desarrollar alternativas a los antimicrobianos veterinarios en el contexto normativo de la Unión Europea**

R. Brunner, J. Straube, S. Richter, C. Huettinger, M. Gambino, T. Thymann, L. Good, A. Middelkoop, L. Guardabassi & K. Hellmann

### **Resumen**

El objetivo de la acción de innovación de la Unión Europea «Alternativas a los Antimicrobianos Veterinarios», conocido como AVANT, era desarrollar nuevas alternativas no antibióticas, específicas para el control de la diarrea posterior al destete en cerdos. El proyecto se centró en cuatro tipos de alternativas: moduladores de la microbiota intestinal para mantener un microbioma saludable, terapias dirigidas al patógeno (*Escherichia coli* enterotóxica), inmunoestimulantes y estrategias de alimentación (formulaciones dietéticas). Hasta la fecha, la orientación y el apoyo normativo para el desarrollo y la autorización de alternativas a los antimicrobianos han sido limitados. La falta de orientación genera incertidumbre con respecto a la obtención de autorización de comercialización en la Unión Europea, ya sea como producto médico veterinario o como aditivo para piensos.

En este artículo, se revisa el marco normativo de siete alternativas específicas a los antibióticos, administradas principalmente por vía oral: simbióticos (combinaciones de probióticos y prebióticos); trasplante de microbiota fecal (TMF)/trasplante de filtrado fecal (TFF); bacteriófagos; polímeros; inmunoestimulantes (orales e inyectables), y estrategias de alimentación. También se evalúa la posible clasificación legal de estas intervenciones, así como los requisitos de datos para su autorización en la Unión Europea. Se presta especial atención al TMF/TFF, que ha arrojado resultados prometedores en la reducción de la prevalencia de la diarrea. Se menciona la necesidad de superar varios obstáculos para facilitar las solicitudes de autorización de comercialización/registro, según corresponda. El desarrollo de intervenciones innovadoras y no antibióticas en la producción ganadera aparece como una prioridad social que requiere apoyo legislativo. El artículo concluye presentando un conjunto de estrategias pragmáticas para facilitar el registro y la aplicación de dichas intervenciones.

### **Palabras clave**

Aditivos para piensos – Alternativas – Antimicrobianos – Autorización – Cerdos – Diarrea posterior al destete – Intervenciones innovadoras – Normativo – Producto médico veterinario.

## References

- [1] Van Boeckel TP, Brower C, Gilbert M, Grenfell BT, Levin SA, Robinson TP, *et al.* Global trends in antimicrobial use in food animals. *Proc. Natl Acad. Sci. USA.* 2015;112(18):5649-54. <https://doi.org/10.1073/pnas.1503141112>
- [2] European Medicines Agency. Sales of veterinary antimicrobial agents in 31 European countries in 2022: trends from 2010 to 2022 – thirteenth ESVAC report. Luxembourg: Publications Office of the European Union; 2023. 94 p. <https://doi.org/10.2809/895656>
- [3] European Medicines Agency (EMA) Committee for Medicinal Products for Veterinary Use and European Food Safety Authority (EFSA) Panel on Biological Hazards, Murphy D, Ricci A, Auce Z, Beechinor JG, Bergendahl H, *et al.* EMA and EFSA joint scientific opinion on measures to reduce the need to use antimicrobial agents in animal husbandry in the European Union, and the resulting impacts on food safety (RONAFA). *EFSA J.* 2017;15(1):e04666. <https://doi.org/10.2903/j.efsa.2017.4666>
- [4] Amezcua R, Friendship RM, Dewey CE, Gyles C, Fairbrother JM. Presentation of postweaning *Escherichia coli* diarrhea in southern Ontario, prevalence of hemolytic *E. coli* serogroups involved, and their antimicrobial resistance patterns. *Can. J. Vet. Res.* 2002;66(2):73-8. Available at: <https://pmc.ncbi.nlm.nih.gov/articles/PMC226986> (accessed on 6 March 2026).
- [5] Tang Q, Lan T, Zhou C, Gao J, Wu L, Wei H, *et al.* Nutrition strategies to control post-weaning diarrhea of piglets: from the perspective of feeds. *Anim. Nutr.* 2024;17:297-311. <https://doi.org/10.1016/j.aninu.2024.03.006>
- [6] European Medicines Agency (EMA) Media and Public Relations. Countries should reduce use of colistin in animals to decrease the risk of antimicrobial resistance. Amsterdam (The Netherlands): EMA; 2016. 3 p. Available at: [https://www.ema.europa.eu/en/documents/press-release/countries-should-reduce-use-colistin-animals-decrease-risk-antimicrobial-resistance\\_en.pdf](https://www.ema.europa.eu/en/documents/press-release/countries-should-reduce-use-colistin-animals-decrease-risk-antimicrobial-resistance_en.pdf) (accessed on 6 March 2026).
- [7] European Medicines Agency (EMA) Veterinary Medicines Division. Questions and answers on veterinary medicinal products containing zinc oxide to be administered orally to food-producing species: outcome of a referral procedure under Article 35 of Directive 2001/82/EC (EMEA/V/A/118). Amsterdam (The Netherlands): EMA; 2017. 2 p. Available at: [https://www.ema.europa.eu/en/documents/referral/zinc-oxide-article-35-referral-questions-and-answers-veterinary-medicinal-products-containing-zinc-oxide-be-administered-orally-food-producing-species\\_en.pdf](https://www.ema.europa.eu/en/documents/referral/zinc-oxide-article-35-referral-questions-and-answers-veterinary-medicinal-products-containing-zinc-oxide-be-administered-orally-food-producing-species_en.pdf) (accessed on 6 March 2026).
- [8] Subramani P, Pirolo M, Haugegaard S, Skarbye AP, Conrady B, Pedersen KS, *et al.* Neomycin resistance in clinical *Escherichia coli* from Danish weaner pigs is associated with recent neomycin use and presence of F4 or F18 fimbriaes. *Prev. Vet. Med.* 2023;212:105852. <https://doi.org/10.1016/j.prevetmed.2023.105852>

- [9] Goodman MR, Amezcua MR, Friendship RM, Farzan A. Investigations into the effects of *Escherichia coli* vaccination and diet composition on post-weaning diarrhea and growth performance in pigs. *Can. Vet. J.* 2023;64(4):329-36. Available at: <https://pmc.ncbi.nlm.nih.gov/articles/PMC10037348> (accessed on 6 March 2026).
- [10] Nadeau É, Fairbrother JM, Zentek J, Bélanger L, Tremblay D, Tremblay C-L, *et al.* Efficacy of a single oral dose of a live bivalent *E. coli* vaccine against post-weaning diarrhea due to F4 and F18-positive enterotoxigenic *E. coli*. *Vet. J.* 2017;226:32-9. <https://doi.org/10.1016/j.tvjl.2017.07.004>
- [11] Vangroenweghe FACJ, Boone M. Vaccination with an *Escherichia coli* F4/F18 vaccine improves piglet performance combined with a reduction in antimicrobial use and secondary infections due to *Streptococcus suis*. *Animals (Basel)*. 2022;12(17):2231. <https://doi.org/10.3390/ani12172231>
- [12] European Union. Regulation (EC) No. 767/2009 of the European Parliament and of the Council of 13 July 2009 on the placing on the market and use of feed, amending European Parliament and Council Regulation (EC) No. 1831/2003 and repealing Council Directive 79/373/EEC, Commission Directive 80/511/EEC, Council Directives 82/471/EEC, 83/228/EEC, 93/74/EEC, 93/113/EC and 96/25/EC and Commission Decision 2004/217/EC. *Off. J. Eur. Union.* 2009;L 229:1-41. Available at: <https://eur-lex.europa.eu/eli/reg/2009/767/2018-12-26> (accessed on 9 March 2026).
- [13] Larsen C, Pirolo M, Rasmussen TS, Brunse A, Mikkelsen CV, Haugaard MM, *et al.* Faecal filtrate transplantation in neonatal pigs induces gut microbiota changes and reduces mortality and diarrhoea. *Gut. Microbiol.* 2026;(in press).
- [14] Middelkoop A, Priem J, Larsen C, Thyman T, Molist F. Faecal filtrate transplantation and dietary fibre supplementation as alternatives to veterinary antimicrobials [abstract]. *Anim. Sci. Proc.* 2025;16(2):311-2. <https://doi.org/10.1016/j.anscip.2025.07.011>
- [15] European Union. Commission Regulation (EU) No. 68/2013 of 16 January 2013 on the catalogue of feed materials. *Off. J. Eur. Union.* 2013;L 29:1-90. Available at: <https://eur-lex.europa.eu/eli/reg/2013/68/2022-07-24> (accessed on 9 March 2026).
- [16] Feed materials register. European Union Feed Chain Task Force; 2026. Available at: <https://www.feedmaterialsregister.eu> (accessed on 8 July 2025).
- [17] European Union. Regulation (EC) No. 1831/2003 of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrition. *Off. J. Eur. Union.* 2003;L 268:1-26. Available at: <https://eur-lex.europa.eu/eli/reg/2003/1831/2021-03-27> (accessed on 9 March 2026).
- [18] European Union. Regulation (EU) 2019/6 of the European Parliament and of the Council of 11 December 2018 on veterinary medicinal products and repealing Directive 2001/82/EC. *Off. J. Eur. Union.* 2019;L 4:1-207. Available at: <https://eur-lex.europa.eu/eli/reg/2019/6/2022-01-28> (accessed on 9 March 2026).
- [19] Ott SJ, Waetzig GH, Rehman A, Moltzau-Anderson J, Bharti R, Grasis JA, *et al.* Efficacy of sterile fecal filtrate transfer for treating patients with *Clostridium difficile* infection. *Gastroenterology.* 2017;152(4):799-811.e7. <https://doi.org/10.1053/j.gastro.2016.11.010>

- [20] Brunse A, Deng L, Pan X, Hui Y, Castro-Mejía JL, Kot W, *et al.* Fecal filtrate transplantation protects against necrotizing enterocolitis. *ISME J.* 2022;16(3):686-94. <https://doi.org/10.1038/s41396-021-01107-5>
- [21] Offersen SM, Mao X, Spiegelhauer MR, Larsen F, Li VR, Sandris Nielsen D, *et al.* Fecal virus-like particles are sufficient to reduce necrotizing enterocolitis. *Gut Microbes.* 2024;16(1):2392876. <https://doi.org/10.1080/19490976.2024.2392876>
- [22] European Union. Regulation (EC) No. 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. *Off. J. Eur. Union.* 2002;L 31:1-50. Available at: <https://eur-lex.europa.eu/eli/reg/2002/178/2026-01-01> (accessed on 9 March 2026).
- [23] Sansom BF, Gleed PT. The ingestion of sow's faeces by suckling piglets. *Br. J. Nutr.* 1981;46(3):451-6. <https://doi.org/10.1079/bjn19810053>
- [24] Aviles-Rosa EO, Rakhshandeh A, McGlone JJ. Preliminary study: depriving piglets of maternal feces for the first seven days post-partum changes piglet physiology and performance before and after weaning. *Animals (Basel).* 2019;9(5):268. <https://doi.org/10.3390/ani9050268>
- [25] European Union. Commission Regulation (EC) No. 429/2008 of 25 April 2008 on detailed rules for the implementation of Regulation (EC) No. 1831/2003 of the European Parliament and of the Council as regards the preparation and the presentation of applications and the assessment and the authorisation of feed additives. *Off. J. Eur. Union.* 2008;L 133:1-78. Available at: <https://eur-lex.europa.eu/eli/reg/2008/429/2021-03-27> (accessed on 9 March 2026).
- [26] European Commission (EC). EudraLex – volume 4: good manufacturing practice (GMP) guidelines. Brussels (Belgium): EC; 2010. Available at: [https://health.ec.europa.eu/medicinal-products/eudralex/eudralex-volume-4\\_en](https://health.ec.europa.eu/medicinal-products/eudralex/eudralex-volume-4_en) (accessed on 9 March 2026).
- [27] Gambino M, Kushwaha SK, Wu Y, van Haastrecht P, Klein-Sousa V, Lutz VT, *et al.* Diversity and phage sensitivity to phages of porcine enterotoxigenic *Escherichia coli*. *Appl. Environ. Microbiol.* 2024;90(7):e00807-24. <https://doi.org/10.1128/aem.00807-24>
- [28] Rydal MP, Gambino M, Jørgensen CB, Poulsen LL, Brøndsted L, Nielsen JP. Pilot study on CHCF1 genotype in a pig challenge model for enterotoxigenic *Escherichia coli* F4ab/ac associated post-weaning diarrhea. *BMC Vet. Res.* 2022;18(1):382. <https://doi.org/10.1186/s12917-022-03474-3>
- [29] European Medicines Agency (EMA) Committee for Veterinary Medicinal Products. Guideline on quality, safety and efficacy of veterinary medicinal products specifically designed for phage therapy. Amsterdam (The Netherlands): EMA; 2023. 36 p. Available at: [https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-quality-safety-and-efficacy-veterinary-medicinal-products-specifically-designed-phage-therapy\\_en.pdf](https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-quality-safety-and-efficacy-veterinary-medicinal-products-specifically-designed-phage-therapy_en.pdf) (accessed on 6 March 2026).

- [30] Champagne-Jorgensen K, Luong T, Darby T, Roach DR. Immunogenicity of bacteriophages. *Trends Microbiol.* 2023;31(10):1058-71. <https://doi.org/10.1016/j.tim.2023.04.008>
- [31] European Directorate for the Quality of Medicines and HealthCare of the Council of Europe. European pharmacopoeia: 5.31. Phage therapy medicinal products. Strasbourg (France): Council of Europe; 2024. 2 p. Available at: <https://www.edqm.eu/documents/52006/277566/European%20Pharmacopoeia%20-%20Phage%20therapy%20medicinal%20products%20%285.31%29.pdf/d9da2e01-e002-32c9-b2eb-8a9360439c05?t=1727862827906> (accessed on 6 March 2026).
- [32] European Food Safety Authority Panel on Additives and Products or Substances used in Animal Feed, Villa RE, Azimonti G, Bonos E, Christensen H, Durjava M, *et al.* Safety and efficacy of a feed additive consisting of the bacteriophages PCM F/00069, PCM F/00070, PCM F/00071 and PCM F/00097 (Bafasal®) for all poultry (Proteon Pharmaceuticals S.A.). *EFSA J.* 2024;22(12):e9132. <https://doi.org/10.2903/j.efsa.2024.9132>
- [33] Kar S, Middelkoop A, Jansman A, Molist F. Early life fibre-enriched diets affect gut functionality of piglets challenged with enterotoxigenic *Escherichia coli* after weaning [abstract]. *Anim. Sci. Proc.* 2022;13(2):221. <https://doi.org/10.1016/j.anscip.2022.03.406>
- [34] Kar SK, Zaccaria E, Binnendijk G, van Wikselaar P, Jansman AJM. Functional ingredients to optimize gut functionality in post weaning piglets [abstract]. *Anim. Sci. Proc.* 2025;16(2):335. <https://doi.org/10.1016/j.anscip.2025.07.061>
- [35] European Union. Commission Regulation (EU) 2020/354 of 4 March 2020 establishing a list of intended uses of feed intended for particular nutritional purposes and repealing Directive 2008/38/EC. *Off. J. Eur. Union.* 2020;L 67:1-31. Available at: <https://eur-lex.europa.eu/eli/reg/2020/354/2025-04-22> (accessed on 9 March 2026).
- [36] European Medicines Agency (EMA), Heads of Medicines Agencies. Faecal microbiota transplantation: EU-IN horizon scanning report. Amsterdam (The Netherlands): EMA; 2022. 18 p. Available at: [https://www.ema.europa.eu/en/documents/report/faecal-microbiota-transplantation-eu-horizon-scanning-report\\_en.pdf](https://www.ema.europa.eu/en/documents/report/faecal-microbiota-transplantation-eu-horizon-scanning-report_en.pdf) (accessed on 9 March 2026).
- [37] Keller JJ, Ooijevaar RE, Hvas CL, Terveer EM, Lieberknecht SC, Högenauer C, *et al.* A standardised model for stool banking for faecal microbiota transplantation: a consensus report from a multidisciplinary UEG working group. *United European Gastroenterol. J.* 2021;9(2):229-47. <https://doi.org/10.1177/2050640620967898>
- [38] Fecal microbiota for transplantation: safety alert – risk of serious adverse events likely due to transmission of pathogenic organisms. Silver Spring (United States of America): United States Food and Drug Administration; 2020. Available at: <https://www.fda.gov/safety/medical-product-safety-information/fecal-microbiota-transplantation-safety-alert-risk-serious-adverse-events-likely-due-transmission> (accessed on 8 July 2025).

- [39] Vecchiato CG, Sabetti MC, Sung CH, Sportelli F, Delsante C, Pinna C, *et al.* Effect of faecal microbial transplantation on clinical outcome, faecal microbiota and metabolome in dogs with chronic enteropathy refractory to diet. *Sci. Rep.* 2025;15(1):11957. <https://doi.org/10.1038/s41598-025-96906-7>
- [40] Liu Q, Akhtar M, Kong N, Zhang R, Liang Y, Gu Y, *et al.* Early fecal microbiota transplantation continuously improves chicken growth performance by inhibiting age-related *Lactobacillus* decline in jejunum. *Microbiome.* 2025;13(1):49. <https://doi.org/10.1186/s40168-024-02021-6>
- [41] Sugita K, Yanuma N, Ohno H, Takahashi K, Kawano K, Morita H, *et al.* Oral faecal microbiota transplantation for the treatment of *Clostridium difficile*-associated diarrhoea in a dog: a case report. *BMC Vet. Res.* 2019;15(1):11. <https://doi.org/10.1186/s12917-018-1754-z>
- [42] Hui Y, Vestergaard G, Deng L, Kot WP, Thymann T, Brunse A, *et al.* Donor-dependent fecal microbiota transplantation efficacy against necrotizing enterocolitis in preterm pigs. *NPJ Biofilms Microbiomes.* 2022;8(1):48. <https://doi.org/10.1038/s41522-022-00310-2>
- [43] Wang X, Tsai T, Zuo B, Wei X, Deng F, Li Y, *et al.* Donor age and body weight determine the effects of fecal microbiota transplantation on growth performance, and fecal microbiota development in recipient pigs. *J. Anim. Sci. Biotechnol.* 2022;13(1):49. <https://doi.org/10.1186/s40104-022-00696-1>
- [44] Winston JA, Suchodolski JS, Gaschen F, Busch K, Marsilio S, Costa MC, *et al.* Clinical guidelines for fecal microbiota transplantation in companion animals. *Adv. Small Anim. Care.* 2024;5(1):79-107. <https://doi.org/10.1016/j.yasa.2024.06.006>
- [45] Tang W, Chen D, Yu B, He J, Huang Z, Zheng P, *et al.* Capsulized faecal microbiota transplantation ameliorates post-weaning diarrhoea by modulating the gut microbiota in piglets. *Vet. Res.* 2020;51(1):55. <https://doi.org/10.1186/s13567-020-00779-9>
- [46] European Medicines Agency (EMA) Committee for Medicinal Products for Veterinary Use. Reflection paper on promoting the authorisation of alternatives to antimicrobial veterinary medicinal products in the EU. Amsterdam (The Netherlands): EMA; 2021. 20 p. Available at: [https://www.ema.europa.eu/en/documents/scientific-guideline/reflection-paper-promoting-authorisation-alternatives-antimicrobial-veterinary-medicinal-products-eu\\_en.pdf](https://www.ema.europa.eu/en/documents/scientific-guideline/reflection-paper-promoting-authorisation-alternatives-antimicrobial-veterinary-medicinal-products-eu_en.pdf) (accessed on 9 March 2026).
- [47] European Medicines Agency (EMA). EMA regulatory science to 2025: strategic reflection. Amsterdam (The Netherlands): EMA; 2020. 79 p. Available at: [https://www.ema.europa.eu/en/documents/regulatory-procedural-guideline/ema-regulatory-science-2025-strategic-reflection\\_en.pdf](https://www.ema.europa.eu/en/documents/regulatory-procedural-guideline/ema-regulatory-science-2025-strategic-reflection_en.pdf) (accessed on 9 March 2026).

[48] European Medicines Agency (EMA) Veterinary Medicines Division. Advice on the implementing measures under Article 93(2) of Regulation (EU) 2019/6 of the European Parliament and of the Council on Veterinary Medicinal Products, as regards the GMP for veterinary medicinal products and active substances used as starting materials. Amsterdam (The Netherlands): EMA; 2023. 124 p. Available at: [https://www.ema.europa.eu/en/documents/other/advice-implementing-measures-under-article-932-reg-eu-2019-6-european-parliament-council-veterinary-medicinal-products-regards-gmp-veterinary-medicinal-products-active-substances-used-starting\\_en.pdf](https://www.ema.europa.eu/en/documents/other/advice-implementing-measures-under-article-932-reg-eu-2019-6-european-parliament-council-veterinary-medicinal-products-regards-gmp-veterinary-medicinal-products-active-substances-used-starting_en.pdf) (accessed on 9 March 2026).

**Table I****Initially proposed AVANT interventions**

<b>Approach</b>	<b>Description</b>	<b>Effect</b>
Modulating the gut microbiota	1) Synbiotic mix of pre- and probiotics	Improvement of intestinal function
	2) On-farm faecal transplantation to maintain gut health	Unspecific stimulation of the immune system and modulation of the microbiome
Target pathogen (ETEC)	1) Phage-based product	Destruction of bacteria by lytic phages
	2) Polymer-mediated mechanical effect on the bacteria	Mechanical destruction of the outer membrane of the targeted bacteria
Stimulate immune response	1) Injectable 2) Oral immunostimulants obtained from bacterial extracts of <i>Ochrobactrum intermedium</i>	Unspecific stimulation of the immune system
Feeding strategy, diet	Optimising nutrient content (fibre, carbohydrates, amino acids and fat)	Impact on the microbiome to improve intestinal function

ETEC: enterotoxigenic *Escherichia coli*

**Table II****Initially intended use and potential classification of the AVANT interventions**

Intervention	Initially intended use within AVANT	Potentially regulated as			Comments
		Feed	Feed additive	VMP	
Faecal microbiota/filtrate transplantation	On-farm procedure for the maintenance of gut health	No	No	Yes	The placing on the market of faeces as feed is legally prohibited
Synbiotic mix of pre- and probiotics	Feed additive for the stabilisation of the gut microbiome during weaning	No	Yes	No	If registered as feed additives, no claims regarding prevention or treatment of disease are allowed, incl. PWD
Phages	Reduction of ETEC diarrhoea	No	Yes	Yes	
Polymers (chitosan)	Reduction of ETEC diarrhoea	No	Yes	Yes	
Injectable immunostimulants	Immunostimulant against respiratory and intestinal disease	No	No	Yes	Treatment of disease claims would be possible if effect can be shown
Oral immunostimulants	Immunostimulant against respiratory and intestinal disease	No	Yes	Yes	Claims depend on classification
Diets	Feeding strategy	Yes	No	No	No claim regarding prevention or treatment of disease permitted, incl. PWD

AVANT: Alternatives to Veterinary Antimicrobials

ETEC: enterotoxigenic *Escherichia coli*

PWD: post-weaning diarrhoea

VMP: veterinary medicinal product