ANSES Opinion
Request No 2013-SA-0122
Related Request Nos 2011-SA-0071 and 2012-SA-0067

The Director General

Maisons-Alfort, 30 October 2018

OPINION of the French Agency for Food, Environmental and Occupational Health & Safety of 16/02/2018, supplemented on 30/10/2018[1], for translation and international dissemination purposes

on the inventory of alternatives to antibiotics

aimed at reducing their use in animal husbandry

Development of a method for assessing

scientific publications and results

ANSES undertakes independent and pluralistic scientific expert assessments.
ANSES primarily ensures environmental, occupational and food safety as well as assessing the potential health risks they may entail.
It also contributes to the protection of the health and welfare of animals, the protection of plant health and the evaluation of the nutritional characteristics of food.

It provides the competent authorities with all necessary information concerning these risks as well as the requisite expertise and scientific and technical support for drafting legislative and statutory provisions and implementing risk management strategies (Article L.1313-1 of the French Public Health Code).

Its opinions are published on its website.
This opinion is a translation of the original French version. In the event of any discrepancy or ambiguity the French language text dated 16/02/2018 shall prevail.

On 22 May 2013, in the framework of the Ecoantibio 1 Plan and its Measure 19, ANSES received a formal request to undertake the following expert appraisal: production of an inventory of alternatives to antibiotics aimed at reducing their use in animal husbandry.

1. BACKGROUND AND PURPOSE OF THE REQUEST

Theme 2 of the "Ecoantibio 1" Plan, published in 2012 with a five-year action plan, provided for the "Development of alternatives to avoid the use of antibiotics". Measure 19 of this same Plan focused on the assessment of the benefits of alternative treatments helping to limit the use of antibiotics. It was in this framework that ANSES received the formal request.

The goal of reducing the use of antibiotics in veterinary medicine can only be achieved if alternative methods to antibiotics are developed in the various health situations encountered in the field. As such, Measure 19 of the Plan supplemented Measures 14 and 15, respectively providing

[1] This Opinion includes the initial Opinion as well as annexes with figures and tables extracted from the related collective expert appraisal report. This supplement was prepared for the purpose of translating a summary document with a view to European dissemination. The additions are described in the table in Annex 4 of this document.

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for the development of tools in favour of preventive health treatment and zootechnical measures, and the promotion of research into immunity and the use of vaccines and autogenous vaccines. This is the context in which “alternatives” (products and substances) should be considered and analysed.

In 2014, ANSES published a report further to an internal request on antimicrobial resistance and the assessment of unsafe practices in the use of antibiotics in veterinary medicine. The collective expert appraisal raised the issue of alternatives to antibiotics but did not provide details about the corresponding products.

Moreover, in 2013, ANSES had issued an Opinion on the use of zinc oxide in the diet of piglets at weaning to reduce the use of antibiotics (No. 2012-SA-0067). This was the first assessment of alternatives as defined in Measure 19, in which a final section devoted to “alternatives” indicated the product classes then being researched, based on recent publications. Following this first Opinion, the DGAL clarified its request regarding the other alternatives to be assessed, which constitutes the current formal request.

This request dealt with:

- Producing an inventory of the products and substances currently used as alternatives to antibiotics with the aim of reducing their use, specifying the destination species and target disease, based on Section 5 of Report No. 2012-SA-0067.
- The overall assessment of the efficacy and safety, to humans (consumers and operators), animals and the environment, of substances that have not undergone a regulatory assessment, after a review of the available literature.

It should be noted that the very notion of alternatives to antibiotics evolved during the discussions, particularly in the framework of the Ecoantibio 1 Plan, moving from a fairly restrictive definition (alternatives used “instead of” antibiotics) to a much broader concept (contributing to reducing the use of antibiotics), as explained in Point 3.1. It was also in this broader context that the Ecoantibio 2 Plan[2] (especially its Theme 1), published on 19 April 2017 by the Ministry of Agriculture, emerged.

2. ORGANISATION OF THE EXPERT APPRAISAL

The expert appraisal was carried out in accordance with French Standard NF X 50-110 "Quality in Expert Appraisals – General Requirements of Competence for Expert Appraisals (May 2003)".

The expert appraisal falls within the sphere of competence of the Expert Committees (CESs) on “Animal feed” and "Animal health and welfare", with the CES on “Animal feed” coordinating this request.

ANSES entrusted the expert appraisal to the Working Group (WG) on "Alternatives to antibiotics". The methodological and scientific aspects of the work were presented to the CESs between 9 July 2013 and 19 September 2017. They were adopted by the CES on "Animal feed" during its meeting on 21 November 2017.

The concomitant existence of a European Working Group, common to EFSA[3] and the EMA[4], whose mandate was to give a scientific opinion on “measures to reduce the need to use antimicrobial agents in animal husbandry in the EU, and the resulting impacts on food safety”, led

[2] http://agriculture.gouv.fr/le-plan-ecoantibio-2-2017-2021 last consulted in October 2017.
[3] EFSA: European Food Safety Authority.
[4] EMA: European Medicines Agency.

ANSES to hold discussions with this Working Group's coordinator, under the terms of Article 30[5] of Regulation (EC) No 178/2002 of the European Parliament and of the Council[6]. An expert from this European Working Group was interviewed by ANSES's WG on 17 June 2016.

ANSES analyses interests declared by experts before they are appointed and throughout their work in order to prevent risks of conflicts of interest in relation to the points addressed in expert appraisals.

The experts' declarations of interests are made public via the ANSES website (www.anses.fr).

[5] Article 30.1*: The Authority shall exercise vigilance in order to identify at an early stage any potential source of divergence between its scientific opinions and the scientific opinions issued by other bodies carrying out similar tasks.*
Article 30.2: *Where the Authority identifies a potential source of divergence, it shall contact the body in question to ensure that all relevant scientific information is shared and in order to identify potentially contentious scientific issues.*

[6] 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.

3. ANALYSIS AND CONCLUSIONS OF THE CES AND THE WG ON "ALTERNATIVES TO ANTIBIOTICS"

3.1. Definition of "alternatives to antibiotics" and the scope of the expert appraisal

The Working Group on "Alternatives to antibiotics" defined the subject of the request as being not only products and substances that can be used to replace antibiotics in animal husbandry but also those that, used preventively, help reduce the frequency of certain bacterial animal diseases, thus potentially reducing the need for antibiotics. The term "alternative to antibiotics" was thus considered in a broad sense to identify products and substances, as well as administration methods to be recorded.
It should be noted that vaccines and autogenous vaccines were not included in the scope of the expert appraisal, as indicated in the formal request.
Alternatives to antibiotics used as growth factors were also excluded from the request, which only took into account scientific evidence regarding products or substances assessed in studies presenting data in connection with animal health, since the goal here was to identify products that may provide preventive or curative solutions in the context of bacterial diseases.
Lastly, parasitic diseases were excluded from the request, which dealt only with bacterial diseases, even though certain products are active against both types of pathogens, there also being abundant literature on the topic of parasites.

3.2. Inventory of "alternatives to antibiotics"

Given the large scope of the request (all animal species, multiple classes of products and substances, several diseases), the inventory of commercial substances and preparations was drawn up based on a review of the trade press in the animal husbandry sector (for 2012, 2013 and 2014).

This inventory work targeted all commercial substances and preparations that directly or indirectly claim to be solutions to limit the use of antibiotics in animals in various animal sectors (ruminants, poultry, pigs, rabbits and fish). Commercial preparations whose claims only relate to the improvement of zootechnical performance were not considered, but any claims more or less directly mentioning a decrease in the use of antibiotics in animal husbandry were included (general health, improvement of digestive disorders, immunity, etc.).

This inventory showed that the most commonly mentioned commercial preparations mainly contain:

- plant extracts
- probiotics
- acids (organic and/or fatty)
- essential oils

Furthermore, the majority of claims focus on the digestive system, regardless of the animal species.

- Pigs are the main recipients of these commercial preparations, with 69 mentions out of 150 in total, i.e. almost 50%. Of the identified claims, 67% involved the balance or restoration of the digestive microbiota, 16% dealt with overall health status, 8.5% were explicit claims as being alternatives to antibiotics and 8.5% combined various types of claims.
- Ruminants were in second position, after pigs, for the number of mentions found in the trade press for the 2012-2014 period. That said, "ruminant" mentions accounted for less than 20% of all mentions, all species combined. Within these "ruminant" mentions, almost 2/3 involved cattle and the remaining third involved goats. Digestive symptoms were also

the major indications for ruminants; commercial preparations targeting the udder, specific to ruminants, were in second position.

- Poultry were in third place in terms of mentions. While chickens are the main target species, the majority of commercial preparations target all poultry, whether for laying or for meat production. Digestive function (or the digestive system) is the main target.
- Rabbits were the fourth species in terms of mentions, with most claims dealing with digestive function.
- The number of commercial preparations intended for aquaculture species (fish and crustaceans) described as having properties corresponding to an alternative to antibiotics remained limited in this inventory.

However, some limitations identified during this inventory should be underlined:

- lack of exhaustiveness, due to the inventory method itself, relying on the trade press over a limited publication period, and to the likely existence of commercial preparations offered to farmers without communication;
- likely non-homogeneous representation of the various animal sectors;
- lack of feedback on efficacy, with the products being included based solely on the fact that they are available for sale;
- unclear composition of the identified commercial preparations.

3.3. Overall assessment of the efficacy and safety of substances and products

While the product inventory relied on a review of the trade press for the animal husbandry sector, the efficacy and safety of these products were assessed using the available scientific literature.

Considering:

- that the issue of alternatives to antibiotics is a relatively recent and evolving area of investigation,
- and that candidate substances for this theme, and even new product classes, are liable to emerge at any time,

the WG chose to develop a generic and long-term analytical methodology capable of taking into account these changes.

The WG thus proposed the development and publication of a method, available for future assessments independently of the time frame of this request, for assessing scientific publications measuring the efficacy of substances leading to a reduction in the use of antibiotics.

This assessment aims to establish a level of evidence for the efficacy claimed in published studies, for the “alternative” substances and products.

The WG also chose a method for assessing the safety of “alternative” products that is compatible with their rich diversity.

The collective expert appraisal report, attached to this Opinion, is therefore a step-by-step description of the established methodology. This was then applied to certain product classes, for various species x disease pairs.

3.3.1. Assessment of efficacy

The literature search identified two main categories of studies:

- ***In vitro* studies**: these were not selected by the experts, who considered them as pre-screening for subsequent animal studies in the framework of research into products and substances as “alternatives to antibiotics”. Where *in vitro* efficacy has been demonstrated, the WG emphasises that it is essential to undertake *in vivo* studies before drawing conclusions for a disease in a given species.

- **Animal studies**: much of the method developed and applied to the scientific publications in the report focused on these studies.

The literature search did not identify any **epidemiological studies** (observational or interventional) undertaken with the aim of demonstrating the efficacy of an antibiotic alternative. However, the WG considers that the concept of these studies should be used in the future to supplement experimental trials on the efficacy of substances aiming to reduce the use of antibiotics, especially in the long term. It was therefore important to specify the strengths and limitations of these studies, and that is why there is a section devoted to them in the report.

The assessment method is broken down into three main steps:

- Methodological scoring of each publication from 1 to 4;
- Determination of the relevant variables in each publication in light of the studied diseases and claimed effects;
- Conclusions that can be drawn from the publications, by compiling them, based on the results of the two previous steps.

These various steps are described in detail in Section 5 of the collective expert appraisal report; only their general principles have been included in this Opinion.

3.3.1.1 Methodological scoring criteria

Relying on ANSES Opinion No. 2011-SA-0150 and on the "Guide to literature analysis and the grading of recommendations"[7] of the HAS[8], the methodological scoring of *in vivo* publications took into account five criteria characterising:

❶ the single-agent or multifactorial aetiology of the bacterial disease.

❷ the infectious model of the study (experimental infection *vs* spontaneous infection, associated to varying degrees with degraded sanitary conditions).

❸ the use (or not) of an antibiotic compared with a tested alternative product, eliminating publications in which antibiotics were used at "growth factor" levels and verifying that the conditions of use of the reference antibiotic were acceptable (dose, duration of administration, choice of antibiotic).

❹ the experimental plan's ability to highlight significant effects.

❺ the general terms of the study including the use (or not) of an "untreated control" batch of inoculated animals (or animals placed in degraded sanitary conditions) expressing the disease, as well as the duration of the study, in its ability to model the duration of the animals' susceptibility to the disease.

The multiple integration of these criteria relied on decision trees presented in Section 5.1.2 of the collective expert appraisal report, resulting in each scientific publication being assigned a methodological score from 1 (highest) to 4 (lowest). See Figures 1 and 2 in Annex 1, page 18.

3.3.1.2 Relevance of the variables measured in the publications

The scientific publications describe various variables, whose change is analysed from one batch of animals to the next, in order to conclude whether or not the tested product has a significant effect.

These variables can be more or less relevant to the studied disease, especially when they are not specific to that disease. Therefore, the analysis of the effects of alternatives to antibiotics should include this parameter.

[7] https://www.has-sante.fr/portail/jcms/c_434715/fr/guide-d-analyse-de-la-litterature-et-gradation-des-recommandations.
[8] HAS: French National Authority for Health.

Based on the various variables studied in the publications, the experts drew up a table of relevance levels for them, for all of the diseases studied in the report (see Table 1 in Annex 1, pages 19-20).

In the context of this approach, the relevance of a variable was not evaluated based on its ability to describe a disease, but mainly on its ability to enable improvements in an animal's health status to be assessed, following treatment with a product. Thus, the experts classified variables measuring the improvement/elimination of clinical signs as highly relevant priorities. They then classified the other variables in relation to these priority variables.

3.3.1.3 Conclusions by publication

Based on the analysis of the publications (summarised in an analysis grid), the WG defined a basis for decision-making enabling an individual conclusion to be drawn for each publication regarding the efficacy of a product against a disease in an animal species, by aggregating information for the three reported criteria:

- Methodological scoring of the publications;
- Relevance of the studied variables;
- Statistical effect obtained and biological magnitude of this effect.

Conclusions were proposed for each publication (Tables 2 and 3 in Annex 1, pages 21-22). These conclusions aimed to distinguish between:

- products for which a comparison with a batch receiving an antibiotic enables their (preventive or curative) efficacy to be evaluated compared with that of the antibiotic (used as metaphylactic or curative treatment),
- products for which a simple comparison with an untreated control batch provides information about their ability to treat or prevent a disease, which cannot however be assessed compared to the action of an antibiotic in a similar situation, making it unclear whether farmers would ultimately need to use antibiotics.

3.3.1.4 Conclusions by class of products

These conclusions by class aimed to estimate whether or not a class of products was an interesting approach for a disease in a given species, considering the terms of the literature search and the analysis.

They are meant to respond to the DGAL which, through this formal request, expected an assessment of alternatives to antibiotics in general, not in an individual context falling within the scope of regulatory provisions on marketing authorisations, but rather with the aim of identifying promising products or classes of products, based on available knowledge.

To that end, summary tables by product class, based on the analysis grid of publications, were created in order to tally the publications, which were separated as follows:

- Studies with the preventive use of a product
  - Basis of comparison: untreated control
  - Basis of comparison: antibiotic
- Studies with the curative use of a product
  - Basis of comparison: untreated control
  - Basis of comparison: antibiotic

These tables provided the experts with a summary working document, giving them an overview of the number of publications and their results before beginning to draw up general conclusions by class of products, for a given animal species and disease.

However, within the same class of products, the experimental studies analysed in the publications differed in terms of the exact nature of the products, the doses used, the durations of use and product combinations. Thus, the results of these studies could not be directly compared with one another due to their high level of heterogeneity. As such, no “meta-analysis” approach was

possible, which led the WG to consider that the establishment of a clear decision-making rule, aiming to combine these publications to draw a general conclusion for a class of products, was not scientifically justified.

The WG therefore chose to prepare a qualitative expert conclusion when the repetition of studies undertaken with products from the same class, using suitable experimental procedures, enabled such an overall conclusion to be considered.

3.3.1.5 Conclusions by product and related confidence levels

The general conclusion for a product needed to take into account, for a given disease and animal species, the individual conclusions of all of the publications dealing with it. Thus, the WG had to define a decision-making rule enabling these individual conclusions to be aggregated, in order to arrive at a general conclusion associated with a confidence level.

This decision-making rule was based on three criteria:

- heterogeneity of the publication results, i.e. coexistence of publications with significant effects reaching the same conclusions, and of others with non-significant or opposite effects;
- methodological scores of the various publications;
- number of publications available for the same product.

These criteria determined whether or not it was possible to draw a conclusion and if so, the confidence level associated with this conclusion.

Two confidence levels were set: low/high.

The decision-making rule proposed methods for combining publications with converging results on the one hand and with diverging results on the other hand, in order to draw a conclusion on the product's efficacy, associated with a low or high confidence level (Figure 3 in Annex 1, page 23).

3.3.1.6 Implementation of the proposed method

In order to test and illustrate the entire method (see diagram of the entire method in Figure 4, Annex 1, page 24), substance class, animal species and disease combination were determined to study them in light of the literature.

- To do this, the WG selected certain classes of substances (those most frequently mentioned in the prior inventory), for which scientific information (publications) was abundant in principle.

  The inventory described in Point 3.2 enabled the most frequently mentioned commercial preparations to be identified. Four classes of substances stood out from the mentions found in the trade press:

  - plant extracts;
  - essential oils;
  - probiotics;
  - organic acids.

  These were followed by enzymes, antimicrobial peptides, plasma, algae, prebiotics, etc.

  Thus, as a priority, the WG chose to analyse the literature on the first four classes of substances. Nonetheless, for certain species and diseases, the experts retained the option of broadening this analysis to include other classes when the scientific literature was abundant and thorough (publications of good scientific quality describing animal studies). Classes of products that were not assessed by the WG have been described in another section entitled “Other approaches”. However, a similar assessment could be considered for them, using the method set out in the report.

- As for animal species, the WG considered that any animal species that can be used for production and be affected by a given bacterial disease should be evaluated using this method, in order to test it and improve it for all species.

  Thus, the following species and groups of species were selected: pigs, poultry, rabbits, cattle, small ruminants, pre-ruminants, fish.

  Horses were also considered in the response to the request, although the section related to the inventory did not include them. This decision was motivated in particular by the occurrence of a bacterial disease in foals, *Rhodococcus equi*, for which antibiotic treatment calls on (for want of alternatives) a combination of antibiotics including rifampicin, which is now classified as an antimicrobial of critical importance but benefits from an exceptional status as an essential substance for equines affected by this disease.

  Due to a lack of time, the WG was not able to broaden its investigation to include pets (domestic carnivores) such as dogs and cats.
- The targeting of bacterial diseases first took into account the diseases most commonly found in the survey of the trade press, i.e. digestive bacterial infections, far ahead of other conditions.

  However, instead of implementing the method in all species for digestive diseases only, the WG decided to cross-check this information with the data in ANSES Opinion No. 2011-SA-0071 on “the risks of emergence of antimicrobial resistance associated with patterns of antimicrobial use in the field of animal health”. Section 4 of this Opinion surveys antibiotic therapy practices in various animal sectors, enabling certain disorders generating more antibiotic prescriptions than others to be identified by animal species.

  On these bases, the WG decided on the following combinations:
  - Digestive disorders: piglets at weaning, rabbits, small ruminants, pre-ruminants, horses
  - Mastitis: dairy cattle
  - Metritis: cows
  - Systemic colibacillosis: poultry
  - Systemic diseases: fish
  - *Rhodococcus equi*: foals

The implementation of the entire method, aiming to establish a level of evidence for claimed efficacy for “alternative” substances and products, appears in Section 6 of the collective expert appraisal report, for each studied substance class, animal species and disease combination. An example of its implementation is given in Annex 2, page 26.

3.4. Assessment of the safety of the identified products and substances

Given the high number of identified products and substances (compounds, plants, plant extracts and micro-organisms), the principle chosen for the literature search regarding their safety was to use the summaries of a leading scientific organisation, the European Food Safety Authority (EFSA), as priority sources without including their analysis of each referenced publication.

Moreover, in the absence of data in these summary documents for certain products, compounds, plants, plant extracts and micro-organisms, the analysis was undertaken on a case-by-case basis, based on individual scientific publications or on scientific evidence available on referenced websites.

The literature search dealt with the safety of various products, compounds, plants, plant extracts and micro-organisms for the target animal species, humans using them (i.e. those handling the products, substances and micro-organisms), humans consuming foodstuffs of animal origin containing possible residues of these products, substances and micro-organisms, and the

environment. The issue of the risk of antimicrobial resistance potentially induced by the identified products and substances was also raised, although this aspect has not yet been studied in depth.

The table listing more than 220 different micro-organisms, substances and products and the available data on their safety for humans, animals and the environment appears in Annex 3, page 32.

Furthermore, the WG cross-checked the list of products appearing as promising in terms of efficacy, in light of the available literature, with the known data on their safety, in order to gain a broader view of these substances (see Table 4 in Annex 1, page 25).

3.5. Discussion on the regulatory positioning of the identified products and substances

After observing the plurality of possible regulatory statuses for the identified products, the WG undertook its expert appraisal work and implemented the established method without taking into account regulatory positioning, focusing only on efficacy and safety data with no connection with the various guidelines for authorisation dossiers (additives and medicinal products in particular).
Even so, the WG launched a discussion on the possible regulatory positioning of these various products, both considering the current regulations and with a view to future regulatory changes (Section 10 of the collective expert appraisal report).
These discussions should necessarily be put into a European context, given the origin of the regulatory provisions governing both animal feed and veterinary medicinal products.

3.6. Conclusions of the WG on "Alternatives to antibiotics" and the CES on "Animal feed"

3.6.1. Background and limitations of the WG's work

1. During the WG's work, the concept of alternatives to antibiotics encompassed two complementary approaches:
   - ✓ in the framework of a curative approach to bacterial animal diseases, alternatives to antibiotics included all substances and products that can totally or partially replace antibiotics used for curative treatment, demonstrating efficacy equivalent (not inferior) or superior to that of the reference antibiotic treatments;
   - ✓ in the framework of a preventive approach to diseases, alternatives to antibiotics included all substances and products that would lead to a reduction in the frequency of certain animal diseases, thus reducing the use of antibiotics.
2. Given the limitations of the *in vivo* literature data, and due especially to the non-feasibility of a meta-analysis approach, the WG first and foremost proposed a methodology to characterise the level of evidence provided by all of the scientific publications for products claiming to have properties as antibiotic alternatives. This methodology was then applied to combinations of a product, animal species and disease.

   This methodology is sufficiently generic (methodological classification of publications, relevance of analysed variables, proposed semi-quantitative compilation of results) that it could be used for other combinations of product, species and disease, or when new scientific data become available on this theme, or even by other Working Groups on other themes, provided some adaptations are made.
3. However, this approach was limited in the WG's work due to the identification of three publication biases that could influence the conclusions:
   - "Selective publication" bias: studies describing a positive effect of a product are likelier to be published than those not demonstrating any efficacy. This probably influenced the

conclusions, largely established based on the number of studies showing the significant positive effects of a product, weighed against those not showing any.

- “Bonus for originality” bias: some studies may not have been accepted for publication due to their lack of originality, insofar as a comparison of the studied product with antibiotics or of its efficacy versus an untreated control had already been described in publications.
- "Privatisation of results" bias: related to the fact that research into these products is often conducted with private funding and that the results are usually not published for reasons of confidentiality and company strategy.

4. In light of the few available scientific publications, meta-analysis approaches, which are the reference methods for characterising the average efficacy and heterogeneity of response of the studied products, were not used. In addition, even when there was a sufficient number of studies for a given class of products (probiotics and essential oils, for example), the studied compounds or strains were highly heterogeneous, with different mechanisms of action, limiting the possibility of grouping together publications for quantitative analysis purposes. Lastly, in the sector of animal health and nutrition, it was unlikely that a large number of publications would deal with the same combination of product, animal species and disease. Therefore, the semi-quantitative approach used by the WG was a suitable analytical method combining the establishment of an analysis grid for the publications based on their methodologies, the definition of relevant variables for characterising the progression of a disease in the presence of a potential alternative substance to antibiotics, and the definition of decision-making rules in the event of publications with potentially heterogeneous results.
5. The methodological scoring grid for the publications led to the establishment of a level of evidence for the claimed efficacy of the alternative products, for each published study, based on a decision tree (score from 1 to 4). Some of the WG's choices in this methodological scoring penalised certain publications whose methodological score decreased from 2 to 3, which influenced the degree of confidence associated with the product conclusions.
6. Out of all of the variables measured in the publications, a limited number were chosen by the experts based on their biological relevance, to characterise changes in the health status of animals having received alternative products to antibiotics. These variables were considered individually, without taking into account the possible multidimensional nature of changes in an animal's health status following treatment (simultaneous improvement in several indicators).
7. The method's primary limitation involved the establishment of conclusion rules for publications with heterogeneous results for the same type of product. Nonetheless, the decision-making rule, which had to be simple in order to be generic, was logically based on the statistical heterogeneity of the results combined with the methodological score of these publications. The agreed decision of the experts was to give priority to publications with high methodological scores (1 & 2), regardless of the number of score 3 & 4 publications, which could induce a bias in the conclusions.
8. An additional challenge in the WG's work was attempting to link the inventory approaches for data from the trade press (Phase 1) and those from the literature (Phase 2). Indeed, in the trade press, a number of products were found resulting from a combination of products in the same class (e.g. mixture of essential oils or micro-organisms) or products in different classes (combination of yeasts and organic acids). In the scientific publications, the studied products were most often simple products, with the aim of demonstrating efficacy associated with mechanisms of action. These publications therefore could not demonstrate potential synergies or antagonisms between products, as can be found in complex products available on the market. The conclusions from the publications thus may not fully reflect the efficacy of product combinations, as observed in the field. It should also be noted that it was not possible to assess combinations of products in terms of their safety as mixtures (antagonistic potential).

   Moreover, it is important to note the gap between the abundance of products found in the initial survey of the trade press and the few scientific publications available for these products.

9. The WG's work focused only on *in vivo* studies. Indeed, while there was a very high number of *in vitro* publications dealing with the potential ability of certain products to replace antibiotics, in the large majority of cases, these were merely preliminary studies aiming to identify antibacterial activities or characterise molecular mechanisms (screening).

3.6.2. Results

1. In Phase 1 of the work, the analysis of the trade press showed that the main mentions involved the digestive tract, regardless of the animal species (with the exception of fish). However, in poultry and pigs, there was recurring difficulty determining the objective of the studies, between alternatives to growth factor antibiotics and prevention/treatment of a bacterial disease.

   The main targeted animal species were, in descending order by number of mentions, pigs, ruminants, poultry and rabbits. The case of fish was different since the consultation of the trade press showed few claims regarding alternative products to antibiotics, these claims dealing mainly with general health status.

   The main identified products and classes of products were plant extracts and essential oils, organic acids and probiotics.
2. The WG relied on these main points of the inventory to conduct Phase 2 dedicated to the analysis of the scientific literature. However, for certain species, when possible based on the scientific literature, other alternatives were considered and included in the analysis. For example, prebiotics were studied in the context of diarrhoea in rabbits and piglets, as was plasma for various animal species.

   Furthermore, other diseases were added when, according to the report on “Assessing the risk of emergence of antimicrobial resistance associated with patterns of antimicrobial use in the field of animal health” (ANSES, 2014), they induce the heavy use of antibiotics. Thus, metritis in cows and clinical and subclinical mastitis in dairy cows were included in the analysis.

   Lastly, horses were included in the Phase 2 analytical work for *Rhodococcus equi* in foals and diarrhoea in adult horses, given the significance of these diseases in terms of the use of antibiotics. In this particular framework, specific products were included in the analysis based on the availability of references.
3. The literature search showed that the availability of information differed between the studied products. For example, all animal species and diseases combined, the collected information primarily involved probiotics, essential oils and plant extracts; there were fewer data on prebiotics and organic acids. Here it should be noted that publications on organic acids were often older and were therefore not all identified during the literature search, which was limited, with exceptions, to a 10-year period. Moreover, except for a few specific diseases, the information in the literature was significantly more abundant for products intended for a preventive approach than for those intended for a curative approach, in the context of the diseases considered in this report. This was not evidence of these products’ lack of efficacy as curative treatments. To learn more, however, studies should be undertaken with this specific objective.
4. The most abundant information in the literature involved the curative and preventive treatment of digestive disorders in young animals at weaning and in adults. For the four main classes of products, the WG compiled data in order to highlight potential trends enabling either the efficacy of these products to be characterised or the product classes seeming the most promising for these disorders to be identified.

   For each product class considered, there were only a few publications directly comparing these products to an antibiotic, since the majority of studies had been undertaken for the

prevention of digestive disorders, not in a curative context. However, there was a slightly larger body of data regarding the use of these four classes as part of a curative or preventive approach to digestive disorders, with no comparison to an antibiotic. When the referenced studies did not undertake a comparison with an antibiotic, the conclusions nonetheless showed that certain classes may provide interesting approaches for the preventive or curative management of these disorders:

- Plant oils and extracts showed some heterogeneity with regards to their effects on digestive disorders in rabbits and piglets, although several publications with high methodological scores suggested a decrease in digestive disorders in these species. Specific products could not be further analysed due to the heterogeneity of the products considered.
- Due to the small number of publications involving organic acids, no conclusions could be drawn regarding the efficacy of these products against digestive disorders. This was probably due to the time window for the selection of publications, these products having been studied in a more distant past.
- Prebiotics are a class of products that, in some cases in rabbits, have been shown to be effective in preventing digestive disorders. In a very limited number of cases, their efficacy was similar to that of antibiotics, in the described experimental conditions.
- Probiotics appear to be a promising class of products, since the majority of the analysed publications suggested the ability to prevent, to a degree, digestive disorders in numerous species and thus contribute to reducing the use of antibiotics, especially in piglets at weaning. Nonetheless, for each animal species or group of species, the heterogeneity of the agents responsible for digestive disorders, and the diversity of health situations (especially in the event of a multifactorial disease) on the one hand and the wide variety of probiotic strains used on the other hand made it impossible to generalise these findings, which were valid only in the experimental conditions of the studies (species x pathogen x strain(s), dose of probiotic, timing of administration, etc.).

5. Regarding other combinations of animal species and diseases, the literature search on systemic diseases in fish identified a fairly large number of studies relating to probiotics, as well as studies dealing with essential oils and plant extracts.

   A majority of the publications analysed indicated that probiotics had a significant positive effect on mortality. However, the magnitude of the described positive effects, even when they were statistically significant, varied considerably.

   Considering all of the analysed publications, it could nonetheless be concluded that certain probiotics have a preventive effect enabling them to reduce fish mortality related to bacterial diseases. This class of products can therefore be considered as promising for reducing the use of antibiotics in fish.

   Regarding essential oils and plant extracts, even though some publications reported some favourable results, their small number made it impossible or very difficult to draw any conclusions regarding the potential curative or preventive efficacy of these classes of products in fish.

6. As for the literature search relating to *Rhodococcus equi* in foals, a fairly large number of publications dealing with hyperimmune plasma were identified. While the observed differences, which were many and varied, made it particularly difficult to compare the findings of these publications, it could still be considered that on the whole, they suggested that administering hyperimmune plasma to foals could be a way to prevent pulmonary infection caused by *Rhodococcus equi*.

   This is a promising class of products whose efficacy should be confirmed through studies targeting specific products.

7. For other combinations of animal species and diseases, such as metritis in cows and clinical and subclinical mastitis in dairy cows, it was not possible to draw conclusions about the

analysed product classes, due mainly to the extremely small number of publications available for them. This observation of a lack of scientific references should encourage research in these areas in order to identify promising products and characterise their effects.

8. The favourable effect of probiotics that is starting to emerge from this analysis is associated with the preventive use of products. This raises the issue of their timing of administration in livestock rearing conditions. Is continuous administration feasible in acceptable economic conditions? Or should it be limited to more vulnerable stages or to degraded livestock rearing conditions? The metaphylactic use of probiotics (replacing a traditional antibiotic treatment) could be beneficial to "protect" disease-free animals in a batch where a disease has been declared. This would mean achieving protective probiotic efficacy over a short administration time and within a potentially infected environment. This is an area worth exploring.
9. Epidemiological approaches: to date, few epidemiological studies have investigated the effects of alternative substances to antibiotics on the use of antibiotics or the risk of disease. The WG nevertheless devoted a section to these studies since they are interesting complements to experimental studies.
10. Toxicological approach: many of the alternative products considered had been assessed for animals, consumers and the environment and were found to be safe. This was the case of numerous yeasts and bacteria with QPS[9] status, antibodies in plasma (after verifying non-contamination by micro-organisms), certain chemically defined substances extracted from plants or plant parts, enzymes, organic acids and prebiotics already favourably evaluated. However, their dermal, respiratory or eye irritant potential requires precautions for users.

    Certain products used in studies (probiotics, especially bacteria) were not assessed for their safety for animals, humans (sometimes) or the environment (in most cases). Thus, some bacteria used may be potentially pathogenic to humans or are characterised by a lack of data regarding their safety. It is therefore necessary, and mandatory under the regulations, to assess these products before any practical use.

    Plants and plant extracts (other than chemically defined substances extracted from plants) are characterised by a high level of heterogeneity regarding the presence or absence of information on their safety for animals, consumers, users or the environment. Therefore, for these products, analyses should be undertaken on a case-by-case basis.

    A final risk should be mentioned related to the use of these products: it involves whether or not they are capable of promoting antimicrobial resistance. For the time being, this aspect has not been widely studied but some scientific publications show that there is a risk, for example with organic acids (used as biocides), probiotics and bacteriophages. Post-authorisation monitoring of use should be ensured as well as the ability to monitor resistance in environmental flora.
11. The current regulatory positioning of the products identified in this analysis is primarily related to the claims described in all of the documents relating to these products. The initial analysis indicates that:
    - many claims found while producing the inventory lead the product to be considered as a veterinary medicinal product (especially the generic claim "alternative to antibiotics");
    - several products used are already authorised as additives in animal feed but for uses that are not necessarily related to that identified by the experts during the inventory;
    - claims such as "contributes to reducing the use of antibiotics" have not been given any particular regulatory classification thus far, making it necessary to explore the need and relevance of creating new European regulatory provisions for these products, if they do not have the status of veterinary medicinal product by function.

[9] QPS: Qualified Presumption of Safety.

3.6.3. Recommendations

1. As stated above, in a large number of cases, the WG was unable to rule as to the efficacy of products as alternatives to antibiotics, for want of sufficient evidence in the literature. This does not necessarily mean that these products are ineffective but highlights the need to encourage research in this area in order to identify promising products and characterise their effects.

   Moreover, based on the literature, it was not possible to study the possible effects of combinations of alternatives on reducing the use of antibiotics in animal husbandry, even though the Phase 1 inventory identified several mentions of combinations. Research into combinations of these alternatives should be undertaken in the future.

   Lastly, while further research is still necessary to identify promising products and provide proof of their efficacy, their conditions of use should also be studied in order to define the most suitable distribution conditions, especially in the context of prevention.
2. The efficacy of a product as an alternative to antibiotics cannot be demonstrated solely through experimental studies as described in the literature. In addition to a potential demonstration arising from the WG's work (taking into account a methodological score, relevance of variables, compilation of publications), the efficacy of these products should also be evaluated in terms of their ability to reduce the probability of antibiotic use or treatment frequency over several farm production cycles. It is in this context that epidemiological (especially interventional) approaches should be combined with these *sensu stricto* experimental approaches, thus making it possible to study aspects that are difficult to take into consideration in the framework of experimental studies, which are nevertheless decisive in determining a long-term reduction in antibiotic use.

   These complementary epidemiological studies would also enable the effects of these alternatives to be analysed jointly with those of other preventive measures, with the goal of reducing antibiotic use in animal husbandry. Indeed, the WG would like to emphasise that these product classes are merely a driver for reducing the use of antibiotics in animal husbandry, and that a systemic approach to this issue, including various animal husbandry parameters, is essential.
3. According to the available literature, the prospects provided by the various classes of products often seem characterised by effects of a smaller magnitude than those of antibiotics. If this situation is not merely a matter of application doses, then it is a major challenge for experimental research, which has to adapt its protocols to the detection of “low-intensity effects”. The WG recommends strongly encouraging research into these new protocols (degraded sanitary conditions, new variables to be measured, combinations of experimental studies and epidemiological investigations, studies of product combinations, etc.).

   All of these research recommendations are fully in line with the objectives of the Ecoantibio 2 Plan, whose Action 2 involves *“acquiring references on alternative treatments helping to limit the prescription of antibiotics”.*

   Furthermore, due to responses for these substances that are usually smaller in magnitude than those of antibiotics, these products alone probably cannot be alternatives to antibiotics in the strict sense in terms of efficacy. The WG therefore emphasises, as in the previous point, the importance of considering these substances as being part of a more integrated approach, combined with other disease control measures (farming practices, biosafety, vaccination, nutrition, etc.).
4. Under the regulations, these products and substances helping to reduce the use of antibiotics can have various legal statuses depending on their function, presentation and/or claim.

   Certain products, due to their presentation or function, have the legal status of veterinary medicinal products. Even so, although products are likened to veterinary medicinal products

by their presentation, especially through the wording of the manufacturers' claims, their function may be different and an alternative presentation could be considered for them:

- through the regulation of additives for animal feed, by proposing a new category of additives or modifying the definition of zootechnical additives, with an appropriate functional group, which would require that the European regulations be amended;
- or, for certain products, through the regulations on particular nutritional purposes (PARNUTs) (modification of certain current purposes or creation of one or more new purposes);
- or by means of claims focused on raw materials (for products that can be considered raw materials for animal feed).

These various possibilities require the exploration of potential changes to the European regulations on animal feed, the definition of new categories of additives or PARNUTs or suitable claims, and the conditions for including expressions such as "reduces (or contributes to reducing) the use of antibiotics" in the various regulations subject to change.

4. AGENCY CONCLUSIONS AND RECOMMENDATIONS

The French Agency for Food, Environmental and Occupational Health & Safety endorses the analysis and conclusions of the CES on "Animal feed" regarding the inventory of alternatives to antibiotics aimed at reducing their use in animal husbandry and the development of a method for assessing scientific publications and their findings.

The Agency notes that a large number of products and substances (compounds, plants, plant extracts and micro-organisms) are used as alternatives to antibiotics. It underlines the heterogeneity and variability of the bibliographic database observed in the expert appraisal, which are limitations for assessing the safety and efficacy of many of these alternatives and their ability to cause resistance to emerge or develop. ANSES stresses the need to initiate a debate to define, for the main animal sectors affected, the classes of alternatives that should be given priority for in-depth research to remove uncertainties as to their efficacy and safety.

The French Agency for Food, Environmental and Occupational Health & Safety draws attention to the importance of determining the legal status of these products, before studies and experiments are undertaken, since this will enable the necessary studies and their terms of implementation to be defined, prior to a product's marketing and use. In the framework of the antimicrobial resistance control plan, ANSES recommends raising the issue of the legal status of these products at European level, so studies may be conducted on the relevance and feasibility of creating a specific status for products inducing a reduction in the use of antibiotics without them being considered as veterinary medicinal products.

Lastly, the Agency underlines various methodological research needs to ensure that demonstrations regarding the efficacy and safety of these products are established on solid scientific bases.

Dr Roger Genet

KEYWORDS

Alimentation animale, alternatives aux antibiotiques, antibiorésistance, probiotique, prébiotique, acide organique, huile essentielle, extrait de plante, peptide antimicrobien, phage, algue, plasma, porc, volaille, vache laitière, ovin, caprin, pré-ruminant, veau, cheval, lapin, poisson, troubles digestifs, colibacillose systémique, entérocolite, rhodococcose, mammite, métrite.

Feed, antimicrobial alternatives, antimicrobial resistance, probiotic, prebiotic, organic acid, essential oil, plant extract, antimicrobial peptide, phage, seaweed, plasma, pig, poultry, dairy cow, sheep, goat, calf, rabbit, fish, horse, digestive disorders, systemic colibacillosis, enterocolitis, rhodococcosis, mastis, metritis.

ANNEX 1: ILLUSTRATIVE FIGURES AND TABLES

Figure 1: Decision tree for the methodological scoring of publications for multifactorial diseases

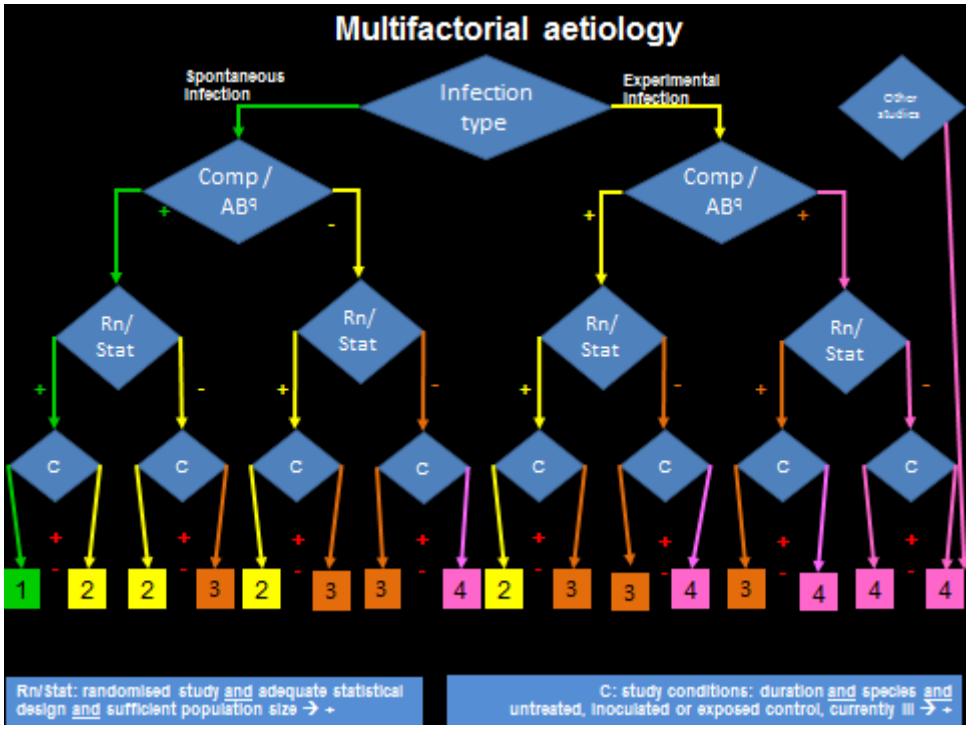

Figure 2: Decision tree for the methodological scoring of publications for single-agent diseases

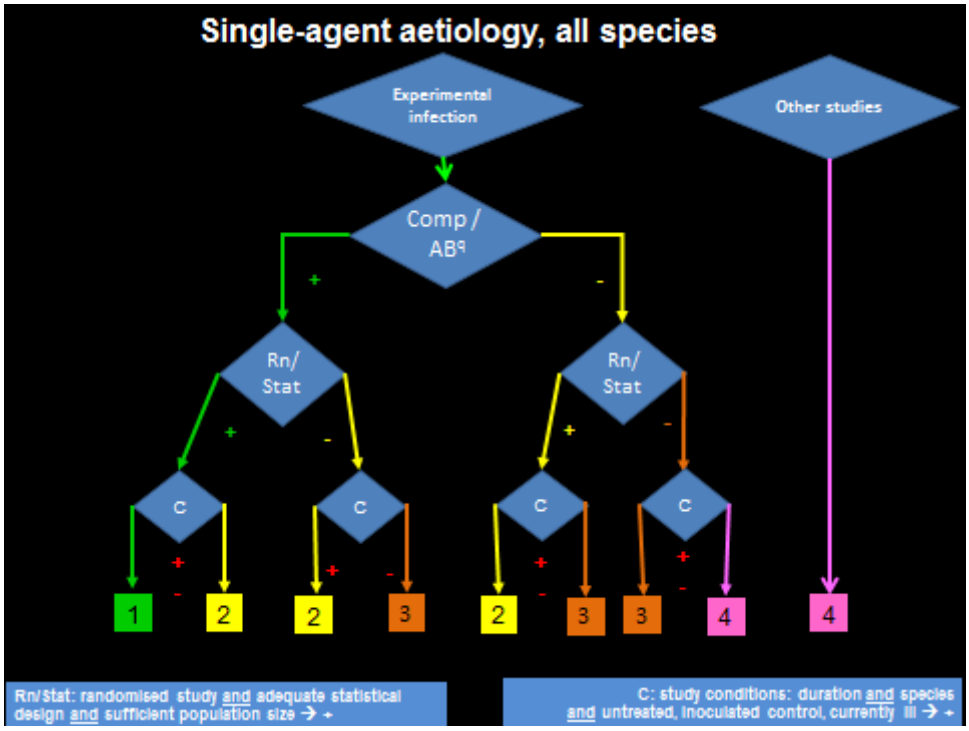

Table 1: Relevance of the variables measured by disease/animal species (two-page table)

0 = no relevance; * = low relevance; ** = relevance; *** = high relevance; NA = not applicable

Variable	Digestive disorders, all species°		Subclinical mastitis, dairy ruminants		Clinical mastitis, dairy ruminants		Fish diseases		Systemic colibacillosis in poultry		Bovine metritis		Equine rhodococcosis		Exceptions
General clinical score	Body temperature	*	Body temperature	*	Body temperature	*	Body temperature	NA	Body temperature	NA	Body temperature	*	Body temperature	*	
	General state, behaviour	**	General state, behaviour	*	General state, behaviour	*	General state, behaviour		General state, behaviour		General state (toxaemia), behaviour	**	General state, behaviour, growth	*	
	Therapeutic intervention (veterinarian, treatment)	**							Therapeutic intervention (veterinarian, treatment)	**			Existence of a treatment and duration	**	
Specific clinical score	Diarrhoea score (excluding poultry)	***	Udder symmetry	*	Udder symmetry	*					Vaginal palpation (vaginal secretions, symmetry of the uterus)	***	Age at disease onset	**	
	Litter condition (poultry)	**	Udder volume/oedema	*	Udder volume/oedema	*					Ultrasound (increased diameter, viscosity of content)	***	Dyspnea/lung auscultation	**	
	Morbidity	**	Udder colour	*	Udder colour	*							Lung x-ray and/or ultrasound	***	
			Pain	*	Pain	*							Respiratory symptoms (cough, discharge, polypnoea)	*	
Cure	Diarrhoea reduction/stoppage (specify observation period)	** (***) depending on disease	Clinical cure	*	Clinical cure	**									
Vital parameters	Mortality	***	Mortality	NA	Mortality	NA	Mortality	***	Mortality	***	Mortality	NA	Mortality	***	
													Age at death	**	
	Survival curves	***	Survival curves	NA	Survival curves	NA	Survival curves	***	Survival curves	***	Survival curves	NA	Survival rate (fatality)	***	
							RLP: related level of protection	***					Survival time	***	
Bacterial counts	Pathogenic bacteria counts in faeces	**	Enumeration of pathogenic bacteria	***	Enumeration of pathogenic bacteria	**					Enumeration of pathogenic bacteria in secretions	**	Enumeration of pulmonary bacteria	**	
	Changes in the profile of the intestinal microbiota (specify the parameter measured)	*													
Histological and histopathological parameters	Height of intestinal villi	*											Extent of fibrosis (total collagen)	*	Equine enterocolitis: lesional, macroscopic or histological criteria, not used in publications
	Depth of intestinal crypts	*													
	Macroscopic observation of lesions	**							Observation of lesions (autopsies)	**			Pulmonary lesion score (% of tissue volume affected or number of abscesses)	***	
	Inflammatory cell infiltration of the intestinal epithelium	*													

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Variable	Digestive disorders, all species°		Subclinical mastitis, dairy ruminants		Clinical mastitis, dairy ruminants		Fish diseases		Systemic colibacillosis in poultry		Bovine metritis		Equine rhodococcosis		Exceptions
Blood parameters	White blood cells	0	Level of macrophages	*	Level of macrophages	*	White blood cells	* if consistent with challenge					leukocytes including neutrophils	**	** salmonellosis in horses
			Myeloperoxidase and phosphatase activity	*	Myeloperoxidase and phosphatase activity	*							VapA-specific IgG and IgG subclasses in serum	*	*** IgA-IgG in sows for pre-weaning diarrhoea in piglets
													VapA-specific IgG and IgG subclasses in broncho-alveolar lavage fluid	*	
	Immunoglobulin levels in serum	0					Immunoglobulin levels in serum		Immunoglobulin levels in serum	0	Immunoglobulin levels in serum	0	Immunoglobulin levels in serum	0	
													Blood gases (pCO2, SO2, tCO2)	0 to *	
	Other blood parameters: cytokines	0					Various biochemical parameters: total proteins, carbohydrates, cholesterol				Another blood parameter of inflammation: haptoglobin	0 to * depending on publication	Fibrinogenaemia	*	
Cytokines and other local parameters	Inflammatory cytokines (assay)	*	Cytokines	**	Cytokines	**							Cytokine panel (at least TH1 pathway)	**	
	Cytokine gene expression	0											Expression/regulation of immune response genes (several cytokines)	*	
													Phagocytosis (alveolar macrophages and/or blood monocytes)	*	
													Proliferation of R equi in macrophages derived from monocytes	*	
													Proliferation of blood monocytes	0	
													Oxidative burst in neutrophils	0 to *	
													% of blood monocytes secreting IN_{γ} and IL4	*	
	Local immunity	*	Level of metallo-proteinases (MMPs)	*	Level of metallo-proteinases (MMPs)	*							Expression/regulation of inflammatory response genes	0	
Cellular parameters of oxidative stress		0		0		0	Oxidative burst (NBT test), SOD and MPO (oxidase) activities	0		0		0		0	
Zootechnical parameters, if reflection of return to good general state	ADG	**	Milk production	**	Milk production	**	Weight gain	*	ADG	**	Fertility rate (calving-AI time)	*	Weight gain	*	
	FCR	*							FCR	*	Milk production/DMI	*			
Secretion parameters			Milk cells (CMT/SCC)	***	Milk cells (CMT/SCC)	**									
			Level of neutrophils	*	Level of neutrophils	*									
			Conductivity of milk	**	Conductivity of milk	**									
			pH of milk	**	pH of milk	**									
			Viscosity/consistency of milk	**	Viscosity/consistency of milk	**									
			Colour of milk	*	Colour of milk	*									
			Odour of milk	*	Odour of milk	*									
			Casein/lactoferrin content of milk	*	Casein/lactoferrin content of milk	*									

Table 2: Conclusions for multifactorial aetiologies

	Infection type	AB[q] in the study	Product comparison vs.	'Biological' effect of the product (statistical and biological)	Conclusion by publication (curative/preventive)	Methodological score
Multifactorial aetiology	Spontaneous infection	yes	Ab[q]	S+ / NS=	Alternative treatment to AB[q] / Contributes to reducing the use of AB[q]	1 to 3
				S-	Is not an alternative treatment to AB[q] / Does not contribute to reducing the use of AB[q]	
			Untreated control	S+	Treats/prevents the disease	
				NS / S-	Does not treat/does not prevent the disease	
	Experimental infection	yes	AB[q]	S+ / NS=	Alternative treatment to AB[q] / Contributes to reducing the use of AB[q]	2 to 4
				S-	Is not an alternative treatment to AB[q] / Does not contribute to reducing the use of AB[q]	
			Untreated control	S+	Treats/prevents the disease	
				NS / S-	Does not treat/does not prevent the disease	
	Spontaneous or experimental infection	no	Untreated control	S+	Treats/prevents the disease	2 to 4
				NS / S-	Does not treat/does not prevent the disease	
	No infection	yes	AB[q]	No conclusion. Publication not subsequently taken into account		4
		no	Untreated control			

S-	Significant difference, against the product
S+	Significant difference, in favour of the product
NS	Non-significant difference
NS=	Non-significant difference, product whose effect can be considered equivalent to that of the antibiotic

Table 3: Conclusions for single-agent aetiologies

	Infection type	AB^q in the study	Product comparison vs.	'Biological' effect of the product (statistical and biological)	Conclusion by publication (curative/preventive)	Methodological score
Single-agent aetiology	Experimental infection	yes	AB^q	S+ / NS=	Alternative treatment to AB^q / Contributes to reducing the use of AB^q	1 to 3
				S-	Is not an alternative treatment to AB^q / Does not contribute to reducing the use of AB^q	
			Untreated control	S+	Treats/prevents the disease	
				NS / S-	Does not treat/does not prevent the disease	
		no	Untreated control	S+	Treats/prevents the disease	2 to 4
				NS / S-	Does not treat/does not prevent the disease	
	No infection	yes	AB^q	No conclusion. Publication not subsequently taken into account		4
		no	Untreated control			

S-	Significant difference, against the product
S+	Significant difference, in favour of the product
NS	Non-significant difference
NS=	Non-significant difference, product whose effect can be considered equivalent to that of the antibiotic

The WG would like to emphasise that that the individual conclusions by publication should be considered as intermediate working conclusions, serving as benchmarks for the experts, with a view to compiling various publications relating to the same combination of product, animal species and disease. These individual conclusions have no significance in terms of a product's efficacy. Only the compilation of several studies can provide knowledge of efficacy.

Figure 3: Decision-making rule for general conclusions by product with a confidence level after combining publications

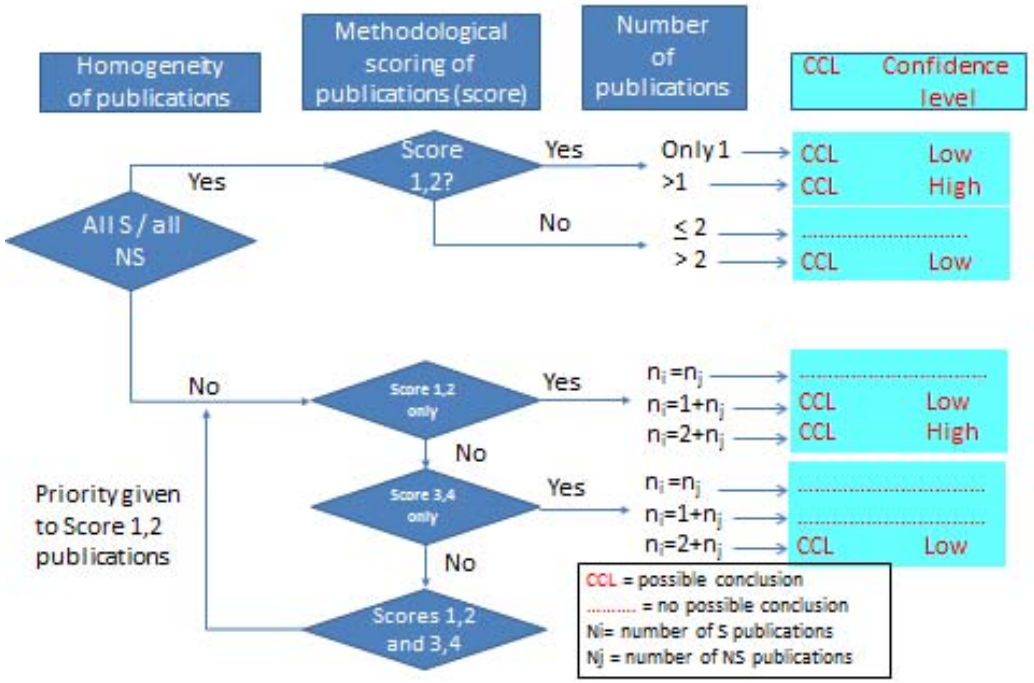

Figure 4: General methodological framework for the assessment of scientific publications in terms of product efficacy

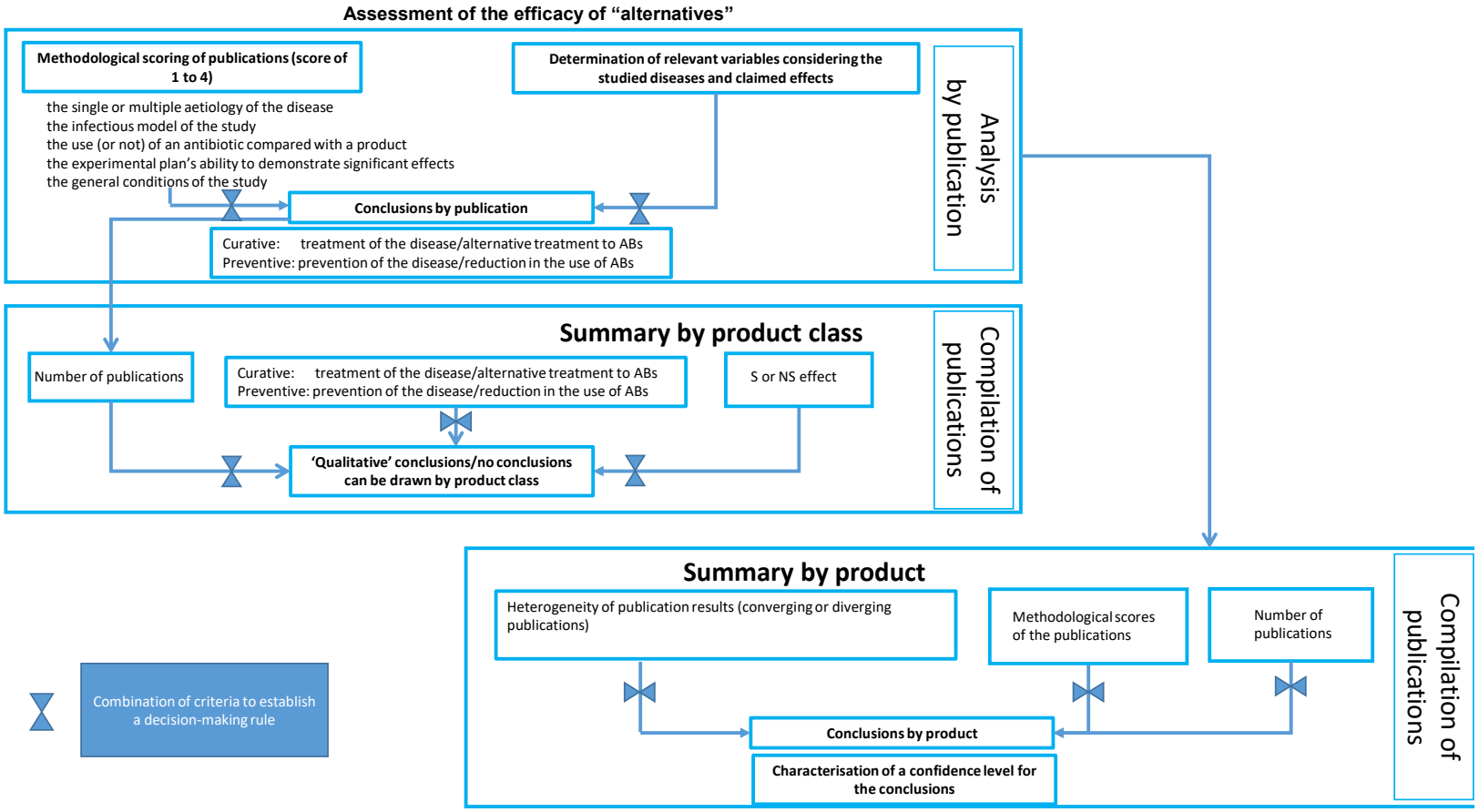

Table 4: Efficacy x safety correlation for promising products and classes of products

Disorder	Product category	Product type	Efficacy: contribution to reducing the use of antibiotics	Safety
Diarrhoea in piglets at weaning	Probiotics	*Saccharomyces cerevisiae, Pediococcus acidilactici, Lactobacillus plantarum, Lactobacillus rhamnosus, Enterococcus faecium*	Preventive treatment (high confidence level)	For the target species, consumers and the environment
	Plasma	Pig plasma	Preventive treatment (high confidence level)	For the target species, consumers and the environment if the product is not contaminated by micro-organisms
	Casein	-	Preventive treatment (high confidence level)	For the target species, consumers and the environment
Systemic colibacillosis in poultry	Probiotics	*Clostridium butyricum*	Preventive treatment (low confidence level)	No for certain strains considered as pathogenic
Digestive disorders in rabbits	Probiotics	*Saccharomyces cerevisiae, Lactobacillus plantarum, Bacillus licheniformis, Bacillus subtilis, Bacillus toyonensis*	Preventive treatment (low confidence level)	For the target species, consumers and the environment EXCEPT *Bacillus toyonensis*
Diarrhoea in calves	Egg immuno-globulin G	-	Preventive and curative treatment (low confidence level)	For the target species, consumers and the environment if the product is not contaminated by micro-organisms
Systemic diseases in fish	Essential oils	*Hesperozygis ringens*, *Ocimum americanum*	Curative treatment (low confidence level)	For the target species
	Plant extracts	Cinnamon extract (cinnamaldehyde), clove	Curative treatment (low confidence level)	For the target species, consumers and the environment but maximum tolerated dose to be verified for fish Skin, eye and respiratory irritant and skin sensitiser for users
	Probiotics	Mainly the *Bacillus* and *Lactobacillus* genera	Preventive treatment (low confidence level)	For the target species, consumers and the environment
Rhodococcus equi in foals	Hyper-immune plasma	Hyper-immune equine plasma	Preventive treatment (low confidence level)	For the target species, consumers and the environment if the product is not contaminated by micro-organisms
	Gallium maltolate	-	Curative treatment (low confidence level)	Few data but yes in principle (anti-tumour properties of gallium in particular)
Mastitis	Plant extracts and essential oils	*Linum usitassimum*, *Atractylodis macrocephalae koidz*, *Ocimum sanctum*, *Eucalyptus globus* + *Glycyrrhiza gloria* + *Curcuma longa* + *Cedrus deodora* + *Paearia foetida*	Curative treatment of subclinical mastitis (low level of evidence)	No data except for *Ocimum*, *Linum* and *Curcuma* (safety for the target species) and *Eucalyptus* (safety for the target species and consumers)
Metritis	Plant extracts	Garlic, *Nigella sativa*	Curative treatment (low confidence level)	No data for the local route
Digestive disorders in goat kids	Plant extracts	*Datura inoxia*	Preventive treatment (low confidence level)	No for the target species, no data for consumers or the environment

ANNEX 2: EXAMPLE OF IMPLEMENTING THE PUBLICATION ASSESSMENT METHODOLOGY

Digestive disorders in piglets at weaning

- ## Analysis grid for publications and comments

Table 1: Analysis of publications relating to digestive disorders in piglets at weaning (two-page table ↔)

Preventive/curative approach	Publication reference	Animal species	Targeted disease, function	Measured variable(s)	Variable's level of relevance	Product class	Product substance(s) or strain(s)	Study conditions
Preventive	Gebru et al., 2010	Pigs	Digestive disorders Salmonella excretion	Salmonella count in faeces	**	Probiotic	*Lactobacillus plantarum*	Experimental infection with *Salmonella* Typhimurium Products distributed in feed, 2 weeks before and 2 weeks after experimental infection 1 untreated control batch; 1 batch receiving 100mg/kg of chlortetracycline; 1 batch receiving $6.5.10^5$ CFU/kg feed of *L. Plantarum*
Preventive	Gebru et al., 2010	Pigs	Digestive disorders Salmonella excretion	Salmonella count in faeces	**	Probiotic	Soya fermented with *Bacillus subtilis*	Experimental infection with *Salmonella* Typhimurium Products distributed in feed, 2 weeks before and 2 weeks after experimental infection 1 untreated control batch; 1 batch receiving 100mg/kg of chlortetracycline; 1 batch receiving 5% of the product in feed
Preventive and curative	Trevisi et al., 2015	Pigs	Digestive disorders	Faecal score	***	Probiotic	*Saccharomyces cerevisiae (SCC)*	Experimental infection with *E. coli* F4ac, 7 days post-weaning 55 pigs at weaning at 24 days divided into 5 groups: untreated control, colistin (1 g/kg feed), SCC 5.10^8 CFU/kg feed for 21 days, SCC 5.10^8 CFU/kg from day 7 to day 11, one shot of 2. 10^9CFU of SCC on the first day of diarrhoea.
				Mortality	***			
Preventive	Lebon et al., 2010	Pigs	Digestive disorders	*E. coli* count	**	Probiotic	*Saccharomyces cerevisiae boulardii* (SCB) + *Pediococcus acidilactici* (PA)	Spontaneous infection (weaning conditions). Eight weaned piglets divided into 2 batches: the untreated control batch and a batch receiving feed supplemented with 10^9 CFU/kg feed of SCB for 6 weeks and then feed supplemented with PA for 3 weeks. Note that no pigs showed clinical signs in the weaning conditions of the study.
Preventive	Kiarie et al., 2012	Pigs	Digestive disorders	Faecal score	***	Probiotic	*Saccharomyces cerevisiae* (SC)	Experimental infection: Ciprofloxacin-resistant *E. coli* K88 on day 7. 90 pigs divided into groups of 3 in 5 pens per treatment, 6 treatments: Batch 1 = untreated control, Batch 2 = SC 0.2%, Batch 3 = antibiotics (chlortetracycline 0.04% + tiamulin 0.004%), Batches 4-5-6: antibiotics + 0.1, 0.2 or 0.4% SC
Preventive	Trckova et al. 1992	Pigs	Digestive disorders	IgA: piglet serum	**	Probiotic	*Saccharomyces cerevisiae*	Experimental infection: inoculation with *E. Coli* O149:K88 the day after weaning. Treatment of sows at the end of gestation and during lactation and of piglets at birth (1g, bolus 3 times a week) and during post-weaning. Untreated control.
Preventive	Hancox et al., 2013	Pigs	Digestive disorders	Diarrhoea score	***	Probiotic	*Saccharomyces cerevisiae*	Spontaneous infection. Monitoring of neo-natal diarrhoea. Administration of a bolus on the day of birth for the treated batch
Preventive	Zanello et al., 2013	Pigs	Digestive disorders	Incidence of diarrhoea	***	Probiotic	*Saccharomyces cerevisiae (SC)*	Natural conditions. Monitoring of neo-natal diarrhoea. The piglets received no solid feed. The sows received the product in feed from 86 days of gestation and during lactation SC: 0.5 or 5 ppm of each of the Sc01, Sc02 and Sb03 strains

Basis of comparison no. 1	Nature of the effect (S/NS)	Magnitude of the effect if S	Basis of comparison no. 2	Nature of the effect (S/NS)	Magnitude of the effect if S	Conclusion	Methodological score
Product vs untreated control	S	Low at 6 days Average 14 days post-challenge	Product vs antibiotic	S^- at 6 days NS at 14 days	Product vs antibiotic effects not different at 14 days	Contributes to reducing the use of antibiotics	2
Product vs untreated control	S	Average at 6 days High 14 days post-challenge	Product vs antibiotic	NS at 6 days S^+ at 14 days	Product vs antibiotic effects not different at 6 days and greater effect with product vs antibiotic at 14 days	Contributes to reducing the use of antibiotics	2
Product vs untreated control	S	For all conditions	Products vs antibiotics	NS	The comparison is relevant with the negative control but complicated with colistin (NS differences but with no demonstration of equivalence)	Prevents the disease Severe model: 70% mortality for the untreated controls. SCC is most effective for prevention	1
	S	Low S for the 21-day distribution. NS for the other conditions					
Product vs untreated control	S					Prevents the disease	4
Products vs untreated control	S with 0.2% SC	Moderate magnitude				Prevents the disease	1
Product vs untreated control	S in sows and/or piglets	Modest effect				Prevents the disease	2
Product vs untreated control	S	Significant but modest effect: diarrhoea persisted until day 7.				Prevents the disease	2
Product vs untreated control	NS	Non-significant tendency to reduce the incidence of diarrhoea				Does not prevent the disease	3

Preventive/curative approach	Publication reference	Animal species	Targeted disease, function	Measured variable(s)	The variable's level of relevance	Product class	Product substance(s) or strain(s)	Study conditions
Preventive	Jin et al., 2016	Pigs	Digestive disorders	Rate of diarrhoea	***	Probiotic and Antimicrobial peptide	*Bacillus subtilis* and plectasin	Spontaneous infection 24 pigs at weaning at 24 days divided into 4 groups: Batch 1 = untreated control, Batch 2 = colistin 60 mg/kg, Batch 3 = Bacillus subtilis > 10^9 CFU, Batch 4 = plectasin 60 mg/kg, monitored for 21 days
				ADG	**			
Preventive	Kiers et al., 2003	Pigs	Digestive disorders	Diarrhoea (incidence, severity, duration)	***	Fermented raw material (probiotic?)	Soya fermented with *Bacillus subtilis* or *Rhizopus microsporus*	Experimental infection with *E. coli* K88 (*per os*). Study undertaken for 4 weeks, challenge 2 days post-weaning. Products distributed in feed, before and after the challenge. 96 weaned piglets divided into 4 batches: - batch 1: feed containing toasted soybeans, acting as control - batch 2: feed containing soybeans fermented with *Rhizopus microsporus* - batch 3: feed containing soybeans fermented with *Bacillus subtilis* - batch 4: feed containing hulled and cooked whole soybeans
Preventive	Liu et al., 2010	Pigs	Digestive disorders	Faecal consistency	***	Prebiotic	Chitooligo-saccharide	Experimental infection with *Escherichia coli* K88 (*per os*). Product distributed in feed, before and after the challenge. 72 piglets divided into 4 batches: batch 1 = non-contaminated, non-supplemented feed batch 2 = contaminated, non-supplemented feed batch 3 = contaminated feed supplemented with the product (160 mg) batch 4 = contaminated feed supplemented with an antibiotic (Cyadox 100 mg/kg)
				Incidence of diarrhoea	***			
				E. coli quantification	**			
Preventive	Zhou et al., 2012	Pigs	Digestive disorders	Incidence of diarrhoea	***	Prebiotic	Chitooligo-saccharides	Natural infection. 120 weaned piglets divided into 4 batches: batch 1: untreated control batch 2: control with antibiotics batch 3: feed with 1g/kg feed of product batch 4: feed with 2g/kg feed of product
				Diarrhoea score	***			
Preventive	Owusu-Asiedu et al., 2003	Pigs	Digestive disorders	Mortality	***	Pig plasma/ egg yolk antibody (hens immunised against ETEC K88)/other products		Experimental infection with *E. coli* K88 (*per os*). Study undertaken for 14 days, challenge at 7 days. Products distributed in feed, before and after the challenge. 90 weaned piglets (early weaning) divided into 6 batches: - batch 1: feed containing pea protein isolate, acting as control - batch 2: feed containing pea protein isolate + egg yolk Ab - batch 3: feed containing pig plasma, with no pea protein isolate - batch 4: feed containing pea protein isolate + fumaric acid - batch 5: feed containing pea protein isolate + antibiotic (Carbadox) - batch 6: feed containing pea protein isolate + zinc oxide
				Diarrhoea score	***			
Preventive	Peace et al., 2011	Pigs	Digestive disorders	Diarrhoea score	***	Dried pig plasma		Spontaneous infection. 48 pigs divided into 3 batches, monitored for 2 weeks post-weaning (18 days) Batch 1 = untreated control Batch 2 = feed containing 2.5% plasma Batch 3 = feed containing 5% plasma
Preventive	Maribo H, 2009	Pigs	Digestive disorders	Reduction in the number of days of diarrhoea treatment	***	Pig plasma		Spontaneous infection. 2 tests: one with 440 piglets, the other with 288 piglets. Weaning at 26 days. Batch 1 = untreated control Batch 2 = feed with 5% plasma Batch 3 = feed with 2500 pm zinc oxide Batch 4 = feed with 5% plasma + 2500 ppm zinc oxide

Basis of comparison no. 1	Nature of the effect (S/NS)	Magnitude of the effect if S	Basis of comparison no. 2	Nature of the effect (S/NS)	Magnitude of the effect if S	Conclusion	Methodological score
Products vs untreated control	70% reduction with plectasin and colistin, 30% with *B. subtilis* but no statistical analysis for this parameter		Products vs antibiotic	Equivalent to the antibiotic with plectasin Lesser effect than the antibiotic with *B. subtilis* No statistical analysis for this parameter		Contributes to reducing the use of antibiotics for plectasin Prevents the disease for *B. subtilis*	1
	NS for *B. subtilis* S for plectasin			S⁻ for *B. subtilis* NS for plectasin	Effect equivalent to that of the antibiotic for plectasin. Lesser effect than the antibiotic for *B. Subtilis*		
Products vs untreated control	S	Statistically significant decrease in the severity of diarrhoea for the batch with *Rhizopus* Decrease in the incidence and duration of diarrhoea, but not significant for the 3 experimental batches				Prevents the disease for soya fermented with *Rhizopus microsporus*	2
Product vs untreated control	S	Low	Product vs antibiotic	NS	Product vs antibiotic effects not different	Contributes to reducing the use of antibiotics	2
	S						
	S	S in the caecum					
Products vs untreated control	S	For the 2 tested doses	Products vs antibiotics	NS	Product vs antibiotic effects not different	Contributes to reducing the use of antibiotics	1
Products vs untreated control	S	All S compared to batch 1	Product vs antibiotic	NS	Product vs antibiotic effects not different	Contributes to reducing the use of antibiotics	2
	S	All S compared to batch 1					
Product vs untreated control	S	Low				Prevents the disease	2
Products vs untreated control	S	Low magnitude of the effect on diarrhoea and only for the first 7 days of life				Prevents the disease	2

Preventive/curative approach	Publication reference	Animal species	Targeted disease, function	Measured variable(s)	The variable's level of relevance	Product class	Product substance(s) or strain(s)	Study conditions	Basis of comparison no. 1	Nature of the effect (S/NS)	Magnitude of the effect if S	Basis of comparison no. 2	Nature of the effect (S/NS)	Magnitude of the effect if S	Conclusion	Methodological score
Preventive	Gebru et al., 2010	Pigs	Digestive disorders Salmonella excretion	Salmonella count in faeces	**	Organic acids	Mixture containing 20% citric ac. 20% fumaric ac. 10% malic ac. 10% phosphoric ac.	Experimental infection with *Salmonella* Typhimurium Products distributed in feed, 2 weeks before and 2 weeks after experimental infection 1 untreated control batch; 1 batch receiving 100mg/kg of chlortetracycline; 1 batch receiving 0.2% of the mixture of organic acids in feed	Product vs untreated control	S	Average at 6 days High 14 days post-challenge	Product vs antibiotic	NS at 6 days and 14 days	Product vs antibiotic effects not different at 6 and 14 days	Contributes to reducing the use of antibiotics	2
Preventive	Hermes et al., 2013	Pigs	Digestive disorders	Incidence of diarrhoea	***	Glyco-macropeptide	Casein	Experimental infection with *E. coli* K88. Distribution of the product in feed 1 week before and after the challenge Batch 1 = untreated challenged control Batch 2 = untreated non-challenged control Batch 3 = challenged, treated Batch 4 = non-challenged, treated	Product vs untreated control	NS	Qualitative improvement				Does not prevent the disease	2
				Enterobacteriaceae count in the ileum	**					NS						
Preventive	Rong et al., 2015	Pigs	Digestive disorders	Diarrhoea score	***	Glyco-macropeptide	Casein	Experimental infection with *E. coli* K88. Distribution of the product in feed 1 week before and after the challenge Batch 1 = untreated challenged control Batch 2 = untreated non-challenged control	Product vs untreated control	S	Significant improvement only after 7 days				Prevents the disease	2
				Enterobacteriaceae count in intestines	**											
Preventive	Bontempo et al., 2014	Pigs	Digestive disorders	Diarrhoea score	***	Plant extract	Green tea leaves + pomegranate	Experimental infection with *E. coli* O149 during treatment with products	Product vs untreated control	S					Prevents the disease	2
				Enterobacteriaceae count in faeces	**											
Preventive	Hansen et al., 2014	Pigs	Digestive disorders	Diarrhoea score	***	Plant extract	Lupin or chicory root or both together	Experimental infection with *B. hyodysenteriae* during treatment with products	Product vs untreated control	S					Prevents the disease	2

The literature search was undertaken with the Google Scholar, PubMed and Web of Science search engines, using the following keywords: “diarrhoea” and (“pig or “swine”) and (“alternatives to antibiotics” or “probiotic” or “prebiotic” or “plant” or “essential oil” or “organic acid”). A search by author was also conducted. For journal articles, some of the articles cited had not previously been identified but were found during the literature search.

This search gave rise to 146 publications from the last 10 years. Studies dealing with additives presented as alternatives to growth factor antibiotics were eliminated.

In the end, 36 publications were subject to the analytical method defined by the WG. There were relatively few scientific publications on alternative treatments to antibiotics in pigs.

- **Conclusions by class**

  - Essential oils (EOs) and plant extracts

The literature searches and sorting operations resulted in a very small number of publications providing data for only three products, of different types and with different roles (see Table 2). The ingestion of a mixture of green tea leaves and pomegranate skin (commercial mixture) in a study with experimental infection but no antibiotics had a significant effect on digestive tract health and thus offset the negative effects observed following *E. coli* infection in piglets at weaning (Bontempo *et al.*, 2014).

Regarding other digestive diseases in pigs, another study investigated the prevention of dysentery in piglets and compared two products: lupin seeds proved ineffective in limiting the negative effects of experimental infection with *Brachyspira hyodysenteriae*, whereas chicory root inulin provided protection against the development of dysentery (Hansen *et al.*, 2014).

Table 2: Summary of publications involving EOs and plant extracts – diarrhoea in piglets

Prevention/AB^q summary table

No of pubs	Scores 1-2	Scores 3-4
S^+ ($P > AB^q$) and NS ($P \approx AB^q$)	0	0
S^- ($P < AB^q$)	0	0

Important! For comparisons with AB^q: S and NS do not lead to the same conclusions as for comparisons with the control and S has been divided into S^+ and S^- depending on the positioning of test product P compared to AB^q or compared to the control

Prevention/control summary table

No of pubs	Scores 1-2	Scores 3-4
S^+ ($P > C$)	2	0
NS ($P \approx C$) and S^- ($P < C$)	1	0

Important! A publication studying both the effect compared to the negative control and the effect compared to an antibiotic should be counted twice: once in each table.

Curative/AB^q summary table

No of pubs	Scores 1-2	Scores 3-4
S^+ ($P > AB^q$) and NS ($P \approx AB^q$)	0	0
S^- ($P < AB^q$)	0	0

Curative/control summary table

No of pubs	Scores 1-2	Scores 3-4
S^+ ($P > C$)	0	0
NS ($P \approx C$) and S^- ($P < C$)	1	0

In light of the few data identified in the scientific literature, the wide variety of products used in studies and their diverging results, it is not possible to draw a positive or negative conclusion as to a generic effect on diarrhoea in piglets at weaning for the class of essential oils and plant extracts, considered as a whole.

It should be noted that none of these studies made comparisons with a group treated with an antibiotic.

- Organic acids

Only one publication was selected for this category. Thus, it was not possible to analyse this class of products in greater detail.

- Probiotics

Table 3: Summary of publications involving probiotics – diarrhoea in piglets

Prevention/AB^q summary table				**Prevention/control summary table**		
No of pubs	Scores 1-2	Scores 3-4		No of pubs	Scores 1-2	Scores 3-4
S^+ ($P > AB^q$) and NS ($P \approx AB^q$)	3	0		S^+ ($P > C$)	7	1
S^- ($P < AB^q$)	1	0		NS ($P \approx C$) and S^- ($P < C$)	0	1

Ten publications were selected for this category. They tested several species of probiotics (*Saccharomyces cerevisiae, Bacillus subtilis, Pediococcus acidilactici, Lactobacillus plantarum*), sometimes in combination. Various strains were tested within a species. In addition, the administration protocols for the animals varied greatly in terms of the animals (sows or piglets) and the duration. Significant effects were demonstrated on reducing the shedding of pathogenic bacteria and in some cases on preventing diarrhoea at weaning. Overall (see Table 3), despite the heterogeneity of the protocols/results, it seems that this approach may contribute to reducing the use of antibiotics.

- Prebiotics

Two publications were selected for prebiotics (chitooligosaccharides), with scores of 1-2, giving interesting results regarding the incidence of diarrhoea, the diarrhoea score and the shedding of *Escherichia coli*. Another, with a score of 3-4, was not selected due to a lack of relevant parameters measured during the study. Note that in one of these publications, the tested product was compared to antibiotics, but their dosage of use was insufficient, thus weakening the comparison.

The second publication established a decrease in the incidence of diarrhoea but did not compare chitooligosaccharides to an antibiotic. However, the tested product did not correct the negative effect of inoculation on growth (see Table 4).

Table 4: Summary of publications involving prebiotics – diarrhoea in piglets

Prevention/AB^q summary table				**Prevention/control summary table**		
No of pubs	Scores 1-2	Scores 3-4		No of pubs	Scores 1-2	Scores 3-4
S^+ ($P > AB^q$) and NS ($P \approx AB^q$)	0	0		S^+ ($P > C$)	2	0
S^- ($P < AB^q$)	0	0		NS ($P \approx C$) and S^- ($P < C$)	0	0

No conclusions can be drawn as to the preventive action of chitooligosaccharides based solely on this work.

- **Conclusions by product**

Some publications dealt with simple, clearly identified products, leading the experts to consider drawing conclusions not by class but by product, using the methodology described in Section 5.6. These products were casein and pig plasma.

o Casein

Two publications with scores of 1-2 were selected. They were controlled studies comparing untreated controls that were or were not inoculated with *Escherichia coli* K88. This product did not give the same results in the two publications, especially regarding parameters related to diarrhoea in piglets (see Table 5).

Table 5: Summary of publications involving casein – diarrhoea in piglets

Prevention/AB^q summary table					**Prevention/control summary table**		
No of pubs	Scores 1-2	Scores 3-4			No of pubs	Scores 1-2	Scores 3-4
S^+ ($P > AB^q$) and NS ($P \approx AB^q$)	0	0			S^+ ($P > C$)	1	0
S^- ($P < AB^q$)	0	0			NS ($P \approx C$) and S^- ($P < C$)	1	0

Additional studies thus need to be undertaken to conclude as to the benefits of casein.

o Pig plasma

The selected publications provided valuable input since they used relevant criteria: diarrhoea score and mortality (see Table 6). A comparison with an antibiotic suggested equivalent efficacy but the statistical power of the comparison was low.

Table 6: Summary of publications involving pig plasma – diarrhoea in piglets

Prevention/AB^q summary table					**Prevention/control summary table**		
No of pubs	Scores 1-2	Scores 3-4			No of pubs	Scores 1-2	Scores 3-4
S^+ ($P > AB^q$) and NS ($P \approx AB^q$)	1	0			S^+ ($P > C$)	3	0
S^- ($P < AB^q$)	0	0			NS ($P \approx C$) and S^- ($P < C$)	0	0

Regarding studies testing plasma (for prevention) not compared to an antibiotic, three publications were identified that had converging results. The application of the decision-making rule in Figure 3 (Annex 1) enabled a conclusion to be drawn with a high confidence level. As these were studies in comparison with an untreated control, the final conclusion for this product is as follows:

"*The available scientific data indicate that pig plasma prevents diarrhoea in piglets at weaning, with a high confidence level. However, given the small number of identified publications (only three), this conclusion remains dependent on the experimental conditions*".

ANNEX 3: SAFETY OF COMPOUNDS POTENTIALLY LIMITING THE USE OF ANTIBIOTICS

Compound(s) or micro-organism(s)	Safety for the target species	Safety for users	Safety for consumers	Safety for the environment	Reference
Saccharomyces cerevisiae	Yes (QPS – EFSA)		Yes (QPS – EFSA)	Yes (QPS – EFSA)	EFSA, 2016
Saccharomyces cerevisiae boulardii	Yes (QPS – EFSA)		Yes (QPS – EFSA)	Yes (QPS – EFSA)	EFSA, 2016
Debaryomyces hansenii	Yes (QPS – EFSA)		Yes (QPS – EFSA)	Yes (QPS – EFSA)	EFSA, 2016
Candida deformans	Considered as low pathogenic	Considered as low pathogenic	Considered as low pathogenic	No data	
Cryptococcus laurentii	Considered as low pathogenic	Considered as low pathogenic (isolated in a lung abscess)	Considered as low pathogenic	No data	https://en.wikipedia.org/wiki/Pathogenic_fungus (consulted on 23/02/17)
Metschnikowia viticola	No data	No data	No data	No data	
Rhodotorula mucilaginosa	Few data but considered by some as an emerging pathogen	Few data but considered by some as an emerging pathogen	Few data but considered by some as an emerging pathogen	No data	Deligios M, Fraumene C, Abbondio M, Mannazzu I, Tanca A, Addis MF, Uzzau S. Draft Genome Sequence of Rhodotorula mucilaginosa, an Emergent Opportunistic Pathogen. Genome Announc. 2015, 3(2)
Yarowia lipolytica	No data	Potentially pathogenic in humans (rare opportunistic infections)	Yeast naturally found in cheese, meat, etc.	No data	Groenewald M, Boekhout T, Neuvéglise C, Gaillardin C, van Dijck PWM, Wyss M. Critical Reviews in Microbiology 2014;40(3):187-206
Lactobacillus plantarum	Yes (QPS – EFSA)		Yes (QPS – EFSA)	Yes (QPS – EFSA)	EFSA, 2016
Lactococcus lactis	Yes (QPS – EFSA)		Yes (QPS – EFSA)	Yes (QPS – EFSA)	EFSA, 2016
Lactobacillus casei	Yes (QPS – EFSA)		Yes (QPS – EFSA)	Yes (QPS – EFSA)	EFSA, 2016
Lactobacillus acidophilus	Yes (QPS – EFSA)		Yes (QPS – EFSA)	Yes (QPS – EFSA)	EFSA, 2016
Lactobacillus reuteri	Yes (QPS – EFSA)		Yes (QPS – EFSA)	Yes (QPS – EFSA)	EFSA, 2016
Lactobacillus johnsonii	Yes (QPS – EFSA)		Yes (QPS – EFSA)	Yes (QPS – EFSA)	EFSA, 2016
Lactobacillus rhamnosus	Yes (QPS – EFSA)		Yes (QPS – EFSA)	Yes (QPS – EFSA)	EFSA, 2016
Lactobacillus sakei	Yes (QPS – EFSA)		Yes (QPS – EFSA)	Yes (QPS – EFSA)	EFSA, 2016
Leuconostoc mesenteroides	Yes (QPS – EFSA)		Yes (QPS – EFSA)	Yes (QPS – EFSA)	EFSA, 2016
Lactobacillus pentosus	Yes (QPS – EFSA)		Yes (QPS – EFSA)	Yes (QPS – EFSA)	EFSA, 2016
Lactobacillus acidophilus	Yes (QPS – EFSA)		Yes (QPS – EFSA)	Yes (QPS – EFSA)	EFSA, 2016
Lactobacillus ruminis	Not QPS but phylogenetically similar to *L. salivarius* which has QPS status				Charalampopoulos D, Rastall RA.

Lactobacillus equi	Not QPS but phylogenetically similar to *L. salivarius* which has QPS status				Prebiotics and Probiotics Science and Technology, Volume 1, 2009, p 607
Bifidobacterium animalis	Yes (QPS – EFSA)		Yes (QPS – EFSA)	Yes (QPS – EFSA)	EFSA, 2016
Bifidobacterium bifidum	Yes (QPS – EFSA)		Yes (QPS – EFSA)	Yes (QPS – EFSA)	EFSA, 2016
Bifidobacterium longum	Yes (QPS – EFSA)		Yes (QPS – EFSA)	Yes (QPS – EFSA)	EFSA, 2016
Bifidobacterium pseudolongum	Yes (QPS – EFSA) for *Bifidobacterium longum*		Yes (QPS – EFSA) for *Bifidobacterium longum*	Yes (QPS – EFSA) for *Bifidobacterium longum*	EFSA, 2016
Bacillus licheniformis	Yes (QPS – EFSA)		Yes (QPS – EFSA)	Yes (QPS – EFSA)	EFSA, 2016
Bacillus coagulans (= lactobacillus sporogenes)	Yes (QPS – EFSA)		Yes (QPS – EFSA)	Yes (QPS – EFSA)	EFSA, 2016
Bacillus mojavensis	Yes (QPS – EFSA)		Yes (QPS – EFSA)	Yes (QPS – EFSA)	EFSA, 2016
Bacillus pumilus	Yes (QPS – EFSA)		Yes (QPS – EFSA)	Yes (QPS – EFSA)	EFSA, 2016
Bacillus subtilis	Yes (QPS – EFSA)		Yes (QPS – EFSA)	Yes (QPS – EFSA)	EFSA, 2016
Bacillus toyonensis (NCIMB 14858)	No (risk of dissemination of encoding genes for resistance to tetracycline and chloramphenicol + toxinogenic potential)				Commission Regulation (EU) No 2015/1399 of 17 August 2015.
Carnobacterium divergens	Yes (QPS – EFSA)		Yes (QPS – EFSA)	Yes (QPS – EFSA)	EFSA, 2016
Enterococcus faecium	Yes for certain strains in calves, piglets, chickens, turkeys, dogs and cats (100 times the dose of 1.0 x 10^9 – 6.6 x 10^9 CFU/kg feed for example for Oralin®)	Yes for certain strains, except respiratory sensitiser	Yes for certain strains	Yes for certain strains	EFSA Journal 2013;11(2):3097 [14 pp.] and EFSA Journal 2014;12(6):3727 [19 pp.]
Pediococcus acidilactici	Yes for the strains subject to expert appraisal, for all livestock animals	Skin and eye irritant, skin and respiratory sensitiser depending on the strain	Yes for the strains subject to expert appraisal (NCIMB 30005)	Yes for the strains subject to expert appraisal	EFSA Journal 2014;12(3):3613 [11 pp.] and EFSA Journal 2016;14(6):4483 [2 pp.]

Propionibacterium acnes	Exclude for QPS	No data	Exclude for QPS	Exclude for QPS	Leuschner GK *et al.,* Trends in Food Science & Technol. 2010; 21:425-435
Bifidobacterium thermophilum	Yes in principle since naturally found in intestines	Yes in principle since naturally found in intestines	Yes since naturally found in intestines	No data	https://en.wikipedia.org/wiki/Bifidobacterium (consulted on 23/02/17)
Aeromonas hydrophila	No since pathogenic in fish, snakes and mammals	So since opportunistic in humans (gastroenteritis)	No since contaminates numerous foods (shellfish, crustaceans, etc.)	No data	http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/aeromonas-hydrophila-eng.php (consulted on 23/02/17)
Aeromonas sobria	Considered as non-pathogenic	Considered as non-pathogenic	Considered as non-pathogenic	No data	https://academic.oup.com/cid/article/30/6/988/436336/Clinically-Relevant-Aeromonas-Species (consulted on 23/02/17)
Brochothrix thermosphacta	Considered as non-pathogenic	Considered as non-pathogenic	Considered as non-pathogenic	No data	http://www.arrowscientific.com.au/index.php?option=com_content&view=article&id=43:brochothrix-thermosphacta&catid=25&Itemid=40 (consulted on 23/02/17)
Clostridium butyricum	Considered by some as an emerging pathogen	Considered by some as an emerging pathogen	Considered by some as an emerging pathogen	No data	Clostridium butyricum: from beneficial to a new emerging pathogen. Cassir N, Benamar S, La Scola B. Clin Microbiol Infect. 2016 Jan;22(1):37-45.
Enterobacter cloacae	No since potentially pathogenic in animals	No since potentially pathogenic in humans	No since potentially pathogenic in humans (found in foods of animal origin)	No data	www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/enterobacter-fra.php (consulted on 23/02/17)
Paenibacillus polymyxa	Not QPS classified by EFSA for *P. macerans* due to a lack of safety data				EFSA, 2016
Micrococcus diversus and *Kocuria spp.*	Considered as non-pathogenic	Considered as non-pathogenic	Considered as non-pathogenic	No data	http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/micrococcus-fra.php
Pseudomonas aeruginosa	No since pathogenic	No since opportunistic in humans	No since food contaminant	No data	http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/pseudomonas-spp-fra.php

Pseudomonas fluorescens	No since few data	No since few data	No since food contaminant (cheese, etc.)	No data	http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/pseudomonas-spp-fra.php
Shewanella spp.	No since few data but low pathogenic	No since few data but low pathogenic	No since food contaminant (fish, etc.)	No data	doi: 10.4103/0974-2727.72150
Vagococcus fluvialis	Considered as potentially pathogenic	Considered as potentially pathogenic	Considered as potentially pathogenic	No data	doi: 10.1128/AEM.01852-06; Al ahmad *et al.*, 2008 (https://www.ncbi.nlm.nih.gov/pubmed/?term=ahmad+AND+vagococcus
Vibrio alginolyticus	No since potentially pathogenic in fish and crustaceans	No since potentially pathogenic in humans with antimicrobial resistance	Potentially found in certain foods, especially seafood products	No data	doi: 10.1016/j.annder.2007.04.010
Vibrio fluvialis	No since potentially pathogenic in crustaceans	No since potentially pathogenic in humans		No data	doi: 10.3389/fmicb.2014.00091
Vibrio lentus	No since potentially pathogenic in octopuses	Considered as non-pathogenic		No data	doi: 10.3389/fmicb.2013.00413
Vibrio proteolyticus	Considered as non-pathogenic but identification of virulence factors (cytotoxicity) and resistance genes	Considered as non-pathogenic but identification of virulence factors (cytotoxicity) and resistance genes	Considered as non-pathogenic but identification of virulence factors (cytotoxicity) and resistance genes	No data	https://en.wikipedia.org/wiki/Vibrio ; Ray *et al.*, 2016 (https://www.ncbi.nlm.nih.gov/pubmed/27460800)
Zooshikela spp.	Considered as non-pathogenic	Considered as non-pathogenic	Considered as non-pathogenic	No data	Ramaprasad E V, Bharti D, Sasikala C, Ramana CHV. Int. J. Syst. Evol. Microbiol., 2015;65:4669-4673
Haemolytic endotoxin Streptolysin O (Streptococci A, C, G): *inactive in the presence of* O_2	No data	No data	No data	No data	
Inactivated *Parapoxvirus ovis*	No data	No data	No data	No data	

Pig or cattle plasma/serum	Yes if product not contaminated by micro-organisms (FMD, PED, etc.)	No data	Yes	Yes if product not contaminated by micro-organisms (FMD, PED, etc.)	EMA/EFSA, 2017 - Pérez-Bosque A *et al.,* Porcine Health Management 2016; 2:16-25
Hyperimmune equine plasma	Yes if product not contaminated by micro-organisms	No data	Yes	Yes if product not contaminated by micro-organisms	EMA/EFSA, 2017
Immunomodulatory fraction of goat serum	Yes if product not contaminated by micro-organisms	No data	Yes	Yes if product not contaminated by micro-organisms	EMA/EFSA, 2017
Egg yolk IgY	Yes if product not contaminated by micro-organisms	No data	Yes	Yes if product not contaminated by micro-organisms	EMA/EFSA, 2017 - Li X, Lili Wang L, Zhen Y, Li S, Xu Y. Chicken egg yolk antibodies (IgY) as non-antibiotic production enhancers for use in swine production: a review. J Anim Sci Biotechnol, 2015; 6(1):40.

Nigella sativa	LD50 (IP mice) = 0.542 ml/kg	Reported as potentially toxic (**seeds**; isoquinoline alkaloids)	Reported as potentially toxic (**seeds**; isoquinoline alkaloids)	Not applicable	EFSA, 2012 Özbek H, Öztürk M, Öztürk A, Ceylan E, Yener Z. Determination of Lethal Doses of Volatile and Fixed Oils of Several Plants. Eastern J Med, 2004; 9(1):4-6
Quillaja saponaria	NOAEL ≥ 2470 mg/kg/day (90-day studies) in rats	Reported as potentially toxic (**bark**; Ca oxalate 11% + triterpenoid saponins)	Reported as potentially toxic (**bark**; Ca oxalate 11% + triterpenoid saponins)	Not applicable	EFSA, 2012 EPA. Saponins of Quillaja saponaria. 2009.

Quillaja japonica	No data	No data	No data	Not applicable	
Thymus vulgaris	LD50 = 2.84 g EO/kg *per os* in rats	No data	No MRL required for *Thymi aetheroleum* or thymol ("MRL/veterinary medicinal product" regulation)	Not applicable	EFSA, 2012 Basch E, Ulbricht C, Hammerness P, Bevins A, Sollars D. Thyme (Thymus vulgaris L.), Thymol. J Herbal Pharmacotherapy, 2004; 4(1):49-68
Gaultheria procumbens	Benign digestive symptoms	Pure EO application sometimes fatal	No data	Not applicable	EFSA, 2012
Glycyrrhiza uralensis	No data	No data	No data	Not applicable	EFSA, 2012
Angelica sinensis	Potential phototoxicity	No data	No MRL required for *Angelicae radix aetheroleum* ("MRL/veterinary medicinal product" regulation)	Not applicable	EFSA, 2012
Ocimum sanctum	Not reported as toxic at 2000 mg/kg or 800 mg/kg/day for 28 days	No data	No data	Not applicable	EFSA, 2012 Gautam MK, Goel RK. Toxicological Study of *Ocimum sanctum* Linn Leaves: Hematological, Biochemical, and Histopathological Studies. J Toxicol. 2014;2014:135654
Atractylodis macrocephalae koidz	No data	No data	No data	Not applicable	
Azadirachta indica	Not reported as toxic at 2 g root/kg for 14 days *per os* Decrease in fertility in males and females and abortions	No data	Decrease in fertility in males and females and abortions	Not applicable	EFSA, 2012 Biswas K, Chattopadhyay I, Banerjee RK, Bandyopadhyay U. Biological activities and medicinal properties of neem (Azadirachta indica). Current Science, 2002; 82(11): 1336-1345.
Stems of *Guatteriopsis friesiana*: 6,6a-dihydrodemethoxyguadiscine, guatteriopsiscine, demethoxyguadiscine, liriodenine, corypalmine, and coreximine	Not reported as toxic	Not reported as toxic	No data	Not applicable	

Bursera aloexylon (leaves)	No data	No data	No data	Not applicable	
Morinda citrifolia (cheese fruit or noni)	Rare hepatotoxicity in humans: animals?	Yes	No data	Not applicable	EFSA Journal, 2009;998
Origanum vulgare (oregano), *Origanum majorana*	Yes in dogs and cats (gastroenteritis)	Rare allergies after ingestion	No data	Not applicable	Poison control centres
Eucalyptus sp	Yes at the maximum dose of 1,8-cineole of 5mg/kg of complete feed	Skin, eye and respiratory irritant, skin sensitiser	No MRL required for eucalyptol ("MRL/veterinary medicinal product" regulation)	Not applicable	EFSA, 2012 - EFSA Journal 2012;10(11):2967
Curcuma longa	Yes in rats, guinea pigs and monkeys at 2.5 g/kg *per os*	Skin sensitiser	No data	Not applicable	EFSA, 2012 Shankar TN, Shantha BNV, Ramesh HP, Murthy IAS, Murthy VS. Toxicity studies on turmeric (Curcuma longa): acute toxicity studies in rats, guinea pigs and monkeys. Indian Journal of Experimental Biology 1980; 18(1):73-75.
Salviae sp	Not reported as toxic except *S. fruticosa* (decrease in fertility in males and females)	No data	Not reported as toxic except *S. fruticosa* (potential decrease in fertility in males and females) No MRL required for *Salviae folium* ("MRL/veterinary medicinal product" regulation)	Not applicable	EFSA, 2012
Panax ginseng	Benign digestive symptoms after ingestion	No data	No data	Not applicable	
Allium cepa (onion)	Yes but <5 g/kgLW in cats and 15/30 g/kgLW in dogs	No data	No MRL required ("MRL/veterinary medicinal product" regulation)	Not applicable	Salgado BS *et al*. The Journal of Venomous Animals and Toxins including Tropical Diseases 2011;17(1):4-11

Allium sativum (vaginal route)	No data (10% solution; intrauterine route)	No data	No data	Not applicable	EFSA, 2012
Allium sativum (oral route)	Lower toxicity than *Allium cepa*	No data	No data	Not applicable	EFSA, 2012
Cuminum cyminum (see cinnamaldehyde)					
Foeniculum vulgare = *Foeniculi aetheroleum* (fennel)	LD50 (IP mice) = 1.038 ml/kg	No data	Not reported as toxic No MRL required ("MRL/veterinary medicinal product" regulation)	Not applicable	EFSA, 2012 Özbek H, Öztürk M, Öztürk A, Ceylan E, Yener Z. Determination of Lethal Doses of Volatile and Fixed Oils of Several Plants. Eastern J Med, 2004;9(1):4-6.
Gentianae, Gentianella sp	No data	No data	No MRL required for *Gentianae radix* ("MRL/veterinary medicinal product" regulation)	Not applicable	
Melissa officinalis	No data	No data	No MRL required ("MRL/veterinary medicinal product" regulation)	Not applicable	
Mentha piperita	No data	No data	Not reported as toxic No MRL required ("MRL/veterinary medicinal product" regulation)	Not applicable	EFSA, 2012
Pimpinella sp, *illicium sp*	LD50 (IP mice) = 0.847	No data	No data	Not applicable	EFSA, 2012

(green and star anise)	ml/kg for *Pimpinella anisum*				Özbek H, Öztürk M, Öztürk A, Ceylan E, Yener Z. Determination of Lethal Doses of Volatile and Fixed Oils of Several Plants. Eastern J Med, 2004;9(1):4-6.
Quercus sp (Oak bark)	Yes for *Q. robur* and *Q. Sessiliflora*: 4 kg dried acorns/adult cattle	No data	Hepatotoxicity if high doses over a long period No MRL required for *Quercus cortex* ("MRL/veterinary medicinal product" regulation)	Not applicable	EFSA, 2012
Syzygium aromaticum (clove) - also see eugenol	No data	No data	No data	Not applicable	EFSA, 2012
Silybum marianum	Indirect toxicity in ruminants (plant rich in potassium nitrate)	Not reported as toxic	No MRL required for homoeopathic medicines ("MRL/veterinary medicinal product" regulation)	Not applicable	EFSA, 2012
Linum usitatissimum (EO)	Yes but ≤ 0.5 to 1kg/day for a bovine, ≤ 0.1 to 0.25kg/day for a sheep, ≤ 5% of the ration in pigs and ≤ 0.1 to 0.3kg/day for a horse	No data	No data	Not applicable	EFSA, 2012
Hesperozygis ringens	Not reported as toxic	No data	No data	Not applicable	
Ocimum americanum	Not reported as toxic	No data	No data	Not applicable	EFSA, 2012
Ocimum gratissimum	Not reported as toxic	No data	No data	Not applicable	EFSA, 2012
Scutellaria baicalensis	Cardiac lesions in rats (120 mg wogonin/kg in the long term)	No data	No data	Not applicable	EFSA, 2012
Citrus (especially contains furocoumarins in EO)	Photosensitisation (certain species such as *Citrus bergamia*)	Photodermatitis	No data	Not applicable	EFSA, 2012
"*Sucupira branca*" bark (*Pterodon emarginatus*	No data	No data	No data	Not applicable	

Vogel)					
Melaleuca alternifolia	Toxic in dogs and cats by the dermal and oral routes (0.1-85 ml EO)	No data	No data	Not applicable	EFSA, 2012 Khan SA, McLean MK, Slater MR. Concentrated tea tree oil toxicosis in dogs and cats: 443 cases (2002–2012). JAVMA, 2014; 244(1):95-99.
Punica granatum L. (pomegranate)	Not toxic in rats by repeated intranasal route at 1.2 mg extract/kg LW; genotoxicity of the fruit hydroalcoholic extract	No data	All organs toxic except seeds (French Pharmacopoeia A List)	Not applicable	Vidal A *et al.* Studies on the toxicity of *Punica granatum* L. (*Punicaceae*) whole fruit extracts. Journal of Ethnopharmacology 89 (2003) 295–300.
Terminalia chebula (myrobalan)	Not toxic in rats by repeated oral route (14 days) at 2000 mg ethanol extract/kg LW	No data	No data	Not applicable	Kim JH *et al.* Mutagenicity and oral toxicity studies of Terminalia chebula. Phytother Res. 2012 Jan;26(1):39-47.
Aegle marmelos	Rats: decrease in T3 (1 g aqueous extract/kg LW/day/7 days *per os*), decrease in spermatogenesis and fertility	No data	By extrapolation, reprotoxic and potential endocrine disruptor	Not applicable	
Berberis aristata	Indian medicinal plant with numerous properties	Indian medicinal plant with numerous properties	Indian medicinal plant with numerous properties	Not applicable	Sharma Komal *et al. Berberis aristata*: a review. IJRAP 2011, 2 (2) 383-388
Datura Innoxia	Anorexia, tremors, mucosal dryness, mydriasis, sometimes blindness, dyspnoea especially in ruminants and horses	No data	No data	Not applicable	
Holarrhena antidysenterica	Contains steroidal alkaloids	No data	No data	Not applicable	

Calendula aventis	Increase in serum urea and transaminases, maternal toxicity during the foetal phase at a repeated high oral dose in rats	No data	Not reported as toxic (potential maternal toxicity during the foetal phase?) No MRL required for *Calendula officinalis* ("MRL/veterinary medicinal product" regulation)	Not applicable	EFSA, 2012
Camphor	Yes at the maximum dose of 0.5 mg/kg feed in cattle, salmonids and pets and of 0.3 mg/kg feed in pigs and poultry	Mainly a respiratory irritant	Yes at the maximum doses considered as "safe" for the target species	Yes at the maximum doses considered as "safe" for the target species	EFSA Journal 2016;14(6):4475
Limonene	Yes, except for male rats, at the maximum dose of 25 mg/kg feed	Irritating to the skin and eyes Respiratory irritant Skin sensitiser	Yes at the maximum dose of 25 mg/kg feed	Yes	EFSA Journal 2015;13(3):4053
Anethole	Yes, except for fish, at doses of 5-25 mg/kg feed with a low safety margin (2 to 6)	Skin sensitiser	Yes at doses of 5-25 mg/kg feed (except for poultry due to a lack of data on metabolism and residues)	Yes	EFSA Journal, 2011;9(12):2440
Carvacrol	Yes at the maximum dose of 5 mg/kg feed sometimes with low safety margins (1 to 5 in pigs and poultry)	Irritating to the skin and eyes Respiratory irritant	Yes at the maximum dose of 5 mg/kg feed	Yes at the maximum dose of 5 mg/kg feed	EFSA Journal 2012;10(2):2573

Thymol	Yes at the maximum dose of 5 mg/kg feed sometimes with low safety margins (1 to 5 in pigs and poultry)	Irritating to the skin and eyes Respiratory irritant	Yes at the maximum dose of 5 mg/kg feed	Yes at the maximum dose of 5 mg/kg feed	EFSA Journal 2012;10(2):2573
Vanillin	Yes at the maximum dose of 25 mg/kg feed in all species	Irritating to the skin and eyes Respiratory irritant Skin sensitiser Toxic if swallowed	Yes at the maximum dose of 5 mg/kg feed	Yes at the maximum dose of 25 mg/kg feed	EFSA Journal 2012;10(7):2785
Linalool	Yes at the maximum dose of 25 mg/kg feed in salmonids, calves, fattening cattle and pets (except cats), 20 mg/kg feed in dairy cows and pigs, 12 mg/kg feed in piglets and 10 mg/kg feed in poultry	Irritating to the skin and eyes Respiratory irritant Skin sensitiser	Yes in mammals (lack of data for poultry and fish)	Yes at the maximum doses considered as “safe” for the target species	EFSA Journal 2012;10(11):2966
Terpinolene, alpha-terpinene and gamma-terpinene	Yes at the maximum dose of 1.5 mg/kg feed (ruminants, salmonids and non-food-producing animals) and 1 mg/kg feed (pigs and poultry)	Irritating to the skin and eyes Respiratory irritant Skin sensitiser	Yes at the maximum dose of 1.5 mg/kg feed (ruminants, salmonids and non-food-producing animals) and 1 mg/kg feed (pigs and poultry)	Yes	EFSA Journal 2015;13(3):4053

Terpineol and 4-terpinenol	Yes at the maximum dose of 5 mg/kg feed	Irritating to the skin and eyes Respiratory irritant Skin sensitiser	Yes at the maximum doses considered as “safe” for the target species	Yes for the terrestrial compartment only, at the maximum doses considered as "safe” for the target species (lack of data for the aquatic compartment)	EFSA Journal 2012;10(11):2966
Alpha-pinene and betapinene	Yes at the maximum dose of 5 mg/kg feed	No specific data	Yes at the maximum doses considered as “safe” for the target species	Yes at the maximum doses considered as “safe” for the target species	EFSA Journal 2015;13(14):4069; EFSA Journal 2016;14(1):4339
Beta-caryophyllene	Yes at the maximum dose of 5 mg/kg feed	No specific data	Yes at the maximum doses considered as “safe” for the target species	Yes at the maximum doses considered as “safe” for the target species	EFSA Journal 2015;13(14):4069; EFSA Journal 2016;14(1):4339
Eudesmol (see Calendula officinalis)			No MRL required (“MRL/veterinary medicinal product” regulation)		
Eugenol	Yes, except for fish, at doses of 5-25 mg/kg feed with a low safety margin (2 to 6)	Irritating to the skin and eyes Respiratory irritant Skin sensitiser	Yes at doses of 5-25 mg/kg feed (except for poultry due to a lack of data on metabolism and residues)	Yes	EFSA Journal, 2011; 9(12):2440
Cinnamaldehyde	Yes at the maximum dose of 30 mg/kg feed in broiler chickens	Irritating to the skin and eyes Respiratory irritant Skin sensitiser	Yes at the maximum dose	Yes at the maximum dose	EFSA Journal 2015;13(2):4011
Hydrolysable tannins (*Castanea sativa* Mill)	No data	Potential skin irritant	Hepatotoxicity (administration of a high dose over a long period)	Not applicable	EFSA, 2012

Citric acid	Yes at the maximum dose of 30,000 mg/kg feed or 10,000 mg/l water	Irritating to the skin and eyes Respiratory irritant	Yes at the maximum doses	Yes at the maximum doses	EFSA Journal 2015;13(2):4009
Fumaric acid	Yes at the maximum dose of 20,000 mg/kg feed (pigs, poultry with a safety margin of 2) and at higher doses in ruminants	Irritating to the eyes and mucous membranes Respiratory irritant	Yes at the maximum doses	Yes at the maximum doses	EFSA Journal 2013;11(2):3102
Malic acid	Yes at the maximum dose of 1600 mg/kg feed (acid or Na or Ca salts) in ruminants, calves, piglets, poultry and dogs	Irritating to the eyes, mucous membranes and skin Respiratory irritant	Yes at the maximum dose of 1600 mg/kg	Yes at the maximum dose of 1600 mg/kg	EFSA Journal 2014;12(2):3563
(Ortho)phosphoric acid	Yes at the dose of 100 to 5000 mg/kg feed	Caustic to the skin and eyes Hazardous to the respiratory tract	Yes	Yes	EFSA Journal 2013;11(1):3043
Sorbic acid	Yes at the maximum dose of 2500 mg/kg feed in all species, except young cattle (6700 mg/kg feed)	Irritating to the skin and eyes Respiratory irritant	Yes at the maximum doses	Yes at the maximum doses	EFSA Journal 2015;13(9):4239
Ascorbic acid	Yes with no maximum dose for any animal species	Irritating to the skin and eyes Respiratory irritant Skin sensitiser	Yes	Yes	EFSA Journal 2013;11(2):3103
Cinnamaldehyde	Yes at the maximum dose of 30 mg/kg feed in broiler chickens	Irritating to the skin and eyes Respiratory irritant Skin sensitiser	Yes at the maximum dose	Yes at the maximum dose	EFSA Journal 2015;13(2):4011
Capric acid [(medium-chain FA (C8, C10)]	Yes for all species at the doses proposed for feed flavourings	Irritating to the skin and eyes Respiratory irritant	Yes at the proposed doses	Yes at the proposed doses	EFSA Journal 2013;11(4):3169

		Skin sensitiser			
Caproic acid	Yes at the maximum dose of 55 mg/kg feed with a safety margin of 1 to 120	Irritating to the skin and eyes Respiratory irritant Skin sensitiser	Yes at the maximum dose of 5 mg/kg feed	Yes at the maximum dose of 5 mg/kg feed	EFSA Journal 2013;11(4):3169
Caprylic acid (=octanoic acid)	Yes for all species at the doses proposed for feed flavourings	Irritating to the skin and eyes Respiratory irritant Skin sensitiser	Yes at the proposed doses	Yes at the proposed doses	EFSA Journal 2013;11(4):3169
Di-tri-octahedral smectite = bentonite (which is a smectite)	Yes at the maximum concentration of 2% in feed for all animal species	Slightly irritating to the eyes Potential respiratory irritant	Yes at the maximum concentration of 2% in feed	Yes at the maximum concentration of 2% in feed	EFSA Journal 2010;10(7):2787
Acid soluble bio-organic substances (SBO)	No data	No data	No data	Not applicable	No data
Beta-glucanase	Yes (numerous additives marketed)	Potential skin and respiratory sensitiser	Yes (numerous additives marketed)	Yes (numerous additives marketed)	Numerous EFSA opinions, e.g. EFSA Journal 2014;12(6):3722
Beta-xylanase	Yes (numerous additives marketed)	Potential skin and respiratory sensitiser	Yes (numerous additives marketed)	Yes (numerous additives marketed)	Numerous EFSA opinions, e.g. EFSA Journal 2014;12(6):3722
Amylase	Yes (numerous additives marketed)	Potential skin and respiratory sensitiser	Yes (numerous additives marketed)	Yes (numerous additives marketed)	Numerous EFSA opinions, e.g. EFSA Journal 2012;10(7):2777
MOS = manno-oligosaccharides	Case-by-case assessment depending on the type of compound				
Galactosyl lactose	Case-by-case assessment depending on the type of compound				
Galacto-oligosaccharides	Case-by-case assessment depending on the type of compound				
Inulin = polysaccharides	Case-by-case assessment depending on the type of compound				

Gallium maltolate	Few data but yes in principle since gallium is used in particular for its anti-tumour properties	Domingo JL, Corbella J. A review of the health hazards from gallium exposure. In: Trace Elements in Medicine, 1991;8 (2):56-64

ANNEX 4: ADDITIONS MADE TO ANSES'S INITIAL OPINION

ANSES Opinion of 16 February 2018	**Supplemented Opinion of 30 May 2018**
3.3.1.1 Methodological scoring criteria (…) The multiple integration of these criteria relied on decision trees presented in Section 5.1.2 of the collective expert appraisal report, resulting in each scientific publication being assigned a methodological score from 1 (highest) to 4 (lowest).	3.3.1.1 Methodological scoring criteria (…) The multiple integration of these criteria relied on decision trees presented in Section 5.1.2 of the collective expert appraisal report, resulting in each scientific publication being assigned a methodological score from 1 (highest) to 4 (lowest). **See Figures 1 and 2 in Annex 1, page 18**.
3.3.1.2 Relevance of the variables measured in the publications (…) Based on the various variables studied in the publications, the experts drew up a table of relevance levels for them, for all of the diseases studied in the report (Table 10, Section 5.2)	3.3.1.2 Relevance of the variables measured in the publications (…) Based on the various variables studied in the publications, the experts drew up a table of relevance levels for them, for all of the diseases studied in the report **(see Table 1 in Annex 1, pages 19-20).**
3.3.1.3 Conclusions by publication (…) Conclusions were proposed for each publication (Tables 11 and 12, Section 5.4).	3.3.1.3 Conclusions by publication (…) Conclusions were proposed for each publication **(Tables 2 and 3 in Annex 1, pages 21-22).**
3.3.3.5 Conclusions by product and related confidence levels (…) The decision-making rule proposed methods for combining publications with converging results on the one hand and with diverging results on the other hand, in order to draw a conclusion on the product's efficacy, associated with a low or high confidence level (see Section 5.6 of the report).	3.3.3.5 Conclusions by product and related confidence levels (…) The decision-making rule proposed methods for combining publications with converging results on the one hand and with diverging results on the other hand, in order to draw a conclusion on the product's efficacy, associated with a low or high confidence level **(Figure 3 in Annex 1, page 23).**
3.3.1.6 Implementation of the proposed method In order to test and illustrate the entire method, substance class, animal species and disease combinations were determined to study them in light of the literature. (…) The implementation of the entire method, aiming to establish a level of evidence for claimed efficacy for "alternative" substances and products, appears in Section 6 of the collective expert appraisal report, for each studied substance class, animal species and disease combination.	3.3.1.6 Implementation of the proposed method In order to test and illustrate the entire proposed method (**see general diagram of the method in Figure 4, Annex 1, page 24**), substance class, animal species and disease combinations were determined to study them in light of the literature. (…) The implementation of the entire method, aiming to establish a level of evidence for claimed efficacy for "alternative" substances and products, appears in Section 6 of the collective expert appraisal report, for each studied substance class, animal species and disease combination. **An example of its implementation is given in Annex 2, page 26.**
3.4. Assessment of the safety of the identified products and substances (…) The table listing more than 220 different micro-organisms, substances and products and the available data on their safety for humans, animals and the environment appears in Annex 4 of the collective expert appraisal report. Furthermore, the WG cross-checked the list of products appearing as promising in terms of efficacy, in light of the available literature, with the known data on their safety, in order to gain a broader view of these substances (Section 9.4 of the report).	**3.4. Assessment of the safety of the identified products and substances** (…) The table listing more than 220 different micro-organisms, substances and products and the available data on their safety for humans, animals and the environment **appears in Annex 3, page 32.** Furthermore, the WG cross-checked the list of products appearing as promising in terms of efficacy, in light of the available literature, with the known data on their safety, in order to gain a broader view of these substances (**see Table 4 in Annex 1, page 25**). **Annexes 1-2-3-4: Figures and tables included in the collective expert appraisal report**