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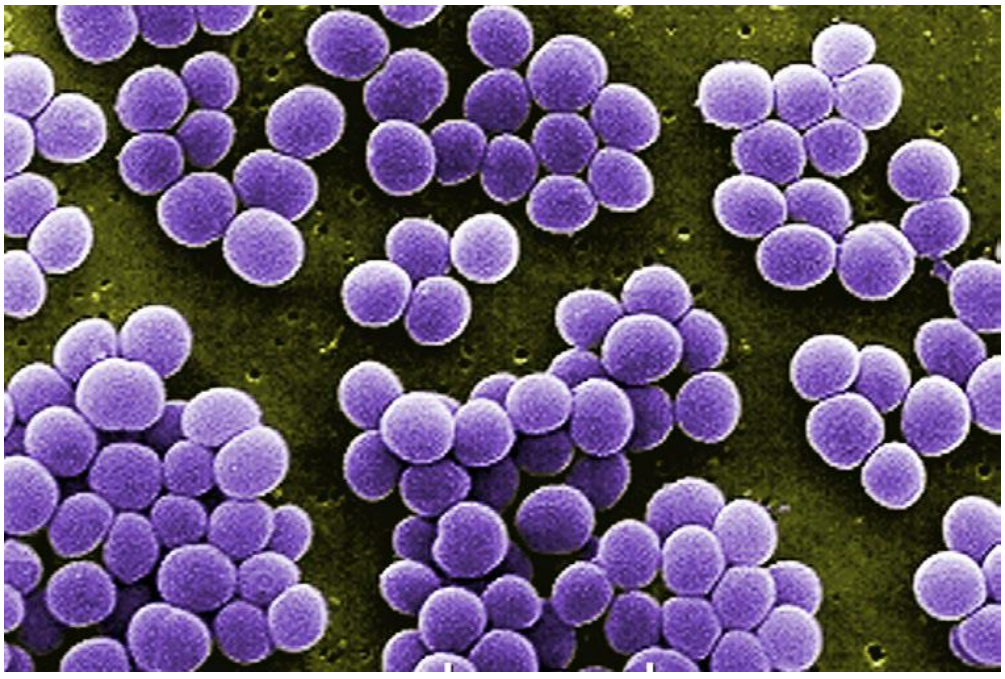
Impact of guidelines and recommendations on the level and patterns of antimicrobial use in livestock and companion animals

SYSTEMATIC REVIEW

Abel Ekiri, Barbara Haesler, Nicholas Mays, Katharina Staerk and Ana Mateus



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Executive Summary

Background: There are currently several voluntary guidelines and recommendations that aim to promote the responsible use of antimicrobials (AMU) and to reduce misuse of these medicines in both food-producing animals and companion animals. They have been developed by a number of organisations and implemented in several European countries with the aim of reducing the impact of AMU on antimicrobial resistance (AMR). However, the extent of implementation of these guidelines and their effectiveness in changing behaviours associated with AMU are unknown in most cases. This review assesses the extent of implementation of guidelines, and the impact of these on levels and patterns of AMU in food-producing animals and companion animals in order to inform the development and implementation of better voluntary approaches for reducing AMU in the animal health sector.

Methods: Databases including Science Direct and MEDLINE were searched for studies assessing the extent of implementation and impact of guidelines on levels and patterns of AMU in food-producing animals and companion animals. Additional searches using reference tracking, snowballing and grey literature were also performed. Quality of evidence and risk of bias assessment were conducted. A narrative synthesis approach was followed to assess and present the evidence gathered across eligible studies.

Results: A total of 784 studies were screened. Fourteen studies were deemed eligible for inclusion. All, apart from three, were conducted in Europe. Several voluntary guidelines on prudent AMU were referred to in these studies, mostly developed by international or regional bodies. There is limited evidence on the extent of implementation and the effectiveness of these guidelines in food-producing animals and companion animals. In food-producing animals, the quality of studies was deemed low as most were cross-sectional and based on convenience sampling. There were differences in uptake of prudent AMU guidelines including use of antimicrobial susceptibility testing (ASTs) and critically important antimicrobials (CIAs) among countries. Voluntary initiatives from levy bodies supporting farmers involving reduction and ban of use of CIAs (e.g. third generation cephalosporins), combined with changes in animal husbandry and farming practices, and improvement of vaccination strategies were deemed amongst the most effective in the swine (UK and Denmark) and poultry (UK) sectors, and to a lesser extent in the dairy cattle production sector (Denmark). There may be lessons to be learnt from these countries for more effective AMU reduction strategies. Nevertheless, there are still scarce data on the potential impact of voluntary interventions on animal health and welfare, and productivity. One of the few countries to have assessed the impact of the promotion of prudent use campaigns, on animal health and welfare, and productivity was Denmark which has recently reported that their impact was low in the short term according to surveillance data, though further assessments are required to assess impact in the long-term. There was even less evidence available for companion animals, and the quality of studies was lower; as consequence, these studies were not deemed suitable for the assessment of impact of prudent use recommendations and guidelines.

Conclusions: Prudent use guidelines are available in most European countries, at different levels: international (Europe-wide); national (countrywide or for members of associations); and local (e.g. at hospital level). In some countries like the UK, the livestock and poultry industries have taken the initiative to reduce the use of AMs by adopting national and international recommendations for the reduction of use the use of critically important antimicrobials. However, there is currently limited quantitative evidence of the impact of the recommendations voluntary interventions in AMU in both food-producing and companion animals, and, particularly, their impact on animal health and welfare,

and productivity. This is due to the lack of systematic assessment of surveillance data and of longitudinal studies to investigate the effectiveness of guidelines in changing antibiotic use in animal populations the promotion of prudent AMU. Targeted adoption of prudent use practices by farmers and veterinarians were reported to be an effective approach to reduce AMU, including CIAs in poultry, swine and dairy cattle.

Recommendations

- Further assessment of the impact of existing guidelines and voluntary initiatives should be promoted to address these gaps in knowledge. This could include analysis of existing surveillance data (as conducted in **Denmark**) collated at national level by government bodies, benchmarking data collated by the food and animal industry and clinical data from veterinary practices.
- Further studies that examine the underlying factors as to why certain countries achieve better uptake of prudent use guidelines – perhaps through the adoption of social sciences research methods, such as the Theory of Behaviour Change – may help to improve the effectiveness, acceptability and thus sustainability of the impact in the medium and long term of existing and new guidelines in countries or particular animal sectors where these would be beneficial.
- Continued provision and promotion of guidelines and relevant education to veterinarians at both under- and postgraduate levels is necessary to further improve uptake of responsible AMU and AST.
- Although this review indicated that CIAs were only used in small quantities in livestock production if compared to non-CIAs, further improvement in uptake of guidelines targeting CIAs are required to reduce prescriptions of these antimicrobials to preserve their therapeutic efficacy. Targeted interventions are needed to reduce the use of CIAs (third and fourth generation cephalosporins, fluoroquinolones) without support of ASTs in animal sectors such as dairy cattle production (dry cow therapy) and companion animals in order to maintain therapeutic efficacy of these substances.
- Where possible, interventions focused on improving animal husbandry and farm management practices, biosecurity and non-antimicrobial disease prevention and control measures should continue to be promoted at farm level. Animal mass treatments should be discouraged but may be required when a confirmed diagnosis has been obtained and under veterinary supervision. Furthermore, veterinarians should be involved in the education of farmers on responsible AMU.

Background

The use of antimicrobials (AMU) is one of the main risk factors associated with the occurrence and spread of antimicrobial resistance (AMR) in humans and animals (1). In both food-producing and companion animals, antimicrobials (AM) are used not only for therapeutic purposes, but also for prophylaxis and metaphylaxis purposes. Prophylaxis in veterinary medicine, is the administration of AMs to an individual animal or to a group of animals deemed to be at risk of infectious disease whilst metaphylaxis corresponds to the treatment with AMs of a group of still healthy animals when disease is already present in part of the population. The aim of metaphylaxis is to contain the spread of infection in animal populations (2).

The emergence and spread of AMR, particularly in Gram negative bacteria to AMs deemed as critically important (CIAs) to preservation of human health, poses a serious public health threat (3). So far, the focus and the bulk of the evidence for AMU and AMR has been in food-producing animals due to the scale of intensive farming systems, and the potential risk of transmission of AMR through the food chain and to the environment (4). Nevertheless, there is evidence that AMU is also associated with AMR in companion animals, and that through the close contact and social interactions with their owners, pets can act as reservoirs for AMR in the community (5).

The risk factors for the emergence and spread of AMR, and the need for measures to prevent and control infection in intensive farming and in veterinary practice are similar to those observed in human hospitals. These are AMU-intensive facilities, often with high animal and patient densities that enable spread of resistant bacteria and genetic traits in these populations (6). Misuse of AMs in animals may occur when: a) AMs are used to treat conditions where a bacterium is not the causative agent (e.g. viral infections, non-infectious diseases), and the risk of secondary bacterial infection is negligible; b) the chosen AM is ineffective in treating a specific condition; c) the incorrect dosage of AMs (i.e. dosage too low or too high) to combat infection is used; d) the incorrect frequency of dosage is selected (e.g. beta-lactam antibiotics that are time-dependent and therefore rely on timely dosing to maintain therapeutic concentrations in the serum); and, e) the duration of treatment is incorrect (i.e. too long or too short), among others (7).

A One Health approach is needed in order to tackle AMR, as it affects humans, animals and the environment. This has resulted in the development of international strategies for the containment of AMR (e.g., the European Union (EU) One Health Action Plan and the joint World Health Organization (WHO), World Animal Health Organization (OIE) and the Food and Agriculture Organization of the United Nations (FAO) strategy for the development of national action plans against AMR) (8, 9).

Many countries have statutory frameworks to license, regulate and restrict AMU in animal populations (10). Scandinavian countries were the first to implement both forms of mandatory initiative to reduce AMU due to their high awareness of AMR through surveillance in animal populations. With the notable exception of Sweden, Norway, Denmark and the Netherlands, other EU countries have only recently introduced mandatory AMU reduction targets. Sweden was the first country in Europe to implement a total ban on the use of AM growth promoters (AGPs) (that were used to increment feed conversion and productivity) n food-producing animals in 1986, as sub-therapeutic dosages of these were associated with the emergence of resistance to CIAs in human medicine (e.g. avoparcin was used as an AGP and associated with the emergence and spread of vancomycin-resistant *Enterococci* or VRE) (11). This ban was effective and had only a minor negative impact on animal health and productivity as it was combined with changes in farming and animal husbandry practices implemented to prevent gastrointestinal (GI) diseases (12). Other Nordic countries (Denmark, Norway) followed Sweden's example and also reported a considerable decrease in AMU for therapeutic purposes after the ban of AGPs (13). The rest of Europe –including the UK- followed suit in January 2006 (11). Antimicrobial growth promoters are still in use in many non-EU countries.

In Denmark, use of fluoroquinolones in animal populations is restricted and can only be used if supported by antimicrobial susceptibility testing (ASTs) since 2002 (10). Furthermore, in 2010, Denmark implemented the mandatory "Yellow Card" system in the pig sector targeting unnecessary AMU by farmers and veterinarians. This resulted in the reduction of the use of third and fourth generation cephalosporins by 99%[1] (14, 15). As a result, there has been a decrease in the overall AMU of 25% between 2009 and 2011 in Danish pigs (15). This reduction was mainly due to the decrease in the number of prescriptions of tetracyclines, macrolides and pleuromutilins for GI diseases through medicated feed or water, often for prophylactic or metaphylactic purposes and higher uptake of vaccination programmes (15).

The Netherlands also implemented a mandatory, stepwise reduction of overall AMU in food-producing animals of 20% in 2011 that was later increased to 50% in 2013. These measures were implemented by the Dutch government in response to the increase observed in multidrug resistant (MDR) bacteria [i.e., extended spectrum beta-lactamase producing *Enterobacteriaceae* or ESBLs in poultry and farm clones of Methicillin-resistant *Staphylococcus aureus* or livestock-associated methicillin-resistant *Staph. aureus* (LA-MRSA)] and the perceived risk to public health. This measure was effective in reducing AMU by 56% in food-producing animals (16) and a reduction on AMR has been observed in both food borne pathogenic and commensal bacteria in animals and foods from animal origin, according to surveillance data (17). In the UK, the Government set a target of achieving a 20% reduction of AMU in food-producing animals by 2018 as part of its five-year AMR Strategy, 2013-18. A reduction of 10% was obtained between 2014 and 2015 (18) but no assessment of the potential impact of this intervention on animal health and welfare and productivity has been undertaken.

In contrast with mandatory restrictions, there is less evidence of the effectiveness of voluntary approaches. Prudent AMU is defined by the European Commission as "*the use which benefits the patient while at the same time minimises the probability of adverse effects (including toxicity and the selection of pathogenic organisms, like Clostridium difficile) and the emergence and spread of AMR*" (19). Guidelines on prudent use of AMs and antimicrobial stewardship programmes have been developed in recent years at various levels in the human and veterinary sectors in order to prevent and control the spread of AMR (20). The effectiveness and other impacts of such voluntary interventions on the reduction of AMU in animals and on changing prescribing behaviours among AM end users (e.g., veterinarians, farmers and pet owners) has not yet been reviewed systematically. It is essential to bring together the evidence of implementation – reflected by compliance – and effectiveness of responsible AMU guidelines in curbing and changing behaviour by end users to assess whether these approaches are worth continuing and, indeed, whether particular efforts should be intensified. To address this need, a systematic review was conducted to assess the extent of implementation and impact of guidelines on levels and patterns of AMU in both food-producing and companion animals.

The review tackles the following research questions:

1. What are the current guidelines for prudent use available at international and national levels and who are the stakeholders involved?
2. What is the extent of implementation of the guidelines for prudent AMU? In which contexts are these more or less likely to be implemented and what are the main drivers (or barriers) associated with the success (failure) of implementation of these?
3. What is the impact of these guidelines for responsible AMU on the extent and patterns of overall AMU and use of CIAs in food-production and companion animals? Are AMs used according to

[1] Note that all reported percentages are rounded to full numbers.

recommendations and is excessive/ unnecessary AMU reduced or prevented in the animal populations of interest? If so, in what manner is AMU being reduced?

Methods

A systematic review of both grey and scientific literature was conducted to assess the extent of implementation and the impact of guidelines on levels and patterns of AMU in food-producing animals and companion animals. The research questions were used to define the PIO (Population, Intervention or Exposure, and Outcome) focus of the review. "Population" was described as food-producing animals (e.g., poultry, sheep, goats, cattle, calves, pigs including seafood produced in aquaculture systems) or companion animals (including exotic pets and horses). "Intervention or Exposure" was described as the implementation or adoption of prudent AMU guidelines, advice, recommendations from a particular competent authority, association or professional body. "Outcomes" of interest were defined as the level of implementation/uptake of these recommendations and/or assessment of changes in the frequency of and AMU patterns in companion and food-producing animal populations. The PIO guided the definition of the search terms of interest that were used to identify potentially eligible studies.

Scoping search

A scope search was conducted in PubMed to explore the range and volume of studies to refine the search criteria of the eventual review. For this purpose, wide search terms covering the theme of interest were used.

Eligibility criteria

Inclusion criteria

Studies in the following categories were considered eligible: a) food-producing animals including; ruminants (cattle, sheep, goats), pigs, poultry species (chicken, turkeys, ducks, etc), and seafood produced under intensive systems (i.e., fish or shellfish produced in aquaculture systems); b) companion animals including; dogs, cats, rabbits and exotic pets (birds, reptiles, small mammals). Horses were also considered as companion animals as this is usually how these animals are kept in the UK, although under EU legislation, horses are categorised as food-producing animals; c) reports, reviews, systematic reviews, meta-analyses, risk analysis and mathematical modelling studies; d) scientific expert opinion reports (e.g. European Food Safety Authority (EFSA), European Medicines Agency (EMA)) deemed relevant to the research questions; and e) observational (e.g. case-control, prospective and retrospective cohort and cross-sectional studies) and experimental studies (e.g. randomised controlled trials, evaluation of interventions at farm level). When a reduction of AMU was observed due to implementation of guidelines, or prudent use recommendations, data were also collated for potential impact observed in animal health and welfare and productivity if provided in eligible studies. At the preliminary stage, only abstracts available in English were considered. If studies reported in other languages (German, Spanish, Portuguese, Italian and French) were deemed to be relevant after careful evaluation of the abstracts, these were considered after a native speaking colleague had been identified to help with translation, and/or eligibility assessment and data extraction.

Exclusion criteria

Studies in the following categories were considered ineligible if: a) the evaluation of voluntary interventions in any animal sector (farm or veterinary practice) –not by existing guidelines, studies in which the impact assessed focused on trends and patterns of AMR in animals, the environment and/or humans; b) interventions focused solely on assessing the impact of voluntary interventions on AMU levels and patterns in humans; and c) focused solely or mainly on the impact of changes in national and/or international legislation implementing statutory bans on specific antimicrobial molecules or groups and/or mandatory reduction of AMU levels in animals.

Search strategy

The search strategy used science database search engines, grey literature websites (e.g., national and international government institutes, industry levy bodies), citation tracking and snowballing to identify potentially relevant studies (**Table 1**).

Table 1- Study search strategy.

Category	Sources
Scientific databases	• Science Direct (http://www.sciencedirect.com/) • PubMed (http://www.ncbi.nlm.nih.gov/pubmed/)
Scientific journals	• Veterinary Record • Journal of Antimicrobial Chemotherapy (JAC)
Reference tracking	Reference lists of all studies selected for inclusion will be searched to identify further relevant studies through snowballing
Grey literature	• http://ec.europa.eu/ (Portal of the European Commission) • http://apua.org/ (APUA- Alliance for the Prudent Use of Antibiotics) • http://www.ruma.org.uk/ (RUMA - Responsible Use of Medicines in Agriculture Alliance) • http://www.epruma.eu/ (EPRUMA - European Platform for the Responsible Use of Medicines in Animals) • https://www.bva.co.uk/ (BVA - British Veterinary Association) • http://www.rcvs.org.uk/home/ (RCVS - Royal College of Veterinary Surgeons) • http://www.oie.int/ (OIE - World Organisation for Animal Health) • http://www.fve.org/ (FVE - Federations of Veterinarians of Europe) • www.phe.gov.uk (Public Health England, UK) • www.efsa.europa.eu (European Food Safety Authority) • www.ema.europa.eu (European Medicines Agency) • www.fao.org (Food and Agriculture Organisation of the United Nations) • http://www.vmd.defra.gov.uk (Veterinary Medicines Directorate, UK) • http://www.fda.gov/ (FDA/CDC, USDA, USA)

The search criteria were piloted by a single researcher to generate the final search strategy**.** All literature searches and criteria used were documented to allow for replication of the method. Free text searches covered both title and abstract, when the latter was available. Searches included MeSH (Medical Subject Headings) and free text terms that covered PIO criteria. The MeSH and free terms were combined with the Boolean operator OR and/or could be combined with AND, at a later stage of the search process, as; population AND intervention AND outcomes (PIO). The combinations of search terms across the PIO groups were extracted separately to produce the final list of search hits from each database. The search terms used were as follows: Search (((((antimicrobial[Title/Abstract]) OR antibiotic[Title/Abstract]) AND (use[Title/Abstract] OR usage[Title/Abstract]))) AND (((impact[Title/Abstract]) OR effect*[Title/Abstract]) OR effective*[Title/Abstract])) AND ((guide*[Title/Abstract]) OR recommendation[Title/Abstract]). The search was conducted in March 2017 and included all potentially eligible studies published until that date. Search interfaces with limited functionality (e.g. those which support single line searches only or limited number of search terms) were initially searched using broad "prudent antimicrobial use guidelines" terms followed by longer search strings or by using "advanced search" modalities if these were available in the interfaces of the databases and websites selected.

Study screening

All search hits were imported into reference management software (i.e., Endnote) to collate the identified literature. All duplicates were removed prior to the first stage sifting process. Identified studies and other relevant literature were screened for eligibility by one member of the team using a three-stage sifting approach based in turn on the title, abstract and full text. A random check of the

excluded studies was conducted by a second reviewer and any discrepancies observed were discussed amongst all reviewers. The numbers of documents identified and excluded were recorded at each stage and are presented in the PRISMA diagram in Figure 1. Reasons for exclusion were recorded during the process.

Data extraction, synthesis and assessment of risk of bias

An Excel template for data extraction was prepared by the research team based on the PIO. The template was piloted prior to implementation. The revised template was then used by the reviewers to collate the relevant data used for the preparation of the review. This enabled the assessment of accuracy and consistency of data extracted by a second reviewer. Study characteristics (e.g. study design, interventions evaluated, sample size, sampling methods amongst others) and outcome(s) of interest were extracted and summarised accordingly. To synthesise the data extracted and evaluate their quality a narrative approach was used according to the framework described by Sargent and O'Connor (21). Assessment of risk of bias as a measure of study quality in non-randomised studies was conducted following the criteria described by Sterne and colleagues (22).

Results

Search results

Searches retrieved a total of 784 studies. Additional searches using snowballing retrieved 23 studies. After removing duplicates, 734 studies were submitted to the three-stage screening process; 34 were met the criteria for full text screening. A total of 14 studies were deemed eligible for inclusion in the systematic review. (**Figure 1**).

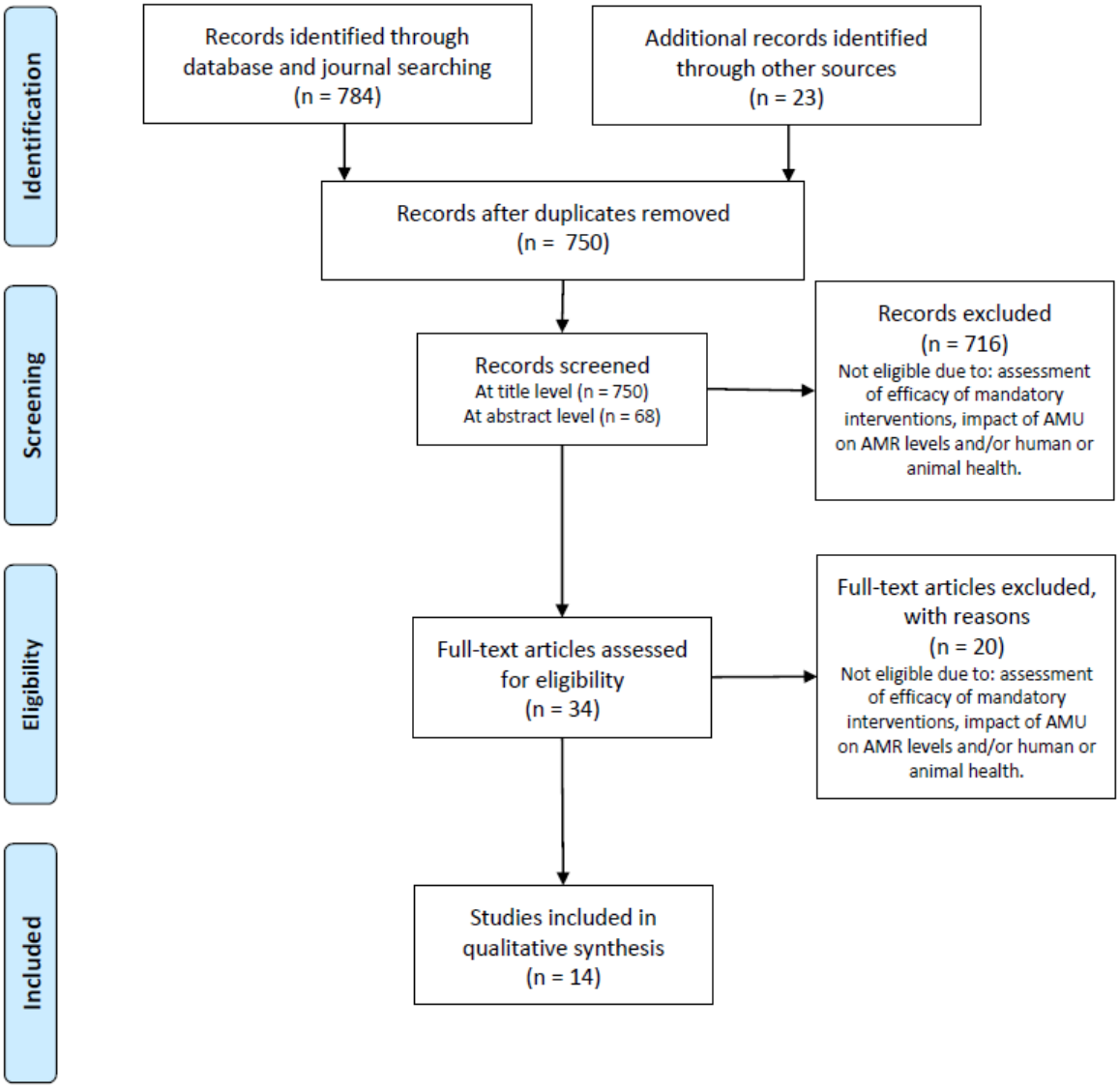

Figure 1- Strategy for screening of studies to be included in the systematic review - adapted from Liberati et al (2009).

Study characteristics

Characteristics of the 14 studies are summarized in **Tables 2** and **3**. The reviewed studies were published between 1999 and 2017. Most studies were conducted in Europe (n= 11) with two studies in Australia and one in the United States (**Table 2**). Some of the studies addressed impact of guidelines in more than one country. The European countries included were; United Kingdom (n= 4 studies), Belgium (n= 3), Germany (n= 2), Switzerland (n= 2), Denmark (n= 2), France (n= 2), Finland (n= 2), Ireland (n= 1), Italy (n= 2), Netherlands (n= 1), Spain (n= 2), Sweden (n= 2), and Norway (n= 1).

Eligible studies were linked to varying animal populations and settings (farm, veterinary practice). Animal populations included were cattle (n=2), dogs and cats (n=5), pigs (n=2), horses (n=3), and mixed (food-producing and companion animals, n=3).

Table 2 - Characteristics of eligible studies (NS = not specified, NA = not applicable).

Study	Country	Study design	Relevant animal population and setting	Sampling unit (number of units)	Sampling approach
Bager et al, 2017	Denmark	Retrospective cohort study (surveillance data)	General/ farm and veterinary practice settings	Demographic data provided as part of surveillance report	Random, probabilistic
Bertulat et al. 2015	Germany	Cross-sectional survey	Cattle/ farm setting	Farmers	Convenience, non-probabilistic
De Bryine et al. 2013	Europe (Belgium, Czech Republic, France, Germany, Spain, Sweden, United Kingdom, Austria, Cyprus, Finland, Latvia, Lithuania, Portugal, Slovakia, Switzerland, Denmark, Ireland, Norway, Italy, Netherlands, Poland, Romania, Iceland, Liechtenstein, Luxembourg, Bulgaria, Estonia, Greece, Hungary, Malta, Slovenia)	Cross-sectional survey	Mixed/ veterinary practice settings	Veterinarians (n=3,004)	Convenience, non-probabilistic
De Bryine et al. 2014	Europe (Belgium, France, Germany, Spain, Sweden, United Kingdom)	Cross-sectional survey	Cattle, pigs, horses, cats, and dogs/ veterinary practice settings	Veterinarians (n=4,500)	Convenience, non-probabilistic
EMA and EFSA, 2017	Belgium, Denmark, France, Netherlands, United Kingdom, Canada	Scientific opinion report	Food-producing animals	Veterinarians and farmers	NA
Escher et al. 2011	Italy	Cross-sectional survey	Dogs and cats/ veterinary practice	Veterinary practice (n=1); (Clinical records of dogs and cats, n=5,804)	Random, probabilistic
Fowler et al. 2016	United States	Cross-sectional survey	Small animals, food-producing animals, zoo animals and wildlife /veterinary practice	Veterinarians (n=300)	Convenience, non-probabilistic
Hardefelt et al, 2017	Australia	Cross-sectional survey	Dogs and cats/ veterinary practice	Veterinarians (n= 721)	Random, probabilistic
Hardefelt et al, 2018	Australia	Cross-sectional survey	Mixed/ veterinary practice	Veterinarians (n= 184)	Convenience, non-probabilistic
Knights et al. 2012	United Kingdom	Cross-sectional survey	Dogs and cats/veterinary practice	Veterinarians (n=2,951)	Convenience, non-probabilistic
Rantala et al. 2004	Finland	Cross sectional survey	Dogs and cats/ veterinary practice	Veterinarians (n=NS)	Convenience, non-probabilistic

Study	Country	Study design	Relevant animal population and setting	Sampling unit (number of units)	Sampling approach
Regula et al. 2009	Switzerland	Cross sectional survey	Mixed/ veterinary practice	Vet practices (electronic records) (n=8)	Convenience, non-probabilistic
Sarrazin et al, 2017	Belgium	Longitudinal study	Dogs and cats/ veterinary practice	Veterinarians & veterinary practices (n= 14)	Convenience, non-probabilistic
Schwechler et al. 2016	Germany, Austria, Switzerland	Cross sectional survey	Equine/ veterinary practice	Veterinarians (n=1,227)	Convenience, non-probabilistic

The most common type of study design was cross-sectional survey (n=11), followed by a retrospective cohort study that assessed surveillance data, one longitudinal study and one scientific opinion report (**Table 2**). Nine of the eligible studies applied a convenience, non-probabilistic sampling approach, whilst only three studies followed a probabilistic sampling approach. The scientific opinion assessed the impact of mandatory and/or guidelines and recommendations in AMU and AMR across several EU Member States.

Table 3- Guidelines for prudent AMU available in countries covered in eligible studies

Study	Country	Intervention(s)/ AMU guidelines assessed	Study aims
Bager et al, 2017	Denmark	Voluntary ban on the use of 3rd and 4th generation cephalosporins by the industry in pigs (implemented in July 2010) and in dairy cattle (implemented in July 2014) Voluntary reduction on the use of antimicrobials for mastitis treatment in Dairy cows by the industry between 2014 and 2016 by 20% Voluntary introduction of hybrid breed of turkeys by the industry that was less likely to develop arthritis Improvement of vaccination strategy against Turkey Rhino Tracheitis Danish National Antimicrobial Treatment Guidelines by the Danish Small Animal Veterinary Association (implemented in November 2012) Introduction of new vaccines and improvement of vaccination strategies by the industry in aquaculture systems	The surveillance data was used to assess the impact of voluntary interventions across on the overall and patterns of AMU across different animal populations (pigs, dairy cattle, turkeys, companion animals and fish).
Bertulat et al. 2015	Germany	Prudent use recommendations/ guidelines issued by FVE.	Survey of dry-off practices practiced by dairy farmers, including antimicrobial dry cow therapy.
De Bryine et al. 2013	Europe (Belgium, Czech Republic, France, Germany, Spain, Sweden, United Kingdom, Austria, Cyprus, Finland, Latvia, Lithuania, Portugal, Slovakia, Switzerland, Denmark, Ireland,	Numerous strategies, recommendations and treatment guidelines on responsible AMU developed by a variety of national, European and international bodies (e.g., EPRUMA 2008, FVE 2012, OIE 2012).	Identification of factors influencing antimicrobial prescribing by veterinarians in Europe.

Study	Country	Intervention(s)/ AMU guidelines assessed	Study aims
	Norway, Italy, Netherlands, Poland, Romania, Iceland, Liechtenstein, Luxembourg, Bulgaria, Estonia, Greece, Hungary, Malta and Slovenia)		
De Bryine et al. 2014	Europe (Belgium, France, Germany, Spain, Sweden, United Kingdom)	Best practice guidance for cattle such as the RUMA guidelines.	In this study, a survey of veterinary practitioners from European countries was conducted to determine the indications for which vets most commonly prescribe antimicrobials according to species and indication.
EMA & EFSA 2017	Belgium, Denmark, France, Netherlands, United Kingdom, Canada	The intervention evaluated was the EC Prudent Use of Antimicrobials in Veterinary Medicine (PUAVM) Guidelines advice that CIAs should only be used when and where the veterinarian has determined that no effective non-CIA is available based on AST and relevant epidemiological data.	Impact of voluntary and statutory interventions in AMU and/or AMR levels.
Escher et al. 2011	Italy	Prudent use recommendations/ guidelines issued by FVE.	Investigation of pattern of AMU and how these were prescribed in the context of prudent use recommendations/ guidelines issued by FVE.
Fowler et al. 2016	United States	American College of Veterinary Internal Medicine (ACVIM) guidelines for responsible AMU.	Survey conducted to assess factors influencing veterinarians' to use ASTs to guide AMU.
Hardefelt et al, 2017	Australia	National Australian Infectious Disease Advisory Panel (AIDAP 2013) and British Small Animal Veterinary Association (BSAVA, 2016) guidelines.	The aim of this study was to investigate self-reported antimicrobial use in a range of surgical conditions in small animal practice in Australia and to assess compliance with AIDAP (Australian Infectious Disease Advisory Panel, 2013) and BSAVA (British Small Animal Veterinary Association 2016) guidelines.
Hardefelt et al, 2018	Australia	Antimicrobial stewardship programmes existing in Australia for veterinary practice.	To assess veterinarians' attitudes to AMR and AMU in animals in Australia. Assessment of extent to which AM stewardship programmes are implemented in veterinary practice in Australia.
Knights et al. 2012	United Kingdom	Recommendations on antimicrobial prophylaxis assessed included: a) restriction of AMU to procedures with a relatively high rate of septic complications or in which the consequences of infection are especially serious; b) use of narrow spectrum antimicrobial substances rather than broad-spectrum, as the former are effective against major anticipated contaminating bacterial species; and, d) the administration of antimicrobials sufficiently in advance of the operation and by such a route of administration	This study examined the attitudes of veterinarians regarding the use of perioperative antimicrobials in cats and dogs in first opinion practice in the UK.

Study	Country	Intervention(s)/ AMU guidelines assessed	Study aims
		that effective tissue concentrations are reached before and maintained during surgery.	
Rantala et al. 2004	Finland	Guidelines for AMU of infectious diseases in dogs by the Finish Ministry of Agriculture and Forest Affairs. These guidelines included "infection- 1st choice antimicrobial/ 2nd choice antimicrobial" as follows: (1) Pyoderma- macrolide or lincosamides, trimethoprim-sulphonamides/ 1st generation cephalosporins; (2) Wound infection- Penicillin V/ beta-lactam and metronidazole; (3) Acute gastrointestinal conditions (vomiting or diarrhoea)- no antimicrobial treatment/ trimethoprim-sulphonamides, metronidazole or amoxicillin-clavulanate; (4) Acute UTI- trimethoprim-sulphonamides/ fluoroquinolones; and (5) Acute bronchitis 'kennel cough'- no antimicrobial treatment/ trimethoprim-sulphonamides, tetra- or doxycycline. Guidelines for AMU prophylaxis in surgical procedures by the internal Hygiene Committee of the Finnish Veterinary Teaching Hospital, which included: a) antimicrobial selected according to surgical site and, b) antimicrobial to be administered intravenously 30 minutes prior to surgery.	In this study, prescriptions of antimicrobial drugs for the treatment of common infectious diseases in dogs at the Finnish Veterinary Teaching Hospital was searched to determine to what extent national guidelines were followed.
Regula et al. 2009	Switzerland	FVE guidelines: a) antimicrobial class used for treatment- an appropriate narrow-spectrum agent should be selected in preference to a broad-spectrum agent; preference should be given to antimicrobial classes with minor relevance to human medicine (non-CIA); b) avoid usage of combinations of antimicrobial substances; and, c) dosage should be in accordance with the recommended dosage regimen to avoid administration of sub-therapeutic doses.	The authors analysed antimicrobial prescriptions by veterinarians to evaluate the appropriateness of AMU compared with published FVE guidelines.
Sarrazin et al, 2017	Belgium	National guidelines for use of antimicrobials produced by the Belgian centre of expertise on Antimicrobial Consumption and Resistance in Animals (AMCRA) in 2014. These guidelines classified some antimicrobial drugs as critically important and highest priority CIAs based on the lists provided by the WHO (2011) and OIE (2015).	The aim of this study was to evaluate the impact of introducing these antimicrobial use guidelines on the prescription habits of veterinarians in small animal practices in Flanders. The hypotheses were that veterinarians would refrain from the prescription of AMs, if not needed according to the guidelines, and that they would prescribe less CIAs.
Schwechler et al. 2016	Germany, Austria, Switzerland	Guidelines on licensed dosages for antimicrobials retrieved from each country's licensing body (Swissmedic—Switzerland, Federal ministry for Health—Germany, and Austrian Agency for Health and Food Safety—	The antimicrobial prescribing practices of equine practitioners in Germany, Austria and Switzerland were evaluated by comparing to licensed dosages for antimicrobials for each

Study	Country	Intervention(s)/ AMU guidelines assessed	Study aims
		Austria). BEVA guidance for AMU was selected as a representative for current scientific recommendations.	country which were retrieved from each country's licensing body. Dosages prescribed by veterinarians were compared with the above to determine under dosing (±10%).

Assessment of risk of bias

Risk of bias was assessed for 13 of the 14 eligible studies, as one of the studies was a scientific opinion report from EFSA assessing impact of both mandatory interventions and guidelines in Member States and therefore was not deemed eligible for this type of assessment (23) (**Table 4**). Overall, risk of bias due to confounding, selection of participants, deviation from intended interventions, and bias in measurement of outcomes were deemed moderate as the majority of the eligible studies were cross-sectional surveys and followed a convenience sampling approach, which made the evaluation of the impact of interventions more difficult. Bias in the classification of interventions was considered low in all eligible studies.

Table 4 - Risk of bias assessment for all studies included (in alphabetical order of the first author). NA- Not Applicable; N- No; NI- No Information; PN- Probably Not; PY- Probably Yes; Y- Yes.

Study ID	Pre-intervention		At intervention	Post- intervention			
	Bias due to confounding	Bias in the selection of participants	Bias in the classification of interventions	Bias due to deviation from intended interventions	Bias due to missing data	Bias in measurement of outcomes	Bias in selection of the reported result(s)/ outcome(s)
Bager et al, 2017	1.1= N Risk of bias judgement = low risk	2.1 = N, 2.4 = N, 2.5=NA. Risk of bias judgement =	3.1=NA, 3.2= NA, 3.3 =N, Risk of bias judgement = low risk	4.3 =NA, 4.4 =Y, 4.5 =Y. Risk of bias judgement = low risk	5.1 =PY, 5.2=NA, 5.3=NA, 5.5=Y, Risk of bias judgement = low risk	6.1=N, 6.2=Y, 6.3=NA, 6.4=N, Risk of bias judgement = low risk	7.1=N, 7.2=N & 7.3=N, Risk of bias judgement = low risk
Bertulat et al 2015.	1.1=Y; 1.2=N; 1.4=N; 1.6=N; 1.7=N Risk of bias judgement = Serious risk	2.1=NA, 2.4=NA. Risk of bias judgement = moderate risk	3.1=NA, 3.2 = NA, 3.3 = NA. Risk of bias judgement = low risk	4.3=NA, 4.4=PY, 4.5=NI. Risk of bias judgement = moderate risk	5.1 = N, 5.2=N, 5.3=N, 5.4=NA, 5.5=NI, Risk of bias judgement = moderate risk	6.1=PN, 6.2=N, 6.3=NA, 6.4=PY. Risk of bias judgement = moderate risk	7.1=PN & 7.2=PN & 7.3=PN. Risk of bias judgement = low risk
De Bryine et al 2013	1.1=Y; 1.2=N; 1.4=N; 1.6=N; 1.7=N Risk of bias judgement = Serious risk	2.1=NA, 2.4=NA. Risk of bias judgement = moderate risk	3.1=NA, 3.2 = NA, 3.3 = NA. Risk of bias judgement =low risk	4.3=NA, 4.4=PY, 4.5=NI. Risk of bias judgement = moderate risk	5.1 = N, 5.2=N, 5.3=N, 5.4=NA, 5.5=NI, Risk of bias judgement =	6.1=PN, 6.2=N, 6.3=NA, 6.4=PY, Risk of bias judgement = moderate risk	7.1=PN & 7.2=PN & 7.3=PN, Risk of bias judgement = low risk

Study ID	Pre-intervention		At intervention	Post- intervention			
	Bias due to confounding	Bias in the selection of participants	Bias in the classification of interventions	Bias due to deviation from intended interventions	Bias due to missing data	Bias in measurement of outcomes	Bias in selection of the reported result(s)/ outcome(s)
					moderate risk		
De Bryine et al 2014.	1.1=Y; 1.2=N; 1.4=N; 1.6=N; 1.7=N Risk of bias judgement = Serious risk	2.1=NA, 2.4=NA. Risk of bias judgement = moderate risk	3.1=NA, 3.2 = NA, 3.3 = NA. Risk of bias judgement = low risk	4.3=NA, 4.4=PY, 4.5=NI. Risk of bias judgement = moderate risk	5.1 = N, 5.2=N, 5.3=N, 5.4=NA, 5.5=NI, Risk of bias judgement = moderate risk	6.1=PN, 6.2=N, 6.3=NA, 6.4=PY. Risk of bias judgement = moderate risk	7.1=PN & 7.2=PN & 7.3=PN. Risk of bias judgement = low risk
Escher et al 2011.	1.1=PN. Risk of bias judgement = low risk	2.1=N, 2.4=NA. Risk of bias judgement = low risk	3.1=Y, 3.2 = Y, 3.3 = NA. Risk of bias judgement = low risk	4.3=NA, 4.4=Y, 4.5=NI. Risk of bias judgement = low risk	5.1 = N, 5.2=N, 5.3=NI, 5.4=NA, 5.5=NI. Risk of bias judgement = moderate risk	6.1=PN, 6.2=N, 6.3=NA, 6.4=PN. Risk of bias judgement = low risk	7.1=PN & 7.2=PN & 7.3=PN. Risk of bias = low risk
Fowler et al 2016.	1.1=PN. Risk of bias judgement = low risk	2.1=N, 2.4=NA, 2.5=NA. Risk of bias judgement = low risk	3.1=Y, 3.2 =Y, 3.3 = NA. Risk of bias judgement = low risk	4.3=NA, 4.4=Y, 4.5=NA. Risk of bias judgement = low risk	5.1 = N, 5.2=N, 5.3=N, 5.4=NA, 5.5=NI. Risk of bias judgement = moderate risk	6.1=PN, 6.2=N, 6.3=NA, 6.4=PN. Risk of bias judgement = low risk	7.1=PN, 7.2=PN, 7.3=PN. Risk of bias judgement = low risk
Hardefelt et al, 2017	1.1=Y; 1.2=N; 1.4=N; 1.6=N; 1.7=N Risk of bias judgement = Serious risk	2.1=N, 2.4=NA. Risk of bias judgement = low risk	3.1 & 3.2 = NA, 3.3 = NA. Risk of bias judgement = low risk	4.3=NA, 4.4=PY, 4.5=NI. Risk of bias judgement = moderate risk	5.1 = N, 5.2=N, 5.3=N, 5.4=NA, 5.5=NI, Risk of bias judgement = moderate risk	6.1=PN, 6.2=N, 6.3=NA, 6.4=PY, Risk of bias judgement = moderate risk	7.1 & 7.2 & 7.3=PN, Risk of bias judgement = low risk
Hardefelt et al, 2018	1.1=Y; 1.2=N; 1.4=N; 1.6=N; 1.7=N Risk of bias judgement = Serious risk	2.1=N, 2.4=NA. Risk of bias judgement = low risk	3.1 & 3.2 = NA, 3.3 = NA. Risk of bias judgement = low risk	4.3=NA, 4.4=PY, 4.5=NI. Risk of bias judgement = moderate risk	5.1 = N, 5.2=N, 5.3=N, 5.4=NA, 5.5=NI, Risk of bias judgement = moderate risk	6.1=PN, 6.2=N, 6.3=NA, 6.4=PY. Risk of bias judgement = moderate risk	7.1 & 7.2 & 7.3=PN. Risk of bias judgement = low risk

Study ID	Pre-intervention		At intervention	Post- intervention			
	Bias due to confounding	Bias in the selection of participants	Bias in the classification of interventions	Bias due to deviation from intended interventions	Bias due to missing data	Bias in measurement of outcomes	Bias in selection of the reported result(s)/ outcome(s)
Knights et al 2012.	1.1=Y; 1.2=N; 1.4=N; 1.6=N; 1.7=N Risk of bias judgement = Serious risk	2.1=N, 2.4=NA. Risk of bias judgement = low risk	3.1 & 3.2 = NA, 3.3 = NA. Risk of bias judgement = low risk	4.3=NA, 4.4=PY, 4.5=NI. Risk of bias judgement = moderate risk	5.1 = N, 5.2=N, 5.3=N, 5.4=NA, 5.5=NI, Risk of bias judgement = moderate risk	6.1=NA, 6.2=PN, 6.3=NA, 6.4=PY. Risk of bias judgement = moderate risk	7.1=PN, 7.2=PN, 7.3=PN. Risk of bias judgement = low risk
Rantala et al 2004.	1.1=PN. Risk of bias judgement = low risk	2.1=N, 2.4=NA, 2.5=NI. Risk of bias judgement = low risk	3.1 & 3.2 = Y, 3.3 = PN. Risk of bias judgement = low risk	4.3=NA, 4.4=Y, 4.5=NA. Risk of bias judgement = low risk	5.1 =Y, 5.2=N, 5.3=N, 5.4=NA, 5.5=NI. Risk of bias judgement = low risk	6.1=N, 6.2=N, 6.3=NA, 6.4=PN. Risk of bias judgement = low risk	7.1=N, 7.2=N, 7.3=N. Risk of bias judgement = low risk
Regula et al 2009.	1.1=PN. Risk of bias judgement = low risk	2.1=N, 2.4=NA, 2.5=NI. Risk of bias judgement = low risk	3.1 & 3.2 = Y, 3.3 = PN. Risk of bias judgement = low risk	4.3=NA, 4.4=Y, 4.5=NA. Risk of bias judgement = low risk	5.1 =Y, 5.2=N, 5.3=Y, 5.4=NA, 5.5=NI. Risk of bias judgement = serious risk	6.1=N, 6.2=PN, 6.3=NA, 6.4=NI. Risk of bias judgement = low risk	7.1=PN, 7.2=N, 7.3=Y. Risk of bias judgement = moderate risk
Sarrazin et al, 2017	1.1=N. Risk of bias judgement = Low risk	2.1=N, 2.4=Y. Risk of bias judgement = low risk	3.1=Y, 3.2 = Y, 3.3 = N. Risk of bias judgement = low risk	4.3=NA, 4.4=PY, 4.5=PN, 4.6=Y. Risk of bias judgement = low risk	5.1 = N, 5.2=NA, 5.3=Y, 5.4=NA, 5.5=PY, Risk of bias judgement = moderate risk	6.1=PN, 6.2=N, 6.3=NA, 6.4=N. Risk of bias judgement = low risk	7.1=N, 7.2=N, 7.3=N. Risk of bias judgement = low risk
Schwechler et al 2016.	1.1=PN. Risk of bias judgement = low risk	2.1=N, 2.4=NA, 2.5=PY. Risk of bias judgement = low risk	3.1 & 3.2 = Y, 3.3 = PN. Risk of bias judgement = low risk	4.3=NA, 4.4=Y, 4.5=NA. Risk of bias judgement = low risk	5.1 =N, 5.2=N, 5.3=Y, 5.4=NA, 5.5=NI. Risk of bias judgement = serious risk	6.1=N, 6.2=N, 6.3=NA, 6.4=PN. Risk of bias judgement = low risk	7.1=PN, 7.2=Y, 7.3=Y. Risk of bias judgement = serious risk

Bias due to missing data was considered moderate in all but two studies, for which risk was deemed serious due to the extent of missing data reported by the authors (27, 28). In the two studies by De

Bryine et al., risk of bias was considered moderate because analysis for some of the participating countries was not conducted due to small sample size (24, 25). The authors of these studies reduced the risk of bias by only conducting analysis at country level when the number of responses was considered to be sufficiently large to be meaningful, nationally representative, and when sufficient data (at least 50 responses per species per country) were available, though no sample size calculation was provided. Consequently, analysis was conducted for only six of the 25 European countries included in the survey (24, 25). In Escher et al., risk of bias due to missing data was considered moderate because for some observations, data on AST were missing and so this variable was not assessed thoroughly (e.g. to assess if chosen antimicrobial was based on ASTs, in compliance with guidelines) (26). In Fowler et al., risk of bias due to missing data was considered moderate because for some outcome parameters of interest (e.g., AST), data were not available for all participants, and it was not clear how risk of bias was dealt with in the analysis (29). In the study by Regula et al., bias was considered serious because 1,579 records were excluded from the analysis due to missing information on animal species or age group treated (27). In Schwechler et al., bias due to missing data was also considered serious because outcome data were not available for all participants; analyses were not performed for macrolides as the number of respondents using these antimicrobials was too low (30).

Bias in selection of the reported results or outcome was considered moderate in two studies (27) and considered serious in another study (28). In Regula et al. (2009), bias in selection of the reported results was considered moderate because exclusion of some groups of participants might have had an effect on reported estimates; 1,579 records were excluded from the analysis due to missing information on animal species or age group treated. In Schwechler et al. (2016), bias in selection of the reported results was considered serious because there were incomplete data for some variables; analyses were not performed for macrolides as the number of respondents using these antimicrobials was too low (30).

Guidelines for prudent use

The reviewed studies cited guidelines for prudent use implemented at national and international (e.g. EU) levels (**Table 3**). Guidelines for prudent use developed in Europe were the most widely cited by eligible studies in as many as 32 European countries. The guidelines available at European level were developed by various bodies including the Federation of Veterinarians of Europe (FVE) (24-27), the European Platform for the Responsible Use of Medicines in Animals (EPRUMA) (24, 25), the UK's Responsible Use of Medicines in Agriculture Alliance (RUMA) (24, 25), European Commission's guidelines for responsible use of veterinary medicines 2011 (24, 25), European Medicines Agency (EMA) and European Food Safety Authority (EFSA) joint scientific opinion on measures to reduce the need to use antimicrobial agents in animal husbandry in the European Union, and the resulting impacts on food safety (RONAFA) (23) and World Organization for Animal Health (OIE) (24, 25). Guidelines available at national level were reported in Finland (31), Germany (28), Austria (28), Switzerland (28) and the US (29).

The EPRUMA's guidelines were first published in 2008 and were aimed at farm veterinarians (32). The revised EPRUMA guidelines (2018) provide a more detailed approach on how to implement responsible AMU for further optimisation of animal health, including guidance on indoor and free-range production, housing, biosecurity and nutrition, amongst others (33).

The FVE 2012 guidelines are targeted to veterinarians across all animal sectors and provide advice for responsible AMU such that: a) prescription of AMs should be guided by findings from clinical examination and diagnosis; b) veterinarians should work closely together with clients to minimize and stop the need for AMs; c) there should be optimisation of use of diagnostic tests, including ASTs, as much as possible; d) AMs should be used correctly, with special attention to new and CIAs as defined by WHO (3); e) veterinarians should avoid off-label use whenever possible; f) veterinarians should

report their prescription data to the national competent authorities if required; and, g) veterinarians should report any adverse effects due to AMU (34).

Previous OIE guidelines focused mainly on promoting responsible AMU in veterinary medicine (35), whilst the revised guidelines now also include a resolution on AMU and were prepared to support the global action plan on AMR that the WHO developed in collaboration with the Food and Agriculture Organization of the United Nations (FAO) and the OIE (35). The resolution focused on combating AMR and promoting the prudent AMU in animals, and resulted in eleven recommendations including: a) the OIE will continue to develop and update standards and guidelines related to AMU and AMR including updating regularly the OIE's List of Antimicrobial Agents of Veterinary Importance; and, b) OIE Member Countries will be required to set up an official harmonised national system, based on OIE standards, for the surveillance of AMR and the collection of data on the AMU in food-producing animals, and actively participate in the development of the OIE global database (35).

The UK's RUMA guidelines focus on the responsible AMU in livestock production, and range from application and responsibilities of the farmer and veterinarian, to strategies for reduced AMU for poultry and game (36), pig (37), cattle (dry cow management, cattle production) (38, 39), sheep (40) and fish farmers (41).

In Belgium, small animal veterinarians were requested to adopt the guidelines for use of antimicrobials produced by the Belgian centre of expertise on Antimicrobial Consumption and Resistance in Animals (AMCRA) in 2014 (42), whilst in Denmark, surveillance data for AMU in dogs and cats between 2012 and 2016 were analysed to assess the impact of the National Antimicrobial Treatment guidelines by the Danish Small Animal Veterinary Association (DSAVA) implemented in November 2012 (43) .

Extent of reported evidence of implementation of general AMU guidelines

Veterinarians in Nordic countries (**Denmark, Sweden**) were found to be the most compliant with guidelines and in taking voluntary initiatives to reduce unnecessary AMU (24, 43). This may be due to the high level of awareness of AMR, and its potential impact on animal and public health. Implementation of recommendations on use of ASTs was variable across countries and animal sectors (24, 28).

Limited implementation of existing recommendations for AM prophylaxis was reported in a postal survey study with 1,121 participants on veterinarian attitudes to the use of perioperative antimicrobials in cats and dogs in first opinion veterinary practice in the **UK** (44). These recommendations included: limitation of AMU to procedures with a relatively high rate of septic complications or in which the consequences of infection are deemed to be especially serious; use of narrow spectrum AMs effective against major anticipated contaminating bacterial strains; and, the administration of AMs sufficiently in advance of the surgical procedure and by such a mode of administration that effective tissue concentrations are reached prior and for the whole duration of the surgery. Findings indicated that participants often used AMs in clean surgeries and clean-contaminated surgeries which contravened recommendations, but used AMs more frequently in contaminated surgeries and dirty surgeries, in compliance existing recommendations (44). Beta-lactam (both beta-lactamase-resistant and beta-lactamase-susceptible beta-lactams) AMs were the most commonly used AM class; from these, potentiated amoxicillin was the most commonly substance selected by participating veterinarians. Other classes of AMs reportedly used to a lesser extent by participating veterinarians were fluoroquinolones (CIAs), lincosamides and nitroimidazoles. Findings indicated that improvements could be made with respect to timing of administration, duration and choice of AM and a more prudent assessment of surgical cases requiring AM prophylaxis should be conducted by veterinarians as recommended by existing guidelines (44).

A low extent of implementation of guidelines on use of perioperative AMs was reported in two other eligible studies included in this review (31, 44, 45). In **Australia**, participating small animal veterinarians stated using routinely AMs in clean surgeries and clean-contaminated surgeries (against current recommendations) (45). Furthermore, in Australia compliance with existing AM prophylaxis guidelines was higher in veterinarians working with small animals than in veterinarians that worked in mixed practices but was lower in recent graduates, and in veterinarians working by themselves or with only another colleague. The same authors also found a low level of implementation (15%) and of awareness (15%) for the existence of antimicrobial stewardship programmes in the workplace (46). In the **US**, a survey by Fowler et al only 17% (26/203) and 36% (56/203) of veterinarians (most of which working in small animal and mixed practice) reported conducting ASTs always (>75% of the time) or often (at least 50% of time) to guide selection of AMs, according to the ACVMI recommendations, respectively (29).

The extent of implementation in clinical practice of guidelines issued by international (FVE, OIE) and/or national professional bodies for prudent use varied in the reviewed studies, and differed according to country of implementation and the type of AMU parameters assessed (e.g., prescription, amount used). In the study by De Bryine et al. (2013), uptake of international guidelines for responsible use developed by EPRUMA (32), FVE (34), and OIE (35) implemented in **selected European countries** was assessed through self-reporting by participating veterinarians in a questionnaire survey. The guidelines included recommendations to use AST to inform effective prescribing at an animal level. The survey suggested that respondents were likely to be implementing and following these recommendations in everyday practice, though this was not further explored by the authors of this study. The extrapolation of these findings to other European countries should be made with caution because findings might have been limited by selection bias and small sample size; there were only 3,004 responses from 25 European countries, representing 1.5% of active veterinarians in Europe, and detailed data were only available for seven countries. Findings suggested that participating veterinarians were aware of existing guidelines but that this was not always reflected in responsible AMU (24).

In an eligible study from **Italy**, the prevalence of prescriptions and pattern of AMU by veterinarians in a small animal teaching hospital were investigated, including how AM substances had been prescribed with respect to the recommendations issued by FVE, (26). The frequent use of last-resort and/or broad-spectrum antimicrobials was associated with a very low uptake of ASTs to support and confirm clinical diagnosis; in fact, only 4.8% of AMs prescribed were associated with any kind of microbiological examination and of these, only 2.1% were selected based on AST findings. Prescriptions of fluoroquinolones were guided by ASTs in only 3.7% of cases, and the recommendation to prescribe "last-resort" AMs (i.e., CIAs) based on ASTs was followed to guide therapy selection in a very low proportion of cases of pyoderma, urinary tract infections (UTIs), gastroenteritis (GI) and respiratory tract infections (RTIs). The frequent use of CIAs without ASTs contravenes the FVE's guidelines (34) and highlights a key area for focus with regards to developing and increasing uptake of prudent use guidelines on CIAs.

A recent cross-sectional survey of 721 **Australian** small animal veterinarians investigated the level of implementation of guidelines for AM prophylaxis across a range of surgical scenarios (i.e., spaying, castration, femoral head and neck resection, femoral fracture repair with a pin, exploratory laparotomy with enterotomy, non-ulcerated dermal mass removal and dental prophylaxis without extractions on a healthy animal). Only a quarter of participants (178/721) stated following guidelines. Twenty-three percent (166/721) of veterinarians reported having AMU protocols in the workplace. The latter were found also to be more likely to consult guidelines for AMU (20% vs 12%, respectively; mean difference, 8.7%; 95% CI, 2.6% to 15%; p= 0.009), and the scientific literature more frequently (41% vs 35%; mean difference, 10%; 95% CI, 2.4% to 18%; p= 0.004) than veterinarians that worked in

practices without protocols. Compliance with existing AMU guidelines for the different surgical scenarios assessed varied from 12% to 67%. The compliance was reportedly higher for routine dental procedures (67%), castrations (65%) and spaying (56%) and non-complicated, non-ulcerated dermal mass removals (50%). However, this was much lower for orthopaedic surgeries (20% to 28%) and exploratory laparotomy (12%); the non-compliance in surgical cases was mainly due to duration of surgery, inappropriate timing for AM administration prior to surgery and duration of AM treatment afterwards. Selection of appropriate AM substances according to recommendations for the most orthopaedic scenarios presented ranged between 57% and 63%. When considering the demographics of participants, it was observed that veterinarians in small animal practice had slightly higher odds of complying with guidelines than those in mixed practice (OR= 1.4, 95% CI: 1.1–1.9). However, recent graduates (graduated from 2012 onwards) had lower odds of compliance than those that had graduated earlier (OR=0.8, 95% CI: 0.6–0.9). Odds of compliance were higher for veterinarians working in practices with more than two veterinarians working full time than for those that worked by themselves or with another colleague (OR= 1.4, 95% CI: 1.1–1.9) (45). A subsequent cross-sectional survey by the same researchers in Australia assessing implementation of AMU stewardship protocols in farm and small animal practices with 184 veterinarians found that only 15% of participants were aware of protocols in their workplace (27/184), whilst 15% did not know if these were implemented in any respect (46). Only 28% (51/184) of respondents stated that they followed any guidelines for responsible AMU, with the Australasian Infectious Disease Advisor Panel and the BSAVA guidelines being the most commonly reportedly used (46).

A study conducted in **Finland** assessed to which extent national guidelines were followed with regards to prescriptions of AMs for the treatment of common infectious diseases in dogs at the Finnish Veterinary Teaching Hospital (31). Recommendations from the Finish Ministry of Agriculture and Forest Affairs on the selection of AMs for treating common infectious diseases in dogs included "infection: first choice antimicrobial/second choice AMs" as follows: a) pyoderma: macrolide or lincosamides, trimethoprim-sulphonamides/first generation cephalosporins; b) wound infection: penicillin V/beta-lactam and metronidazole; c) acute GI conditions (vomiting or diarrhoea): no antimicrobial treatment/ trimethoprim-sulphonamides, metronidazole or amoxicillin-clavulanate; d) acute UTI: trimethoprim-sulphonamides/fluoroquinolones; and e) acute bronchitis 'kennel cough': no antimicrobial treatment/ trimethoprim-sulphonamides, tetracycline or doxycycline. Other guidelines for AMU for surgical prophylaxis were provided by the Hygiene Committee of the Finnish Veterinary Teaching Hospital. These included antimicrobial prophylaxis in surgical procedures with AM substances selected according to the surgical site and administered intravenously 30 minutes before the operation. Findings showed that selection of the AM substances for the treatment of acute UTIs was in agreement with the national guidelines and that use of fluoroquinolones- classified as CIAs by WHO - was adequately controlled. In some cases, however, the use of AMs was not justified and was against these guidelines; for example, AMU in clean or clean-contaminated surgical procedures, and for the treatment of acute GI conditions. AMs were also used excessively in the treatment of minor injuries, such as small wounds or skin traumas (31).

The compliance with voluntary guidelines for responsible AMU by veterinarians in mixed practice was assessed in **Switzerland** (27). The guidelines related to the following FVE recommendations: a) AM class used for the treatment (i.e. an appropriate narrow-spectrum substance should be selected instead of broad-spectrum ones, and preference should be given to AM classes with minor relevance to human medicine, e.g. non-CIAs); b) discourage the use of combinations of AM substances due to the risk of increased toxicity, pharmacological antagonism and AMR selection; and, c) appropriate dosage to avoid administration of sub-therapeutic doses, which can lead to a lack of efficacy and, in some cases, may increase the risk of emergence of AMR. The authors analysed patterns of AM prescriptions in clinical records of eight veterinary practices to evaluate the appropriateness of use compared with the guidelines of interest. A dosage within +10% of the recommended dose compared

against drug manufacturer's recommendations was classified as correct according to recommendations. Forty-five per cent of the dosages were deemed to be according to existing recommendations. In 8% and 31% of the records, dosages were below (for aminoglycosides, fluoroquinolones and sulphonamides) and above (tetracyclines and penicillins/cephalosporins) recommended dosages for the condition and species treated, respectively (27).

Guidelines on recommended dosages for AMs published by national licensing bodies , and the British Equine Veterinary Association (BEVA) were assessed in a study that evaluating the AM prescribing practices of equine practitioners in **Switzerland, Germany, Austria** (28). Seventy-seven (38%) respondents treated uncomplicated strangles with AMs. Third and fourth generation cephalosporins were selected by 11% (23/203), fluoroquinolones by 4% (8/203) and streptomycin by 21% (44/203) of respondents. This was against the current guidelines for treatment of strangles that advise against AM treatment and recommend drainage and flushing of abscesses instead (47). Inappropriate prescribing practices of AMs in Switzerland, Germany and Austria were evidenced by selection of AMs without confirmed diagnosis, extended perioperative use, inappropriate AMU and the use of third and fourth generation cephalosporins as first line AMs, instead of being used only when supported by ASTs as second line choice AMs, according to current recommendations. Third and fourth generation cephalosporins and fluoroquinolones were used in 2/104 (4%) cases for perioperative AM prophylaxis. Level of compliance varied when assessing compliance to recommended dosages from national guidelines[12% (15/130)] and the BEVA recommendations 72% [(109/151)] by participating veterinarians across the three countries (28).

Veterinarians from **Sweden** requested AST significantly more often (p <0.001) than veterinarians from **six other EU countries** (**Belgium, Czech Republic, France, Germany, Spain and the UK**), which suggests a higher awareness of guidelines for responsible AMU. The odds of performing ASTs more frequently increased, on average, by 1.31 times (95% CI: 2.0 to 1.71, p= 0.047) comparing equine practitioners with farm animal veterinarians, whilst compared with small animal veterinarians the odds of performing ASTs more frequently increased, on average, by 2.14 times (95% CI 1.66 to 2.75, p <0.001), but it was not clear if this was due to the impact of guidelines or if due to differences in case management between the animal sectors.

The high and inappropriate use of AMs in cattle and for mastitis and specific use of CIAs suggest AMU usage for mastitis or dry cow management is a key area that should be targeted when developing and implementing strategies to reduce AMU in cattle (48). In **the Netherlands**, the current mandatory reduction of AMU in food-producing animals, together with the public concerns due to an increase of AMR awareness has led to a reduction on AM dry cow treatments by farmers (49).

Drying-off practices on dairy farms in northern **Germany** were assessed in the context of the current FVE recommendations in a study that examined preparation strategies before drying-off, dry period length, AM dry cow treatment and the effect of milk yield on decisions related to the drying-off procedure (48). Results of a survey of 200 farmers indicated that blanket AM dry cow treatment was conducted on 80% of the farms. Bacteriological examinations of milk samples of all cows before drying-off were conducted on only 7% of the farms, and 24% of the farmers mentioned performing bacteriological examination for selected cases such as high milk-yielding cows. A total of 65% of all AM dry cow treatments were conducted without preceding bacteriological examination, and selective dry cow treatment was not mentioned by any of the participating farmers, in contravention of the FVE guidelines. According to the international guidelines assessed in this study, AMU should be limited and the AST profile of pathogens determined before dry-off treatment in order to conduct a more targeted AMU in cows at higher risk of developing clinical mastitis (34, 48). This would avoid

unnecessary AMU through blanket therapy which is currently a common practice in the dairy sector (48).

Implementation of AMU guidelines targeting CIA

Favourable uptake of guidelines regarding use of CIAs was observed in mixed practices in **Switzerland** (27), and across most animal sectors according to **Danish** surveillance data (43). Although findings suggest there was uptake of guidelines regarding use of CIAs to some extent in mixed practices and that the use of these substances was low, it was also identified by authors that there was room for improvement in uptake in order to further reduce usage of CIAs and amounts of AMs used for mass prophylaxis, metaphylaxis and treatment of groups of animals through water and feed.

Implementation of guidelines including use of CIAs and appropriate dosage was also reported in a study that evaluated the AM prescribing practices of equine practitioners in **Germany, Austria and Switzerland** by comparing dosages prescribed by veterinarians to recommendations by drug manufacturers for each country (30). The variation in use of CIAs across species and countries may in part be due to preferences, national custom and practice, but may also be a reflection on availability of AMs and alternative approaches (25). The use of CIAs without prior guidance of AST results contravenes the recommendations on use of CIAs (34). Results revealed that 38% of respondents selected to treat uncomplicated strangles with AMs for which systemic AM therapy is not recommended by current guidelines (47). Inappropriate AM prescribing practices of equine veterinarians including the use of third and fourth generation cephalosporins as first-line antimicrobials, under-dosing by equine practitioners, selection of AMs without a laboratory-confirmed diagnosis, extensive perioperative AMU, and selection and inappropriate AMU were observed (30).

Uptake of guidelines related to use of CIAs was reported in another study that assessed how veterinarians in a small animal teaching hospital in **Italy** prescribed AMs with respect to prudent AMU recommendations issued by the FVE. The use of CIAs including fluoroquinolones, 3rd generation cephalosporins and macrolides predominated in several conditions affecting the genitourinary system, ear, respiratory system, and musculoskeletal systems (26). The frequent use of last-resort (i.e., CIAs) and/or broad-spectrum AMs was associated with a low uptake of AST, particularly for conditions such as pyoderma, UTIs, gastroenteritis and RTIs (26). Implementation of recommendations on the use of CIAs by veterinarians was varied; suboptimal uptake was reported in equine, mixed and small animal practice (25, 27, 28, 31), and poor uptake of guidelines on use of CIAs and AST was also reported in small animal settings (26). The use of CIAs without prior guidance of AST results contravenes the CIAs use guidelines (34) and highlights a key area for focus with regards to developing and increasing uptake of guidelines on use of CIAs as 2nd line antimicrobials. Extrapolation of findings should be interpreted with caution because this study might have been limited by selection bias, use of a convenience sampling approach and a small sample size (only 203 equine veterinarian respondents in the three countries). Although findings suggested uptake of certain guidelines such as use of AST results as an important criterion for antimicrobial selection, findings also highlighted non-compliance and a need for improvement in uptake of guidelines on use of CIAs and responsible antimicrobial dosing in participating countries.

Factors associated with responsible AMU

In the **European** survey conducted by De Bryine et al. (2013) the most important self-reported factors influencing prescribing habits were ASTs, work experience, risk of AMR and ease of administration. Results showed that important criteria for AM selection included anticipated therapeutic effectiveness, results of ASTs, lack of significant adverse effects and route of administration. Official information, such as public assessment reports (official reports from regulators on authorised veterinary medicines) were not ranked as an important information source to guide AMU. Practitioners reported using also the drug label and the package information leaflet (PIL) -which are

based on the SPC (Summary of Product Characteristics)- for information (24). AST parameters and risk factors contributing to the emergence of AMR were deemed some of the most important factors influencing prescribing habits (24). Furthermore, veterinarians have stated in a recent study in Australia that one of the barriers for adoption of guidelines was if these were produced or endorsed by pharmaceutical companies due to the perceived conflict of interests (46).

Impact of guidelines on extent and patterns of AMU

In 2012, the **UK**'s levy for the poultry industry sector, the British Poultry Council (BPC) adopted the guidelines on the use of CIAs from the WHO and introduced a voluntary ban on the use of third and fourth generation cephalosporins under its Antibiotic Stewardship Scheme, together with a commitment to reducing the use of fluoroquinolones in one day-old broilers (52). In 2013, there was a general increase in AMU in the poultry sector and particularly of fluoroquinolones use between 2012 and 2014, which was attributed to poor harvest and hence poor feed quality. The BPC scheme members revised their guidelines and between 2014 and 2015, the use of fluoroquinolones decreased by 48% in poultry. A 96% reduction was achieved in the chicken sector; smaller reductions in other poultry sectors (e.g. turkeys) were attributed to the poor availability of alternative licensed drugs to treat infectious diseases in these species (53).

The adoption of farmer-led strategies in collaboration with farm veterinarians resulted in a decrease of 47% in antibiotic usage in dairy cattle in the Netherlands between 2009 and 2015, including a decrease in the use of CIAs (50). This is supported also by other studies that have emphasised the importance of raising the awareness and educating farmers for the risks derived from AMU (51).
A scientific assessment conducted by ECDC and EFSA of the current voluntary and mandatory strategies **across different Member States in the EU** to reduce the AMU in food-producing animals, and its impact on AMR patterns. Following examination of the available evidence, findings of the scientific report indicated that AMU reduction strategies had been implemented successfully in several countries (e.g., **Denmark, the Netherlands, UK**) (23). Different national and international guidelines, cited by studies reviewed by EMA & EFSA (2017) were reported to have an impact on AMU in European countries (23). Voluntary guidelines in **Denmark** were reported to be linked to a reduction in AMU. The use of cephalosporins was reported to be minor compared to overall AMU in pigs, and since the voluntary ban in 2010 it has been extremely low – i.e., one kg in 2015 and close to zero in 2016 (43), according to recent surveillance data.

In companion animals, **Denmark** reported a reduction of AMU of 14% between 2012 (1,483 kgs active substance) and 2016 (1,323 kgs), with a significant decrease observed in the use of cephalosporins (36%, from 272 kg down to 137 kg during the same period), after the introduction of the first National Antimicrobial Treatment guidelines developed the Danish Small Animal Veterinary Association (DSAVA) in 2012 (43). The Danish surveillance data also indicated a shift in the use of AM substances after introduction of the DSAVA guidelines from broad spectrum AMs (i.e., cephalosporins, trimethoprim-sulphonamides) to narrow spectrum ones (i.e., penicillin).

In a study recently conducted in **Belgium** by Sarrazin et al. (42), the impact of guidelines implemented for a period of 20 days in the frequency of AM prescriptions was assessed in 14 small animal practices in the Flanders region. Although a decrease of overall AMU was observed, this was not statistically significant (p= 0.71). The corresponding values for Cohen's kappa (0.09 and 0.20 before and after, respectively) that were used to assess concordance between recommended AMs and actual AMU per indication showed only a slight agreement between these (42). Furthermore, unnecessary AMU was still detected after the introduction of the guidelines [45% (110/247)] not very dissimilar to the levels observed prior to their implementation [54% (106/198)]. Nevertheless, a significant difference after

implementation of guidelines was observed for some AM substances, two of which were CIAs: amoxicillin clavulanate (-15%, $p < 0.001$), cefovecin (+11%, $p < 0.001$) and doxycycline (+6%, $p < 0.001$). There was a significant change in the prescription patterns of veterinarians of highest priority CIAs (+12%, $p = 0.02$) of first- (+4%), second- (-16%) and third-choice (+8%) AMs as well as off-label (i.e., not licensed for the species or condition treated) AM preparations (+3%) after the guidelines were implemented ($p = 0.02$).

Discussion

A total of 14 studies assessing the implementation and/or impact of voluntary guidelines on prudent AMU were eligible and evaluated in this systematic review. Responsible AMU guidelines and voluntary interventions based on these were available in many **European countries**, and to a lesser extent elsewhere, e.g. in **Australia** and the **US**. The quality of most studies – mainly cross-sectional surveys – was deemed moderate for the purpose of establishing the implementation of guidelines and interventions to raise awareness. However, they were generally not suitable for assessing their impact, with the notable exception of three longitudinal studies using surveillance data.

The impact of AMU guidelines in **Belgium** was promising with a significant reduction of the use of 2nd line AMs and of last resort CIAs, but the period of implementation was too short to be able to evaluate properly changes in overall AMU by participating veterinarians (42). Findings from three of the eligible studies suggest that further strategies are required to improve uptake of guidelines particularly on use of perioperative AMs and of AMs for treatment of minor injuries for which topical therapy (antimicrobial or otherwise) are recommended. These strategies could include; continuous education courses for veterinarians already in practice but also at undergraduate level (46, 54) and educational campaigns for members of the public (e.g., pet owners and farmers) as end-users (54).

The existence of integrated surveillance programmes for AMU and AMR in humans, animals and food (43, 55) and the existence of both industry and government initiatives – some of which mandatory – to promote responsible AMU and contain use of CIAs may have contributed to the increased awareness of AMR in the veterinary profession. Furthermore, surveillance programmes are vital for the evaluation of effectiveness of interventions but also to guide the development of policies when new patterns of resistance are detected in pathogens and commensal bacteria of public health interest (56). However, results from studies described above may not be transferable to other countries or settings. It would be relevant to explore through qualitative research methodologies or through mixed methods, the factors influencing the variation in adoption of guidelines by veterinarians across animal health sectors and between countries in order to develop more sustainable interventions in the future.

The implementation of recommendations made in AMU guidelines and other voluntary approaches were reported to be influenced by a wide range of factors, including: a) veterinarians' preferences in relation to different AMs, often based in previous experience rather than based on evidence (57); b) availability/ licensing of AMs for veterinary use and/or alternative approaches across animal species and countries (25); c) economic sustainability (e.g., cost of diagnostic testing, fear of loss of clients); d) farmer or owner expectations of AM prescription; e) limited use or access to diagnostic tests including ASTs; f) perceived safety and effectiveness of AMs (particularly broad-spectrum substances) (57); g) gaps in scientific knowledge on AMU and AMR mechanisms (20); h) different patterns of infectious diseases between animal species relevant for a practice; i) different types of production systems used by the clients and for specific livestock species (25); and, j) differences in under- and postgraduate veterinary training (57). Lack of appropriate training at undergraduate and post-graduate levels has been identified in previous studies as a factor associated with low levels of compliance with responsible use guidelines among clinicians in human healthcare (58). These factors may influence the level of uptake of AMU guidelines so it is important to consider these aspects when

developing and implementing AMU guidelines in the veterinary profession by engaging with these stakeholders early on in the development stage of the guidelines in order to deal with their expectations and assess the challenges faced in their everyday practice so as to ensure that future guidelines and interventions stand a better chance of being implemented in routine practice.

Many of the guidelines assessed in this review were available free of access to the veterinary profession through websites supported by organisations and professional bodies. Voluntary initiatives by the industry in food-producing animals were usually well-accepted by farmers and this may reflect the level of trust towards levy bodies. These organisations often provide support to farmers by dissemination of knowledge and advice on disease prevention and control, good animal husbandry and farming practices, and represent their interests when lobbying policy-makers and government bodies. There would therefore be benefit in involving these levy bodies in the early stages of policy development by government authorities as their involvement will be key to ensure the commitment of farmers and other animal production stakeholders.

According to the recent Scientific Opinion published by EFSA and EMA (23) that assessed effectiveness of both statutory and voluntary interventions to reduce AMU and AMR in food-producing animals across different **EU Member States**, a multifaceted, integrated approach should be adopted flexibly with adaptations to each country's context. This is essential, as the concept of "one size fits all" cannot be applied to guidelines for responsible AMU due to differences in production systems, socioeconomic and legislative framework contexts, access to veterinary care and resources of veterinary government services. All these factors should be taken in consideration by relevant stakeholders and policy makers when developing and implementing stewardship programmes, guidelines and initiatives for responsible AMU. Recommended options included amongst others: a) development of national strategies for reduction of AMU with set targets agreed with the animal production sector; b) harmonised surveillance systems for monitoring both AMU and AMR in animal populations in an integrated manner; c) adoption of on-farm health plans (including vaccination programmes) to prevent and control disease in animal populations in a more efficient manner, reducing the need for AMs; d) increase the responsibility of veterinarians for AM prescribing, so that this is more evidence-based and supported by adequate diagnostic testing; e) train, educate and raise public awareness for responsible AMU (including end users like farmers and pet owners); f) increase the availability of rapid and reliable diagnostics to support selection of AMs for animal therapy; and, g) improve/ change animal husbandry and farm management practices, including biosecurity for effective disease prevention and control (23).

The findings from this review should be interpreted carefully as the quality of most of eligible studies was low and results may be biased. For example, self-reported usage studies are prone for bias due to responses potentially being influenced by perceived social approval ("social desirability bias") and recall bias when participants were required to discuss their selection of AMs in common clinical conditions and compliance with existing guidelines (59). Therefore, such studies were not deemed as appropriate to assess the impact of interventions, but were useful to ascertain awareness of veterinarians of existing guidelines and to explore the maximum likely extent of implementation due to social desirability.

Based on the publications available for this review, there is currently limited evidence of the impact of voluntary recommendations and guidelines across all animal production sectors as well as for pets. Furthermore, most of the studies considered in this review relied on self-reporting by participants in cross-sectional surveys which are suitable to assess implementation of recommendations and initiatives for responsible AMU but are not adequate to assess the impact of these measures. Particularly for companion animals, evidence of the extent of the implementation of good practice guidelines and their impact on prudent prescribing and resistance prevention is lacking. Small animal

practice electronic databases could be used for surveillance of AMU and AMR but also for assessment of effectiveness of interventions and guidelines for responsible AMU (60). This may be due to lower emphasis on companion animals in the current strategy and the perceived higher risk to public health related to misuse of AMs in food-production animals (4). Also, there has been less effort at international level to assess the implementation and effectiveness of guidelines for companion animals.

Conclusions

Existing evidence of the level of implementation and impact of responsible AMU guidelines were limited for both food-producing and companion animals. The most effective strategies appeared to be those where end users, such as farmers and industry bodies, were fully engaged and took responsibility for the interventions. This was observed with the poultry and pig industry (e.g., Denmark and the UK) and to a lesser extent in dairy cattle (e.g. Denmark). However, there is currently little evidence on the extent to which guidelines result in change of practice. Evidence was particularly scarce for small animal practice. Most of the studies were based on self-reporting by veterinarians which can lead to bias in the assessment of the impact of guidelines as other factors could have influenced changes in AMU behaviour. Causal inferences cannot be drawn due to the lack of controlled trials. Also, it was not always possible to assess the individual contribution of guidelines if these were applied simultaneously with other interventions. To minimise AMU, a multifaceted integrated approach should be implemented, adapted to local circumstances. The findings of this review suggest that it is important to tailor prudent AMU strategies to the animal production characteristics, the veterinary profession, regulatory frameworks and the socioeconomic and cultural context of target countries. These findings are in line with the recommendations made by the WHO which currently promotes the development of National Action Plans as part of the International Health Regulations (9).

Recommendations

- Further assessment of the impact of existing guidelines and voluntary initiatives should be promoted to address these gaps in knowledge. This could include analysis of existing surveillance data (as conducted in **Denmark**) collated at national level by government bodies, benchmarking data collated by the food and animal industry and clinical data from veterinary practices.
- Further studies that examine the underlying factors as to why certain countries achieve better uptake of prudent use guidelines – perhaps through the adoption of social sciences research methods, such as the Theory of Behaviour Change – may help to improve the effectiveness, acceptability and thus sustainability of the impact in the medium and long term of existing and new guidelines in countries or particular animal sectors where these would be beneficial.
- Continued provision and promotion of guidelines and relevant education to veterinarians at both under- and postgraduate levels is necessary to further improve uptake of responsible AMU and AST.
- Although this review indicated that CIAs were only used in small quantities in livestock production if compared to non-CIAs, further improvement in uptake of guidelines targeting CIAs are required to reduce prescriptions of these antimicrobials to preserve their therapeutic efficacy. Targeted interventions are needed to reduce the use of CIAs (third and fourth generation cephalosporins, fluoroquinolones) without support of ASTs in animal sectors such as dairy cattle production (dry cow therapy) and companion animals in order to maintain therapeutic efficacy of these substances.

- Where possible, interventions focused on improving animal husbandry and farm management practices, biosecurity and non-antimicrobial disease prevention and control measures should continue to be promoted at farm level. Animal mass treatments should be discouraged but may be required when a confirmed diagnosis has been obtained and under veterinary supervision. Furthermore, veterinarians should be involved in the education of farmers on responsible AMU.

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Appendix 1

Risk of bias assessment (Adapted from ROBIS I tool – Risk of bias tool for non-randomised studies of interventions)

https://sites.google.com/site/riskofbiastool/welcome/home

Study ID	Pre-intervention		At intervention	Post- intervention				Overall risk of bias judgement (+ short justification)
	Bias due to confounding	Bias in the selection of participants	Bias in the classification of interventions	Bias due to deviation from intended interventions	Bias due to missing data	Bias in measurement of outcomes	Bias in selection of the reported result(s)/ outcome(s)	

Instructions:

Responses **underlined in green** are potential markers for **low risk of bias**, and responses in **red** are potential markers for a **risk of bias**. Where questions relate only to sign posts to other questions, no formatting is used.

N= No; **PN**= Probably No; **Y**= Yes; **PS**= Probably Yes; **NA**= Not Applicable; **NI**= No Information

A) At pre-intervention stage

Signalling questions	Response options
Bias due to confounding	
1.1 Is there potential for confounding of the effect of intervention in this study? **If N/PN to 1.1:** the study can be considered to be at **low risk of bias** due to confounding and no further signalling questions need be considered	NA / Y / PY / PN / N / NI
If Y/PY to 1.1: determine whether there is a need to assess **time-varying confounding**:	
1.2. Was the analysis based on splitting participants' follow-up time according to intervention received? **If N/PN**, answer questions relating to baseline confounding (1.4 to 1.6) **If Y/PY**, go to question 1.3.	NA / Y / PY / PN / N / NI
1.3. Were intervention discontinuations or switches likely to be related to factors that are prognostic for the outcome? **If N/PN**, answer questions relating to baseline confounding (1.4 to 1.6) **If Y/PY**, answer questions relating to both baseline and time-varying confounding (1.7 and 1.8)	NA / Y / PY / PN / N / NI

Questions relating to baseline confounding only	Response options
1.4. Did the authors use an appropriate analysis method that controlled for all the important confounding domains?	NA / Y / PY / PN / N / NI
1.5. **If Y/PY to 1.4**: Were confounding domains that were controlled for measured validly and reliably by the variables available in this study?	NA / Y / PY / PN / N / NI
1.6. Did the authors control for any post-intervention variables that could have been affected by the intervention?	NA / Y / PY / PN / N / NI
Questions relating to baseline and time-varying confounding	

1.7. Did the authors use an appropriate analysis method that controlled for all the important confounding domains and for time-varying confounding?	NA / Y / PY / PN / N / NI
1.8. **If Y/PY to 1.7**: Were confounding domains that were controlled for measured validly and reliably by the variables available in this study?	NA / Y / PY / PN / N / NI
Risk of bias judgement	Low / Moderate / Serious / Critical / NI
Optional: What is the predicted direction of bias due to confounding?	Favours experimental / Favours comparator / Unpredictable

Bias in selection of participants into the study	**Response options**
2.1. Was selection of participants into the study (or into the analysis) based on participant characteristics observed after the start of intervention? **If N/PN to 2.1:** go to 2.4	Y / PY / PN / N / NI
2.2. **If Y/PY to 2.1**: Were the post-intervention variables that influenced selection likely to be associated with intervention?	NA / Y / PY / PN / N / NI
2.3 **If Y/PY to 2.2**: Were the post-intervention variables that influenced selection likely to be influenced by the outcome or a cause of the outcome?	NA / Y / PY / PN / N / NI
2.4. Do start of follow-up and start of intervention coincide for most participants?	Y / PY / PN / N / NI
2.5. **If Y/PY to 2.2 and 2.3, or N/PN to 2.4**: Were adjustment techniques used that are likely to correct for the presence of selection biases?	NA / Y / PY / PN / N / NI
Risk of bias judgement	Low / Moderate / Serious / Critical / NI
Optional: What is the predicted direction of bias due to selection of participants into the study?	Favours experimental / Favours comparator / Towards null /Away from null / Unpredictable

B) At intervention stage

Bias in classification of interventions	Response options
3.1 Were intervention groups clearly defined?	Y / PY / PN / N / NI
3.2 Was the information used to define intervention groups recorded at the start of the intervention?	Y / PY / PN / N / NI
3.3 Could classification of intervention status have been affected by knowledge of the outcome or risk of the outcome?	Y / PY / PN / N / NI
Risk of bias judgement	Low / Moderate / Serious / Critical / NI
Optional: What is the predicted direction of bias due to classification of interventions?	Favours experimental / Favours comparator / Towards null /Away from null / Unpredictable

C) At post-intervention stage

Bias due to deviations from intended interventions	Response options
If your aim for this study is to assess the effect of assignment to intervention, answer questions 4.1 and 4.2	
4.1. Were there deviations from the intended intervention beyond what would be expected in usual practice?	Y / PY / PN / N / NI
4.2. **If Y/PY to 4.1**: Were these deviations from intended intervention unbalanced between groups *and* likely to have affected the outcome?	NA / Y / PY / PN / N / NI
If your aim for this study is to assess the effect of starting and adhering to intervention, answer questions 4.3 to 4.6	
4.3. Were important co-interventions balanced across intervention groups?	Y / PY / PN / N / NI
4.4. Was the intervention implemented successfully for most participants?	Y / PY / PN / N / NI
4.5. Did study participants adhere to the assigned intervention regimen?	Y / PY / PN / N / NI
4.6. **If N/PN to 4.3, 4.4 or 4.5**: Was an appropriate analysis used to estimate the effect of starting and adhering to the intervention?	NA / Y / PY / PN / N / NI
Risk of bias judgement	

Bias due to deviations from intended interventions	**Response options**
Optional: What is the predicted direction of bias due to deviations from the intended interventions?	

Bias due to missing data	**Response options**
5.1 Were outcome data available for all, or nearly all, participants?	Y / PY / PN / N / NI
5.2 Were participants excluded due to missing data on intervention status?	Y / PY / PN / N / NI
5.3 Were participants excluded due to missing data on other variables needed for the analysis?	Y / PY / PN / N / NI
5.4 **If PN/N to 5.1, or Y/PY to 5.2 or 5.3**: Are the proportion of participants and reasons for missing data similar across interventions?	NA / Y / PY / PN / N / NI
5.5 **If PN/N to 5.1, or Y/PY to 5.2 or 5.3**: Is there evidence that results were robust to the presence of missing data?	NA / Y / PY / PN / N / NI
Risk of bias judgement	Low / Moderate / Serious / Critical / NI
Optional: What is the predicted direction of bias due to missing data?	Favours experimental / Favours comparator / Towards null /Away from null / Unpredictable

Bias in measurement of outcomes	**Response options**
6.1 Could the outcome measure have been influenced by knowledge of the intervention received?	Y / PY / PN / N / NI
6.2 Were outcome assessors aware of the intervention received by study participants?	Y / PY / PN / N / NI
6.3 Were the methods of outcome assessment comparable across intervention groups?	Y / PY / PN / N / NI
6.4 Were any systematic errors in measurement of the outcome related to intervention received?	Y / PY / PN / N / NI
Risk of bias judgement	Low / Moderate / Serious / Critical / NI

Optional: What is the predicted direction of bias due to measurement of outcomes?	Favours experimental / Favours comparator / Towards null /Away from null / Unpredictable

Bias in selection of the reported result	**Response options**
Is the reported effect estimate likely to be selected, on the basis of the results, from...	
7.1. ... multiple outcome *measurements* within the outcome domain?	Y / PY / PN / N / NI
7.2 ... multiple *analyses* of the intervention-outcome relationship?	Y / PY / PN / N / NI
7.3 ... different *subgroups*?	Y / PY / PN / N / NI
Risk of bias judgement	Low / Moderate / Serious / Critical / NI
Optional: What is the predicted direction of bias due to selection of the reported result?	Favours experimental / Favours comparator / Towards null /Away from null / Unpredictable

Overall bias	**Response options**
Risk of bias judgement	Low / Moderate / Serious / Critical / NI
Optional: What is the overall predicted direction of bias for this outcome?	Favours experimental / Favours comparator / Towards null /Away from null / Unpredictable

Guidance for risk of bias judgements- Pre- and at intervention levels

Judgement	Bias due to confounding	Bias in selection of participants into the study	Bias in classification of interventions
Low risk of bias (the study is comparable to a well-performed randomized trial with regard to this domain)	No confounding expected.	(i) All participants who would have been eligible for the target trial were included in the study; *and* (ii) For each participant, start of follow up and start of intervention coincided.	(i) Intervention status is well defined; *and* (ii) Intervention definition is based solely on information collected at the time of intervention.
Moderate risk of bias (the study is sound for a non-randomized study with regard to this domain but cannot be considered comparable to a well-performed randomized trial):	(i) Confounding expected, all known important confounding domains appropriately measured and controlled for; *and* (ii) Reliability and validity of measurement of important domains were sufficient, such that we do not expect serious residual confounding.	(i) Selection into the study may have been related to intervention and outcome; *and* The authors used appropriate methods to adjust for the selection bias; *or* (ii) Start of follow up and start of intervention do not coincide for all participants; *and* (a) the proportion of participants for which this was the case was too low to induce important bias; *or* (b) the authors used appropriate methods to adjust for the selection bias; *or* (c) the review authors are confident that the rate (hazard) ratio for the effect of intervention remains constant over time.	(i) Intervention status is well defined; *and* (ii) Some aspects of the assignments of intervention status were determined retrospectively.

Guidance for risk of bias judgements- Pre- and at intervention levels (cont)

Judgement	Bias due to confounding	Bias in selection of participants into the study	Bias in classification of interventions
Serious risk of bias (the study has some important problems);	(i) At least one known important domain was not appropriately measured, or not controlled for; *or* (ii) Reliability or validity of measurement of an important domain was low enough that we expect serious residual confounding.	(i) Selection into the study was related (but not very strongly) to intervention and outcome; *and* This could not be adjusted for in analyses; *or* (ii) Start of follow up and start of intervention do not coincide; *and* A potentially important amount of follow-up time is missing from analyses; *and* The rate ratio is not constant over time.	(i) Intervention status is not well defined; *or* (ii) Major aspects of the assignments of intervention status were determined in a way that could have been affected by knowledge of the outcome.
Critical risk of bias (the study is too problematic to provide any useful evidence on the effects of intervention);	(i) Confounding inherently not controllable *or* (ii) The use of negative controls strongly suggests unmeasured confounding.	(i) Selection into the study was very strongly related to intervention and outcome; *and* This could not be adjusted for in analyses; *or* (ii) A substantial amount of follow-up time is likely to be missing from analyses; *and* The rate ratio is not constant over time.	(Unusual) An extremely high amount of misclassification of intervention status, e.g. because of unusually strong recall biases.
No information on which to base a judgement about risk of bias for this domain.	No information on whether confounding might be present.	No information is reported about selection of participants into the study or whether start of follow up and start of intervention coincide.	No definition of the intervention or no explanation of the source of information about intervention status is reported.

Guidance for risk of bias judgements- post-intervention level

Judgement	Bias due to deviations from intended intervention	Bias due to missing data	Bias in measurement of outcomes	Bias in selection of the reported result
Low risk of bias (the study is comparable to a well-performed randomized trial with regard to this domain)	**Effect of assignment to intervention:** (i) Any deviations from intended intervention reflected usual practice; *or* (ii) Any deviations from usual practice were unlikely to impact on the outcome. **Effect of starting and adhering to intervention:** The important **co-interventions** were balanced across intervention groups, and there were no deviations from the intended interventions (in terms of **implementation or adherence**) that were likely to impact on the outcome.	(i) Data were reasonably complete; *or* (ii) Proportions of and reasons for missing participants were similar across intervention groups; *or* (iii) The analysis addressed missing data and is likely to have removed any risk of bias.	(i) The methods of outcome assessment were comparable across intervention groups; *and* (ii) The outcome measure was unlikely to be influenced by knowledge of the intervention received by study participants (i.e. is objective) or the outcome assessors were unaware of the intervention received by study participants; *and* (iii) Any error in measuring the outcome is unrelated to intervention status.	There is clear evidence (usually through examination of a pre-registered protocol or statistical analysis plan) that all reported results correspond to all intended outcomes, analyses and sub-cohorts.

Guidance for risk of bias judgements- post-intervention level (cont.)

Judgement	Bias due to deviations from intended intervention	Bias due to missing data	Bias in measurement of outcomes	Bias in selection of the reported result
Moderate risk of bias (the study is sound for a non-randomized study with regard to this domain but cannot be considered comparable to a well-performed randomized trial):	**Effect of assignment to intervention:** There were deviations from usual practice, but their impact on the outcome is expected to be slight. **Effect of starting and adhering to intervention:** (i) There were deviations from intended intervention, but their impact on the outcome is expected to be slight. *or* (ii) The important co-interventions were not balanced across intervention groups, or there were deviations from the intended interventions (in terms of implementation and/or adherence) that were likely to impact on the outcome; *and* The analysis was appropriate to estimate the effect of starting and adhering to intervention, allowing for deviations (in terms of implementation, adherence and co-intervention) that were likely to impact on the outcome.	(i) Proportions of and reasons for missing participants differ slightly across intervention groups; *and* (ii) The analysis is unlikely to have removed the risk of bias arising from the missing data.	(i) The methods of outcome assessment were comparable across intervention groups; *and* (ii) The outcome measure is only minimally influenced by knowledge of the intervention received by study participants; *and* (iii) Any error in measuring the outcome is only minimally related to intervention status.	(i) The outcome measurements and analyses are consistent with an *a priori* plan; or are clearly defined and both internally and externally consistent; *and* (ii) There is no indication of selection of the reported analysis from among multiple analyses; *and* (iii) There is no indication of selection of the cohort or subgroups for analysis and reporting on the basis of the results.

Guidance for risk of bias judgements- post-intervention level (cont.)

Judgement	Bias due to deviations from intended intervention	Bias due to missing data	Bias in measurement of outcomes	Bias in selection of the reported result
Serious risk of bias (the study has some important problems);	**Effect of assignment to intervention:** There were deviations from usual practice that were unbalanced between the intervention groups and likely to have affected the outcome. **Effect of starting and adhering to intervention:** (i) The important co-interventions were not balanced across intervention groups, or there were deviations from the intended interventions (in terms of implementation and/or adherence) that were likely to impact on the outcome; *and* (ii) The analysis was not appropriate to estimate the effect of starting and adhering to intervention, allowing for deviations (in terms of implementation, adherence and co-intervention) that were likely to impact on the outcome.	(i) Proportions of missing participants differ substantially across interventions; *or* Reasons for missingness differ substantially across interventions; *and* (ii) The analysis is unlikely to have removed the risk of bias arising from the missing data; *or* Missing data were addressed inappropriately in the analysis; *or* The nature of the missing data means that the risk of bias cannot be removed through appropriate analysis.	(i) The methods of outcome assessment were not comparable across intervention groups; *or* (ii) The outcome measure was subjective (i.e. vulnerable to influence by knowledge of the intervention received by study participants); *and* The outcome was assessed by assessors aware of the intervention received by study participants; *or* (iii) Error in measuring the outcome was related to intervention status.	(i) Outcomes are defined in different ways in the methods and results sections, or in different publications of the study; *or* (ii) There is a high risk of selective reporting from among multiple analyses; *or* (iii) The cohort or subgroup is selected from a larger study for analysis and appears to be reported on the basis of the results.