13 February 2014
EMA/CVMP/AWP/257904/2013-Rev.1
Committee for Medicinal Products for Veterinary Use

Overview of comments received on 'Reflection paper on use of pleuromutilins in food-producing animals in the European Union: development of resistance and impact on human and animal health' (EMA/CVMP/SAGAM/119489/2012)

Interested parties (organisations or individuals) that commented on the draft document as released for consultation.

Stakeholder no.	Name of organisation or individual
1	Association of Veterinary Consultants
2	Novartis Animal Health Inc.
3	IFAH-Europe
4	Federation of Veterinarians of Europe (FVE)

The overview of comments was initially published on 7 November 2013. Later on it was identified that comments from IFAH-Europe and the FVE had not been addressed. The overview of comments has now been revised to take into account those comments.

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1. General comments – overview

Stakeholder no. *(See cover page)*	General comment (if any)	Outcome (if applicable)
1	The Association of Veterinary Consultants (AVC) welcomes the opportunity to comment on this reflection paper on the pleuromutilins, a class of antibiotics of great importance to the pig and poultry industries. Overall, this reflection paper gives a very comprehensive overview of the current situation, regarding pleuromutilin use and the potential for resistance development both in animals and man. In animals, Brachyspira hyodysenteriae and to a lesser extent other Brachyspira spp. have been shown to develop resistance to the pleuromutilins. However, there is little to no additional resistance development reported in the EU in other target pathogens, for which it is licensed, such as Mycoplasma spp. in both pigs and poultry or Lawsonia intracellularis in pigs. Reduced susceptibility to B. hyodysenteriae has undoubtedly occurred in some member States (MS), as judged by susceptibility patterns (Rohde et al, 2004, but to date there is no standardised method of determining with precision, what the clinical breakpoints should be. Minimum inhibitory concentration (MIC) determinations can give variable results depending on the method employed, such as broth or agar dilution. Furthermore the dose rate/inclusion rate of the drug administered varies between 40-200ppm tiamulin and 25-200ppm valnemulin in feed depending on its use either for prevention or for treatment. A pharmacokinetic/pharmacodynamic (PK/PD) approach could clarify some of these issues, as well as determine	Antimicrobial susceptibility testing of *Lawsonia intracellularis* is difficult as this obligate intracellular bacterium needs complicated cell culture systems to grow and published data on their in-vitro susceptibility are very scarce and include only a very limited number of isolates (McOrist, Mackie et al. 1995; Wattanaphansak, Singer et al. 2009; Yeh, Lee et al. 2011). There are no international accepted standards for susceptibility testing of Mycoplasma spp. and *Lawsonia intracellularis*. In addition, internationally accepted interpretative criteria for both Mycoplasma spp. and *Lawsonia intracellularis* are lacking (Clinical Laboratory Standards Institute (CLSI) 2012). *Mycoplasma gallisepticum* mutants with decreased susceptibility to pleuromutilins could be selected by serial passages of the parent strains in sub inhibitory concentrations of tiamulin or valnemulin in vitro (Li et al. 2011). The same could potentially occur in vivo. Generally reports on susceptibility testing of Mycoplasma spp. against pleuromutilins are scarce and therefore knowledge on the prevalence of resistance is very limited. Reports available in the literature on *Mycoplasma spp*. are on small numbers of isolates, often from outside of Europe and also often on Mycoplasma species which are not target organisms of pleuromutilins. For *Lawsonia intracellularis* no resistance to pleuromutilins has been reported, but only very few isolates have been investigated and accepted methods and interpretative criteria for such

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	whether there is a bacteriostatic or bactericidal effect likely to be achieved. As a result, it is currently impossible to say what is precisely resistant or not, as the parameters have not been determined.	susceptibility testing are lacking (McOrist, Mackie et al. 1995; Wattanaphansak, Singer et al. 2009). Therefore it is greatly unknown whether or not resistance to pleuromutilins is a problem in Mycoplasma spp. and *Lawsonia intracellularis*.
	Limiting or restricting the use of pleuromutilins to swine dysentery use only, in some respects would make no sense, as there is little to no development of resistance in other bacteria or mycoplasma associated with the other indications or species for which the pleuromutilins are approved.	As explained above, reports on susceptibility testing to other bacteria for which pleuromutilins are licenced (*Lawsonia intracellularis, Mycoplasmata*) are scarce. This does not mean that there is no resistance; this means that this has not been investigated to any significant extent.
	It is suspected to be the repeated, high level use of tiamulin for swine dysentery treatment that has been the main cause of the resistance development. Following the removal of other therapeutics, such as dimetridazole and ronidazole, the banning of growth promoters, such as carbadox, olaquindox and salinomycin, the last, both because it has activity against B. hyodysenteriae but also because it is contra-indicated with the therapeutic use of the pleuromutilins.	Generally the risk of developing resistance is greater at low (sub inhibitory) concentrations. It is unclear what is meant by “high level use”. If this means repeated use or prolonged use of pleuromutilins, we agree. Every use, preventive or therapeutic of antimicrobials, including pleuromutilins, can potentially lead to the development of resistance.
	Increased generic competition and resultant price reduction has increased the use of tiamulin for the treatment of swine dysentery. Previously, it was often reserved as the drug of last resort, because of price and incompatibilities and so lincomycin was the primary	See above

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	product used. Lincomycin and in some cases the macrolide tylvalosin are considered potential alternatives in many MS (Hidalgo et al, 2011). The use of pleuromutilins for other approved indications appears to have little or no impact on resistance development against those other target pathogens. A potential problem/hazard regarding resistance genes in Staphylococcus aureus, whether methicillin resistant (MRSA) or methicillin susceptible (MSSA) has been highlighted but none of these are specifically pleuromutilin resistance genes but multiple resistance genes e.g. the vga gene – streptogramin A (virginiamycin), lincosamides (lincomycin) and pleuromutilin (tiamulin or valnemulin) and the cfr gene – oxazolidinones (linezolid), phenicols (florfenicol, thiamphenicol), streptogramin A, lincosamides, pleuromutilins. The cfr gene is primarily selected for by linezolid use and as the binding site is close in the ribosome, co-selects for resistance against other families of drugs that have adjacent binding sites but they do not select for linezolid resistance (Miller et al, 2008). It is uni-directional.	The *cfr* and *vga* genes are important emerging mechanisms of resistance to several antimicrobials, including pleuromutilins. We do not agree with the statement that the *cfr* gene is not selected for by antimicrobials other that linezolid. The *cfr* gene has mainly been detected in *Staphylococcus* isolates from animals and linezolid is not used in animals and its reservoir is thought to be coagulase-negative staphylococci in animals (Witte & Cuny, 2011). The *cfr* gene is selected for by all antimicrobials to which it confers resistance e.g. phenicols such as florfenicol, lincosamides and pleuromutilins, which are all used in animals as well as oxazolidones and streptogramin A, which are used in humans. The reference by Miller et *al.* (2008) stating that there is no cross resistance between tiamulin and linezolid and resistance to linezolid is uni-directional is on resistance due to chromosomal mutations, not on resistance due to horizontal transfer mediated by the *cfr* gene. Miller et *al.* (2008) clearly state that the *cfr* gene does cause cross resistance to linezolid and pleuromutilins due to methylation of residue A2503 in 23S rRNA.

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	There is continuous expression of potential hazard but no relation to risk, which is increasingly being recognised as human use of antibiotics for humans resistance and animal use for animal resistance and the actual transmission rate between the two is relatively low. For example, the transmission rate of livestock associated (LA) MRSA CC398 from pigs to man can be determined using the Danish Danmap reports (2011 & 2012) data. Transmission from pigs to pig farm workers is high but on a human population basis (5.5 million), MRSA cases (including carriage and clinical cases) were 0.02% in Denmark and LA MRSA cases accounted for approximately 10% or 0.002%. The Danes produce 10 times more pigs/capita than the EU average of approximately 0.5 pigs/capita and therefore may be considered as having a comparatively high risk of exposure. In addition, 90% of cases were attributed to direct pig contact (Danmap 2011, 2012) and recent reports have found 88% of slaughter pigs carrying MRSA. Therefore the estimated risk to the general population with no direct pig contact is 0.0002% or 0.2 people/100,000 population, which is remarkably low. Reports from other MS, such as Germany (Blaha et al, 2009), the normal human carriage of non-pig associated MRSA is put at 1-2% or 1-2,000/100,000 population. Therefore the risk of any gene transmission from pigs to the human population in the EU is also comparatively low, especially in the light of human over-use of antibiotics and direct human to human contact, especially in the healthcare environment. (Blaha, T., Eckmans, T., Cuny, C., Witte, W. And Meemken, D. (2009) Methicillin-resistant Staphylococcus aureus (MRSA) in veterinarians and meat inspectors. Proceedings of the 14th	Although transmission of MRSA CC398 into the general population is still relatively low, there are an increasing number of reports on MRSA CC398 of unknown origin. This means in people without known risk factors such as contact with livestock. MRSA CC398 are also reported increasingly from companion animals and horses. The situation in Denmark is different from the situation in other EU MS. In the Netherlands LA-MRSA accounted for 37% of all first MRSA isolates sent to the National Institute for Public health and the Environment. Danish authorities for public health have recently classified farmers and other people working with pigs as a risk group in health care, similar to what has been done in The Netherlands. LA-MRSA has also been found in poultry, including multidrug-resistant isolates carrying the vga genes. Wendlandt S, Kadlec K, Feßler AT, Monecke S, Ehricht R, van de Giessen AW, Hengeveld PD, Huijsdens X, Schwarz S, van Duijkeren E. Resistance phenotypes and genotypes of methicillin-resistant *Staphylococcus aureus* isolates from broiler chickens at slaughter and abattoir workers. J Antimicrob Chemother. 2013 Jun 24. [Epub ahead of print]

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	International Society for Animal Hygiene (ISAH) Congress, Vechta, Germany, vol 2, pp 645-648.) The debate over treatment and prophylactic use of antimicrobials is still ongoing and the European Commission may decide on this in the near future. However, there are two approaches for consideration regarding prophylactic use. The first is low level use/inclusion rate, which provides concentrations of drug at target sites, which inhibits the bacterium from growing but does not eliminate infection. The second is prevention use at high treatment levels to clear out the infection before clinical disease develops. This is also referred to as metaphylactic use or 'early treatment'. Both approaches have a role in veterinary medicine, usually based on previous experiences of earlier production batches i.e. what diseases are endemic on the farm and depending on the source of animals, if purchased in for finishing. Low level use can be effectively used in pigs, which are known to be carrying an infection, particularly B. hyodysenteriae. If the number of bacteria is kept low below 102, then mutation rates can be very low and the development of resistance is also low (Drlica, 2003). However, if there are other disease problems, such as pneumonia and appetite is depressed and the drug concentrations fall too low, then the organisms are not inhibited and frank disease can develop. There is a risk then of mutations potentially occurring but this appears to be rare, in the case of pleuromutilins. (Drlica, K. (2003). The mutant selection window and antimicrobial resistance. Journal of Antimicrobial Chemotherapy, 52, 11-17.)	Every use of antimicrobials, whether preventive, metaphylactic or therapeutic may and will lead to the development and spread of resistance. It must also be kept in mind that development of resistance is not limited to target organisms; it concerns the whole microbiome of the treated individual, especially in the case of oral mass medication. As explained in the reflection paper, chromosomal mutations are not the only mechanisms of resistance. The mechanism of resistance depends on the microorganism involved. Therefore the discussion on the numbers of bacteria seems irrelevant here. We note that the reference quoted refers to fluroroquinolones, not pleuromutilins.

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	With high level prophylactic/early treatment use one is depending on sufficient drug concentrations being achieved to kill off the bacterium that might be carried in the animal or bird. The ideal situation is to eliminate the infection but as pleuromutilins are primarily bacteriostatic drugs and are co-dependent on time and concentration they may not completely eliminate the organism and this may grow up later, after a suitable incubation period. These pigs may require treatment and it is the high number of organisms (>106) that is the problem, as this increases the chance of mutations occurring. In a way, repeated treatments are possibly the worst case scenario for the chances for resistance mutations to occur. This is confirmed by Sperling et al (2011) where 'An analysis of epidemiological data showed that multiresistant B. hyodysenteriae were more often detected on fattening farms (59%), compared with farms with other types of production.' Poor hygiene between batches will also compound the infection. Regarding poultry Mycoplasma spp infections, the preventive use of many antibiotics including the pleuromutilins, macrolides and lincosamides focus on preventing the disease developing. Once mycoplasmal infections do develop in chickens and turkeys, they are commonly also infected with Escherichia coli, as a secondary bacterial invader, against which these antibiotics have no action. This then leads to the increased use of antibiotics with E. coli action, in particular the fluoroquinolones (FQs), which are active against both mycoplasma and E. coli, hence their popularity in practice to treat complicated chronic respiratory disease (CCRD). Since the	

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	introduction of generic FQs at a low cost and the lack of suitable alternatives to treat Gram-negative pathogens, their use has increased substantially in practice.	See comment above.
	There are a number of issues around responsible use of antimicrobials, which are mentioned in the reflection paper and it is felt that there is a need to develop veterinary understanding of how antimicrobial drugs work and how they can be best used, which is to be commended. It also touches on improvement in the health status of animals and this is absolutely critical for controlling swine dysentery and the corresponding use of antibiotics to control the disease. In an ideal world, breeding companies should be free of swine dysentery and the selling of weaners/growers from known infected breeding farms to finisher farms would also be forbidden. These are the major ways that the disease is spread from farm to farm. It is outside the scope of this paper to make recommendations here but it is hoped that other responsible agencies might look into this further. For example, M. gallisepticum has been largely eradicated from broiler-breeder producers and broiler production is largely free in many MSs. Eradication of swine dysentery infected farms is important but it is often only when they have multiple-drug resistance, that individual farmers take that major final step of depopulation. Possibly, other agencies might consider inducements to encourage this in the future.	We agree that there is an urgent need to develop understanding of veterinarians and farmers on how antimicrobials work, on how resistance develops and spreads and what responsible use of antimicrobials means. In the Netherlands, a reduction of more than 50% in the consumption of antimicrobials in veterinary medicine has been achieved in a relatively short period of time and without a significant decrease in animal production or increase of disease and mortality. This shows that there has been a significant overconsumption of antimicrobials. A significant reduction of consumption without negative consequences for animal production will very likely also be possible in other MS with high consumption. We agree that eradication of diseases caused by *M. gallisepticum* or *B. hyodysenterieae* is possible and should be encouraged.
2	The paper provides a general overview on the use of Pleuromutilins in pigs and poultry, the resistance prevalence vs. target veterinary	Reviewing all indications for pleuromutilins mentioned in textbooks and national treatment guidelines was out of the scope of this reflection paper. As mentioned in the title, the

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	bacteria, zoonotic and commensal bacteria. The paper's focus is the situation on MIC`s of tiamulin and valnemulin against Brachyspira hyodysenteriae. The importance of the use of the Pleuromutilins against respiratory pathogens like Mycoplasmas (*Mycoplasma hyopneumoniae*, *Mycoplasma synoviae, Mycoplasma hyorhinis)* and Actinobacillus (*Actinobacillus pleuropneumoniae*) and other enteric pathogens (*Brachyspira pilosicoli*, *Lawsonia intracellularis*) is not considered of similar importance. In many textbooks and national treatment guidelines the Pleuromutilins, tiamulin and valnemulin, are recommended for many indications in food animals (Burch et al. 2008, Löhren et al. 2008, Denmark, the Netherlands (http://wvab.knmvd.nl/wvab/formularia/formularia). The importance of this first choice application against different common porcine and avian infections and the corresponding reasons is not considered in the paper; we believe this to be important. The pharmacokinetic characteristics of tiamulin in the case of application in pigs and poultry and of valnemulin in the case of pig are not considered. Those data are important to justify and evaluate the possible effect of Pleuromutilins against enteric and respiratory pathogens. PK/PD relationships are not considered. Clinical trial data at range of different concentrations (infeed application) for swine dysentery treatment and prevention are not considered (Taylor, 1980 & 1982 (tiamulin); Burrows et al, 1996a	development of resistance and the impact of this resistance on animal and human health was the scope of this reflection paper. The pharmacokinetic characteristics of pleuromutilins were also out of the scope of this reflection paper. Published literature on other target pathogens is mentioned, but published data are scarce. Reports are either on other Mycoplasma species, are from countries outside the EU and concern very small numbers of isolates. In addition, no internationally agreed methods and breakpoints are available for most of these pathogens, making interpretation and comparison of available data difficult.

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	and b (valnemulin)). These historic data are not mentioned. The European Commission decision on the Referral procedure for **Tiamutin premix (2010)** based on the Committee for Medicinal Products for Veterinary Use (CVMP) agreement on harmonized indications, posology and withdrawal periods for the concerned premix products is not mentioned (COMMISSION DECISION (C(2010)5372 CORR) of 27.7.2010 concerning the marketing authorisations for “Tiamutin premix and associated names”) In the context of the Referral procedure **harmonized SPCs** for Tiamutin Premix formulations are approved based on the SPC proposals of Novartis Animal Health. The EU Commission decision-approved SPCs are based on most recent MIC data, clinical data and residue data and justify the use of tiamulin for treatment and prevention purposes and for a variety of substantiated disease indications. Similarly, there is no mention of the successful application for a marketing authorisation for Econor Oral Powder for Pigs, which was approved in 2011 (EMEA/V/C/042/X/033), where many of these issues were previously and thereby recently addressed. Great amounts of information on susceptibility of *B.hyodysenteriae* strains to tiamulin and valnemulin, on resistance mechanisms in different bacterial species and the impact on animal and human health are given. Nevertheless sensitivity information of other pathogens in pig and poultry are not always documented. Historic and recent data of resistance to other target pathogens are generally lacking in most cases, other than for *B. hyodysenteriae*.	

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	It is a well written paper, however, in addition to the above comments, a number of factual errors require specific consideration and clarification.	
2	We agree that for an antibiotic substance, claims related to production enhancement are not appropriate and not consistent with existing legislation in the EU. However, the paper seems to be putting some fundamental principles under question, which is of concern: Treatment and prevention: for swine dysentery, prevention as defined in EMA/CVMP/414812/2011 is of critical importance also in the absence of an eradication programme. When claims have been substantiated in accordance with guidance in place and recently endorsed as was the case for valnemulin (EMA, 2011), their withdrawal should not be considered lightly. Veterinary medicine availability and treatment needs should be carefully evaluated, also in the light of a "One Health" approach. Dose and treatment duration for any given pathogen on an approved label have been determined and confirmed in studies conducted according to guidance in place, and with the final formulation of any given product. These parameters can thus differ depending on the pathogen, and on the formulation (which can e.g. influence bioavailability). Therefore, direct comparisons between products with the aim of establishing an optimal dose for all, in terms of active ingredient, are not necessarily valid.	The outcome of the Tiamutin Premix referral (2010) recommended as follows: "*preventative treatment with tiamulin should only be initiated after confirmed infection with B hyo and then as part of a program including measures aiming to eradicate or control infection in the herd*." In regards to the approval for a new formulation for valnemulin referenced (EMA, 2011), no new clinical data were provided as cross-reference was made to studies submitted for the Econor premix formulation in support of the claim for prevention that was approved in **2000** (CVMP/031/99). Since this time there have been increasing reports of AMR development and repeated use of antimicrobials is a concern in this respect. We are pleased about the support harmonised monitoring of susceptibility in *Brachyspira*. Indeed, as many countries now report subpopulations with increased MICs, it is now urgent to monitor for further (undesired) trends. Inclusion of isolated from herds with therapy failure is important, as this makes it possibly to detect highly resistant stains as early as possible (early warning).

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	We welcome the proposal of resistance monitoring in *B. hyodysenteriae* and in fact this would be useful for other target pathogens as well. We understand from the data presented in the paper that it appears that baseline monitoring in *B. hyodysenteriae* for pleuromutilins is no longer applicable since decreased susceptibility has already been reported since 1996 (Molnar, 1996 (Hungary); Gresham et al, 1998 (UK) however, we are concerned that some of the generated data is biased by selection of the tested isolates, as acknowledged by some authors of the respective publications. We support initiatives to install harmonized target pathogen susceptibility monitoring across the EU based on a single protocol and harmonization of MIC testing methodology developed for all countries. *Burch, D.G.S., Duran, C.O. and Aarestrup, F.M. (2008) Chapter 7: Guidelines for antimicrobial use in swine. In Guide to Antimicrobial Use in Animals. Eds Guardabassi, L., Jensen, L.B and Kruse, H., Blackwell Publishing Ltd, Oxford, UK, pp 102-125.* *Löhren,U., Ricci, A., Cummings,T.S. (2008) Chapter 8: Guidelines for antimicrobial use in poultry, In: Guardabassi, L., Jensen,L.B., Kruse, H. (Eds.) Guide to Antimicrobial Use in Animals. Blackwell Publishing Ltd., Oxford, UK, pp126-142.* *Taylor, D.J. (1980) Tiamulin in the treatment and prophylaxis of experimental swine dysentery. Veterinary Record, 106, 526-528.* *Taylor, D.J. (1982) In feed medication with tiamulin in the treatment of experimental swine dysentery. Proceedings of the International Pig*	

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	Veterinary Society Congress, Mexico City, Mexico, p 47. *Burrows, M.R., Morgan, J.H., Burch, D.G.S. and Ripley, P.H. (1996) The comparison of a new compound SDZ PMD 296 (Econor – Sandoz Ltd) and tiamulin for the treatment of swine dysentery. Proceedings of the International Pig Veterinary Society Congress, Bologna, Italy, p 284.* *Burrows, M.R., Morgan, J.H., Burch, D.G.S. and Ripley, P.H. (1996) The comparison of a new compound SDZ PMD 296 (Econor – Sandoz Ltd) and tiamulin for the prevention of swine dysentery. Proceedings of the International Pig Veterinary Society Congress, Bologna, Italy, p 283.* *Molnar, L. (1996) Sensitivity of strains of Serpulina hyodysenteriae isolated in Hungary to chemotherapeutic drugs. Veterinary Record, 138, 158-160.* *Gresham, A., Hunt, B. and Dalziel, B. (1998) Treatment of swine dysentery – problems of antibiotic resistance and concurrent salmonellosis. Veterinary Record, 143, 619.*	
3	IFAH-Europe welcomes the opportunity to comment on this reflection paper. The paper provides a general overview on the use of Pleuromutilins in pigs and poultry, the resistance prevalence vs. target veterinary bacteria, zoonotic and commensal bacteria. The focus of the paper is the situation of the MIC's of tiamulin and valnemulin against Brachyspira hyodysenteriae. The use of the Pleuromutilins against respiratory pathogens like Mycoplasmas (*Mycoplasma hyopneumoniae*, *Mycoplasma synoviae, Mycoplasma*	As mentioned above, reviewing all indications for pleuromutilins mentioned in textbooks and national treatment guidelines was out of the scope of this reflection paper.

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	hyorhinis) and Actinobacillus (*Actinobacillus pleuropneumoniae*) and other enteric pathogens (*Brachyspira pilosicoli*, *Lawsonia intracellularis*) is not considered of similar importance. In many textbooks and national treatment guidelines the Pleuromutilins, tiamulin and valnemulin, are recommended for many indications in food animals (Burch et al. 2008, Löhren et al. 2008, Denmark, the Netherlands (http://wvab.knmvd.nl/wvab/formularia/formularia). The importance of this first choice application against different common porcine and avian infections, and the corresponding reasons for it, are not considered in the reflection paper; we believe this to be an important omission. The pharmacokinetic characteristics of tiamulin in the case of application in pigs and poultry and of valnemulin in the case of pigs are not considered. Likewise, clinical trial data at a range of different concentrations (infeed application) for swine dysentery treatment/control are not considered (Taylor 1992; Burrows et al 1996) and historical data are not mentioned. These data are important to justify and evaluate the possible effect of Pleuromutilins against enteric and respiratory pathogens. The European Commission decision on the Referral procedure for **Tiamutin premix (2010)** based on the Committee agreement on harmonized indications, posology and withdrawal periods for the concerned premix products is not mentioned (COMMISSION DECISION (C(2010)5372 CORR) of 27.7.2010 concerning the marketing authorisations for “Tiamutin premix and associated	As mentioned above, the pharmacokinetic characteristics of pleuromutilins were also out of the scope of this reflection paper.

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	names”). A large amount of information on the susceptibility of *B.hyodysenteriae* strains to tiamulin and valnemulin, on resistance mechanisms in different bacterial species and on the impact on animal and human health are given. Nevertheless the sensitivity information of other pathogens in pig and poultry are not always documented. Historical data of resistance and non-resistance are lacking in certain cases. Despite the omissions mentioned above, in general it is a well written paper. However, in addition to the above comments, a number of factual errors require specific consideration and clarification in the “Specific Comments” section.	As mentioned above, published literature on other target pathogens is mentioned, but published data are scarce. Reports are either on other Mycoplasma species, are from countries outside the EU and concern very small numbers of isolates. In addition, no internationally agreed methods and breakpoints are available for most of these pathogens, making interpretation and comparison of available data difficult. Thank you, those comments have been taken into account.
3	We agree that for an antibiotic substance, claims related to production enhancement are not appropriate and not consistent with existing legislation in the EU. However, the paper seems to be putting some fundamental principles under question, which is of concern: Treatment and prevention: for swine dysentery, prevention as defined in EMA/CVMP/414812/2011 is also of critical importance in the absence of an eradication programme. When claims have been substantiated in accordance with guidance in place and recently endorsed as seems to be the case for valnemulin (EMA, 2011), their withdrawal should not be considered lightly. Veterinary medicine availability and treatment needs should be carefully evaluated, in the light of the “One Health” approach. The negative impact of unforeseen and sudden product withdrawals on the willingness of	See comments provided to Stakeholder number 2.

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	companies to make major investment in product research should also be considered. Dose and treatment duration for any given pathogen on an approved label have been determined and confirmed in studies conducted according to guidance in place, and with the final formulation of any given product. These parameters can thus differ depending on the pathogen, and on the formulation (which can influence *e.g.* bioavailability). Therefore, direct comparisons between products with the aim of establishing an optimal dose for all, in terms of active ingredient, are not necessarily valid. We welcome the proposal of resistance monitoring in *B. hyodysenteriae* and in fact this would be useful for other target pathogens as well. We understand from the data presented in the paper that apparently baseline monitoring in *B. hyodysenteriae* for pleuromutilins is no longer applicable since decreased susceptibility has already been reported since 2001. However, we are concerned that some of the generated data is biased by selection of the tested isolates, as acknowledged by some authors of the respective publications. Initiatives to install harmonized target pathogen susceptibility monitoring exist and IFAH-Europe is actively involved in these efforts; support from the CVMP is welcomed.	
4	FVE welcomes this EMA reflection paper, aiming at ensuring efficiency of pleuromutilins for the future. We recognise that there is a strong need for harmonisation of SPCs across EU, as well as for more research in the areas of susceptibility testing, new active compounds for use in animals, vaccines and alternatives to medicines. Research	Thank you, we agree.

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	towards that direction shall be encouraged and supported.	

2. Specific comments on text

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
15	1	**Comment:** Pleuromutilin group 'is the sole treatment option' this is incorrect **Proposed change:** 'is an important treatment option along with the lincosamides and the macrolide tylvalosin...'	Partly accepted. The sentence indicates that it is the sole treatment option in isolates **resistant** to macrolides. Therefore, tylvalosin is not a treatment option. We changed the sentence into: is the sole treatment option …..in isolates resistant to lincosamides and macrolides.
19-20	1	**Comment:** 'There are several products available on the EU market and most of the use is for group medication in feed or water.' This supports 'sole' is incorrect in L15 but also there are injectable products for treatment **Proposed change:** 'There are several products available on the EU market and most of the use is for group medication in feed or water, but some can be used by injection.'	Not accepted. The word "most' already indicates that there are other kinds of products. In addition this is mentioned elsewhere in the reflection paper (line 118) and figure 3. From figure 3 it is clear that injectables account for only 1% of the sales of pleuromutilins.
25-27	1	**Comment:** Following the Referral for Tiamutin premix and associated names (EMA, 2010) this has been already included. It is possibly up to other licence holders to harmonise their SPCs accordingly. *(EMA/118068/2010)* **Proposed change:** 'are used preventively without	Not accepted. Partially accepted, the following text will be added at the end of the sentence "…e.g. as specified in … the CVMP Tiamutin Premix referral."

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		applying adequate control programmes, as specified in the Tiamutin premix Referral (EMA/118068/2010)'	
28-31	1	**Comment:** This risk assessment has been largely done (Burch, 2013) based on Danish data and the risk of spread to humans is 0.002% but as 90% were associated with pig production, spread to the general public is 0.0002% or 0.2 people/100,000 human population. This can be considered exceptionally low even in a high risk country like Denmark, where 10 times as many pigs are produced/capita population than the EU average of 0.5 pigs/capita. **Proposed change:** Change line 30-31 '....the risk for spread of resistance from animals to humans is considered low.' *(Burch, D. (2013) Perché la resistenza agli antibiotici nei suini non è così importante perla salute pubblica?(Why is antimicrobial resistance in pigs not so important for public health?). Proceedings della Societa Italiana di Patologia Ed Allevamento dei Suini, Vol XXXIX, pp 45-64. English version -* http://www.octagon-services.co.uk/articles/ABresistanceSIPAS2012.pdf*)*	Not accepted. The Danish situation may not reflect the situation in all MS. In addition, the epidemiology of LA-MRSA might change in the future. We do not agree that the risk for spread of resistance from animals to humans is considered low.
38-39	1	**Comment:** The use of pleuromutilins for treatment and prevention has been justified in the recent Tiamutin Premix	Not accepted. The approval for the oral powder formulation of Econor was based on extrapolation of the clinical data provided for the

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		referral (EMA, 2010) as explained above and has been included in the Premix SPCs e.g. *"Preventative treatment with tiamulin should only be initiated after confirmed infection with B. hyodysenteriae and then as part of a programme including measures to eradicate or control the infection in the herd"*. Econor Premixes and Oral Powder, following the latter's registration approval (EMA, 2011) also carries similar warning statements *"Long term preventative use of valnemulin should be avoided by improving management practice and thorough cleansing and disinfection. Consideration should be given to the eradication of infection from the farm".* Other licence holders should carry similar warnings on their tiamulin-based products. **Proposed change:** Change to 'Pleuromutilins should only be used for treatment and prevention of disease in well defined clinical situations and eradication programmes, including swine dysentery.	Premix, approved in 2000. Since this time, reports of resistance have increased and this text, along with that of SPC s for other products, should be brought into alignment with that agreed for the Tiamutin Premix referral. Not agreed, see above.
41	1	**Comment:** The sentence seems contradictory 'Approved indications for unspecified prevention of disease should be withdrawn.' Either a specific indication is approved or not and therefore cannot be unspecified? **Proposed change:** Delete L 41	Partly agreed. We changed this sentence into: Approved indications such as prevention of disease other than during eradication programmes should be withdrawn.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
47-51	1	**Comment:** The meaning of this paragraph is not quite clear. Following the Tiamutin Premix referral (EMA, 2010) there was a harmonisation of indications, dose rates and inclusion rates, for all indications. In some cases there was a range of doses and inclusion rates, which depended on the indication and the susceptibility of the organism, especially *B. hyodysenteriae*. There are variations in doses and inclusion rates in some other national registrations belonging to other licence holders. Is this what is meant? **Proposed change:** Clarify the wording and meaning of the paragraph.	This paragraph has now been deleted and reference is made to the Tiamutin premix referral with a recommendation that SPCs for generic products should be brought into alignment.
52-68	1	**Comment:** These statements are largely supported. There are quite a large number of publications on antimicrobial resistance to *B. hyodysenteriae* but there is no standardised method or standardised interpretation of clinical breakpoints. **Proposed change:** No change proposed	No change proposed
117	1	**Comment:** Tiamulin is also available in some MS as a water soluble granule and oral powder. **Proposed change:** '...premix, a solution and water soluble powder for	Accepted.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		medicating drinking water and as an oral powder in some MS for adding to feed and/or water.	
196	1	**Comment:** The *vga* genes are resistant to pleuromutilins, streptogramin A and lincosamides. It must be remembered that in a number of reports, outside the EU, e.g. Mendes et al (2011), virginiamycin is still extensively used as a growth promoter and antibiotics are used primarily off-veterinary prescription. In line196 it just refers to pleuromutilin-streptogramin genes. In the light of the Hauschild et al (2012) data where all the isolates were also spectinomycin resistant (*spc* gene) and lincomycin is largely sold in combination with spectinomycin, I think it may be misleading to just refer to this as a pleuromutilin-streptogramin gene. **Proposed change:** Change line 196 to 'There are 7 known streptogramin, lincosamide and pleuromutilin resistance genes: ....'	Partly accepted. Genes as such are not resistant, bacteria carrying these genes are. The *vga* genes do not confer resistance to spectinomycin. The fact that the *vga* gene can be present together with other resistance genes, such as the *spc* gene, is beyond the scope of this reflection paper. However, we do agree that the vga gene encodes resistance to pleuromutilins, lincosamides and streptogramin A. Proposed change accepted.
240-257	1	**Comment:** Most of this data is developed in China, outside the EU. It highlights the potential hazard of resistance gene development but it must be remembered that antimicrobials are often locally produced and easily available. There is no need for a veterinary prescription. Human and animal waste is often mixed	Not accepted. Lines 240-257 is a review of described resistance mechanisms, not of the prevalence of resistance in specific regions. Therefore the country where the isolates originated from or the animal species from which the isolate was cultured is not important. The risk of resistance genes, which are often present on

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		together and used as fertilizer directly on fields, especially for rice growing. Although of interest, not too much emphasis should be put on it, as non-EU data. In Wendlandt et al (2012) it states "*In Enterococcus faecalis and Streptococcus agalactiae, the genes lsa(A) and lsa(C), respectively, which also encode ABS transporters, mediate a similar resistance phenotype*." This phenotype is similar to vga genes. However, the isolates were from human origin, rather than animal. Hart et al (2004) showed that 96% (90/94) of Enterococci isolates from pigs were resistant to tiamulin, 60% to lincomycin and 0% to virginiamycin, suggesting that they were inherently resistant to tiamulin, rather than acquiring resistance and certainly not by the *lsa* gene. **Proposed change:** Change – 'In *Enterococcus faecalis*, in humans resistance to pleuromutilins, streptogramin A and lincosamides may be mediated by *lsa*(A) genes (Wendlandt et al, 2012) but generally enterococci appear inherently resistant to tiamulin (Hart et al, 2004).' *(Hart, W.S., Heuzenroeder, M.W and Barton, M.D. (2004) Antimicrobial resistance in Campylobacter spp., Escherichia coli and Enterococci associated with pigs in Australia. Journal Of Veterinary Medicine, B 51, 216-*	mobile elements, is not only resistance in the bacteria were it was first detected, but the fact that they might be transferable to other kinds of bacteria. This has already been reported for the enterococcal ABC transporter gene lsa (E) that has been found in MSSA and MRSA strongly suggesting its transfer between Enterococcus spp. and *Staphylococcus aureus.* As opposed to *Enterococcus faecalis* that is intrinsically resistant to lincosamides, streptogramins A, and pleuromutilins (LS_AP phenotype) by production of the ABC protein lsa(A), *Enterococcus faecium* is naturally susceptible. In fact, BC-3781 is developed to treat human infections including those caused by vancomycin resistant *Enterococcus faecium*. Therefore we do not agree to the suggested changes.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		221.)	
251 & 259	1	**Comment:** Rather than *Isa* gene it should be *lsa* – after lincosamides and streptogramins A **Proposed change:** Change *Isa* to *lsa on line 251 and 259*	Agreed.
259	1	**Comment:** In Malbruny et al (2011) there could be some confusion regarding the derivation of *S. agalactiae,* as it is a common bovine pathogen. This reference is purely related to human beings and the isolate was from a human vaginal swab, where it is a common human infection. **Proposed change:** Change L 259 'Expression of this gene in *S. agalactiae*, isolated from a human vaginal swab, ……..'	In line 261 it is already stated that the isolates originated from humans. No change needed.
298-301	1	**Comment:** Setting clinical breakpoints for *B. hyodysenteriae* is difficult (Burch, 2005) even when basing them on PK/PD relationships. However, in the Vyt and Hommez (2006) paper only one sensitive isolate (MIC 0.06µg/ml) was reported not to work in their **survey** of 17 farms (88% success rate otherwise) and one with an intermediate MIC between >1-≤4µg/ml, but the treatment route or inclusion rate was not reported.	Agreed: We changed study to survey.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		Anderson et al (1994) reported concentrations in colonic contents of 8.05μg/ml when included in feed at 220ppm but only 2.84μg/ml at 110ppm, so it is important to define these criteria also. Conversely, Vyt and Hommez (2006) showed that in a **trial** lincomycin included in feed at 110ppm treated successfully an isolate with an MIC of 64μg/ml but not ≥128μg/ml. It is possible that the lincomycin concentration in colonic contents at 110ppm has been mis-reported (Degeeter et al, 1980) as at 220ppm concentrations reach 101μg/ml. These matters need to be resolved. **Proposed change:** Change - 'On the basis of a field **survey** of clinical efficacy……' *(Anderson, M.D., Stroh, S.L. and Rogers, S. (1994) Tiamulin (Denagard ®) activity in certain swine tissues following oral and intramuscular. Proceedings of the American Association of Swine Practitioners, Chicago, Illinois, USA, pp115-118.* *DeGeeter, M.J., Barbiers, A.R. and Stahl, G.L. (1980) Concentration of lincomycin in body tissues and fluids fed diets fortified with the antibiotic. Proceedings of the 6th International Pig Veterinary Society Congress, Copenhagen, Denmark, p 283.)*	
304-307	1	**Comment:** Burch (2005) also suggested Clinical inhibitory breakpoints for 200ppm tiamulin in feed of >1.0μg/ml	Agreed

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		for micro-broth methods and >2.0µg/ml for agar plate tests. This inclusion rate has been approved following the Tiamutin premix referral (2010) across the EU now. **Proposed change:** Add -'.... respectively, and double these figures for 200ppm inclusion in feed (Burch 2005).'	
374-375	1	**Comment:** In Witte and Cuny (2011) it clearly states "*Veterinary use of both florfenicol and tiamulin, very likely selected for the emergence of cfr in animal isolates of coagulase negative staphylococci where it was first detected*."Page 926. However, it must be remembered that these are multi-resistant, co-resistant genes to lincosamides, streptogramins and especially oxazolidinones. Additionally, this finding is extremely rare with only 1/436 isolates of ST398 being linezolid resistant. **Proposed change:** Change line 374 to 'It has been suggested that the use of florfenicol and tiamulin very likely selects for the emergence of *cfr*.....'	Agreed
396	1	**Comment:** 'Therefore, pleuromutilins are the only remaining treatment option for this indication.' This statement is inaccurate and should be withdrawn. The clinical	It is true that clinical break-points for the antimicrobials in question have not been agreed. To our knowledge, there is no published evidence that treatment with the alternatives

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		breakpoints for the anti-swine dysentery drugs have not been set, so precise resistance determinations cannot be made. Hidalgo et al (2011) in Spain has shown that the macrolide Tylvalosin has an almost completely susceptible susceptibility pattern (94% at a breakpoint of >16μg/ml) in stark contrast to the other macrolide Tylosin (page 3333). The lincomycin pattern using >32μg/ml as a breakpoint is 70% susceptible and using the clinical breakpoint from Vyt and Hommez (2006) of 64μg/ml it is closer to 85%. In Sperling et al (2011) the tiamulin-resistant Czech isolates were 66% lincomycin susceptible and in the large-scale farm isolates 76% were susceptible. Degeeter et al, 1980 showed that concentrations of 101μg/ml could be achieved in colon contents at 220ppm lincomycin in feed. **Proposed change:** Remove the sentence 'Therefore, pleuromutilins are the only remaining treatment option for this indication.'	(macrolides, lincosamides) at authorized doses is effective against strains with clearly elevated MICs (non-wild type), e,g, above the MICs of strains from efficacy trials. To clarify that the antibiograms may vary, we have changed the sentence to: "When resistance occur to alternative antimicrobials, pleuromutilins remain the only ...."
406	1	**Comment:** Olaquindox is not approved for use in the United States of America **Proposed change:** Delete 'or olaquindox'	Accepted.
426-428	1	**Comment:**	

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		BC-3781 is being developed for human use, however its high plasma-protein binding of 87%, or plasma-free fraction 13% (Zeitlinger et al, 2011), similar to that of valnemulin, may have a limitation on its antibacterial activity with regard to systemic infections, especially in immuno-compromised patients and bactericidal concentrations may not be achieved (Drews et al, 1975). **Proposed change:** After 'Sader et al, 2012b).' Insert – 'However, high plasma protein binding might limit its antibacterial activity in systemic infections, especially in immuno-compromised patients. *(Zeitlinger, M., Obermayr, F., Burian, A., Badreslam, R., Burian, B., Mueller, M., Strickmann, D.B., Novak, R. and Prince, W. (2011) The pharmacokinetics of BC-3781 in muscle and adipose tissue in healthy subjects. Poster 51st Interactive Conference on Antimicrobial Agents and Chemotherapy, Chicago, Il. USA, A2 024.* http://www.nabriva.com/fileadmin/user_upload/A1-1761a_MD_BC-3781_ICAAC_2011.pdf *Drews, J.,Georgopoulos, A., Laber, G., Schuetze, E. and Unger, J.(1975) Antimicrobial activities of 81.723 hfu, a new pleuromutilin derivative.Antimicrobial agents and Chemotherapy, 7, 5, 507-516.)*	Not agreed We do not think this information is relevant here, if this hypothesis is true it will be evident in later stages of the development.
428-429	1	**Comment:** Lotesta et al, (2011) have been working on the activity	

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		of pleuromutilins against *M. tuberculosis* but have not yet identified candidates, which were superior to tiamulin and that had a relatively high MIC of 12-25µg/ml. **Proposed change:** After 'Lotesta et al, 2011)'. Insert – 'To date, no suitable candidates for further development have been reported.	Not agreed. Comment is regarded irrelevant.
450-452	1	**Comment:** Although the presence of a *vga* (A) gene and *tet* (M) were described in the Mendes et al, 2011 paper and tiamulin and tetracycline were being used at the time of sampling, virginiamycin is widely used in the US as a growth promoter as well as lincomycin as a feed additive. In Cuny et al (2009) transmission of MRSA CC398 appears to be common in Germany between infected pigs and pig farmers but there was no evidence of spread beyond family members into the general population. Only a few members of the public may have been infected in Denmark (Danmap 2011, 2012), which were not in contact with pigs. **Proposed change:** Insert – 'transmission may occur (Mendes et al, 2011) but the risk of spread to the general public appears to be very low.' *(Cuny. C., Nathaus. R., Strommenger. B., Altmann.*	Not accepted. See previous comments on *vga* and MRSA.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		D., Witte. W.: 2009. Nasal colonization of humans with methicillin-resistant Staphylococcus aureus (MRSA) CC398 with and without exposure to pigs. PLoS ONE, August: pp e6800.)	
489-491	1	**Comment:** 'Another option to reduce the use of pleuromutilins would be to reserve this class of antimicrobials for the treatment of swine dysentery as alternative treatments for the other indications are available.' It has been shown that it is the widespread treatment of *B. hyodysenteriae* by tiamulin that has caused the resistance problem to develop. Reserving it for just this indication will have no helpful impact. The use in other species such as poultry and rabbits does not interfere with resistance development to *B. hyodysenteriae* and resistance in other pig indications are minimal, regarding *Mycoplasma* spp and *Lawsonia intracellularis*. '**Proposed change:** Delete - 'Another option to reduce the use of pleuromutilins would be to reserve this class of antimicrobials for the treatment of swine dysentery, as alternative treatments for the other indications are available.'	Not agreed. Widespread use of pleuromutilins has caused resistance to develop and this is a concern. Resistance has not only developed in *Brachyspira hyodysenteriae*, but also in other microorganisms, such as staphylococci and enterococci, which is a concern and not only in pigs, but in all animal species and humans.
507-511	1	**Comment:** Specifically in line 509 'and in such situations there is a **high potential** that the use of pleuromutilins for the	Partly agreed.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		treatment or prevention of other disease such as swine dysentery further selects for pleuromutilin resistant staphylococci, including MRSA.' The data demonstrates there is a potential but it is very small. **Proposed change:** Change to: 'and in such situations there is a potential that the use of pleuromutilins for the treatment or prevention of other diseases like swine dysentery may select for pleuromutilin resistant staphylococci, although this has not been described (Rubin et al, 2011). Regarding MRSA ST398, there appears to be a potential for co-selection (Rubin et al, 2011), possibly linked to multi-resistance plasmids carrying *vga*(A) or *vga*(C) resistance genes (Kadlec et al, 2010)."	We changed the sentence into: and in such situations there is a potential that the use of pleuromutilins for the treatment and prevention…
517-518	1	**Comment:** 'The emergence of these resistant genes in animals poses a potential threat to human medicine as they might compromise empirical treatment of human MRSA infections.' **Proposed change:** Insert after the above sentence – 'However, the potential threat currently appears very low.'	Not agreed. This is not a full risk assessment. Quantifying the risk is difficult and beyond the scope of this reflection paper.
15 & 16	2	**Comment:** Other effective antibiotics are available for SD treatment and are alternative choices beside Pleuromutilins like Tylvalosin (Vyt 2010, Vyt et al 2012, Hildago et al 2011) and Lincomycin (Herbst et al	Partly accepted. See comment to stakeholder 1. The sentence indicates that it is the sole treatment option in isolates resistant to macrolides. Therefore, tylvalosin is not a treatment option.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		2008, Vyt & Hommez 2006). Suggest to change 2nd part of sentence; delete “the sole” and “resistant to macrolides”. It must be understood that the data from Hildago et al. 2011 show that not all macrolides have equal activity against *B. hyodysenteriae* and strains resistant to tylosin can still have low MICs against tylvalosin (MICs (MICs ≤4 µg/mL) Suggest to add “respiratory infections caused by *Mycoplasma* species in both pigs and poultry”. Resistance of *Mycoplasma hyopneumoniae* strains to macrolides, fluoroquinolones and lincosamides are reported (Maes et al. 2007, Vicca et al 2004, Kobayashi et al. 2008). In those cases Pleuromutilins can be recommended to be used based on low MIC values. **Proposed change:** So it would read “ … it is one of the treatment options for enteritis caused by *Brachyspira hyodysenteriae* (swine dysentery) and respiratory infections caused by *Mycoplasma* species in both pigs and poultry”. *Vyt , P. 2010. Antimicrobial susceptibility of Belgian Brachyspira hyodysenteriae isolates. Proceedings 21st IPVS Congress, Vancouver, Canada, O.205. p.238.* *Vyt , P., L.Vandepitte, A.Dereu, M.Roozen 2012. Elimination of swine dysentery on a single-site, farrow –to-finish farm using tylvalosin (Aivlosin). Proceedings Vol II 22nd IPVS Congress, Jeju, Korea, BP-272 p.629.* *Hildago,A., Carvajal,A., Vester,B., Pringle, M., Naharro, G., Rubio, P. 2011. Trends towards lower antimicrobial*	We changed the sentence into: is the sole treatment option … in isolates resistant to lincosamides and macrolides.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		susceptibility and characterization of acquired resistance among clinical isolates of Brachyspira hyodysenteriae in Spain. Antimicrobial Agents and Chemotherapy, p.3330-3337. *Herbst, W., Schlez, K., Heuser, J., Baljer,G. (2008). Detection of Brachyspira hyodysenteriae in pigs with and without diarrhoea and drug resistance of German B.hyodyenteriae field isolates. Proceedings 20th IPVS Congress, Durban, South Africa, P03.021 p.241.* *Vyt,P., Hommez,J. (2006). Antimicrobial susceptibility of Brachyspira hyodysenteriae isolates compared with the clinical effect of treatment. Flem. Vet.J.75, 279-285.* *Maes,D., Vicca,J., Stakenborg,T., Butaye,P., De Kruit,A., Haesebruck,F. (2007). In vitro susceptibility of Mycoplasma hyopneumoniae field isolates. Vlaams Diergeneeskundig Tijdschrift, 76, 300-305.* *Kobayashi,H., Kanazaki,M., Kajiwara,K. (2008). Macrolid, tiamulin and valnemulin susceptibility of Mycoplasma hyopneumoniae strains isolated in various parts of Japan. Proceedings 20th IPVS Congress, Durban, South Africa, P02.002 p.187.* *Vicca,J., Stakenborg,T., Maes,D., Butaye,P.,peetrs,J., DeKruit,A., Haesebrouck,F.(2004). In vitro susceptibility of Mycoplasma hyopneumoniae field isolates. Antimicrobial Agents and Chemotherapy, Vol.48, No.11, 4470-4472*	

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
16,17,18	2	**Comment:** Suggest to change sentence; delete "The negative consequences in case such a pathogen becomes pleuromutilin resistant would thus be considerable" **Proposed change:** Replace by the following sentence "It is highly important to use Pleuromutilins prudently to contain resistance development against these major pathogens from an economical and animal welfare perspective".	Not agreed. The phrase is intended to provide a clear message about the consequences of *Brachyspira hyodysenteriae* becoming resistant.
38	2	**Comment:** The fact that pleuromutilins should only be used for treatment of disease does not consider the field condition with animals being at different infection status. Thus the treatment should cover these different stages of the infection. Early treatment is of particular consideration since it has been shown that this correspond to low "inoculum" and thus to increased efficacy and lower risk for resistance development (Ferran et al, Vet Microbiology, 2011). It should be stated that the use of Pleuromutilins should be restricted for indications for which efficacy has been proven with solid clinical data. **Proposed change:** replace the first sentence of the paragraph by "Pleuromutilins should be used according to the responsible use principles and for treatment and prevention of disease for which efficacy has been proven " *Ferran,A.A., Toutain,P.-L., Bousquet-Melou,A. (2011). Impact of early versus later fluoroquinolone treatment*	The perception on what prudent or responsible use of antimicrobials varies. Preventive use of antimicrobials is not the same as early treatment. The reference on treatment of mice and fluoroquinolones in a mouse-lung model of *Pasteurella multicida* seems irrelevant here as this is a reflection paper on pleuromutilins not fluoroquinolones and *Pasteurella multocida* is not a target pathogen. Not agreed. Pleuromutilins should not be used for prevention of disease other than under strict conditions in eradication programmes.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		on the clinical, microbiological and resistance outcomes in a mouse-lung model of Pasteurella multocida infection. Veterinary Microbiology 148, 292-297.	
38 & 39	2	**Comment:** Pleuromutilins are important drugs in eradication programmes for swine dysentery, ileitis and enzootic pneumonia (Sperling et al. 2012, Kixmoeller et al. 2010, Kamp et al. 2010, Burch & Howells 2010, Pico et al. 2008, Rajska et al. 2008, Giger et al. 2006, Nielsen 2004). Based on the results of sensitivity testing they are identified as the drug of choice for eradication of different diseases on specific farms. Suggest to modify the sentence and delete "The exception would be" and add "… for swine dysentery, ileitis and enzootic pneumonia based on MIC testing results". " Pleuromutilins should be used in well-defined eradication programmes for swine dysentery, ileitis and enzootic pneumonia based on MIC testing results " **Proposed change:** It should read " … Pleuromutilins should be used in well-defined eradication programmes for swine dysentery, ileitis and enzootic pneumonia based on MIC testing results " *Sperling,D. Malasek,J., Cizek,A., Smola,J., Hasman,P. Swine dysentery eradication with long-term administration of valnemulin on a large-scale pig farm (2012). Proceedings 22nd IPVS Congress, Jeju, Korea, BP-273, p.630.* *Kixmoeller,M., Kmiec,M., Szancer, J. Attempt to*	Not agreed. Pleuromutilins should not be used in eradication programmes other than those for swine dysentery.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		eradicate Lawsonia intracellularis during establishment of a new breeding herd by combined strategic medication with tiamulin (Denagard) and cleaning/disinfection (2010). Proceedings 21st IPVS Congress, Vancouver, Canada, P.402. p.708. *Kamp,J., Schuttert-Wilps, R., Kars-Hendricksen, S.(2010). Swine dysentery eradication by strategic medication without depopulation. Proceedings 21st IPVS Congress, Vancouver, Canada, P.427. p.733.* *Burch, D., Howells M.J.(2010). Eradication of swine dysentery in an outdoor breeding herd and its production pyramid. Proceedings 21st IPVS Congress, Vancouver, Canada, P.426. p.732.* *Pico, L.,Szancer, J., Pique, J., Vidal, A. (2008). Swine dysentery eradication programme in a large farm with Three site Production by strategic management and medication. Proceedings 20th IPVS Congress, Durban, South Africa, OR.03.16. p.131.* *Rajiska, M.,Kempa, W., Wilczynski, K. (2008). Experiences with control programme of swine dysentery in a typical polish pig unit. Proceedings 20th IPVS Congress, Durban, South Africa, P.03.031. p.250.* *Giger, TG., Schmid, W., Klein, U. (2006). Eradication of M.hyopneumoniae in breeding herds without restocking or partial depopulation. Proceedings 19th IPVS Congress, Copenhagen, Denmark, O.68-02 p.314.*	

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		Harm Nielsen, L. (2004). Attempt to eradicate Lawsonia intracellularis by medication in 3 sow herds. Proceedings 18th IPVS Congress, Hamburg, Germany,Vol.1, p.281.	
41	2	**Comment: "**Approved indications for unspecified prevention of disease should be withdrawn". This is a rather confusing. Either the indication is approved or it is not, therefore unspecified prevention of disease does not make sense. Currently, prevention is permitted as an indication for a disease claim and was included in the recent EU Referral for Tiamutin premixes (EU Commission Decision 2010). **Proposed change:** This sentence should be removed.	Partly agreed. The sentence has been changed into: Approved indications such as prevention of disease other than during eradication programmes should be withdrawn.
42, 43	2	**Comment:** Suggest to introduce "without a solid clinical basis" **Proposed change:** so it would read "General indications against infections in general without a solid clinical basis should be avoided".	Text revised. The following has been added: "e.g. those that do not include named target pathogens".
62, 63 Lines 62 to 68	2	**Comment:** Suggest to change 2nd part of sentence "… monitoring data on the susceptibility of *Brachyspira hyodysenteriae* … add "against Pleuromutilins and other therapeutics" The text in lines 62 to 68 is strongly supported and we suggest adding the importance of sampling, culturing and MIC testing based on a standard protocol. Diversity of results based on a ring test conducted to assess diagnostic and antimicrobial susceptibility	The word "harmonized" already includes sampling strategies The following has been added: "for pleuromutilins and other relevant antimicrobial"

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		testing work of Brachyspira species in different European labs have been found and reported by Rasbeck et al., (2005). **Proposed change:** "… harmonized monitoring data on the susceptibility of *Brachyspira hyodysenteriae* against pleuromutilins and other therapeutics. "… therefore recommend responsible bodies to create such a monitoring system including sampling, culturing and MIC testing work based on a standard protocol, first to allow baseline data …" *Rasbeck,T., Fellstroem,C., Bergsjo,B., Cizek,A., Collin,K., Gurrarson,A., Jensen,S.M., Mars,A., Thomson,J., Vyt,P., Pringle,M. (2005). Assessment of diagnostics and antimicrobial susceptibility testing of Brachyspira species using a ring test. Veterinary Microbiology 109, 229-243.*	
106-117	2	**Comment:** It should be emphasized that during the Referral procedure for **Tiamutin premix** (Commission Decision (C(2010)5372 CORR) of 27.7.2010) there was a harmonization of indications, dose rates, inclusion rates for all registered pig, poultry and rabbit indications established in the EU. Based on the Referral procedure treatment and prevention application was substantiated based on available clinical data for pig, poultry and rabbits in accordance with EU Guidelines (EMA/CVMP/414812/2011). Valnemulin was also recently (2011) re-examined for a marketing authorisation for Econor Oral Powder for	See comments above on referrals. The application for Econor Oral Powder was an extension and cross-referred to the clinical data for the premix formulation which was approved in 2000. Therefore the treatment claims were not fully addressed in the recent procedure.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		Pigs (EMEA/V/C/042/X/033), where all the treatment claims for ileitis and enzootic pneumonia were assessed, in addition to swine dysentery. **Proposed change:** Line 117 after "enteropathy[1]." Insert sentence. Both tiamulin and valnemulin have recently been reviewed by CVMP (2011) and both prevention and therapeutic claims were approved, in addition to other indications and species as well as to swine dysentery in pigs.	
119, 120	2	**Comment: "**Pleuromutilins are also used off-label to treat the polyaetiological disease porcine respiratory disease complex (PRDC), and more rarely, leptospirosis". This has been resolved in the EU Referral process (EU Commission Decision 2010). Tiamulin is highly effective against *Mycoplasma hyopneumoniae* and *Mycoplasma hyopneumoniae* is part of the PRDC disease complex. Leptospirosis is not a registered indication for tiamulin. **Proposed change:** the sentence should be adapted according to approved SPC text based on the EU Referral and EU Commission decision (2010) or preferably deleted.	Agreed
122, 123	2	**Comment:** This reference can be argued to be inappropriate because it only refers to Denmark. Why has the ESVAC report not been cited from 2010 where Table 6 shows that pleuromutilin use across Europe is highly variable. It also shows (Table 6) that Denmark uses disproportionately more than any other European	Agreed to partially change the text to: *"In some countries pleuromutilins are frequently used in the treatment of swine, especially in weaner pigs and finisher pigs (Jensen et al., 2012)."* At the end of the chapter the following has been added:

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		country. Figure 7 of this report shows pleuromutilin sales across Europe in mg/PCU to be almost the lowest of all classes. Also the report shows only 6% of premix sales were attributed to pleuromutilins and 6% as oral solutions, both being the lowest of all reported antimicrobial classes. The reflection paper considers the ESVAC data in lines 139 to 164 but does not put in context of overall sales of antimicrobial classes. **Proposed change:** consider the ESVAC report (2010) and change the sentence accordingly.	*"Sales of pleuromutilins in 19 countries, expressed as mg/PCU, were 3.5% of total sales of antimicrobials to food producing animals (ESVAC, 2012b)."* This is line with the mentioned ESVAC report (table 6). Table 6 in the ESVAC report does not show that Denmark uses "*disproportionately more*", it shows that the percentage of the total sales is higher but as the total Danish sales is not on the high end, the actual use is not among the highest. This is clearly shown in Figure 2 of the reflection paper. Some other countries report considerably higher figures. The figure of 3.5% contradicts the comment that pleuromutilins are the lowest of all reported antimicrobial classes.
139-164	2	**See comment above re lines 122, 123**	Agreed
171-180	2	**Comment:** We support the text but it is important to consider the following, we suggest that this paragraph is added: Studies by Long et al (2006) have shown that different pleuromutilin derivates are similarly anchored in the binding pocket by the common tricyclic mutilin core. However, varying effects are observed at positions U2584 and U2585, indicating that the side chain extensions adopt distinct conformations within the cavity and thereby affect the rRNA conformation differently. The data clearly demonstrate that there is a differential response for tiamulin and valnemulin with valnemulin being better able to withstand an altered rRNA binding surface around the mutilin core and	Not agreed. This is unnecessary long and adds little to the overall aim of the paper.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		retain its' activity. This is likely due to additional interactions made between the specific side chain extension of valnemulin and the rRNA binding site. This clearly impacts upon cross resistance and helps to understand that resistance to tiamulin does not necessarily mean an isolate will be resistant to valnemulin. The fact that the pleuromutilin drugs footprint at the peptidyl transferase and inhibit the peptidyl transferase reaction defines their inhibitory mechanism to some degree. The basis for explaining the inhibitory mechanism of pleuromutilins is steric hindrance of the peptidyl transferase reaction. In brief, tiamulin selectively inhibits bacterial protein synthesis by binding to the ribosome with high affinity, inhibiting ribosomal peptidyl transferase activity and partially inhibiting the binding of the initiator tRNA substrate to the ribosomal P-site. This effect will not necessarily be the same for other pleuromutilin drugs, either valnemulin or others that are in development. *Long KS, Hansen LH, Jakobsen L, Vester B (2006) Interaction of pleuromutilin derivatives with the ribosomal peptidyl transferase center. Antimicrobial Agents and Chemotherapy 50, 1458-1462.*	
181-188	2	**Comment:** We support the text, however, we argue that there is an important issue that is not described and we suggest that the following text is inserted after Line 188: Pringle *et al* (2004) show that all of the laboratory-	Not agreed. The suggested paragraph misquotes or misunderstands the cited studies. In Pringle et al (2004), the authors did not postulate as suggested in the comment. Further, in lines 181-190 there are two references quoted and the second connect the in vitro data with field data. One of the mutations initially described by Pringle et *al* (2004),

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		derived strains with an increased MIC relative to the parent strain contained mutations in either the ribosomal protein L3 or domain V of 23S rRNA. Although this is not direct proof that the mutations cause the increase in MICs, it strongly predicts them as resistance determinants. The isolates selected in the laboratory, however, contained different combinations of mutations and none of the combinations were identical. This illustrates that changes in susceptibility to tiamulin can be obtained in various ways. The strains with the highest tiamulin MICs contained three mutations, indicating that one mutation is not sufficient to cause a high level of resistance. The two strains with the highest tiamulin MICs (>128 µg/mL) were the only strains with the T2504G mutation. The authors postulated that the absence of mutations in 23S rRNA at the peptidyl transfer centre in the field isolates could be because unlike the laboratory derived mutants they had not been exposed to the high concentration of tiamulin and the long exposure time. Alternatively, the described mutations could have had a fitness cost and led to decreased strain viability, with the consequence that such mutants could not survive in the field and thus could only be isolated under more supportive conditions in the laboratory. Clearly this finding suggests that laboratory based resistance data needs to be interpreted with care. *Pringle M, Poehlsgaard J, Vester B, Long KS (2004) Mutations in ribosomal protein L3 and 23S ribosomal*	G2032A, was associated with high MICs (>128 mg/l). This mutation was found in Spanish field isolates of *B. hyodysenteriae* connecting results from the *in vitro* study with isolates being exposed to tiamulin in the pig. We have slightly amended the text to clarify this very important point, see revised reflection paper with the following text: Hidalgo et al. (2011) show that one of the mutations, G2032A, that was present in the *B. hyodysenteriae* strain with the highest tiamulin MIC (>128 µg/mL), K4R, is associated to high tiamulin MICs in Spanish field isolates of *B. hyodysenteriae* connecting results from the *in vitro* study with isolates being exposed to tiamulin in the pig.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		RNA at the peptidyl transferase centre are associated with reduced susceptibility to tiamulin in Brachyspira spp. isolates. Molecular Microbiology 54, 1295-1306.	
188	2	**Comment:** In the absence of harmonised testing methodology for Brachyspira it is considered we must be careful in interpreting comparative data between tiamulin and valnemulin. Long et al (2006) have demonstrated that there is a differential binding response for tiamulin and valnemulin at the active site with valnemulin being better able to withstand an altered rRNA binding surface around the mutilin core and retain its' activity. **Proposed change:** Whilst the MICs for valnemulin seemingly follow those for tiamulin in most cases but are generally a few dilution steps lower (Pringle et al., 2012) it is accepted that binding of tiamulin and valnemulin at the active is different and in the absence of understanding more about potential resistance genes, it is inappropriate to read too much into MIC data without any agreement on internationally agreed harmonised susceptibility testing methodology. *Long KS, Hansen LH, Jakobsen L, Vester B (2006) Interaction of pleuromutilin derivatives with the ribosomal peptidyl transferase center. Antimicrobial Agents and Chemotherapy 50, 1458-1462.* *Pringle, M., A. Landen, H.E. Unnerstad, B. Molander, and B. Bengtsson. 2012. Antimicrobial susceptibility of porcine Brachyspira hyodysenteriae and Brachyspira*	Not agreed. The issue of the problems of susceptibility testing are mentioned elsewhere (line 273-309) and therefore the insertion is unnecessary. In Hidalgo et *al* (2011) the Spanish field isolates with high tiamulin MICs and described mutations associated to these also have high valnemulin MICs. In Karlsson et al. 2001 both tiamulin and valnemulin MICs increase in parallel in laboratory derived mutants. In Rohde et al. (2004) the valnemulin MIC is following the tiamulin MIC for more than 300 clinical isolates if *B. hyodysenteriae*. *Karlsson, M., Gunnarsson, A., Franklin, A., 2001, Susceptibility to pleuromutilins in Brachyspira (Serpulina) hyodysenteriae. Animal Health Research Reviews 2, 59-65.* *Rohde, J., Kessler, M., Baums, C.G., Amtsberg, G., 2004, Comparison of methods for antimicrobial susceptibility testing and MIC values for pleuromutilin drugs for Brachyspira hyodysenteriae isolated in Germany. Vet Microbiol 102, 25-32.*

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		pilosicoli isolated in Sweden between 1990 and 2010. Acta Vet Scand 54:54.	
191-236	2	**Comment:** We support this review of the literature, however, we believe it does not consider all the available data and as such we argue that the additional information needs to be added after line 236 The issue of co-resistance is extremely complex and the data needs to be considered most carefully. This is best exemplified by the study of Miller *et al* (2008); these workers investigated whether other mechanisms of resistance to linezolid in clinical isolates of *S. aureus* might also confer cross-resistance to pleuromutilins. Despite early predictions that linezolid would display a low potential for the development of resistance, resistant clinical isolates of *S. aureus* have, unfortunately, emerged following prolonged therapy with the agent. As oxazolidinone and pleuromutilin antibiotics are currently used in the treatment of staphylococcal infections and as both antibiotics inhibit protein synthesis and have overlapping binding regions on 23S rRNA, the potential for co-resistance between the two classes through target site mutations is an obvious possibility. Miller *et al* (2008) selected mutants of *S. aureus* resistant to linezolid and found them to exhibit cross-resistance to tiamulin. However, resistance was unidirectional because mutants of *S. aureus* selected for resistance to tiamulin did **not** exhibit co-resistance to linezolid. This contrasts with the described PhLOPS$_A$ phenotype, which confers	Not agreed, see answer to stakeholder 1. No additional information is needed after line 236, because this refers to cross resistance of linezolid and pleuromutilins mediated by *cfr*, not to resistance due to chromosomal mutations which is what Miller et al (2008) describes.

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		resistance to both oxazolidinones and pleuromutilins. The genotypes responsible for the phenotypes observed by Miller *et al* (2008) were examined and selection with tiamulin resulted in recovery of mutants with changes in the single-copy *rplC* gene (Gly155Arg, Ser158Leu, or Arg149Ser), whereas selection with linezolid led to recovery of mutants with changes (G2576U in 23S rRNA) in all five copies of the multicopy operon *rrn.* In contrast, cross-resistance to linezolid was exhibited by tiamulin-resistant mutants generated in single-copy *rrn* knockout strains of *E. coli*, illustrating that the copy number of 23S rRNA is the limiting factor in the selection of 23S rRNA tiamulin-resistant mutants. The significance of this finding was clearly expounded by the authors when they made the point that the potency, low potential for development of resistance, and favourable pharmacokinetics of oxazolidinones and pleuromutilins make them ideal anti-staphylococcal drugs. However, the unidirectional cross-resistance between linezolid and tiamulin observed in linezolid -resistant *S. aureus* could limit the systemic use of pleuromutilins in patients for which linezolid treatment has been unsuccessful. Clearly this is not an issue of resistance developing to pleuromutilins but rather to linezolid. The authors concluded that whilst acquisition of the $PhLOPS_A$ phenotype confers cross-resistance between linezolid and pleuromutilins, the emergence of pleuromutilin-resistant isolates by point mutation should have little impact on susceptibility to linezolid. This is because	

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		resistance to linezolid is not observed with tiamulin-resistant *S. aureus* mutants due to the preferential selection of single-copy *rplC* mutations rather than 23S rRNA mutations on the multicopy operon *rrn*. Cross-resistance to linezolid is observed with tiamulin-resistant mutants derived from a single-copy *rrn* knockout strain of *E. coli*, illustrating that the copy number of 23S rRNA genes is the limiting factor in the selection of 23S rRNA tiamulin-resistant mutants. Miller K, Dunsmore CJ, Fishwick CWG, Chopra I (2008) Linezolid and tiamulin cross-resistance in *Staphylococcus aureus* mediated by point mutations in the peptidyl transferase center. Antimicrobial Agents and Chemotherapy 52, 1737-1742.	
171-272	2	**Comment:** We believe it is important to add a comment attempting to put this section into context as the issue is not simply to report all publications considering the identification of resistance genes. **Proposed change:** to insert the following sentences after line 272: Even in the light of current understanding of the described resistance mechanisms it is important to establish that the prevalence of these newly described resistance mechanisms is currently low. For example Long et al (2006) stated that whilst the cfr gene can, in principle, be easily disseminated among staphylococci, surveillance studies in Germany have identified only 6 cfr-carrying staphylococcal strains	Not agreed. The paragraph is mainly on mechanism of resistance not on the prevalence of resistance.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		during the past 17 years (Kehrenberg & Schwarz, 2006). *Long KS, Poehlsgaard J, Kehrenberg C, Schwarz S, Vester B (2006a) The Cfr rRNA methyltransferase confers resistance to phenicols, lincosamides, oxazolidinones, pleuromutilins, and streptogramin A antibiotics. Antimicrobial Agents and Chemotherapy 50, 2500-2505.* *Kehrenberg C, Schwarz S (2006) Distribution of florfenicol resistance genes fexA and cfr among chloramphenicol-resistant Staphylococcus isolates. Antimicrobial Agents and Chemotherapy 50, 1156-1163.*	
216-236	2	**Comment:** While the dissemination of the cfr gene should be avoided, the references taken into consideration in that paragraph are essentially dealing with human pathogens. This explains that the dissemination of the gene is already occurring in human medicine as a result of a man to man "contamination". This being particularly obvious for spreading in hospitals. Indeed Quiles-Melero et al (2013) have reported that the diversity of linezolid resistant species and clones found suggests that linezolid resistance among Gram-positive cocci in Spanish hospitals has resulted from several independent selection events followed by the expansion of a few clones. The authors suggest that both antibiotic selection	Not agreed. This paragraph is a review of described resistance mechanisms to pleuromutilins. Resistance mechanisms were described in human as well as in animal isolates. The origin of the isolates is of less importance, as mechanisms that have been detected in China can also occur in the European Union.

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		pressure and cross transmission have played a role in the local emergence and spread of the resistant clones. Additionally after reaching a maximum in 2009, the number of resistant isolates decreased in 2010 and 2011. The reasons for this rapid decrease are unclear, but it is considered improved linezolid use in the hospital might have been an important factor. Thus the real contribution of the veterinary field in an already established human mechanism of resistance can be questioned. With that regard it is to be emphasised that Kehrenberg et al. limited the risks of spreading to swine farming. The relevance of the findings of Wang et al (2012c) in China can be questioned since no programme of responsible use in this place in this country in contrast to the EU. *Quiles-Melero I, Gómez-Gil R, Romero-Gómez MP, Sánchez-Díaz AM, de Pablos M,* *García-Rodriguez J, Gutiérrez A, Mingorancea J (2013) Mechanisms of Linezolid Resistance among Staphylococci in a Tertiary Hospital. Journal of Clinical Microbiology 51, 998-1001* *Wang, Y., W. Zhang, J. Wang, C. Wu, Z. Shen, X. Fu, Y. Yan, Q. Zhang, S. Schwarz, and J. Shen. 2012c. Distribution of the multidrug resistance gene cfr in Staphylococcus species isolates from swine farms in China. Antimicrobial agents and chemotherapy 56:1485- 1490.*	

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
247-251	2	**Comment:** Same as previous. The resistance mechanism is found in veterinary isolates from China. The conditions of use of antibiotics in the EU would likely limit the occurrence of this mechanism of resistance and the data suggests that this is not happening as it is in China.	Not agreed. See previous answers. It is unknown if this mechanism occurs in Europe or not, as this is not monitored.
296	2	**Comment:** The CLSI reference does not seem to be cited in the reference listing **Proposed Change:** CLSI (2008) Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals; Approved Standard – Third Edition. CLSI Document M31-A3	Agreed. Reference to the latest version (2013) added.
295 to 309	2	**Comment:** This section is considered to be of limited value because it does not make the point that the break point is related to administered dose and takes into account the susceptibility distribution of the target pathogen, the drug concentrations achieved at the site of infection and the clinical response. Only the Burch publications address these issues although even in this case the data has not been presented to an independent breakpoint committee. With respect to Brachyspira we have no internationally harmonised susceptibility testing methodology so cannot with confidence describe susceptibility distributions but of greater importance to this section is there is no mention of drug concentrations at the site of infection, which in turn will be influenced by administered dose.	Not agreed. We do not agree that this section is of limited value. On the contrary, we think that it is very important. The lack of accepted interpretation criteria is pointed out elsewhere in the comments so we believe that we have good support for reviewing this topic.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		It is important to emphasise the point that epidemiological cut-off values cannot be used to predict clinical response. **Proposed Change:** Replace the paragraph with the following text: Internationally accepted interpretative criteria are lacking except for tiamulin for *Actinobacillus* species (Clinical Laboratory Standards Institute (CLSI) 2008). To date, no tiamulin or valnemulin breakpoints have been established for *Brachyspira* species. Whilst breakpoints have been proposed few have addressed the susceptibility distribution of the target pathogen, drug concentrations achieved at the site of infection and the clinical response. Epidemiological cut-off values cannot be used to predict clinical response but may be of use in monitoring changes in susceptibility although harmonised susceptibility testing methodology needs first to be in place before this data can be properly evaluated.	
329-333	2	**Comment:** Given the issues associated with sensitivity testing, reporting should be more nuanced: methodology, link with clinical efficacy? Without this additional information and given the proposed breakpoints (which are dependent on methodology), this would imply that all these isolates would be resistant.	It is clear from the multicenter study of Råsbäck et al that disk diffusion and “Quick-MIC” (testing only a few concentrations) is unreliable. However, agar and broth dilution performed well, considering the fastidious nature of the organism. We have amended section 4 to clarify this. In the paragraph in question, we have been careful to select only studies performed with agar or broth dilution. We have amended the paragraph to clarify that.
332, 333	2	**Comment:** The development of the MIC values has	The values cited are as given in the quoted reference. No

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		not been summarized correctly in both sentences. Suggest to change the first sentence " … (1989-1993) to 2-8 µg/ml (2000-2002)" and the second sentence " … (1989-1993) to 2-4 µg/ml (2000-2002) (Rohde et al. 2004). **Proposed change:** so sentence 1 would read " … (1989-1993) to 8.0 µg/ml (2000) and decreased to 2.0 µg/ml (2002)". Sentence 2 would read "For valnemulin the MIC90 increased from 0.063 µg/ml (1989-1993) to 4.0 µg/ml (2000) and decreased to 2.0 µg/ml (2002). *Rohde, J., M. Kessler, C.G. Baums, and G. Amtsberg. 2004. Comparison of methods for antimicrobial susceptibility testing and MIC values for pleuromutilin drugs for Brachyspira hyodysenteriae isolated in Germany. Veterinary microbiology 102:25-32*	change is needed.
Next sentence after sentence 333	2	**Comment:** It is apparent that situations in different EU member states greatly differ, regarding the susceptibility of *Brachyspira hyodysenteriae* pathogens against Pleuromutilins. In many countries no resistance against tiamulin was found or a low resistance, below 10%. Suggest to add the following sentence to the text to reflect those studies " … in antimicrobial susceptibility studies conducted in Italy (Magistrali et al., 2010), in Belgium (Vyt 2010), in Poland (Zmudzki J. et al, 2012), in Sweden (SVARM 2011) and in Germany (Herbst W. et al., 2008) no resistance or low resistance (Palzer et al. 2008, Ritzmann et al. 2009, Pridmore 2008, Williamson et	Not agreed, no change. Considering the problems with methodology in susceptibility testing we cannot quote conference abstracts where such aspects are mostly not described in sufficient detail. That leaves only Sweden (SVARM, or better Pringle 2012) and possibly the studies from Germany and Poland (Ritzman et al 2009). Further, as there are no universally agreed interpretation criteria we chose to describe changes in the MIC distributions over time. Also in Pringle et al, an increase in MICs is seen from 1990-2003. Therafter, the situation has stabilized. Interestingly, the sales of pleuromutilins has decreased from around 1000 kg in 2003 to below 100 kg in

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		al., 2010) of B.hyodysenteriae strains against tiamulin was found." **Proposed change:** "In antimicrobial susceptibility studies conducted in Italy (Magistrali et al., 2010), Belgium (Vyt 2010), Poland (Zmudzki J. et al, 2012), Sweden (SVARM 2011) and in Germany (Herbst W. et al., 2008) no resistance or low resistance (Palzer et al. 2008, Ritzmann et al. 2009, Pridmore 2008, Williamson et al., 2010) of B.hyodysenteriae strains against tiamulin was found". *Magistrali, C.F., Cucco, L., D`Avino,N., Tentellini, M., Pezzotti, G. (2010). Antimicrobial susceptibility of Brachyspira hyodysenteriae isolates from cases of swine dysentery in Italy recovered by different sampling procedures. Proceedings 21st IPVS Congress, Vancouver, Canada, P.422. p.728.* *Vyt , P. 2010. Antimicrobial susceptibility of Belgian Brachyspira hyodysenteriae isolates. Proceedings 21st IPVS Congress, Vancouver, Canada, O.205. p.238.* *Zmudzki,J., Szczotka,A., Nowak, A., Strzelecka, H., Grzesiak, A., Pejsak, Z. (2012). Polish Journal of veterinary Sciences Vol.15, No.2, 259-265.* *SVARM 2011. Swedish Veterinary Antimicrobial Resistance Monitoring SVA* *Herbst, W., Schlez, K., Heuser, J., Baljer,G. (2008). Detection of Brachyspira hyodysenteriae in pigs with and without diarrhoea and drug resistance of German*	2012 (SVARM 2012).

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		B.hyodysenteriae field isolates. Proceedings 20th IPVS Congress, Durban, South Africa, P03.021 p.241. *Palzer,A., Ritzmann,M., Rohde,J., Verspohl,J., Heinritzi, K. (2008). Sensitivity of Brachyspira species in Germany against tiamulin. Proceedings of the 20th IPVS Congress, Durban, S. Africa, Vol 2, p 448.* *Ritzmann,M., Palzer,A., Verspohl,J., Baier,S., Schulte-Wülwer,J., Nienhoff,H., Schulze Grotthoff,W. (2009). Deutschlandweites monitoring zum nachweis von Brachyspira-Species aus Durchfallproben vom Schwein und zur Sensitivität von Brachyspira hyodysenteriae sowie anderer Brachyspira-Species gegenüber Tiamulin. Der Praktische Tierarzt Heft 1,90.Jahrgang, 78-* *Pridmore, A. (2008). Report to Novartis 'Report No DWS/037/05 – Antibacterial activity of tiamulin, valnemulin, tylosin and lincomycin against Brachyspira and Mycoplasma isolates: determination of minimum inhibitory concentration (MIC).'* *Williamson,S., Rogers, J. Hunt, B., Teale,C. (2010). Preliminary results for Brachyspira MIS assessment of isolates form England. Presentation at Pig Veterinary Society Meeting, Norwich, UK*	
333	2	**Comments:** We suggest that some comment is made that addresses at least in part the variable MIC data seen across Europe, relating to isolate source. The reviewed data does not seem to have been moderated	Not agreed. The difficulties regarding susceptibility testing have been discussed elsewhere. We have been careful to select studies using agar or broth dilution, and we use the term occurrence

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		for isolate type. **Proposed change:** include the following text after line 333 It may be argued that not all of the reports of decreased susceptibility are necessarily due to resistance development but there is likely to be a contribution from ill defined testing methodology. Of even greater significance are the source of isolates and an understanding of the status of the disease in the respective countries. In some cases for example, isolates were all clinical isolates from farms exhibiting clinical treatment failure in which the authors accepted that the data may have been influenced by the choice of *B. hyodysenteriae* isolates Lobová *et al* (2004). It is accepted by OIE (Franklin 2001) that when designing sampling programmes, results from diagnostic submissions may not reflect the resistance situation in the animal population, as these types of submissions tend to include specimens from severe and/or recurrent clinical cases, including therapy failures. *Franklin A, Acar J, Anthony F, Gupta R, Nicholls T, Tamura Y, Thompson S, Threlfall EJ, Vose D, van Vuuren M, White DG, Wegener HC & Costarrica ML (2001). Antimicrobial resistance: harmonisation of national antimicrobial resistance monitoring and surveillance programmes in animals and in animal-derived food. Revue scientifique et technique (International Office of Epizootics) 20, 859-870. Lobová D, Smola J & Cizek A (2004). Decreased*	and not prevalence. Inclusion of isolates from, e.g. therapeutic failure are important for “early warning”. See previous comments.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		susceptibility to tiamulin and valnemulin among Czech isolates of Brachyspira hyodysenteriae. Journal of Medical Microbiology 53, 287-291.	
Sentence before sentence 334 333	2	**Comment:** We suggest in section 5, information on the pharmacokinetic characteristics of tiamulin for the treatment of swine dysentery and its pharmacodynamic relationships should be considered which justify the tiamulin use for treatment and prevention purposes. PK PD relationships described by Burch (2005, 2012) based on pharmacokinetic studies (Anderson et al. 1994) indicated that Tiamulin at 38.5, 110 and 220ppm in feed inhibits achieved colonic contents concentrations of approximately 1.0, 2.8 and 8.1µg/g, respectively. EU approved concentrations would be expected to reach at 40, 100 and 200ppm similar results of 1.0, 2.6 and 7.3µg/g. *B. hyodysenteriae* with MICs of 2.0 and >4.0µg/ml may well be inhibited by the higher concentrations of 100 and 200ppm tiamulin in feed respectively and have a strong bactericidal or even eliminatory effect at MICs of 0.5 and 1.0µg/ml, respectively. At 40ppm tiamulin there is a likely inhibitory effect on all wild types with an MIC of 0.5µg/ml and below. **Proposed change:** Insert at the end of line 333 “However, estimated colon contents concentrations of tiamulin reach 7.3µg/g when administered in feed at the maximum level of 200ppm.”	Not agreed. PK/PD and pharmacokinetics are beyond the scope of this reflection paper.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		Anderson,M.D., Stroh,S.L. and Rogers,S. (1994) Tiamulin (Denagard®) activity in certain swine tissues following oral and intramuscular administration. Proceedings of the American Association of Swine Practitioners Meeting, Chicago, Illinois, USA, pp115-118 *Burch, D.G.S. (2005). Pharmacokinetic, pharmacodynamic and clinical correlations relating to the therapy of colonic infections in the pig and breakpoint determinations. The Pig Journal, 56, 8-24.* *Burch D.G.S. (2012) Fellowship Thesis of the Royal College of Veterinary Surgeons: "Examination of the pharmacokinetic/pharmacodynamic (PK/PD) relationships of orally administered antimicrobials and their correlation with the therapy of various bacterial and mycoplasmal infections in pigs." Chapter 3, pp 95-112.*	
Sentence before sentence 334 Sentence before 334	2	**Comment:** Resistance to the Pleuromutilins tiamulin and valnemulin has not been reported in porcine and avian Mycoplasma species (Makhanon et al. 2012, Thongkamkoon et al. 2010). Pleuromutilins can be used as alternatives to macrolides and lincosamides in case of development of resistance to these substances (Maes et al. 2007, Vicca et al. 2004, Kobayashi et al. 2008, Löhren et al. 2008). Suggest to add the sentence " No resistance has been reported in porcine *Mycoplasma* species and Pleuromutilins can be used as alternatives to macrolides and lincosamides in the case	Not agreed. The reports on resistance by Makhanon et al. 2012 and Thongkamkoon et al. 2010 are on Mycoplasmata from Thailand. This seems to have little relevance to this reflection paper. Reviewing all treatment option is beyond the scope of this reflection paper.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		of resistance to these substances” **Proposed change:** “ No resistance has been reported in porcine *Mycoplasma* species and Pleuromutilins can be used as alternatives to macrolides and lincosamides in the case of resistance to these substances (Maes et al. 2007, Vicca et al. 2004, Kobayashi et al. 2008, Löhren et al. 2008, Thongkamkoon et al. 2010, Makhanon et al. 2012)” *Maes,D., Vicca,J., Stakenborg, T., Butaye, P., De Kruif,A., Haesebrouck,F. (2007). Gevoeligheih van Belgische Mycoplasma hyopneumoniae- isolaten voor antimicrobiele middelen. Vlamms Diergeneeskundig Tijdschrift, 76, 300-305.* *Vicca,J., Stakenborg,Maes,D.,Butaye, P., De Kruif,A., Haesebrouck,F. (2004). In vitro susceptibilities of Mycoplasma hyopneumoniae field isolates. Antimicrobial Agents and Chemotherapy, 48, 11, 4470-4472.* *Kobayashi,H., Kanazaki, M., Kajiwara,K. (2008) Macrolid, tiamulin and valnemulin susceptibility of Mycoplasma hyopneumoniae strains isolated in various parts of Japan. Proceedings 20th IPVS Congress, Durban, South Africa, P02.002 p.187.* *Löhren,U., Ricci, A., Cummings,T.S. (2008) Guidelines for antimicrobial use in poultry, In: Guardabassi, L., Jensen,L.B., Kruse, H. (Eds.) Guide to antimicrobial use in animals. Blackwell Publishing Ltd., Oxford, UK,*	

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		pp126-142. *Thongkamkoon,P., Prapasarakul,N., Makhanon,M., Talummuk,S., Klein,U. (2010) In vitro susceptibility of Porcine Mycoplasmas to antimicrobial agents during 2008-2009. Proceedings 21st IPVS Congress, Vancouver, Canada, P.638.* *Makhanon, M., Thongkamkoon,P., Prapasarakul,N. (2012) In vitro susceptibility study of porcine Mycoplasmas in Thailand. Proceedings 22nd IPVS Congress, Jeju, South Korea, P.710.*	
335	2	**Comment:** APP resistance to tiamulin not reported in pan-European MIC project VetPath II (Felmingham 2009). Suggest adding the sentence "No resistance to tiamulin in Actinobacillus pleuropneumoniae has been reported in a pan-European MIC testing project (Felmingham 2009)." **Proposed change:** Add the following sentence "No resistance to tiamulin in *Actinobacillus pleuropneumoniae* has been reported in a pan-European MIC testing project (Felmingham 2009)." *Felmingham,D. (May 2009) Quotient Bioresearch Ltd., Study number IV257-31-05; A report to CEESA AISBL (Brussels, Belgium) "Determination of the antimicrobial susceptibility of the VetPath II (2004-2006) collection of bacterial pathogens".*	Unfortunately, the study does not seem to be published in a peer reviewed journal. We are unable to assess and quote the study.
352,353, 354	2	**Comment:** The treatment against ileitis (*Lawsonia intracellularis*) with Pleuromutilins is an important	As mentioned before, only a limited number of isolates has been tested and neither approved methods, not accepted

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		indication based on the importance of this disease in the field and the wide range of MIC distribution shown for macrolide and lincosamide antibiotics in the limited number of MIC data available. Wattanaphansak et al. (2009) tested the activity of tiamulin and valnemulin, among other antimicrobials, against 10 isolates of *L. intracellularis*. The narrow range of MIC distribution for tiamulin and valnemulin indicated occurrence of high susceptibility of the tested strains from Europe and USA. Tiamulin and valnemulin were identified as the most active antimicrobials tested in this study. Considering the low MICs PK/PD relationship data on tiamulin (Burch & Klein 2008) and on valnemulin (Burch & Klein 2010) verify that effective drug concentrations are achieved in the intestine for prevention and treatment of ileitis infections when tiamulin is added to feed at 150ppm and valnemulin at 75ppm. Concentrations of 1.59µg/g of tiamulin and 0.49µg/g valnemulin, respectively, are achieved in relation to an iMIC for *L. intracellularis* of ≤0.12µg/ml for both antibiotics (Burch, 2005). We suggest adding the following sentence "Wattanaphansak et al. (2009) tested the activity of tiamulin and valnemulin, among other antimicrobials, against 10 isolates of *L.intracellularis*. The narrow range of MIC distribution for tiamulin and valnemulin indicated occurrence of high susceptibility of the tested strains from Europe and USA".	interpretative criteria exist, making interpretation of susceptibility testing of *Lawsonia* difficult.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		Proposed change: to add in line 354 the sentence "Wattanaphansak et al. (2009) tested the activity of tiamulin and valnemulin, among other antimicrobials, against 10 isolates of *L.intracellularis*. The narrow range of MIC distribution for tiamulin and valnemulin indicated occurrence of high susceptibility of the tested strains from Europe and USA and estimated ileal contents concentration of both tiamulin and valnemulin were well in excess of the reported iMICs". *Wattanaphansak, S., R. S. Singer and C. J. Gebhart.2009. In vitro antimicrobial activity against 10 North American and European Lawsonia intracellularis isolates. Veterinary Microbiology 134: 305–310* *Burch,D., Klein,U. 2008.Pharmacokinetic/pharmacodynamic relationships of tiamulin (Denagard) for Proliferative Enteropathy ileitis. Proceedings 20th IPVS Congress, Durban, South Africa, OR.03.44, p.241.* *Burch,D., Klein,U. 2010.Pharmacokinetic/pharmacodynamic relationships of valnemulin (Econor) and Lawsonia intracellularis the cause of ileitis. Proceedings 21st IPVS Congress, Vancouver, Canada, p.986.* *Burch, D.G.S. (2005) Pharmacokinetic, pharmacodynamic and clinical correlation relating to the therapy of Lawsonia intracellularis infections, the cause of porcine proliferative enteropathy (ileitis) in*	

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		the pig. Pig Journal, 56, 25-44.	
358	2	**Comment: "**Lack of authorized and effective drugs for treatment of swine dysentery has increased the use of pleuromutilins, and this probably explains the emergence of resistant strains". Different effective drugs are available for swine dysentery treatment. It should be mentioned that the removal of growth promoters that are incompatible with tiamulin or valnemulin such as salinomycin, the increasing generic competition and the price erosion has increased the use of pleuromutilins. **Proposed change:** replace the original sentence by the following sentence "Removal of incompatible growth promoters such as salinomycin, increasing generic competition and price erosion has increased the use of pleuromutilins. …"	Addition of this information does not seem necessary.
358 to 392	2	**Comment:** The reflection paper has clearly stated that there are no published breakpoints for B. hyodysenteriae yet within this section discusses "resistant" isolates. In the absence of breakpoints it is not correct to discuss whether isolates are resistant or not. This is especially true for infections where the target pathogens can be exposed to relatively high drug concentrations within the GI tract. In this context the data presented in lines 360 to 364 is to be applauded because it relates MICs to clinical outcome. However, lines 364 to 366 should not discuss "resistant" isolates because these studies have no	It is true that internationally accepted clinical breakpoints are lacking, but the observations in the study by Vyt and Hommez, 2006 – see line 758 for full reference – indicate that decreased susceptibility (non wild type) is associated with decreased clinical efficacy. We are not aware of any studies showing the opposite at authorised doses. In i.e. Hidalgo et *al* 2011, isolates with MICs of timalulin as high as 32 mg/L are described as are the associated mutations. Wild-type isolates typically have MICs or 0.25 or lower, i.e. normally more than ten times lower than the described isolates. This said, we have modified "resistance" to decreased

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		basis for ascribing isolates as “resistant”. Clearly it is appropriate to state that the isolates carry resistance genes but this is not necessarily the same thing as being “clinically resistant” **Proposed Change:** delete lines 364-367	susceptibility where appropriate.
367 to 370	2	**Comment:** Whilst pleuromutilin use is indeed high in Spain, Portugal and Czech Republic it is also high in Denmark (ESVAC 2011) yet no comment is made. **Proposed change:** Add Denmark to the list and make appropriate comments about susceptibility. *ESVAC. 2012. European Medicines Agency, 2012. 'Sales of veterinary antimicrobial agents in 19 EU/EEA countries in 2010' (EMA/88728/2012). Available from the European Medicines Agency web page (http://www.ema.europa.eu/).*	From figure 2 it is obvious that Denmark is far from the top 3 of countries with the highest sales of pleuromutilins expressed as PCU and similar to Latvia. No change.
370	2	**Comment:** The Lobova (2004) data is skewed, the authors state in the publication that the data presented is skewed, this point was acknowledged by the authors because sampling in 2000 and 2001 was from clinical samples. **Proposed change**: change to read: Generally the use of pleuromutilins is high in Spain, Portugal, Czech Republic and Denmark; relatively high percentages of *Brachyspira* isolates not susceptible to pleuromutilins have also been reported from Spain (Hidalgo et al., 2011) and Czech Republic (Lobova et	In the publication by Lobova (2004), isolates from 2000 and 2001 were from farms that experienced lack of efficacy of pleuromutilins. This lack of clinical efficacy in the past was confirmed by the high MIC values. The addition of this information seems unnecessary.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		al., 2004; Sperling et al., 2011) although some of these data are skewed because of the isolate source. *Hidalgo, A., A. Carvajal, B. Vester, M. Pringle, G. Naharro, and P. Rubio. 2011. Trends towards lower antimicrobial susceptibility and characterization of acquired resistance among clinical isolates of Brachyspira hyodysenteriae in Spain. Antimicrobial agents and chemotherapy 55:3330-3337.* *Lobova, D., J. Smola, and A. Cizek. 2004. Decreased susceptibility to tiamulin and valnemulin among Czech isolates of Brachyspira hyodysenteriae. Journal of medical microbiology 53:287-291.* *Sperling, D., J. Smola, and A. Cizek. 2011. Characterisation of multiresistant Brachyspira hyodysenteriae isolates from Czech pig farms. Vet Rec 168:215.*	
374	2	**Comment:** This sentence, "It has been suggested that the use of pleuromutilins very likely selects for the emergence of *cfr* in animal isolates of staphylococci (Witte and Cuny, 2011) is misleading. The publication actually states, "Veterinary use of both florfenicol and tiamulin, very likely selected for the emergence of *cfr* in animal isolates of coagulase negative staphylococci" and it is argued that this also can be considered misleading. As has already been discussed *Cfr* was first discovered in 2000 from a bovine strain of *Staphylococcus sciuri* (Schwarz *et al*, 2000) and confers a resistance phenotype referred to as $PhLOPS_A$	Partly agreed. All antimicrobials to which the *cfr* gene confers resistance will select for its emergence. We changed the sentence accordingly. The manuscript by Miller et al. is on resistance due to chromosomal mutations, not on the *cfr* gene.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		conferring resistance to Phenicols, Lincosamides, Oxazolidinones, Pleuromutilins, and Streptogramin A antibiotics. In this context it can be argued that other antimicrobial classes could have played a role. What is not made clear, however, is the observation of Miller *et al* (2008). These workers being aware of the observation that the $PhLOPS_A$ phenotype conferred resistance to both linezolid and tiamulin in *S. aureus,* selected mutants of *S. aureus* resistant to linezolid and found them to exhibit cross-resistance to tiamulin but resistance was unidirectional, mutants of *S. aureus* selected for resistance to tiamulin did **not** exhibit co-resistance to linezolid. **Proposal:** Replace lines 374 & 375 with: "It has been suggested that the use of both florfenicol and tiamulin very likely selected for the emergence of *cfr* in animal isolates of staphylococci (Witte and Cuny, 2011), however, this observation is not necessarily supported by other data. Miller *et al* (2008) selected mutants of *S. aureus* resistant to linezolid and found them to exhibit cross-resistance to tiamulin but resistance was unidirectional, mutants of *S. aureus* selected for resistance to tiamulin did **not** exhibit co-resistance to linezolid; it is important to consider phenotypes and genotypes when considering pleuromutilin and linezolid resistance. *Witte, W., and C. Cuny. 2011. Emergence and spread of cfr-mediated multiresistance in staphylococci: an interdisciplinary challenge. Future microbiology 6:925-*	

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		931. *Schwarz, S., C. Werckenthin, and C. Kehrenberg. 2000. Identification of a plasmid-borne chloramphenicol-florfenicol resistance gene in Staphylococcus sciuri. Antimicrobial agents and chemotherapy 44:2530-2533.* *Miller, K., C.J. Dunsmore, C.W. Fishwick, and I. Chopra. 2008. Linezolid and tiamulin cross-resistance in Staphylococcus aureus mediated by point mutations in the peptidyl transferase center. Antimicrobial agents and chemotherapy 52:1737-1742.*	
Lines 374 to 392	2	**Comment:** Whilst we largely accept the presented text it does not address anything of prevalence of the described resistance genes nor does it explain the context of the data. We believe there needs to be additional comment. **Proposed change:** add the following text within this section: Whilst the data clearly demonstrates that the *cfr* gene can be disseminated between bacteria little consideration has been given to how frequently this is likely to occur. The evidence suggests that this is a relatively rare event. Kehrenberg & Schwarz (2006) reported only 6 *cfr*-carrying staphylococcal strains isolated during the 17 years prior to 2006, i.e. 1989-2006, none of which were isolated from swine. Kehrenberg *et al* (2009)	Not agreed. This paragraph is a review on resistance mechanisms, not on prevalence of resistance. Therefore no changes are needed.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		specifically investigated *S. aureus* strains of porcine origin for the presence of *cfr*-positive strains. They screened nasal swabs from 846 swine from 367 farms all over Germany during 2007; they additionally screened 90 porcine coagulase-positive and coagulase-variable staphylococci collected all over Germany from diseased swine in the BfT-GermVet study 2004-2006 and 56 nonrelated porcine *S. aureus* strains provided by veterinary diagnostic laboratories from all over Germany collected mainly in 2008. In total, 2 staphylococcal strains of porcine origin displayed a resistance phenotype indicative of the presence of *cfr*. Both strains originated from swine farms in different geographic areas of northern Germany and were isolated in 2004 and 2007, respectively, and carried the gene *cfr*. Clearly the data suggests that the prevalence of *cfr* in Germany is very low. Whilst MRSA ST9 isolates are commonly found in pigs in China, *cfr*-positive MRSA isolates have rarely been reported in China (Kehrenberg *et al* (2009). Whilst *cfr* is largely expressed in Gram-positive staphylococci, it is known that it can be expressed in *E. coli*. Wang *et al* (2011) described the presence of the *cfr* gene in a naturally occurring *Proteus vulgaris* isolate of porcine origin and subsequently screened 1230 *E. coli* isolates from individual pigs, chickens and ducks in Shandong (n=491; pigs n=189, ducks n=77 and chicken n=225) and Sichuan provinces (n=739; pigs n=218, ducks n=66 and chicken n=455) during 2008–10 (**Wang *et al,* 2012a**). The analysis of 1230 *E. coli* isolates	

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		revealed the presence of the *cfr* gene in a single isolate, obtained from the nasal swab of a pig in a slaughterhouse in Shandong province in 2010. The authors concluded that whilst *cfr* could be found in *E. coli*, the very low prevalence (0.08%) suggest that the detection of the *cfr* gene represents only sporadic incidence. *Kehrenberg, C., and S. Schwarz. 2006. Distribution of florfenicol resistance genes fexA and cfr among chloramphenicol-resistant Staphylococcus isolates. Antimicrobial agents and chemotherapy 50:1156-1163.* *Kehrenberg, C., C. Cuny, B. Strommenger, S. Schwarz, and W. Witte. 2009. Methicillin-resistant and - susceptible Staphylococcus aureus strains of clonal lineages ST398 and ST9 from swine carry the multidrug resistance gene cfr. Antimicrobial agents and chemotherapy 53:779-781.* *Wang, Y., C.M. Wu, S. Schwarz, Z. Shen, W. Zhang, Q. Zhang, and J.Z. Shen. 2011. Detection of the staphylococcal multiresistance gene cfr in Proteus vulgaris of food animal origin. The Journal of antimicrobial chemotherapy 66:2521-2526.* *Wang, Y., T. He, S. Schwarz, D. Zhou, Z. Shen, C. Wu, L. Ma, Q. Zhang, and J. Shen. 2012a. Detection of the staphylococcal multiresistance gene cfr in Escherichia coli of domestic-animal origin. The Journal of*	

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		antimicrobial chemotherapy 67:1094-1098.	
390-392	2	**Comment:** The statement that "Antibiotic usage records for Chinese pig farms indicate that multiple antimicrobial drugs, including florfenicol, lincomycin and tiamulin have been used on farms where *cfr* positive isolates have been found, suggesting that selective pressure might have played a role" is misleading because all cited studies do not support this statement. In the **Wang *et al* (2012b)** study there was indeed information regarding antimicrobial therapy on the farm. This information indicated that a number of antimicrobial agents, including penicillin, florfenicol, trimethoprim/sulfamethoxazole, kanamycin, streptomycin, oxytetracycline and tylosin had been used for treating or preventing bacterial infections. It is important to note that pleuromutilins had **not** been reported as being used. It was only in the paper describing cfr in the Gram-negative Proteus vulgaris that tiamulin was reported as being used. **Proposal: Amend to read** Antibiotic usage records for Chinese pig farms indicate that pleuromutilins have not always been used on farms where *cfr* positive isolates have been found although other antimicrobials including florfenicol and lincomycin have been used. *Wang, Y., S. Schwarz, Z. Shen, W. Zhang, J. Qi, Y. Liu, T. He, J. Shen, and C. Wu. 2012b. Co-location of the multiresistance gene cfr and the novel streptomycin*	Not agreed. As indicated in a previous comment, all antimicrobials to which *cfr* confers resistance will select for it. Therefore the use of florfenicol will thus also select for the emergence of resistance. The references cited are correct, no changes are needed.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		resistance gene aadY on a small plasmid in a porcine Bacillus strain. The Journal of antimicrobial chemotherapy 67:1547-1549	
394, 395, 396	2	**Comment: "**For most indications for which pleuromutilins are authorized there are alternative substances available except for swine dysentery …". "Therefore pleuromutilins are the only remaining treatment option for this indication." It is not correct that pleuromutilins are the only remaining treatment option for swine dysentery treatment. Alternative substances are available for swine dysentery treatment. Vyt et al (2012) have shown that in cases of decreased susceptibility to tiamulin and valnemulin Tylvalosin can be used as an alternative to pleuromutilins for elimination of dysentery in pig farms. The same author (2010) reported that in nearly half of the cases with pleuromutilin resistance tylvalosin can be used as an alternative treatment. In a field study on spontaneous infection of pigs caused by B.hyodysenteriae it was concluded that *in vitro* susceptibility testing of B.hyodysenteriae (for lincomycin) only partially predicted the clinical effect of treatment (Vyt and Hommez, 2006). Lincomycin has shown efficacy in those field studies as a swine dysentery treatment option. Herbst et al. (2008) reported on a resistance rate of 4.3% of German Brachyspira hyodysenteriae strains showing resistance against lincomycin.	See previous comments. It is true that clinical break-points for the antimicrobials in question have not been agreed. To our knowledge, there is no published evidence that treatment with the alternatives (macrolides, lincosamides) at authorized doses is effective against strains with clearly elevated MICs (non-wild type), e.g., above the MICs of strains from efficacy trials. To clarify that the antibiograms may vary, we have changed the sentence to: "When resistance occur to alternative antimicrobials, pleuromutilins remain the only …."

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		The following text changes are therefore suggested "For most indications for which pleuromutilins are authorised alternative substances are available. In the case of swine dysentery different treatment options beside both Pleuromutilin products can be used as recommended in different publications (Vyt et al 2012, Vyt 2010, Vyt & Hommez 2006, Herbst et al. 2008). The sentence "Pleuromutilins are the only remaining treatment option for this indication" should be deleted. *Vyt , P. 2010. Antimicrobial susceptibility of Belgian Brachyspira hyodysenteriae isolates. Proceedings 21st IPVS Congress, Vancouver, Canada, O.205. p.238.* *Vyt , P., L.Vandepitte, A.Dereu, M.Roozen 2012. Elimination of swine dysentery on a single-site, farrow –to-finish farm using tylvalosin (Aivlosin). Proceedings Vol II 22nd IPVS Congress, Jeju, Korea, BP-272 p.629.* *Vyt,P., Hommez,J. (2006). Antimicrobial susceptibility of Brachyspira hyodysenteriae isolates compared with the clinical effect of treatment. Flem. Vet.J.75, 279-285.* *Herbst, W., Schlez, K., Heuser, J., Baljer,G. (2008). Detection of Brachyspira hyodysenteriae in pigs with and without diarrhoea and drug resistance of German B.hyodysenteriae field isolates. Proceedings 20th IPVS Congress, Durban, South Africa, P03.021 p.241.* **Proposed change:** Suggest the following sentences "For most indications for which pleuromutilins are	

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		authorised alternative substances are available. In the case of swine dysentery different treatment options beside both Pleuromutilin products can be used as recommended in different publications (Vyt et al 2012, Vyt 2010, Vyt & Hommez 2006, Herbst et al. 2008).	
401, 402	2	**Comment:** "In most EU Member States there are no national programmes for control of swine dysentery" But in Sweden a programme for control of swine dysentery was launched in 2000 and in several EU countries eradication programmes are established (Denmark, UK, Spain, Germany) based on initiatives by big integrators. **Proposed change:** The contents of the sentence have to be changed accordingly.	Not agreed. The word "most" already indicates that there are exceptions.
407, 408, 409	2	**Comment:** "In many cases pleuromutilins are the only potentially effective choice among antimicrobials with swine dysentery as authorized indication." This is not correct. Tylvalosin (Aivlosin) is centrally authorized in the EU for oral administration indicated in swine for treatment and prevention of swine dysentery. Lincomycin is also registered for swine dysentery treatment and can be used as an alternative treatment option (see comments to line numbers 394, 395, 396). **Proposed change:** Suggest the following sentence " Pleuromutilins are one of the effective choices among antimicrobials for swine dysentery based on authorised indications in EU".	Not agreed. Occurrence of resistance to macrolides and lincosamides is common. The sentence must be read in its context. See also previous comments to comments

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
409	2	**Comment:** It is apparent that the situation on susceptibility of *Brachyspira hyodysenteriae* strains greatly differ among the EU countries. The diversity of the results are based on differences in *Brachyspira* species diagnosis and MIC testing procedure as shown in ring tests all over Europe (Rasbeck et al. 2005) In many countries (Denmark, Germany, Italy, Spain, Sweden, Ireland) high susceptibility of *Brachyspira hyodysenteriae* strains to tiamulin and valnemulin are reported. We suggest adding sentences, which describe the susceptibility situation of *Brachyspira hyodysenteriae* realistically and which explain the reasons of diversity of MIC testing results. **Proposed change:** We suggest to add the following sentences: "It is apparent that the situation on susceptibility of *Brachyspira hyodysenteriae* strains greatly differs among the EU countries. The diversity of the results are most likely based on differences in *Brachyspira* species diagnosis and MIC testing procedure, as shown in ring tests all over Europe (Rasbeck et al. 2005). In many countries (Denmark, Germany, Italy, Spain, Sweden, Ireland) high susceptibilities of *Brachyspira hyodysenteriae* strains to tiamulin and valnemulin are reported. Pleuromutilins are therefore recommended in textbooks and national treatment guidelines. Isolates	The ring test study made by Råsbäck et al. 2005 showed that disc diffusion and "quick MIC" were not suitable methods for susceptibility testing of *Brachyspira* spp. Agar and broth dilution performed well, considering the fastidious nature of the organism. We have therefore chosen only to include studies that were performed with agar dilution and broth dilution. A clarification of this has been added to the text. Further, the information above has been added to section 4. *Råsbäck,T., Fellström,C., Bergsjö,B., Cizek,A., Collin,K., Gunnarson,A., Jensen,S.M., Mars,A., Thomson,J., Vyt,P., Pringle,M. (2005). Assessment of diagnostics and antimicrobial susceptibility testing of Brachyspira species using a ring test. Veterinary Microbiology 109, 229-243.*

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		with reduced susceptibility to Pleuromutilins have emerged among *B.hyodysenteriae* in several countries …" *Rasbeck,T., Fellstroem,C., Bergsjo,B., Cizek,A., Collin,K., Gurrarson,A., Jensen,S.M., Mars,A., Thomson,J., Vyt,P., Pringle,M. (2005). Assessment of diagnostics and antimicrobial susceptibility testing of Brachyspira species using a ring test. Veterinary Microbiology 109, 229-243.*	
415	2	**Comment:** The loss of Pleuromutilins beside macrolid products like tylvalosin and the lincosamid lincomycin as effective tools to treat swine dysentery would present a considerable threat to pig health, welfare and productivity. Therefore prudent use of these antibiotics based on MIC testing is needed. The following sentence should be modified "To summarize, the loss of pleuromutilins as effective tools to treat swine dysentery because of a further increase of resistance or as a consequence of restrictions would present a considerable threat to pig health, welfare and productivity". **Proposed change:** Suggest changing the sentence as follows: "The loss of Pleuromutilins beside macrolide products like tylvalosin and the lincosamide lincomycin as effective tools to treat swine dysentery would present a considerable threat to pig health, welfare and productivity. Therefore prudent us of these	Not agreed. This is a reflection paper on pleuromutilins, not lincosamides or macrolides. Therefore the focus is on pleuromutilins.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		antibiotics based on MIC testing is needed."	
422	2	**Comment:** It would be appropriate to make some comment about the indications for retapamulin. **Proposed change:** To date only one product containing pleuromutilins (retapamulin) is authorised for humans for the topical treatment of impetigo due to susceptible strains of *Staphylococcus aureus* or *Streptococcus pyogenes*, the two most common types of bacteria in this kind of infection. Retapamulin does not have an indication for MRSA. The European assessment report makes the point that, "*In clinical studies of secondarily infected open wounds, the efficacy of retapamulin was inadequate in patients with infections caused by methicillin-resistant Staphylococcus aureus (MRSA).*	Not agreed. There is no need to make remarks on indications for retapamulin.
426	2	**Comment:** It should be stated that BC-3781 is a lead compound and not a finished product, currently in Phase II trials tested.	Partly agreed. We will add that BC-3781 was tested successfully during phase II trials.
440 to 445	2	**Comment:** There are important points from the Morales et al paper that are not included. 1. The animal strains carry cfr on a plasmid whilst many of the human *cfr*-positive *S. aureus* strains studied to date have the *cfr* gene integrated in the genome 2. There were no new cases reported in the hospital for at least 2 years after the initial outbreak **Proposed change:** Insert line 445. Human strains differ from animal strains in that cfr is often integrated	Not agreed. Genes can move from plasmids to the chromosome and vice versa. *Cfr* can be located on a transposon Tn558. Therefore this information seems irrelevant. In addition, generally human strains do not differ from animal strains, mostly *cfr* genes are carried on a plasmid in isolates from animals as well as humans.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		in the chromosome whereas animal strains carry cfr on a plasmid.	
445 to 447	2	**Comment:** Retapamulin does not have indication for MRSA. The European assessment report makes the point that, "*In clinical studies of secondarily infected open wounds, the efficacy of retapamulin was inadequate in patients with infections caused by methicillin-resistant Staphylococcus aureus (MRSA).* **Proposed change:** Revise to say: "Retapamulin demonstrated excellent in vitro activity against MSSA and MRSA strains, but not against MRSA isolates harbouring the *cfr* gene (Candel et al., 2011). Furthermore retapamulin does not have indication for MRSA as it was not efficacious in clinical studies." *Candel, F.J., G. Morales, and J.J. Picazo. 2011. In vitro activity of retapamulin against linezolid and methicillin-resistant Staphylococcus aureus isolates. Revista espanola de quimioterapia : publicacion oficial de la Sociedad Espanola de Quimioterapia 24:127-130.*	Not agreed, the text does not make reference to the authorisation of retapamulin for MRSA indications.
422 to 456	2	**Comment:** This section does not attempt to make any summary of the potential impact upon human health, as the reflection paper has cited the review of Novak (2011) it is considered appropriate to also include his summary and a comment from Sanchez Garcia et al (2010) **Proposed change:** Include the following paragraph (from Novak) after line 452.	In our opinion this does not belong in the summary assessment.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		"So far, the *cfr* genotype is only rarely encountered in human bacterial isolates, (despite use of tiamulin in veterinary medicine since 1979) as illustrated by the most recent LEADER study 2009, which monitored linezolid resistance in the United States. The study found in only four strains (two *S. aureus*, one each of *Staphylococcus epidermidis* and *Staphylococcus capitis*) from four different states the *cfr* genotype, suggesting persistence but limited potential for dissemination. Despite the potential mobility of the *cfr*-resistance determinant, surveillance studies in Germany have identified only six c*fr* carrying staphylococcal strains in animals during the past 17 years. However, because *cfr* has been encountered in some outbreaks, involving mainly *S. epidermidis* but also in one occasion *S. aureus,* careful monitoring of resistance development is clearly warranted. Additionally Sanchez Garcia et al (2010) addressing the hospital outbreak of MRSA carrying cfr concluded that a combination of the emergence of linezolid resistance in *S aureus* with clonal spread and use of linezolid was responsible for the LRSA outbreak. Successful early control of the LRSA outbreak by infection-control measures and reduction of linezolid use was achieved." *Novak, R. 2011. Are pleuromutilin antibiotics finally fit for human use? Annals of the New York 700 Academy of Sciences 1241:71-81.* *Sanchez Garcia, M., M.A. De la Torre, G. Morales, B.*	

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		Pelaez, M.J. Tolon, S. Domingo, F.J. Candel, R. 733 Andrade, A. Arribi, N. Garcia, F. Martinez Sagasti, J. Fereres, and J. Picazo. 2010. Clinical 734 outbreak of linezolid-resistant Staphylococcus aureus in an intensive care unit. JAMA : the 735 journal of the American Medical Association 303:2260-2264.	
462-471	2	**Comment:** This might be illustrating that responsible use initiatives could be best coordinated at country level taking into account country-specific husbandry/population densities/disease prevalence/medicines availability situations, rather than generically at EU-level.	We believe that initiatives at country level as well as EU level are needed.
467,468, 469	2	**Comment:** The prevalence of resistance of porcine Brachyspira species against the macrolide Tylosin is very high in many European countries. This is in contrast to the macrolide Tylvalosin which can be considered as effective against swine dysentery and colitis based on the available MIC testing results. We suggest changing therefore the second part of the sentence. Delete “the macrolides” **Proposed change:** So it would read “ … and a high prevalence of resistance to alternative antimicrobials used to treat swine dysentery, e.g. the macrolide tylosin in countries with the highest use.”	Not agreed. To our knowledge, there are no publically available studies providing evidence for acceptable clinical efficacy of tylvalosin at authorised doses for strains with higher MICs.
479	2	**Comment:** The impact of resistance development to all currently effective antibiotics (tiamulin, valnemulin, tylvalosin and lincomycin) in *Brachyspira*	As this is a reflection paper on pleuromutilins, the focus is on pleuromutilins.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		hyodysenteriae has to be considered for currently running or planned resistance monitoring programmes. Therefore it is suggested to change the first part of the sentence "… Given potential impact of resistance to currently effective antibiotics in B.hyodysenteriae **Proposed change:** So it would read "… Given potential impact of resistance to currently effective antibiotics (tiamulin, valnemulin, tylvalosin and lincomycin) in *B. hyodysenteriae* on pig health, welfare and production, there is a need to include *B.hyodysenteriae* in national resistance monitoring programmes."	
489-490	2	**Comment:** Pleuromutilins are useful in other indications and not only in swine dysentery. Diversity of treatments in the frame of a rational usage should be kept in order to dilute the selection pressure by using just one (swine dysentery) or limited number of antibiotic families (other indications) (Livermore D, Lancet Infect. Dis, 2005)	The use of pleuromutilins for other indications should be discouraged.
489,490, 491	2	**Comment:** In many textbooks and national treatment guidelines the Pleuromutilins tiamulin and valnemulin are recommended for many indications in food animals (Burch et al. 2008, Löhren et al. 2008, Denmark, the Netherlands (http://wvab.knmvd.nl/wvab/formularia/formularia). This is based on resistance development not only of *Brachyspira* species but also porcine and avian Mycoplasma species (Migaki et al. 1993, Maes et	Not agreed, the reflection paper does not intend to review treatment guidelines.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
491		al.2007, …) to macrolide and lincosamide antibiotics. It is important to use Pleuromutilins according to label against all bacterial pathogens listed in the product SPC. Reservation of Pleuromutilins for treatment of swine dysentery is illogical as it is over use for this indication in some MSs which have selected for resistant *B. hyodysenteriae*. **Proposed change**: Insert in line 491 after "…are available" the sentence, "However, this would not resolve the problem of over use of the pleuromutilins for the treatment of swine dysentery, which has resulted in increasing resistance in B. hyodysenteriae seen in some Member States." *Burch, D.G.S., Duran, C.O. and Aarestrup, F.M. (2008) Chapter 7: Guidelines for antimicrobial use in swine. In Guide to Antimicrobial Use in Animals. Eds Guardabassi, L., Jensen, L.B and Kruse, H., Blackwell Publishing Ltd, Oxford, UK, pp 102-125.* *Löhren,U., Ricci, A., Cummings,T.S. (2008) Chapter 8: Guidelines for antimicrobial use in poultry, In: Guardabassi, L., Jensen,L.B., Kruse, H. (Eds.) Guide to Antimicrobial Use in Animals. Blackwell Publishing Ltd., Oxford, UK, pp126-142.* *Migaki,T.T., Avakian,A.P., Bomes,H.J., Ley,D.H., Tanner,A.C., Majonyle,R.A. (1993). Efficacy of danofloxacin and tylosin in the control of mycoplasmosis in chicks infected with tylosin-susceptible or tylosin-resistant field isolates of*	

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		Mycoplasma gallisepticum. Avian Diseases 37: 508-514. *Maes,D., Vicca,J., Stakenborg,T., Butaye,P., De Kruit,A., Haesebruck,F. (2007). In vitro susceptibility of Mycoplasma hyopneumoniae field isolates. Vlaams Diergeneeskundig Tijdschrift, 76, 300-305.*	
511 to 515	2	**Comment:** This section does not address prevalence of resistance genes **Proposed Change**: Please add the following sentence to line 515. "The data suggests that prevalence of these resistance genes is low".	We prefer not to qualify the prevalence of resistance.
517, 518	2	**Comment:** The reflection paper cites the study of Morales et al; these workers identified a linezolid resistant MRSA carrying cfr, however, all the strains were susceptible to tigecycline, vancomycin, and daptomycin. **Proposed Change:** The emergence of these resistance genes in animals poses a potential threat to human medicine as they might compromise empirical treatment of human MRSA infections although data shows alternative antibiotics including tigecycline, vancomycin, and daptomycin remain active.	We do not think mentioning all antimicrobials to which isolates remain susceptible is relevant in the context of this reflection paper.
520, 521, 522	2	**Comment:** The phrase, "As the pleuromutilin resistant isolates are often multidrug-resistant" is confusing as it does not specify what resistance genes are being considered. If it is cfr, for example, then comment needs to be made about the relatively low prevalence.	Partly agreed. This sentence refers to resistance by *vga* and *cfr.*

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		Additionally other classes of antimicrobials in human and veterinary medicine may also co-select for cfr. **Proposed Change:** Please clarify the sentence.	
522 to 525	2	**Comment:** As existing data regarding prevalence of resistance genes suggests low prevalence we strongly support the need for added surveillance	Agreed.
526, 527, 528	2	**Comment:** The sentence "Co-selection for pleuromutilins with many different antimicrobials can potentially occur due to multidrug resistance genes ……..." requires clarification as this statement is now true for all classes of antimicrobial whether in animal or human medicine. As it stands it is of limited value and in terms of public health it is argued that Pleuromutilins are the class least likely to impact public health; this is acknowledged by WHO as pleuromutilins are not classified as "critically important".	The presence of resistance genes on mobile elements is a concern in human and animal medicine and indeed concerns all antimicrobials, as on these mobile elements, often many different resistance determinants can be present.
19,20	2	**Comment:** Suggest to add "herd/flock medication" **Proposed change:** so it would read "… most of the use is for group and herd/flock medication in feed or water".	Agreed
21	2	**Comment:** Suggest to replace "varies" by "vary" **Proposed change:** … approved indications vary considerably.	Agreed
23	2	**Comment:** Suggest to replace "is" by "are" **Proposed change:** …where pleuromutilins are used	Agreed

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		but there is likely additional …	
27	2	**Comment:** Add "of all licence holders" **Proposed change:** so it would read " … use principles are outlined in the SPCs for approved products of all licence holders"	Not agreed, the clarification is unnecessary in this context
45	2	**Comment:** Suggest to introduce "effective" into the sentence **Proposed change:** so it would read "… time needed for effective cure of diseases".	As ineffective cure does not exist, adding effective is unnecessary.
90	2	**Comment:** "Tiamulin was approved for use in veterinary medicine in 1979, followed by …" Tiamulin was approved 1978 in Ireland and mostly in 1979 in different European countries and globally. **Proposed change:** no specific change. The question is if approval in Ireland is considered or more general "the birthdate" of the product in 1979.	No change needed.
117, 118	2	**Comment:** Tiamulin is also available as a water soluble granulate for drinking water medication. Suggest to introduce "and water soluble granulate" ; delete reference Islam et al. 2009 **Proposed change:** so it would read "… and as a solution and water soluble granulate for medication in drinking water".	Partially agreed.
134	2	**Comment:** Suggest to change 2nd part of sentence "… if such recommendation is included in the SPC for	Not agreed, the information on the EMA web page lists the products involved on the referral.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		other products containing tiamulin. Add “for other licence holders” **Proposed change:** so it would read “…if such recommendation is included in the SPC for other licence holders for other products containing tiamulin”.	
291	2	**Comment:** Suggest to add the word “testing” to the sentence **Proposed change:** so it would read “Antimicrobial susceptibility testing of Lawsonia intracellularis is difficult as this obligate intracellular bacterium …”	Agreed
307-309	2	**Comment:** Agar or broth dilution? This should be specified.	This is specified.
323	2	**Comment:** Specify unit (µg/mL)	Agreed
15 & 16	3	**Comment:** Other effective antibiotics are available for SD treatment and are alternative choices beside Pleuromutilins like Tylvalosin (Vyt 2010, Vyt et al 2012, Hildago et al 2011) and Lincomycin (Herbst et al 2008, Vyt & Hommez 2006). **Proposed change:** “it is ~~the sole~~ one of the treatment options for enteritis in pigs caused by *Brachyspira hyodysenteriae* (swine dysenteria) ~~resistant to macrolides~~ and respiratory infections caused by Mycoplasma species.” *Vyt, P. 2010. Antimicrobial susceptibility of Belgian Brachyspira hyodysenteriae isolates. Proceedings 21st*	Partly accepted. See comments to stakeholder number 1 & 2.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		IPVS Congress, Vancouver, Canada, O.205. p.238. *Vyt, P., L.Vandepitte, A.Dereu, M.Roozen 2012. Elimination of swine dysentery on a single-site, farrow –to-finish farm using tylvalosin (Aivlosin). Proceedings Vol II 22nd IPVS Congress, Jeju, Korea, BP-272 p.629.* *Hildago,A., Carvajal,A., Vester,B., Pringle, M., Naharro, G., Rubio, P. 2011. Trends towards lower antimicrobial susceptibility and characterization of acquired resistance among clinical isolates of Brachyspira hyodysenteriae in Spain. Antimicrobial Agents and Chemotherapy, p.3330-3337.* *Herbst, W., Schlez, K., Heuser, J., Baljer,G. (2008). Detection of brachyspira hyodysenteriae in pigs with and without diarrhoea and drug resistance of German B.hyodsenteriae field isolates. Proceedings 20th IPVS Congress, Durban, South Africa, P03.021 p.241.* *Vyt, P., Hommez, J. (2006). Antimicrobial susceptibility of Brachyspira hyodysenteriae isolates compared with the clinical effect of treatment. Flem. Vet.J.75, 279-285.*	
16,17,18	3	**Comment:** This sentence would be improved by reference to prudent use; we would suggest rewording the sentence. **Proposed change:** Delete "The negative consequences in case such a pathogen becomes pleuromutilin resistant would thus be considerable" Replace by the following sentence "It is very important	Not agreed. The phrase is intended to provide a clear message about the consequences of *Brachyspira hyodysenteriae* becoming resistant.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		from an economical and animal welfare perspective to use Pleuromutilins responsibly to contain resistance development against these major pathogens."	
38	3	**Comment:** The fact that pleuromutilins should only be used for treatment of disease does not consider the field condition with animals being at different infection status. Thus the treatment should cover these different stages of the infection. Early treatment is of particular consideration since it has been shown that these correspond to a low "inoculum" and thus to increased efficacy and lower risk for resistance development (Ferran et al, Vet Microb, 2010). In addition, their use should be restricted to indications for which efficacy has been proven with clinical data. **Proposed change:** Please amend the sentence as follows "Pleuromutilins should only be used for treatment and prevention for which efficacy has been proven, and in accordance with the principles of responsible use ~~of disease~~."	See previous comments to Stakeholder no 2: the perception on what prudent or responsible use of antimicrobials varies. Preventive use of antimicrobials is not the same as early treatment. The reference on treatment of mice and fluoroquinolones in a mouse-lung model of *Pasteurella multicida* seems irrelevant here as this is a reflection paper on pleuromutilins not fluoroquinolones and *Pasteurella multocida* is not a target pathogen. Not agreed. Pleuromutilins should not be used for prevention of disease other than under strict conditions in eradication programmes.
38	3	**Comment:** Pleuromutilins are important veterinary medicines in eradication programmes for swine dysentery, ileitis and enzootic pneumonia (Kixmoeller et al. 2010, Kamp et al. 2010, Burch & Howells 2010, Pico et al. 2008, Rajska et al. 2008, Giger et al. 2006, Nielsen 2004). Based on the results of sensitivity testing they are identified as the drug of choice for eradication on specific farms. **Proposed change:** We would suggest modifying the	Not agreed. See comments to Stakeholder 2, Pleuromutilins should not be used in eradication programmes other than those for swine dysentery.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		sentence as follows: "~~The exception would be~~ Pleuromutilins should be used in well-defined eradication programmes for swine dysenteria, ileitis and enzootic pneumonia based on MIC testing results." *Kixmoeller,M., Kmiec,M., Szancer, J. Attempt to eradicate Lawsonia intracellularis during establishment of a new breeding herd by combined strategic medication with tiamulin (Denagard) and cleaning/disinfection (2010). Proceedings 21st IPVS Congress, Vancouver, Canada, P.402. p.708.* *Kamp,J., Schuttert-Wilps, R., Kars-Hendricksen, S.(2010). Swine dysentery eradication by strategic medication without depopulation.Proceedings 21st IPVS Congress, Vancouver, Canada, P.427. p.733.* *Burch, D., Howells M.J. (2010). Eradication of swine dysentery in an outdoor breeding herd and its production pyramid. Proceedings 21st IPVS Congress, Vancouver, Canada, P.426. p.732.* *Pico, L., Szancer, J., Pique, J., Vidal, A. (2008). Swine dysentery eradication programme in a large farm with Three site Production by strategic management and medication. Proceedings 20th IPVS Congress, Durban, South Africa, OR.03.16. p.131.* *Rajiska, M., Kempa, W., Wilczynski, K. (2008). Experiences with control programme of swine dysentery in a typical polish pig unit. Proceedings 20th*	

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		IPVS Congress, Durban, South Africa, P.03.031. p.250. *Giger, TG., Schmid, W., Klein, U. (2006). Eradication of M.hyopneumoniae in breeding herds without restocking or partial depopulation. Proceedings 19th IPVS Congress, Copenhagen, Denmark, O.68-02 p.314.* *Harm Nielsen, L. (2004). Attempt to eradicate Lawsonia intracellularis by medication in 3 sow herds. Proceedings 18th IPVS Congress, Hamburg, Germany, Vol.1, p.281. …*	
41	3	**Comment: "**Approved indications for unspecified prevention of disease should be withdrawn". This sentence is rather confusing. The indication is either approved or it is not. Therefore unspecified prevention of disease does not make sense. Currently, prevention is permitted as an indication for a disease claim and was included in the recent EU Referral for Tiamutin premixes (EU Commission Decision 2010). **Proposed change:** This sentence should be deleted.	Partly agreed. The sentence has been changed into: Approved indications such as prevention of disease other than during eradication programmes should be withdrawn.
42, 43	3	**Comment:** "without a solid clinical basis" **Proposed change:** so it would read "General indications against infections in general without a solid clinical basis should be avoided".	Seen comments to stakeholder 2.
45-46	3	**Comment:** It is in line with the responsible use principles that the duration of an antibiotic treatment is limited to the time needed for a cure of the disease. Duration of treatment is evaluated in the clinical trials	The basis and criteria would be that established by guidance on clinical trials of antimicrobials.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		for the registration dossier and thus justified by the analysis of the clinical data. In this context what would be the basis for a review of "unnecessarily long treatment duration" and what criteria would be used in its determination?	
62, 63 Lines 62 to 68	3	**Comment:** This paragraph is strongly supported and we would suggest adding the importance of sampling based on a standard protocol and the need to monitor the susceptibility to other therapeutic agents. **Proposed change:** Please amend the sentence to read: **"**harmonised monitoring data ~~for pleuromutilin resistance in~~ on the susceptibility of *Brachyspira hyodysenteriae* to pleuromutilins and other therapeutics. CVMP would recommend responsible bodies to create such a monitoring system including sampling based on a standard protocol, first to allow baseline data to be collected"	Partially agreed and reflected on the revised reflection paper. The phrase: "including sampling based on a standard protocol", has now been added to the reflection paper
119, 120	3	**Comment: "**Pleuromutilins are also used off-label to treat the polyetiological disease porcine respiratory disease complex (PRDC), and more rarely, leptospirosis". This has been resolved in the EU Referral process (EU Commission Decision 2010). Tiamulin is highly effective against *Mycoplasma hyopneumoniae* and *Mycoplasma hyopneumoniae* is part of the PRDC disease complex. Leptospirosis is not a registered indication for tiamulin. **Proposed change:** The sentence should be adapted according to approved SPC text based on the EU	Agreed.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		Referral and EU Commission decision (2010).	
122, 123	3	**Comment:** This reference can be argued to be inappropriate because it only refers to Denmark. Please refer instead to the ESVAC report from 2010 where Table 6 shows that pleuromutilin use across Europe is highly variable. It also shows (Table 6) that Denmark uses disproportionately more than any other European country. Figure 7 of this report shows pleuromutilin sales across Europe in mg/PCU to be almost the lowest of all classes. Also the report shows only 6% of premix sales were attributed to pleuromutilins and 6% as oral solutions, both being the lowest of all reported antimicrobial classes. The reflection paper considers the ESVAC data in lines 139 to 164 but does not put in context of overall sales of antimicrobial classes. **Proposed change:** Please reference the ESVAC report (2010) and change the sentence accordingly.	Partially agreed, see comment to stakeholder 2.
139 to 164	3	**Comment:** Please see the comment above for lines 122, 123	Agreed.
171 to 180	3	**Comment**: We support the text but it is important to consider the following paragraph, which we suggest is added: “Studies by Long *et al* (2006) have shown that different pleuromutilin derivates are similarly anchored in the binding pocket by the common tricyclic mutilin core. However, varying effects are observed at positions U2584 and U2585, indicating that the side chain extensions adopt distinct conformations within the cavity and thereby affect the rRNA conformation differently. The data clearly demonstrate that there is a differential response for tiamulin and valnemulin with	Not agreed, seem comment to stakeholder 2.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		valnemulin being better able to withstand an altered rRNA binding surface around the mutilin core and retain its' activity. This is likely due to additional interactions made between the specific side chain extension of valnemulin and the rRNA binding site. This clearly impacts upon cross resistance and helps to understand that resistance to tiamulin does not necessarily mean an isolate will be resistant to valnemulin. The fact that the pleuromutilin drugs footprint at the peptidyl transferase and inhibit the peptidyl transferase reaction defines their inhibitory mechanism to some degree. The basis for explaining the inhibitory mechanism of pleuromutilins is steric hindrance of the peptidyl transferase reaction. In brief, tiamulin selectively inhibits bacterial protein synthesis by binding to the ribosome with high affinity, inhibiting ribosomal peptidyl transferase activity and partially inhibiting the binding of the initiator tRNA substrate to the ribosomal P-site. This effect will not necessarily be the same for other pleuromutilin drugs, either valnemulin or others that are in development." *Long KS, Hansen LH, Jakobsen L, Vester B (2006) Interaction of pleuromutilin derivatives with the ribosomal peptidyl transferase center. Antimicrobial Agents and Chemotherapy 50, 1458-1462.*	
181 to 188	3	**Comment:** We support the text, however, we argue that there is an important issue that is not described and we suggest that the following text is inserted after Line 188: "Pringle *et al* (2004) show that all of the laboratory-derived strains with an increased MIC relative to the parent strain contained mutations in either the ribosomal protein L3 or domain V of 23S rRNA. Although this is not direct proof that the mutations cause the increase in MICs, it strongly predicts them as resistance determinants. The isolates selected in the	Not agreed, see comments to stakeholder 2.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		laboratory, however, contained different combinations of mutations and none of the combinations were identical. This illustrates that changes in susceptibility to tiamulin can be obtained in various ways. The strains with the highest tiamulin MICs contained three mutations, indicating that one mutation is not sufficient to cause a high level of resistance. The two strains with the highest tiamulin MICs (>128 µg/mL) were the only strains with the T2504G mutation. The authors postulated that the absence of mutations in 23S rRNA at the peptidyl transfer centre in the field isolates could be because unlike the laboratory derived mutants they had not been exposed to the high concentration of tiamulin and the long exposure time. Alternatively, the described mutations could have had a fitness cost and led to decreased strain viability, with the consequence that such mutants could not survive in the field and thus could only be isolated under more supportive conditions in the laboratory. Clearly this finding suggests that laboratory based resistance data needs to be interpreted with care." *Pringle M, Poehlsgaard J, Vester B, Long KS (2004) Mutations in ribosomal protein L3 and 23S ribosomal RNA at the peptidyl transferase centre are associated with reduced susceptibility to tiamulin in Brachyspira spp. isolates. Molecular Microbiology 54, 1295-1306.*	
188	3	**Comment:** In the absence of harmonised testing methodology for….. care must be taken interpreting comparative data between tiamulin and valnemulin. Long et al (2006) have demonstrated that there is a differential binding response for tiamulin and valnemulin at the active site with valnemulin being better able to withstand an altered rRNA binding surface around the mutilin core and retain its' activity.	Not agreed, see comment to stakeholder 2.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		Proposed change: Please amend the sentence as follows: "Whilst t~~T~~he MICs for valnemulin follow those for tiamulin in most cases but are generally a few dilution steps lower (Pringle et al., 2012) it is accepted that binding of tiamulin and valnemulin at the active is different and in the absence of understanding more about potential resistance genes, it is inappropriate to read too much into MIC data without any agreement on internationally agreed harmonised susceptibility testing methodology."	
191 to 236	3	**Comment:** We support this review of the literature. However, we believe it does not consider all the available data and we would suggest that the following additional information needs to be inserted after line 236: "The issue of co-resistance is extremely complex and the data needs to be considered most carefully. This is best exemplified by the study of Miller *et al* (2008); these workers investigated whether other mechanisms of resistance to linezolid in clinical isolates of *S. aureus* might also confer cross-resistance to pleuromutilins. Despite early predictions that linezolid would display a low potential for the development of resistance, resistant clinical isolates of *S. aureus* have, unfortunately, emerged following prolonged therapy with the agent. As oxazolidinone and pleuromutilin antibiotics are currently used in the treatment of staphylococcal infections and as both antibiotics inhibit protein synthesis and have overlapping binding regions	Not agreed see answer to stakeholder 1 and 2.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		on 23S rRNA, the potential for co-resistance between the two classes through target site mutations is an obvious possibility. Miller *et al* (2008) selected mutants of *S. aureus* resistant to linezolid and found them to exhibit cross-resistance to tiamulin. However, resistance was unidirectional because mutants of *S. aureus* selected for resistance to tiamulin did not exhibit co-resistance to linezolid. This contrasts with the described $PhLOPS_A$ phenotype, which confers resistance to both oxazolidinones and pleuromutilins. The genotypes responsible for the phenotypes observed by Miller *et al* (2008) were examined and selection with tiamulin resulted in recovery of mutants with changes in the single-copy *rplC* gene (Gly155Arg, Ser158Leu, or Arg149Ser), whereas selection with linezolid led to recovery of mutants with changes (G2576U in 23S rRNA) in all five copies of the multicopy operon *rrn.* In contrast, cross-resistance to linezolid was exhibited by tiamulin-resistant mutants generated in single-copy *rrn* knockout strains of *E. coli*, illustrating that the copy number of 23S rRNA is the limiting factor in the selection of 23S rRNA tiamulin-resistant mutants. The significance of this finding was clearly expounded by the authors when they made the point that the potency, low potential for development of resistance, and favourable pharmacokinetics of oxazolidinones and pleuromutilins make them ideal anti-staphylococcal drugs. However, the unidirectional cross-resistance between linezolid and tiamulin observed in linezolid -resistant *S. aureus* could limit	

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		the systemic use of pleuromutilins in patients for which linezolid treatment has been unsuccessful. Clearly this is not an issue of resistance developing to pleuromutilins but rather to linezolid. The authors concluded that whilst acquisition of the $PhLOPS_A$ phenotype confers cross-resistance between linezolid and pleuromutilins, the emergence of pleuromutilin-resistant isolates by point mutation should have little impact on susceptibility to linezolid. This is because resistance to linezolid is not observed with tiamulin-resistant *S. aureus* mutants due to the preferential selection of single-copy *rplC* mutations rather than 23S rRNA mutations on the multicopy operon *rrn*. Cross-resistance to linezolid is observed with tiamulin-resistant mutants derived from a single-copy *rrn* knockout strain of *E. coli*, illustrating that the copy number of 23S rRNA genes is the limiting factor in the selection of 23S rRNA tiamulin-resistant mutants. " *Miller K, Dunsmore CJ, Fishwick CWG, Chopra I (2008) Linezolid and tiamulin cross-resistance in Staphylococcus aureus mediated by point mutations in the peptidyl transferase centre. Antimicrobial Agents and Chemotherapy 52, 1737-1742.*	
171 to 272	3	**Comment:** We believe it is important to add an explanation to place this section into context as the issue is not simply to report all publications considering the identification of resistance genes. **Proposed change:** Please insert the following sentences after line 272:	Not agreed. See comments to stakeholder 2.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		Even in the light of current understanding of the described resistance mechanisms it is important to establish that the prevalence of these newly described resistance mechanisms is currently low. For example Long et al (2006) stated that whilst the cfr gene can, in principle, be easily disseminated among staphylococci, surveillance studies in Germany have identified only 6 cfr-carrying staphylococcal strains during the past 17 years (Kehrenberg & Schwarz, 2006). *Long KS, Poehlsgaard J, Kehrenberg C, Schwarz S, Vester B (2006a) The Cfr rRNA methyltransferase confers resistance to phenicols, lincosamides, oxazolidinones, pleuromutilins, and streptogramin A antibiotics. Antimicrobial Agents and Chemotherapy 50, 2500-2505.* *Kehrenberg C, Schwarz S (2006) Distribution of florfenicol resistance genes fexA and cfr among chloramphenicol-resistant Staphylococcus isolates. Antimicrobial Agents and Chemotherapy 50, 1156-1163.*	
216-236	3	**Comment:** While the dissemination of the cfr gene should be avoided, the references considered in this paragraph are essentially dealing with human pathogens. They explain that the dissemination of the gene is already occurring in human medicine as a result of a man to man “contamination”. This is particularly obvious for hospitals. Indeed Quiles-Melero et al (2013) have reported that the diversity of linezolid resistant species and clones found suggests	Not agreed, see comments to stakeholder 2.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		that linezolid resistance among Gram-positive cocci in Spanish hospitals has resulted from several independent selection events followed by the expansion of a few clones. The authors suggest that both antibiotic selection pressure and cross transmission have played a role in the local emergence and spread of the resistant clones. Additionally after reaching a maximum in 2009, the number of resistant isolates decreased in 2010 and 2011. The reasons for this rapid decrease are unclear, but it is considered that improved linezolid use in the hospital might have been an important factor. Thus the real contribution of the veterinary field in an already established human mechanism of resistance can be questioned. It should be emphasised that Kehrenberg *et al*. limited the risks of resistance spreading to swine farming. The relevance of the findings of Wang et al (2012c) in China can be questioned since no programme of responsible use in this place in this country in contrast to the EU. *Quiles-Melero I, Gómez-Gil R, Romero-Gómez MP, Sánchez-Díaz AM, de Pablos M,* *García-Rodriguez J, Gutiérrez A, Mingorancea J (2013) Mechanisms of Linezolid Resistance among Staphylococci in a* *Tertiary Hospital. Journal of Clinical Microbiology 51, 998-1001*	
247-251	3	**Comment:** As for comments on lines 216-236. The resistance mechanism is found in veterinary isolates from China. The conditions of use of antibiotics in the EU would likely limit the occurrence of this mechanism	Not agreed, see comments to stakeholder 2.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		of resistance and the data suggests that this is not happening as it is in China.	
296	3	**Comment:** The CLSI reference does not seem to be cited in the reference listing **Proposed Change:** Please add full reference "CLSI (2008) Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals; Approved Standard – Third Edition. CLSI Document M31-A3"	Agreed. Reference to the latest version (2013) added.
295 to 309	3	**Comment:** This section is considered to be of limited value because it does not make the point that the break point is related to administered dose and takes into account the susceptibility distribution of the target pathogen, the drug concentrations achieved at the site of infection and the clinical response. Only the Burch publications address these issues, although even in this case the data has not been presented to an independent breakpoint committee. With respect to Brachyspira we have no internationally harmonised susceptibility testing methodology so cannot with confidence describe susceptibility distributions; but of greater importance to this section is there is no mention of drug concentrations at the site of infection which in turn will be influenced by administered dose. It is important to emphasise the point that epidemiological cut-off values cannot be used to predict clinical response. **Proposed Change:** Replace the paragraph with the following text: Internationally accepted interpretative criteria are	Not agreed, see comments to stakeholder 2.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		lacking except for tiamulin for *Actinobacillus* species (Clinical Laboratory Standards Institute (CLSI) 2008). To date, no tiamulin or valnemulin breakpoints have been established for *Brachyspira* species. Whilst breakpoints have been proposed few have addressed the susceptibility distribution of the target pathogen, drug concentrations achieved at the site of infection and the clinical response. Epidemiological cut-off values cannot be used to predict clinical response but may be of use in monitoring changes in susceptibility although harmonised susceptibility testing methodology needs first to be in place before this data can be properly evaluated.	
329-333	3	**Comment:** Given the issues associated with sensitivity testing, the reporting here should be more nuanced: methodology, link with clinical efficacy *etc.* Without this additional information and given the proposed breakpoints (which are dependent on methodology), it would imply that all these isolates would be resistant.	See comments to stakeholder 2.
332, 333	3	**Comment:** The development of the MIC values has not been summarised correctly in both these sentences. **Proposed change:** Please amend the sentences as follows to more accurately summarise the development: In Germany MIC90 for tiamulin increased from 0.125 µg/ml (1989-1993) to ~~2-~~8 µg/ml (2000~~-2002~~) **and then decreased to 2.0 µg/ml (2002).** For valnemulin the MIC90 increased from 0.063 µg/ml (1989-1993) to ~~2-~~4 µg/ml (2000~~-2002~~) **and then**	No changes required. See comments to stakeholder 2.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		decreased to 2.0 µg/ml (2002) (Rohde et al., 2004).	
333	3	**Comment:** It is apparent that situation in different EU member states differs greatly regarding the susceptibility of *Brachyspira hyodysenteriae* pathogens to Pleuromutilins. In many countries no resistance or low levels of resistance (<10%) against tiamulin were found. Therefore an additional sentence should be added to the text to reflect those studies. **Proposed change:** To properly reflect the situation the following sentence should be added: "In antimicrobial susceptibility studies conducted in Italy (Magistrali et al., 2010), Belgium (Vyt 2010), Poland (Zmudzki J. et al, 2012), Sweden (SVARM 2011) and in Germany (Herbst W. et al., 2008) no resistance or low resistance (Williamson et al., 2010 Pridmore 2008) of *B.hyodysenteriae* strains against tiamulin was found". *Magistrali, C.F., Cucco, L., D`Avino,N., Tentellini, M., Pezzotti, G. (2010). Antimicrobial susceptibility of Brachyspira hyodysenteriae isolates from cases of swine dysentery in Italy recovered by different sampling procedures. Proceedings 21st IPVS Congress, Vancouver, Canada, P.422. p.728.* *Vyt, P. 2010. Antimicrobial susceptibility of Belgian Brachyspira hyodysenteriae isolates. Proceedings 21st IPVS Congress, Vancouver, Canada, O.205. p.238.* *Zmudzki, J., Szczotka, A., Nowak, A., Strzelecka, H.,*	Not agreed. See comments to stakeholder 2.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		Grzesiak, A., Pejsak, Z. (2012). Polish Journal of veterinary Sciences Vol.15, No.2, 259-265. *SVARM 2011. Swedish Veterinary Antimicrobial Resistance Monitoring SVA* *Herbst, W., Schlez, K., Heuser, J., Baljer,G. (2008). Detection of brachyspira hyodysenteriae in pigs with and without diarrhoea and drug resistance of German B.hyodyenteriae field isolates. Proceedings 20th IPVS Congress, Durban, South Africa, P03.021 p.241.* *Williamson, S., Rogers, J. Hunt, B., Teale, C. (2010). Preliminary results for Brachyspira MIS assessment of isolates form England. Presentation at Pig Veterinary Society Meeting, Norwich, UK*	
333	3	**Comments:** We suggest that some comment is made that addresses at least in part the variable MIC data seen across Europe, relating to isolate source. The reviewed data does not seem to have been moderated for isolate type. **Proposed change:** Please include the following text after line 333: It may be argued that not all of the reports of decreased susceptibility are necessarily due to resistance development but there is likely to be a contribution from ill defined testing methodology. Of even greater significance are the source of isolates and an understanding of the status of the disease in the respective countries. In some cases for example, isolates were all clinical isolates from farms exhibiting	Not agreed. See comments to stakeholder 2.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		clinical treatment failure in which the authors accepted that the data may have been influenced by the choice of *B. hyodysenteriae* isolates Lobová *et al* (2004). It is accepted by OIE (Franklin 2001) that when designing sampling programmes, results from diagnostic submissions may not reflect the resistance situation in the animal population, as these types of submissions tend to include specimens from severe and/or recurrent clinical cases, including therapy failures. *Franklin A, Acar J, Anthony F, Gupta R, Nicholls T, Tamura Y, Thompson S, Threlfall EJ, Vose D, van Vuuren M, White DG, Wegener HC & Costarrica ML (2001). Antimicrobial resistance: harmonisation of national antimicrobial resistance monitoring and surveillance programmes in animals and in animal-derived food. Revue scientifique et technique (International Office of Epizootics) 20, 859-870.* *Lobová D, Smola J & Cizek A (2004). Decreased susceptibility to tiamulin and valnemulin among Czech isolates of Brachyspira hyodysenteriae. Journal of Medical Microbiology 53, 287-291.*	
Sentence before sentence 334	3	**Comment:** Resistance to the Pleuromutilins tiamulin and valnemulin has not been reported in porcine and avian Mycoplasma species (Makhanon et al. 2012, Thongkamkoon et al. 2010). Pleuromutilins can be used as alternatives to macrolides and lincosamides in case of development of resistance to these substances (Maes et al. 2007, Vicca et al. 2004, Kobayashi et al.	Not agreed. See comments to stakeholder 2.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		2008, Löhren et al.2008). **Proposed change:** To adequately reflect the comment please insert the following sentence: “No resistance has been reported in porcine and avian Mycoplasma species and Pleuromutilins can be used as alternatives to macrolides and lincosamides in the case of resistance to these substances (Maes et al. 2007, Vicca et al. 2004, Kobayashi et al. 2008, Löhren et al. 2008, Thongkamkoon et al. 2010, Makhanon et al. 2012)” *Maes, D., Vicca, J., Stakenborg, T., Butaye, P., De Kruif, A., Haesebrouck, F. (2007). Gevoeligheih van Belgische Mycoplasma hyopneumoniae- isolaten voor antimicrobiele middelen. Vlamms Diergeneeskundig Tijdschrift, 76, 300-305.* *Vicca, J., Stakenborg, Maes, D., Butaye, P., De Kruif, A., Haesebrouck, F. (2004). In vitro susceptibilities of Mycoplasma hyopneumoniae field isolates. Antimicrobial Agents and Chemotherapy, 48, 11, 4470-4472.* *Kobayashi, H., Kanazaki, M., Kajiwara, K. (2008) Macrolid, tiamulin and valnemulin susceptibility of Mycoplasma hyopneumoniae strains isolated in various parts of Japan. Proceedings 20th IPVS Congress, Durban, South Africa, P02.002 p.187.* *Löhren, U., Ricci, A., Cummings, T.S. (2008) Guidelines for antimicrobial use in poultry, In:*	

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		Guardabassi, L., Jensen, L.B., Kruse, H. (Eds.) Guide to antimicrobial use in animals. Blackwell Publishing Ltd., Oxford, UK, pp126-142. *Thongkamkoon, P., Prapasarakul, N., Makhanon, M., Talummuk, S., Klein, U. (2010) In vitro susceptibility of Porcine Mycoplasmas to antimicrobial agents during 2008-2009. Proceedings 21st IPVS Congress, Vancouver, Canada, P.638.* *Makhanon, M., Thongkamkoon, P., Prapasarakul, N. (2012) In vitro susceptibility study of porcine Mycoplasmas in Thailand. Proceedings 22nd IPVS Congress, Jeju, South Korea, P.710.*	
334	3	**Comment:** *Actinobacillus pleuropneumoniae* resistance to tiamulin was not reported in the pan-European MIC project VetPath II (Felmingham 2009). **Proposed change:** Please add the following sentence: "No resistance to tiamulin in Actinobacillus pleuropneumoniae has been reported in a pan-European MIC testing project (Felmingham 2009)." *Felmingham, D. (May 2009) Quotient Bioresearch Ltd., Study number IV257-31-05; A report to CEESA AISBL (Brussels, Belgium) "Determination of the antimicrobial susceptibility of the VetPath II (2004-2006) collection of bacterial pathogens".*	Thanks for the information, but as *Actinobacillus pleuropneumoniae* is not a target pathogen there is no need to include such phrase.
352,353, 354	3	**Comment:** The treatment against ileitis (Lawsonia intracellularis) with Pleuromutilins is an important indication based on the importance of this disease in	See comments above to stakeholder 2.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		the field and the wide range of MIC distribution shown for macrolide and lincosamide antibiotics in the limited number of MIC data available. Wattanaphansak et al. (2009) tested the activity of tiamulin and valnemulin, among other antimicrobials, against 10 isolates of *L.intracellularis*. The narrow range of MIC distribution for tiamulin and valnemulin indicated the occurrence of high susceptibility of the tested strains from Europe and the USA. Tiamulin and valnemulin were identified as the most active antimicrobials tested in this study. We suggest adding the following sentence "Wattanaphansak et al. (2009) tested the activity of tiamulin and valnemulin, among other antimicrobials, against 10 isolates of L.intracellularis. The narrow range of MIC distribution for tiamulin and valnemulin indicated occurrence of high susceptibility of the tested strains from Europe and USA". **Proposed change:** We suggest adding the following sentence after line 354: "Wattanaphansak et al. (2009) tested the activity of tiamulin and valnemulin, among other antimicrobials, against 10 isolates of *L.intracellularis*. The narrow range of MIC distribution for tiamulin and valnemulin indicated occurrence of high susceptibility of the tested strains from Europe and USA". *Wattanaphansak, S., R. S. Singer and C. J. Gebhart.2009. In vitro antimicrobial activity against 10 North American and European Lawsonia intracellularis isolates. Veterinary Microbiology 134: 305–310*	

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
358	3	**Comment: "**Lack of authorized and effective drugs for treatment of swine dysentery has increased the use of pleuromutilins, and this probably explains the emergence of resistant strains". Different effective drugs are available for swine dysentery treatment. It should be mentioned that the removal of growth promoters that are incompatible with tiamulin or valnemulin such as salinomycin, the increasing generic competition and the price erosion has increased the use of pleuromutilins. **Proposed change:** Please amend the sentence as follows: "~~Lack of authorized and effective drugs for treatment of swine dysentery~~ removal of incompatible growth promoters such as salinomycin, increasing generic competition and price erosion has increased the use of pleuromutilins, and this probably explains the emergence of resistant strains".	Addition of this information does not seem necessary.
358 to 392	3	**Comment:** The reflection paper clearly states that there are no published breakpoints for *B. hyodysenteriae* yet within this section discusses "resistant" isolates. In the absence of breakpoints it is not correct to discuss whether isolates are resistant or not. This is especially true for infections where the target pathogens can be exposed to relatively high drug concentrations within the GI tract. In this context the data presented in lines 360 to 364 is to be applauded because it relates MICs to clinical outcome. However, lines 364 to 366 should not discuss "resistant" isolates because these studies have no	See comment to stakeholder 2.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		basis for ascribing isolates as “resistant”. Clearly it is appropriate to state that the isolates carry resistance genes but this is not necessarily the same thing as being “clinically resistant” **Proposed change:** please delete lines 364-367	
367 to 370	3	**Comment:** Whilst pleuromutilin use is high in Spain, Portugal and Czech Republic it is also high in Denmark (ESVAC 2011) yet no comment is made. **Proposed change:** Please add Denmark to the list and make appropriate comments about susceptibility.	No change, see comments to stakeholder 2.
370	3	**Comment:** The Lobova (2004) data is skewed which the authors acknowledge in the publication because sampling in 2000 and 2001 was from clinical samples. **Proposed change**: Please amend as follows: “been reported from Spain (Hidalgo et al., 2011) and Czech Republic (Lobova et al., 2004; Sperling et al., 2011) **although some of these data are skewed because of the isolate source.**”	The addition of this information seems unnecessary; see detailed comments to stakeholder 2.
374	3	**Comment:** The statement, “It has been suggested that the use of pleuromutilins very likely selects for the emergence of *cfr* in animal isolates of staphylococci (Witte and Cuny, 2011)” is misleading. The publication actually states, “Veterinary use of both florfenicol and tiamulin, very likely selected for the emergence of *cfr* in animal isolates of coagulase negative staphylococci” and it is argued that this in itself can be considered misleading. As has already been discussed *Cfr* was first discovered in 2000 from a bovine strain of	

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		Staphylococcus sciuri (Schwarz *et al*, 2000) and confers a resistance phenotype referred to as $PhLOPS_A$ conferring resistance to Phenicols, Lincosamides, Oxazolidinones, Pleuromutilins, and Streptogramin A antibiotics. In this context it can be argued that other antimicrobial classes could have played a role. What is not made clear, however, is the observation of Miller *et al* (2008). These workers being aware of the observation that the $PhLOPS_A$ phenotype conferred resistance to both linezolid and tiamulin in *S. aureus,* selected mutants of *S. aureus* resistant to linezolid and found them to exhibit cross-resistance to tiamulin but resistance was unidirectional, mutants of *S. aureus* selected for resistance to tiamulin did **not** exhibit co-resistance to linezolid. **Proposal:** Please amend the sentence as follows**:** "It has been suggested that the use of ~~pleuromutilins~~ florfenicol and tiamulin very likely selects~~s~~ed for the emergence of *cfr* in animal isolates of coagulase negative staphylococci (Witte and Cuny, 2011), however, this observation is not necessarily supported by other data. Miller et al (2008) selected mutants of S. aureus resistant to linezolid and found them to exhibit cross-resistance to tiamulin but resistance was unidirectional; mutants of S. aureus selected for resistance to tiamulin did not exhibit co-resistance to linezolid; it is important to consider phenotypes and genotypes when considering pleuromutilin and linezolid resistance."	

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
374 to 392	3	**Comment:** Whilst we largely accept the presented text, it fails to address the prevalence of the described resistance genes nor does it explain the context of the data. We believe there needs to be additional comment. **Proposed change:** add the following text within this section: Whilst the data clearly demonstrates that the *cfr* gene can be disseminated between bacteria little consideration has been given to how frequently this is likely to occur. The evidence suggests that this is a relatively rare event. Kehrenberg & Schwarz (2006) reported only 6 *cfr*-carrying staphylococcal strains isolated during the 17 years prior to 2006, i.e. 1989-2006, none of which were isolated from swine. Kehrenberg *et al* (2009) specifically investigated *S. aureus* strains of porcine origin for the presence of *cfr*-positive strains. They screened nasal swabs from 846 swine from 367 farms all over Germany during 2007; they additionally screened 90 porcine coagulase-positive and coagulase-variable staphylococci collected all over Germany from diseased swine in the BfT-GermVet study 2004-2006 and 56 nonrelated porcine *S. aureus* strains provided by veterinary diagnostic laboratories from all over Germany collected mainly in 2008. In total, 2 staphylococcal strains of porcine origin displayed a resistance phenotype indicative of the presence of *cfr*. Both strains originated from swine farms in different geographic areas of northern Germany and were isolated in 2004 and 2007, respectively, and carried	Not agreed, see comments to stakeholder 2.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		the gene *cfr*. Clearly the data suggests that the prevalence of *cfr* in Germany is very low. Whilst MRSA ST9 isolates are commonly found in pigs in China, *cfr*-positive MRSA isolates have rarely been reported in China (Kehrenberg *et al* (2009). Whilst *cfr* is largely expressed in Gram-positive staphylococci, it is known that it can be expressed in *E. coli*. Wang *et al* (2011) described the presence of the *cfr* gene in a naturally occurring *Proteus vulgaris* isolate of porcine origin and subsequently screened 1230 *E. coli* isolates from individual pigs, chickens and ducks in Shandong (n=491; pigs n=189, ducks n=77 and chicken n=225) and Sichuan provinces (n=739; pigs n=218, ducks n=66 and chicken n=455) during 2008–10 (Wang *et al,* 2012a). The analysis of 1230 *E. coli* isolates revealed the presence of the *cfr* gene in a single isolate, obtained from the nasal swab of a pig in a slaughterhouse in Shandong province in 2010. The authors concluded that whilst *cfr* could be found in *E. coli*, the very low prevalence (0.08%) suggest that the detection of the *cfr* gene represents only sporadic incidence.	
390-392	3	**Comment:** The statement “Antibiotic usage records for Chinese pig farms indicate that multiple antimicrobial drugs, including florfenicol, lincomycin and tiamulin have been used on farms where *cfr* positive isolates have been found suggesting that selective pressure might have played a role” is misleading because all the cited studies do not support	Not agreed, see comments to stakeholder 2.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		this statement. In the **Wang *et al* (2012b)** study there was indeed information regarding antimicrobial therapy on the farm. This information indicated that a number of antimicrobial agents, including penicillin, florfenicol, trimethoprim/sulfamethoxazole, kanamycin, streptomycin, oxytetracycline and tylosin had been used for treating or preventing bacterial infections. It is important to note that pleuromutilins were **not** reported as being used. It was only in the paper describing cfr in the Gram-negative Proteus vulgaris that tiamulin was reported as being used. **Proposal:** Please amend as follows: "Antibiotic usage records for Chinese pig farms indicate that ~~multiple antimicrobial drugs, including florfenicol, lincomycin and tiamulin~~ pleuromutilins have not always been used on farms where *cfr* positive isolates have been found **<u>although other antimicrobials have been used.</u>** ~~suggesting that selective pressure might have played a role~~"	
394, 395, 396	3	**Comment: "**For most indications for which pleuromutilins are authorized there are alternative substances available except for swine dysentery …" "Therefore pleuromutilins are the only remaining treatment option for this indication." It is not correct that pleuromutilins are the only remaining treatment option for swine dysentery treatment. Alternative substances are available for swine dysentery treatment. Vyt et al (2012) have	See previous comments.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		shown that in cases of decreased susceptibility to tiamulin and valnemulin Tylvalosin can be used as an alternative to pleuromutilins for elimination of dysentery in pig farms. The same author (2010) reported that in nearly half of the cases with pleuromutilin resistance tylvalosin can be used as an alternative treatment. In a field study on spontaneous infection of pigs caused by *B.hyodysenteriae* it was concluded that *in vitro* susceptibility testing of *B.hyodysenteriae* (for lincomycin) only partially predicted the clinical effect of treatment (Vyt and Hommez, 2006). Lincomycin has shown efficacy in those field studies as a swine dysentery treatment option. Herbst et al. (2008) reported on a resistance rate of 4.3% of German *Brachyspira hyodysenteriae* strains. The following text changes are therefore suggested "For most indications for which pleuromutilins are authorised alternative substances are available. In the case of swine dysentery different treatment options beside both Pleuromutilin products can be used as recommended in different publications (Vyt et al 2012, Vyt 2010, Vyt & Hommez 2006, Herbst et al. 2008). The sentence "Pleuromutilins are the only remaining treatment option for this indication" should be deleted. **Proposed change:** please delete: "For most indications for which pleuromutilins are authorised there are alternative substances except for swine dysentery where high prevalence of resistance against alternative antimicrobials exists in many Member	

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		States. Therefore, pleuromutilins are the only remaining treatment option for this indication". And replace with: "For most indications for which pleuromutilins are authorised alternative substances are available. In the case of swine dysentery different treatment options beside both Pleuromutilin products can be used as recommended in different publications (Vyt et al 2012, Vyt 2010, Vyt & Hommez 2006, Herbst et al. 2008)." *Vyt, P. 2010. Antimicrobial susceptibility of Belgian Brachyspira hyodysenteriae isolates. Proceedings 21st IPVS Congress, Vancouver, Canada, O.205. p.238.* *Vyt, P., L.Vandepitte, A.Dereu, M.Roozen 2012. Elimination of swine dysentery on a single-site, farrow –to-finish farm using tylvalosin (Aivlosin). Proceedings Vol II 22nd IPVS Congress, Jeju, Korea, BP-272 p.629.* *Vyt, P., Hommez, J. (2006). Antimicrobial susceptibility of Brachyspira hyodysenteriae isolates compared with the clinical effect of treatment. Flem. Vet.J.75, 279-285.* *Herbst, W., Schlez, K., Heuser, J., Baljer,G. (2008). Detection of Brachyspira hyodysenteriae in pigs with and without diarrhoea and drug resistance of German B.hyodysenteriae field isolates. Proceedings 20th IPVS Congress, Durban, South Africa, P03.021 p.241.*	
401, 402	3	**Comment:** "In most EU Member States there are no national programmes for control of swine dysentery" But in Sweden a programme for control of swine	Not agreed. The word "most" already indicates that there are exceptions.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		dysentery was launched in 2000 and in several EU countries eradication programmes are established (Denmark, UK, Spain, Germany) based on initiative of big integrators. **Proposed change:** Please amend the sentence as follows: "In ~~most~~ many EU Member States there are no national programmes for control of swine dysentery. However, in Sweden a programme for the control of swine dysentery was launched in 2000 and eradication programmes are also established in Denmark, UK, Spain, and Germany."	
407 – 409	3	**Comment:** "Thus in many cases pleuromutilins are the only potentially effective choice among antimicrobials with swine dysentery as authorized indication." This is incorrect. Tylvalosin (Aivlosin) is centrally authorized in the EU for oral administration indicated in swine for the treatment and prevention of swine dysentery. Lincomycin is also registered for swine dysentery treatment. **Proposed change:** Suggest the following sentence "~~Thus in many cases~~ pPleuromutilins are ~~the only potentially~~one of the effective choices among antimicrobials with swine dysentery as authorized indication in the EU."	Not agree, see comments to stakeholder 2.
409	3	**Comment:** It is apparent that the situation on the susceptibility of *Brachyspira hyodysenteriae* strains differs greatly among the EU countries. The diversity of the results is based on differences in Brachyspira	See comments to stakeholder 2.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		species diagnosis and MIC testing procedure as shown in ring tests all over Europe (Rasbeck et al. 2005) In many countries (Denmark, Germany, Italy, Spain, Sweden, Ireland) high susceptibility of *Brachyspira hyodysenteriae* strains to tiamulin and valnemulin are reported. **Proposed change:** We suggest adding text which describes the susceptibility situation of *Brachyspira hyodysenteriae* realistically and which explains the reasons for the diversity of MIC testing results. We would propose the following text: "It is apparent that the situation on susceptibility of *Brachyspira hyodysenteriae* strains differs greatly among the EU countries. The diversity of the results are most likely based on differences in Brachyspira species diagnosis and MIC testing procedure as shown in ring tests all over Europe (Rasbeck et al. 2005). In many countries (Denmark, Germany, Italy, Spain, Sweden, Ireland) high susceptibilities of Brachyspira hyodysenteriae strains to tiamulin and valnemulin are reported. Pleuromutilins are therefore recommended in textbooks and national treatment guidelines." Isolates with reduced susceptibility to Pleuromutilins have emerged among B.hyodysenteriae in several countries …"	
418-420	3	**Comment:** The loss of Pleuromutilins beside macrolid products like tylvalosin and the lincosamid lincomycin as effective tools to treat swine dysentery would	This reflection paper addresses the use of pleuoromutilins, and discussion on the use of other antimicrobials is outside the scope of this reflection paper. Recommendations for

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		present a considerable threat to pig health, welfare and productivity. Therefore responsible use of these antibiotics based on MIC testing is needed. **Proposed change:** "To summarise, the loss of pleuromutilins beside macrolid products like tylvalosin and the lincosamid lincomycin as effective tools to treat swine dysentery because of a further increase in resistance or as a consequence of restrictions would present a considerable threat to the pig health, welfare and productivity. Therefore responsible use of these antibiotics based on MIC testing is needed."	responsible use should be made somewhere else in the text.
422	3	**Comment:** It would be appropriate to make some comment about the indications for retapamulin. The European assessment report makes the point that, "In clinical studies of secondarily infected open wounds, the efficacy of retapamulin was inadequate in patients with infections caused by methicillin-resistant Staphylococcus aureus (MRSA)." **Proposed change: "**To date only one product containing pleuromutilins (retapamulin) is authorised for humans for the topical ~~use only~~ treatment of impetigo due to susceptible strains of *Staphylococcus aureus* or *Streptococcus pyogenes*, the two most common types of bacteria in this kind of infection. Retapamulin does not have an indication for MRSA."	Not agreed. There is no need to make remarks on indications for retapamulin.
426-428	3	**Comment:** It should be stated that BC-3781 is a lead compound currently in Phase II trials and not a finished product. IFAH-Europe strongly believes that guidance should not be influenced by products in development that may never reach the market.	Partly agreed. We will add that BC-3781 was tested successfully during phase II trials.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
445 to 447	3	**Comment:** As stated in the comment to line 442, Retapamulin does not have indication for MRSA. The European assessment report makes the point that, "*In clinical studies of secondarily infected open wounds, the efficacy of retapamulin was inadequate in patients with infections caused by methicillin-resistant Staphylococcus aureus (MRSA).* **Proposed change:** Please revise to say: "Retapamulin demonstrated excellent in vitro activity against MSSA and MRSA strains, but not against MRSA isolates harbouring the cfr gene (Candel et al., 2011). Furthermore retapamulin does not have indication for MRSA as it was not efficacious in clinical studies."	Not agreed, the text does not make reference to the authorisation of retapamulin for MRSA indications.
422 to 456	3	**Comment:** This section fails to make a summary of the potential impact upon human health; as the reflection paper has already cited the review of Novak (2011) it is considered appropriate to also include his summary and a comment from Sanchez Garcia et al (2010) **Proposed change:** Please include the following paragraph (from Novak) after line 452: "So far, the *cfr* genotype is only rarely encountered in human bacterial isolates, (despite use of tiamulin in veterinary medicine since 1979) as illustrated by the most recent LEADER study 2009, which monitored linezolid resistance in the United States. The study found in only four strains (two *S. aureus*, one each of *Staphylococcus epidermidis* and *Staphylococcus capitis*) from four different states the *cfr* genotype,	In our opinion this does not belong in the summary assessment.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		suggesting persistence but limited potential for dissemination. Despite the potential mobility of the *cfr*-resistance determinant, surveillance studies in Germany have identified only six c*fr* carrying staphylococcal strains in animals during the past 17 years. However, because *cfr* has been encountered in some outbreaks, involving mainly *S.epidermidis* but also in one occasion *S. aureus,* careful monitoring of resistance development is clearly warranted. Additionally Sanchez Garcia et al (2010) addressing the hospital outbreak of MRSA carrying cfr concluded that a combination of the emergence of linezolid resistance in *S aureus* with clonal spread and use of linezolid was responsible for the LRSA outbreak. Successful early control of the LRSA outbreak by infection-control measures and reduction of linezolid use was achieved." *Novak, R. 2011. Are pleuromutilin antibiotics finally fit for human use? Annals of the New York 700 Academy of Sciences 1241:71-81.* *Sanchez Garcia, M., M.A. De la Torre, G. Morales, B. Pelaez, M.J. Tolon, S. Domingo, F.J. Candel, R. 733 Andrade, A. Arribi, N. Garcia, F. Martinez Sagasti, J. Fereres, and J. Picazo. 2010. Clinical 734 outbreak of linezolid-resistant Staphylococcus aureus in an intensive care unit. JAMA: the 735 journal of the American Medical Association 303:2260-2264.*	
462-471	3	**Comment:** This illustrates the need for EU responsible use initiatives to be tailored to the needs of individual	We believe that initiatives at country level as well as EU level

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		countries taking into account country-specific husbandry/population densities/disease prevalence/medicines availability situations.	are needed.
467 – 469	3	**Comment:** The prevalence of resistance of porcine Brachyspira species against the macrolide Tylosin is very high in many European countries. This is in contrast to the macrolide Tylvalosin which can be considered as effective against swine dysentery and colitis based on the available MIC testing results. **Proposed change:** Please amend the sentence as follows: “and a high prevalence of resistance to alternative antimicrobials used to treat swine dysentery, *e.g*. the macrolid~~es~~ tylosin in countries with the highest use.”	Not agreed, see comments to stakeholder 2.
479	3	**Comment:** The impact of resistance development to all currently effective antibiotics (tiamulin, valnemulin, tylvalosin and lincomycin) in *Brachyspira hyodysenteriae* has to be considered for current or planned resistance monitoring programmes. **Proposed change:** Please amend as follows: “Given the potential impact of resistance to ~~pleuromutilins~~ currently effective antibiotics (tiamulin, valnemulin, tylvalosin and lincomycin) in *B. hyodysenteriae* on pig health, welfare and production, there is a need to include *B. hyodysenteriae* in national resistance monitoring programmes.“	As this is a reflection paper on pleuromutilins, the focus is on pleuromutilins.
489-490	3	**Comment:** Pleuromutilins are useful in other indications and not only in swine dysentery. Diversity of treatments in the frame of a rational usage should be kept in order to dilute the selection pressure caused	The use of pleuromutilins for other indications should be discouraged.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		by using just one (swine dysentery) or limited number of antibiotic families (other indications) (Livermore D, Lancet Infect. Dis, 2005).	
489 – 491	3	**Comment:** In many textbooks and national treatment guidelines the Pleuromutilins tiamulin and valnemulin are recommended for many indications in food animals (Burch et al. 2008, Löhren et al. 2008, Denmark, the Netherlands (http://wvab.knmvd.nl/wvab/formularia/formularia). This is based on resistance development not only of Brachyspira species but also porcine and avian Mycoplasma species (Migaki et al. 1993, Maes et al.2007) to macrolid and lincosamid antibiotics. It is important that Pleuromutilins are used according to the label against all bacterial pathogens listed in the product SPC. Maintenance of Pleuromutilins for treatment of swine dysentery is critical.	Not agreed, the reflection paper does not intend to review treatment guidelines.
511 to 515	3	**Comment:** This section does not address prevalence of resistance genes. **Proposed Change**: Please add the following sentence to line 515: "The data suggests that prevalence of these resistance genes is low."	We prefer not to qualify the prevalence of resistance.
517, 518	3	**Comment:** The reflection paper cites the study of Morales et al; these workers identified a linezolid resistant MRSA carrying cfr, however, all the strains were susceptible to tigecycline, vancomycin, and daptomycin. **Proposed Change:** Please amend as follows: "The emergence of these resistance genes in animals	Not agreed, see comments to stakeholder 2.

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		poses a potential threat to human medicine as they might compromise empirical treatment of human MRSA infections although data shows alternative antibiotics including tigecycline, vancomycin, and daptomycin remain active."	
520-522	3	**Comment:** The phrase, "As the pleuromutilin resistant isolates are often multidrug-resistant" is confusing as it does not specify which resistance genes are being considered. If it is cfr, for example, then comment needs to be made about the relatively low prevalence. Additionally other classes of antimicrobials in human and veterinary medicine may also co-select for cfr. **Proposed Change:** Please clarify the sentence.	Partly agreed. This sentence refers to resistance by *vga* and *cfr.*
522 – 525	3	**Comment:** As existing data regarding prevalence of resistance genes suggests low prevalence we strongly support the need for added surveillance**.**	Agreed,
526 – 528	3	**Comment:** The sentence "Co-selection for pleuromutilins with many different antimicrobials can potentially occur due to multidrug resistance genes ……..." requires clarification as this statement is true for all classes of antimicrobial whether in animal or human medicine. As it stands it is of limited value and in terms of public health it is argued that Pleuromutilins as a class are the least likely to impact public health; this is acknowledged by WHO by whom pleuromutilins are not classified as "critically important".	See comments to stakeholder 2.
19,20	3	**Comment:** Suggest to add "herd/flock medication" **Proposed change:** so it would read "… most of the use is for group and herd/flock medication in feed or	Agreed.

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		water".	
21	3	**Comment:** Suggest to replace "varies" by "vary" **Proposed change:** … approved indications var~~ies~~y considerably.	Agreed.
23	3	**Comment:** Suggest to replace "is" by "are" **Proposed change:** …where pleuromutilins ~~is~~ are used but there is likely additional …	Agreed.
27	3	**Comment:** Add "of all licence holders" **Proposed change:** so it would read " … use principles are outlined in the SPCs for approved products of all licence holders"	Not agreed, the clarification is unnecessary in this context,
45	3	**Comment:** Suggest to introduce "effective" into the sentence **Proposed change:** so it would read "… time needed for effective cure of diseases".	As ineffective cure does not exist, adding effective is unnecessary.
90	3	**Comment:** "Tiamulin was approved for use in veterinary medicine in 1979, followed by …" Tiamulin was approved in 1978 in Ireland and mostly in 1979 in different European countries and globally. **Proposed change:** No specific change. The question is if approval in Ireland is considered or more general "the birthdate" of the product in 1979.	No change needed.
117, 118	3	**Comment:** Tiamulin is also available as a water soluble granulate for drinking water medication. The Islam *et al.* reference is unnecessary at this point. **Proposed change:** Please amend as follows: Tiamulin is available as medicated feed premix and as a solution or water soluble granulate for medication in drinking water ~~(Islam et al., 2009~~).").	Partially agreed.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
134	3	**Comment:** Suggest to change 2nd part of sentence "… if such recommendation is included in the SPC for other products containing tiamulin." **Proposed change:** Please add "for other licence holders" "…if such recommendation is included in the SPC for other licence holders for other products containing tiamulin".	
291	3	**Comment:** Suggest to add the word "testing" to the sentence **Proposed change:** Please amend as follows: "Antimicrobial susceptibility testing of Lawsonia intracellularis is difficult as this obligate intracellular bacterium …"	Agreed.
307 – 309	3	**Comment:** Please specify if this is an agar or broth dilution?	This is specified.
323	3	**Comment:** Please specify unit (µg/mL)	Agreed.
20-22	4	**Comment**: It is important to look into harmonizing indications and withdrawal periods throughout Europe, based on relevant scientific evidence.	We agree.
23-24	4	**Comment**: Difference in prevalence of a disease could be a result of different practices applied in practice in the different European countries. This fact should be taken also into consideration.	Agreed, but as the phrase addresses the differences in use, which includes practices, there is no need for further reference.
32-33	4	**Comment**:	Agreed.

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		FVE agrees that promotion of responsible use of antimicrobials and other best practices is a valuable means for the prevention of antimicrobial resistance.	
38-39	4	**Comment**: Close collaboration of farmers with their veterinarian is crucial part for the success of such a programme. **Proposed change (if any)**: …well-defined eradication programme for swine dysenteria, agreed by both the veterinarian and the farmer.	Agreed the CVMP recommendation has been changed to add at the end:" that have been established by the veterinarian in conjunction with the farmer."
51	4	**Comment**: **Proposed change (if any):** Add a sentence: "Alternative strategies for the control of swine dysentery e.g. development of new antimicrobials, development of vaccines, increased hygiene and better management could be explored."	The text is supported. However, the CVMP decided that as the sentence is already in the body of the text, there is no need to repeat it again in the conclusions.
62-63	4	**Comment**: Put a monitoring system in place for monitoring development of pleuromutilins resistance in B. hyodysenteriae.	Agreed, already included in the recommendations.
109-110	4	**Comment**: Try to harmonise SPCs Europe wide.	Agreed but resources should be considered for such task.
157	4	**Comment**:	Thanks.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		A survey done by HMA and FVE amongst 3017 practitioners gave the same results.	
160-166	4	**Comment**: Prefer oral medication, as administration is much more accurate than administration in premixes. Additionally, it can discourage preventive use in feed routinely.	Discussions on administration via oral powder or oral solution versus premix are outside the scope of this reflection paper.
278-279	4	**Comment**: A survey done by HMA and FVE amongst 3017 practitioners showed that the frequency of sensitivity testing is very much linked to their availability, quality and price, and with policy and culture in the country concerned. The survey indicates that the two most important factors which could influence a greater uptake of testing are the ability to get rapid results and at lower cost. In some countries another factor would be access to help to interpret the results of sensitivity tests. A common remark concerned validity and efficiency of sensitivity tests. They suggested indicated a need for reliable test results relevant to the clinical situation which are directly relevant to the antibiotics used to treat animals rather than those more usually used to treat people.	Thanks.
396	4	**Comment**: Encourage the development of new active compounds for use in animals, vaccines and alternatives to medicines, in order to be in a position to tackle similar situations in the future. Research towards that	Agreed.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		direction shall be encouraged and supported.	
479-483	4	**Comment**: National resistance monitoring programmes for B. hyodysenteriae should be in place.	Agreed, already recommended in the reflection paper.
493	4	**Comment**: **Proposed change (if any):** Sentence "…better management *COULD* be explored.", has to be changed in "…better management *MUST* be explored. "	We agree on the paramount importance of better management, but the main scope of the reflection paper is the use of pleuromutilins, not management for which reason we prefer to be cautious.