Divergent position on a CVMP opinion on an Article 34 of Directive 2001/82/EC

For Girolan and its associated name Apralan (apramycin sulfate)

Procedure no: EMEA/V/A/122

We, the undersigned, have a divergent position to the outcome of the Article 34 referral for Girolan and its associated name Apralan (apramycin sulfate).

Apramycin is a broad-spectrum aminocyclitol antibiotic produced by a strain of *Streptomyces tenebrarius*. Apramycin is recognized in the WHO's list of critically important antibiotics for veterinary use and also selects for resistance towards gentamicin, which is a critically important antibiotic for human use, as the sole or limited therapy for enterococcal endocarditis and multidrug resistant (MDR) tuberculosis (WHO Advisory Group on Integrated Surveillance of Antimicrobial Resistance (AGISAR) 2016 Critically Important Antimicrobials for Human Medicine, 5th Revision). Apramycin, only approved for animal use, was introduced to veterinary medicine in the early 1980s in several European countries. The gene, *aac*(*3*)-*IV*, is the primary identified gene causing enzymatic cross-resistance between apramycin and gentamicin (Chaslus-Dancla *et al*., 1991 Genetic homology between plasmids of human and animal origins conferring resistance to the aminoglycosides gentamicin and apramycin. *Antimicrob Agents Chemother* **35**:590–593.). Apramycin resistance associated with the *aac*(*3*)-*IV* gene in *E. coli* and *S. Typhimurium* was initially reported in 1981 in France, and disseminated rapidly within animal reservoirs in France, Belgium and Great Britain (Chaslus-Dancla *et al*., 1986 Emergence of aminoglycoside 3-*N*-acetyltransferase IV in *Escherichia coli* and *Salmonella typhimurium* isolated from animals in France. *Antimicrob Agents Chemother* **29**:239–243.). In 1986, the gene was first detected in *Enterobacteriaceae* isolated from human patients (Chaslus-Dancla *et al*., 1989 Detection of apramycin resistant *Enterobacteriaceae* in hospital isolates. *FEMS Microbiol Lett* **52**:261–265.). In the 1990's, several studies from Great Britain have shown that approximately 26% of the gentamicin-resistant pathogenic *E. coli* strains from humans were carrying the *aac*(*3*)-*IV* gene (Hunter *et al*., 1993 Human isolates of apramycin-resistant *Escherichia coli* which contain the genes for the AAC(3)IV enzyme. *Epidemiol Infect* **110**:253–259.; Johnson *et al*., 1994 Gentamicin resistance in clinical isolates of *Escherichia coli* encoded by genes of veterinary origin. *J Med Microbiol* **40**:221–226.). In Germany, Livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) in pigs and cattle have been described containing the apramycin-resistant gene, *apmA* (Feßler *et al*., 2011 Novel Apramycin Resistance Gene *apmA* in Bovine and Porcine Methicillin-Resistant *Staphylococcus aureus* ST398 Isolates. *Antimicrobial Agents and Chemotherapy* **55(1)**:373–375.). Furthermore, resistance to apramycin and other aminoglycosides is usually transmissible, encoded on conjugative R-plasmids, and often linked with resistance to other antimicrobials (Chaslus-Dancla *et al*., 1991). The other major concern would be the propagation of newer 16S rRNA methylases, conferring high-level aminoglycoside resistance to a range of aminoglycosides.

Salmonella Dublin is a cattle-adapted *Salmonella* serotype that causes severe and antimicrobial drug–resistant infections in humans (zoonosis) and cattle, where the incidence of human cases are on the rise (Harvey *et al*., 2017 Epidemiology of *Salmonella enterica* Serotype Dublin Infections among Humans, United States, 1968–2013. *Emerging Infectious Diseases* **23(9)**:1493-1501).

Danish human infections are reported (link). *Salmonella* Dublin outbreaks in cattle can be associated with a number of clinical syndromes. For example, abortion is the more common clinical expression in adult cattle, whereas pre-weaned calves are the most susceptible to *Salmonella* infection. In calves, for which this Article 34 procedure have specified for treatment with apramycin, septicemia is the most common clinical expression, including pyrexia, pneumonia, inappetence, bloody or watery diarrhoea, joint infections and nervous signs. Acute infections can progress to chronic cases, characterized by failure-to-thrive, diarrhoea and, in some cases, terminal dry gangrene. Calves can become infected via the conjunctiva or the respiratory tract, but the most common route is colonization of the intestinal tract, where *S.* Dublin attaches to, and invades intestinal cells associated with Peyer's patches. From there the bacteria pass into the lymphatic system and enter macrophages where they colonize lymph nodes. From lymph nodes, *S.* Dublin enters the bloodstream and spreads throughout the body. With this knowledge then it is unclear as to how oral apramycin products, with known poor oral bioavailability, can provide an effective treatment for septicemic forms of *S.* Dublin, commonly expressed in calves. For example, in one study oral bioavailability of apramycin in calves was 11% (Ziv G, Bor A, Soback S, Elad D, Nouws JF 1985 Clinical pharmacology of apramycin in calves. *J Vet Pharmacol Ther* **8(1)**:95-104.). In this study, the product used was apramycin soluble powder, at doses of 20, 30, and 40 mg/kg to ten, nine and ten calves, respectively. Maximum plasma levels were 2 µg/ml, which is less than the MIC for *S.* Dublin. Furthermore, *Salmonella* are facultative intracellular bacteria that survive in the phagolysosome of macrophages, as well as being facultative anaerobic bacteria. As an aminoglycoside, apramycin does not possess the necessary PK/PD properties to eliminate all *Salmonella* spp. from food animals, namely:

- Unable to accumulate intra-cellularly to effective MIC concentrations.
- Unable to kill facultative anaerobic bacteria in anaerobic conditions.
- Unable to affect bacteria growing in low pH conditions.

No scientific references could be found in support of an oral apramycin product for the treatment of *Salmonella* Dublin in calves. A marked difference between *S.* Dublin and other salmonellas is the tendency to persist in carrier cattle (latent carriers), without clinical signs (Radostits *et al*., 2007 *Veterinary Medicine: A textbook of the diseases of cattle, horses, sheep, pigs and goats*. Elsevier, Oxford; House *et al*., 1993 Enzyme-linked immunosorbent assay for serologic detection of *Salmonella* Dublin carriers on a large dairy. *American Journal of Veterinary Research,* **54**:1391-1399.). The organism persists in lymph nodes and other internal organs, and only periodically sheds in milk and/or feces. Furthermore, *S.* Dublin is difficult to control since the pathogen can survive for up to 9 months in the environment (*Wray C, Davies RH. Salmonella infections in cattle. In: Wray C, Wray A, editors. Salmonella in domestic animals. Oxon: CABI Publishing; 2000. Chapter 10.*). Active carrier animals can excrete the pathogen for several months or even years. The use of antimicrobials for the treatment of clinical salmonellosis is controversial for two main reasons. The first is that treatment is only potentially useful in the early stages of infection, and from an antimicrobial with PK/PD characteristics that can be effective. Secondly, antimicrobial therapy comes with the risk/s of inducing 'carrier' status in animals as well as encouraging AMR *Salmonella*. Non-clinical carrier animals entering the food chain are a major concern for public health. In Denmark, in response to the specific threat to human and animal health posed by *Salmonella* Dublin infections, the Danish government passed legislation intended to eradicate this serotype, in 2006. Their policy actions included heightened surveillance for cattle and abattoirs, voluntary interventions to reduce environmental contamination and disease spread within infected herds, economic sanctions for producers who do not control *Salmonella* Dublin in their herds, and closing of infected herds to live-animal trade (Nielsen LR & Nielsen SS 2011 A structured approach to control of *Salmonella* Dublin in 10 Danish dairy herds based on risk scoring and test-and-manage procedures. *Food Research International* **45**:1158–65.).

Colibacillosis in chickens is an acute fatal septicemia or subacute pericarditis and airsacculitis caused by *E. coli* (Merck veterinary Manual, 2016). The main portal of entry is either through the respiratory tract or oviduct. The oral bioavailability of apramycin is very low (2.50±0.02%) in chickens (Elbadawy M & Aboubakr M 2017 Pharmacokinetic, bioavailability and tissue residues of apramycin in broiler chickens. *International Journal of Pharma Sciences* **7(4)**:1826-1831.). Therefore, no systemic 'treatment' effect could be expected for oral apramycin (Aziza MA *et al*., 1994 Pharmacokinetic profile and tissue distribution of apramycin in chickens. *Veterinary Medical Journal of Giza*. **42**:93-97.). Thus, it is unclear as to how a treatment indication can be approved for oral apramycin in chickens for collibacillosis. Theoretically, oral apramycin could prevent *E. coli* entering the systemic circulation via the gastrointestinal tract, but this is a preventative effect and not a treatment. The main risk of acquiring colibacillosis in chickens is via *E. coli* contaminated dust levels. For example, *E. coli* faecal contamination of dust create an ideal portal of entry either through the respiratory tract or oviduct. Oral apramycin can result in a general depopulation of apramycin/gentamicin-susceptible *E. coli* from the gastrointestinal tract, with the spin-off of reducing *E. coli* faecal contamination of dust. However, this is a non-prudent use preventative effect and not a treatment. Also, oral apramycin increases the risk (i.e. probability) of a shift towards apramycin/gentamicin resistant and multidrug-resistant *E. coli* subpopulations, because of the well-known mechanism of co-selection of other resistance traits.

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