Opinion of the Scientific Committee for Animal Nutrition, 10 July 1998

on the immediate and longer-term risk to the value of Streptogramins in Human Medecine posed by the use of Virginiamycin as an animal growth promoter (produced at the request of the Commission in response to the action taken by Denmark under a safeguard clause to ban virginiamycin as feed additive)

1. TERMS OF REFERENCE (June 1998)

The Scientific Committee for Animal Nutrition (SCAN) is requested to give an opinion on:

1. Whether the following conclusions in the Danish report are scientifically justified:

a. that the use of virginiamycin as a growth promoter for pigs and broilers selects for virginiamycin resistant *Enterococcus faecium*;
b. that resistance determinants conferring resistance to virginiamycin in *E. faecium* and staphylococci were shown to confer cross-resistance to streptogramins such as pristinamycin and synercid used for human therapy;
c. that the *satA* gene conferring streptogramin resistance was detected in virginiamycin resistant *E. faecium* from animals; that this gene has also been found in streptogramin resistant *E. faecium* causing infections in humans;
d. that virginiamycin resistance could be transferred *in vitro* from resistant *E.faecium* strains from animals to a sensitive strain of *E. faecium*;
e. that virginiamycin resistant *E. faecium* were detected in food and in faecal samples from healthy humans;
f. that animals and humans can harbour identical strains of virginiamycin resistant *E. faecium*, showing that virginiamycin resistant *E. faecium* can be transmitted from animals to man;
g. that the *vat B* gene conferring streptogramin resistance has been detected in virginiamycin resistant staphylococci from broilers; that this gene has also been found in staphylococci from human infections.

2. Whether or not streptogramin resistant *E. faecium* and staphylococci selected by the use of virginiamycin as a growth promoter constitute a public health risk at present or could they constitute such a risk if streptogramins take a pivotal role for treatment of serious human infections in the future, notably infections with vancomycin resistant *E. faecium* and multiresistant staphylococci.

2. BACKGROUND

In accordance with the provisions of Council Directive 70/524/EEC, the use of virginiamycin (E 711) is authorised at Community level (see Table 1) and at national level (see Table 2) as an antibiotic in feedingstuffs.

Table 1

EEC No	Additive	Chemical formula, description	Species or category of animal	Maximum age	Minimum content	Maximum content	Other provisions
					mg/kg of complete feedingstuffs		
E 711	Virginia-mycin	I $C_{28}H_{35}O_7N_3$ II $C_{43}H_{49}O_{10}N_7$	Turkeys	26 weeks	5	20	--

			Other poultry, excluding ducks, geese, laying hens, pigeons	16 weeks	5	20	--
			Piglets	4 months	5	50	--
			Pigs	6 months	5	20	--
			Calves	16 weeks	5	50	--
				6 months	5	20	--
				6 months	5	80	Milk replacers only
			Laying hens	--	20	20	--
			Cattle for fattening	--	15	40	Indicate in the instructions for use: "The quantity of virginiamycin in the daily ration must not exceed 140mg for 100kg bodyweight and 6mg for each additional 10kg bodyweight".

Table 2

EEC No	Additive	Chemical formula, description	Species or category of animal	Maxi-mum age	Mini-mum content	Maxi-mum content	Other provi-sions	Period of authori-sation
					mg/kg of complete feedingstuffs			
30	Virginiamycin	I $C_{28}H_{35}O_7N_3$ II $C_{43}H_{49}O_{10}N_7$	sows	--	20	40	--	3.6.1998

On 13 January 1998, the Danish Minister for Food, Agriculture and Fisheries informed the Commission that Denmark had applied the safeguard clause for virginiamycin, for all categories of animals and enclosed a report on "The effect of the growth promoter virginiamycin on antimicrobial resistance development" which justifies, according to Denmark, the application of the safeguard clause.

This was followed in March by two manuscripts containing work in support of the initial judgement. Finally a report "The effect of virginiamycin on bacterial antimicrobial resistance development" was received in April, from the Danish Veterinary Laboratory. This contained, in addition to the Danish case, four short technical reports, results from the DANMAP survey not documented previously and selected publications from the open literature.

3. OPINION OF THE COMMITTEE

Streptogramin antibiotics and the mechanism of resistance to the action of streptogramins

Virginiamycin belongs to the streptogramin group of antibiotics and is the only member allowed for use as growth promoter in livestock. Other members of the group, notably pristinamycin, are only used in human medicine. Quinupristin-dalfopristin (Synercid)

is a newly developed product shortly to be licensed for human clinical use with options for use against glycopeptide-resistant enterococci, and other resistant Gram-positive bacteria.

All streptogramin antibiotics are a mixture of two structurally distinct cyclic peptides, collectively referred to as Type A and Type B, both of which share an affinity for the 50S subunit of the ribosome where they inhibit protein synthesis. The mechanism of inhibition is different for each component, however binding of Type A leads to a conformational change in the 50S subunit which potentiates the action of Type B streptrogramin. Individually the molecules are only bacteriostatic, but together they act synergistically and are bacteriocidal for most Gram-positive bacteria.

The most common form of resistance to the Type B streptogramin results from target modification. Resistant bacteria express a methylase enzyme able to introduce two methyl groups into the 23S rRNA component of 50S subunit which is sufficient to prevent binding by the antibiotic. This form of resistance was first noted after the introduction of the macrolide erythromycin and the designation *erm* (erythromycin resistance methylase) has been retained to describe the most common family of genes coding for this enzyme activity. As this implies, there is considerable cross resistance between streptogramin B and two other groups of antibiotics, the macrolides (M) and the lincosamides (L), and the MLS_B resistance phenotype is widely distributed in nature. A second, apparently less common, form of resistance to Type B streptogramins involves inactivation of the antibiotic by a lactonase encoded by *vgb*, a gene initially isolated from a *Staphylococcus* sp. but subsequently detected in *Enterococcus faecium*.

The action of type A streptogramins is not affected by the structural modifications which prevent binding by streptogramin B but is resisted by mechanisms which involve inactivation by acetyl transferases or, in the case of some staphylococci, by enhanced efflux (*vga*). Aceyl transferases conferring resistance are encoded by *vat* or *vatB* in staphylococci and by *satA* in *E. faecium*. Only resistance to Type A streptogramin or to both the A and B components is responsible for any marked loss of antibiotic action.

The nature of resistance to the streptogramins is not fully understood and mechanisms other than those described above may operate.

Conclusions on which the Danish safeguard action was based

The Report from the Danish Veterinary Laboratory contains the totality of the evidence which led the Danish authorities to instigate a safeguard action in January 1998. The authors of the Report present ten key conclusions derived in part from previously published data but largely from research results newly acquired in Danish laboratories. Because of the importance of these conclusions, each of which relates to a link in a chain of events said to tie the use of virginiamycin as growth promoter in animals to possible effects on the future value of streptogramins in human therapy, the validity of each is considered separately.

Conclusion 1 "Virginiamycin used as a growth promoter for pigs and broilers selects for virginiamycin resistant Enterococcus faecium ."

The specific data relating to this conclusion was collected during 1995/96 as part of an on-going surveillance (DANMAP) of resistance to antibiotics amongst the gut flora of food animals in Denmark. Results from the DANMAP survey showed that 43% (23/54) and 47% (274/583) of E. faecium isolates from poultry and pigs were resistant to virginamycin. The contrast between these figures and that for calves of 8% (1/13) where virginiamycin has not been used for growth promotion purposes was noted. Advantage was also taken of the fact that virginiamycin has not been used as growth promoter in Finland and Norway since 1990 and 1991 respectively, to compare the incidence of resistance in these countries with that of Demark. Using a breakpoint of 4 mg L^{-1} 69% (145/209) of isolates of E. faecium from Danish broilers and 49.1% (27/55) from Danish pigs were defined as resistant. The corresponding figures from Finland were 20% (11/55) of isolates from broilers and 2% (1/45) from pigs. No resistant isolates were found in the Norwegian samples, but the authors of the report state that, as selection of E. faecium was made on vancomycin-containing plates, "results are not necessarily comparable with results from Denmark and Finland."

Comment

The evidence for a causal relationship between use of virginiamycin and the development of resistance to this antibiotic is not as clear cut as is implied in these documents. Overall the number of strains examined during the surveillance exercise was small and the method of antibiotic testing used, which is unique to Denmark, is not easy to relate to definitions of resistance used elsewhere. Differences of 1mm in the diameter of the zone of inhibition distinguishes between resistance and susceptibility. Limitations to the selected methodology may explain why figures for resistance to pristinamycin, normally little different from those for virginiamycin, differed significantly amongst the isolates examined. More importantly, a far higher proportion of E. faecium isolated during the surveillance exercise demonstrated resistance to macrolides than to virginiamycin, which, given the potential for cross resistance amongst the MLS_B group of antibiotics, makes any conclusion about the origins or mechanisms of resistance difficult to unravel. Nonetheless SCAN accepts the commonly held view that the pressure created by constant exposure to an antibiotic, will select initially in favour of those organisms demonstrating intrinsic resistance and latterly for acquired resistance to that antibiotic provided that the appropriate resistance genes are present in the population. The evidence provided in the Danish Report, while doing little to substantiate this view, is consistent with this general proposition.

Conclusion 2 "Resistance determinants conferring resistance to virginiamycin in E. faecium and staphylococci were shown to confer cross-resistance to streptogramins used for human therapy."

The proportion of enterococci resistant to virginiamycin and to pristinamycin was similar amongst the isolates obtained during the DANMAP survey of pigs and poultry and meat products derived from these species. Although it is likely that resistance resided with the same isolates, this was not documented. More conclusive is the observation that all of the Danish isolates (209 from broilers and 55 from pigs) of virginiamycin-resistant strains of enterococci and staphylococci selected for the study of resistance mechanisms were also resistant to pristinamycin. Similarly, all of the 51 strains of E faecium isolated in the Netherlands and selected for study on the basis of their resistance to virginiamycin proved resistant to Synercid.

Comment

The data presented supports the general conclusion about cross-resistance between streptogramins expressed above but not the more specific statement found in the body of the Danish Report (p 10) and Conclusion 3 below, that the actual resistance determinants are the same and can be specified.

Conclusion 3 "The satA gene conferring streptogramin resistance was detected in virginiamycin resistant E faecium from animals. This gene was also found in streptogramin resistant E. faecium causing infections in humans."

The satA gene was detected by PCR in 25% (22/89) of virginiamycin-resistant E. faecium isolates from pigs and poultry in Denmark in 1995/6 (ref). This observation was supported by a second Danish study (Technical Report 2) of the genetic background to virginiamycin-resistance in E. faecium isolated in the Netherlands from healthy suburban residents, farmers and from chickens and pigs. Amongst the human isolates, 58% (14/24) were found to be satA positive compared to 18.5% (5/27) of the animal isolates. One isolate from a farmer was also found to carry the vgb gene. Both genes have been reported in clinical isolates of E. faecium.

Comment

The Danish evidence and that already available in the open literature establishes the presence of a pool of antibiotic resistance genes (satA) within the bacteria of the digestive tract of pigs and poultry of a type known to occur in some streptogramin-resistant organisms causing infections in humans. SCAN notes, however, that the presence of satA was found only in a minority of animal strains in both studies but was associated with a far greater proportion of streptogramin-resistant human isolates. This difference may be an artefact reflecting the relatively low number of isolates examined, isolations made from farms which did not use virginiamycin, the quality of the PCR primer used to detect satA or the presence of other, yet unrecognised, resistance factors. Also possible is that the constant use of a low concentration of virginiamycin in farm animals primarily selects for intrinsic resistance of a type that is almost universal amongst the related E. faecalis strains and that this provides the greatest source of resistance to streptogramins. In contrast, in humans where there is no selection pressure for intrinsic resistance, resistance is of the acquired type. Intrinsic resistance is less readily transferred than acquired resistance.

Conclusion 4 "Virginiamycin resistance could be transferred in vitro from resistant E. faecium strains from animals to a sensitive strain of E. faecium . Transferable resistance was associated with the transfer of plasmids harbouring the satA gene as well as plasmids harbouring hitherto uncharacterised resistance gene(s)."

A short report (Technical Report 4) documents the observation that, in filter-mating experiments with 15 virginiamycin-resistant E. faecium strains, resistance transfer to a single laboratory recipient strain (BM4105) occurred in 12/15 cases. Nine of the donor strains were shown to contain satA and, of these, transfer was shown to be positive in seven. In each case, PFGE patterns were reported to indicate the transfer of a plasmid to the recipient strain. Transfer frequencies were measured in an entirely separate experiment involving further matings between the recipient stain which had acquired resistance and BM4105 without a resistance phenotype.

Comment

Enterococci are known to be promiscuous and exchange of genetic information between similar strains is a common occurrence (Clewell et al., 1995). This experiment confirms that such conjugations can involve plasmids carrying resistance genes including satA.

However the data presented on frequency is misleading and is, at best, an indication of the maximum rate possible. The likelihood of a mating occurring is directly related to the similarity of the genetic background between donor and recipient strains. The use of a single strain acting both as donor and recipient, and one selected on the basis of its aptitude for conjugation, is artificial. Data on the frequency of matings between the initial isolates, assuming that these were of animal origin, and the recipient strain would have been of greater value.

Conclusion 5 "Virginiamycin resistant E. faecium was detected in food in Denmark and in faecal samples from healthy humans in

the community in the Netherlands and in Denmark."

The DANMAP survey of foods reported that the 54% (38/71) E. faecium isolates from poultry meat and 22% (5/23) from pig meat in 1995/96 were resistant to virginiamycin. During the same period, resistant strains in dairy, fish and vegetables represented 10% or less of all isolates. Technical Report 2 describes virginiamycin-resistant strains of E. faecium that had been isolated from healthy individuals in the Netherlands. Since streptogramins are not currently used for human clinical purposes in Denmark, resistance to streptogramins was not included in the analysis of the community samples.

Comment

E. faecium resistant to virginiamycin could be detected in Danish food samples, particularly those of poultry origin.

The limited information provided, indicates that there are genetic factors (satA) for virginiamycin resistance existing within the human population in the Netherlands. However, in the absence of data on prevalence, this information is of limited value. No corresponding data for the Danish population is presented. Reference to Danish faecal samples in Conclusion 5 is made on the basis of a single unsubstantiated statement made in the Danish Report (p7) commenting on data from the DANMAP survey yet to be published and so not available for evaluation.

Conclusion 6 "Animals and humans can harbour identical strains of virginiamycin resistant E. faecium showing that virginiamycin-resistant E. faecium can be transmitted between animals and man."

Comment

This conclusion is on a single observation by Bogaard and co-workers cited in Technical Report 2 as a personal communication that two virginiamycin-resistant strains of E. faecium, one isolated from a Dutch farmer and the other from his poultry, had an identical PFGE fingerprint. Even assuming that the strain isolated from the farmer was not an odd transient strain and that it represented a significant colonisation of the gut, this generalisation from the particular remains unsound and without foundation.

Conclusion 7 "The vatB gene was detected in virginiamycin resistant staphylococci from broilers. This gene has also been found in staphylococci from human infections."

An analysis of 52 staphyloccoci isolates from clinical samples taken from broilers demonstrated the presence of the vatB gene in two of these (Technical Report 3). Both were identified as Staph. xylosus and both were resistant to pristinamycin and virginiamycin. The presence of vatB was not detected in other isolates, the majority of which were Staph aureus (69%), nor was vat or vga found in any isolate examined. All three genes have been reported in human clinical isolates of Staphyloccocus aureus and Staph. epidermidis and are recognised to confer resistance to pristinamycin.

Comment

SCAN finds it difficult to see the relevance of this Technical Report. The staphylococci isolated were not of gut origin but were from clinical samples; the two strains proving positive for vatB having originated from liver or from joints. No clinical history was provided and so it is not known whether isolates were from birds receiving virginiamycin as a growth promoter or other therapeutic antibiotic able to co-select for the R plasmid. The species in which vat B was detected is not known to colonise humans, while the species commonly found in humans, apparently, remained sensitive to streptogramins. The author of the Technical Report claims that vatB previously has been found to transfer between different staphylococcal species citing Allignet et al. (1996) as authority. Amongst human isolates, vat B has been found infrequently in France and only in Staph aureus. As Allignet and co-workers found, transfer could be demonstrated only with other Staph aureus strains and with Staph epidemidis. Finally the author included in the discussion the speculation of Allignet et al. (1996) that the presence of genes encoding streptogramin resistance in humans might result from the use of virginiamycin as a growth promoter in animals, but failed to include the comment that, as the plasmids carry other resistance genes, selection pressure may have been exerted by other antibiotics (Allignet et al., 1996).

Conclusion 8 "Streptogramins are expected to take a pivotal role for treatment of serious human infections in the future, notably infections with vancomycin resistant E. faecium and staphylococci."

Streptogramins are neither essential nor used for the treatment of human infections in Denmark at present. Danish concerns derive from the experience in the USA and other parts of Europe where nosocomial infections involving staphylococci and enterococci have increased significantly. Staphylococcus aureus infections are normally treated with ß-lactams, but methicillin-resistant strains of Staph. aureus (MRSA) are being isolated with increasing frequency, particularly in parts of France and Southern Europe. Synercid, an injectable streptogramin currently completing phase III clinical trials, offers a possible, if untried, treatment of last resort. Enterococci are ranked second as a source of nosocomial infections and are normally controlled with ampicillin or, as resistance develops, with the glycopeptide antibiotic vancomycin. However, vancomycin-resistant strains (VRE) are an issue of concern for public health. In 1993 VRE accounted for 8% of all enterococcal infections in the USA and 14% of those occurring in intensive care

units. Most vancomycin-resistant strains of E. faecium are susceptible or moderately susceptible to Synercid. The drug is well tolerated in patients and rapid in action which is thought to limit the emergence of resistant strains (Rubinstein and Keller, 1996)

Comment

Data provided in the DANMAP survey shows that in 1995/6, the latest information presented, none of the enteroccoci or coagulase-negative staphylococci isolated from blood cultures in Denmark were resistant to vancomycin. Most were also susceptible to penicillin or its semi-synthetic derivatives. In fact Denmark appears to have one of the lowest recorded incidence of methicillin-resistance amongst Staphylococcus aureus strains at < 1%, compared to 3% in the Netherlands, 8% in the UK, 10% in the USA and 30% in France. Thus, at present, existing strategies for coping with hospital infections caused by enteroccoci or staphylococci remain successful in Denmark and the Danish report contains no evidence that existing therapies are likely to be compromised in the short term.

Conclusion 9 "Minimising the occurrence of virginiamycin resistant E. faecium and staphylococci in animals and food could be critical to preserve the effect of streptogramins for human therapy."

Comment

The validity of this conclusion depends on establishing a link between a pool resistance factors held within the bacteria comprising the animal gut flora and their transfer to the human flora. No new evidence is provided in this Report to indicate the frequency of such transfers or, indeed, whether they occur at all.

Transfer of enteropathogens from animals and their food products to the human population evidently is a regular event. Based on the UK data for reported outbreaks of food poisoning, which in 1997 exceeded 100,000 cases, and estimating that reported cases represent only 10% of total cases, over one million transfer events must have occurred within a population of 60 million. Enteropathogens represent only a small fraction of the total animal flora whose passage can detected by the onset of clinical symptoms in humans. Most gut bacteria are commensals, strains of which occur in the digestive tract of animals and humans without adversely affecting the host. Making the reasonable assumption that transfer of commensals occurs at least as frequently as enteropathogens and taking account of the relative size of the bacterial sub-populations, on average individuals could be exposed to bacteria originating in animals several hundred times a year. While this would provide the opportunity for the transfer of resistance factors, the likelihood of this occurring will be dependent on the proportion of introduced bacteria carrying resistance factors on mobile elements, the ability to multiply, their residence time in the human digestive tract and their similarity to the recipient strain. Crucial to the probability of gene transfer is whether resistant strains become established in the human gut, whether naturally or under selective pressure.

There is some evidence of host specificity amongst the commensal flora which would argue against permanence. Biotyping of Staphylococcus aureus has shown that human strains are rarely encountered in livestock and conversely biotypes specific to livestock are not found in humans. Since organisms mate more frequently with genetically closely related strains this also would mitigate against conjugations between the human biotype and transients of animal origin. At present there is no evidence that the strains of enterococci show a similar host specificity.

Despite the potential for transfer of resistance factors, virgiamycin does not appear to have greatly compromised the value of pristinamycin in those countries which allow the use of streptogramins as both growth promoter and human therapeutics. After more than 20 years use of both streptogramins in France, resistance to pristinamycin amongst staphylococci remains low at around 5% of isolates. More importantly, in a survey of nearly 1000 MRSA collected from hospitals throughout France, 98.5% were found susceptible to both pristinamycin and Synercid (Gazagne et al., 1998). Unfortunately, corresponding data for E. faecium in France is not available. However, evidence from the USA, where a survey of 1000 strains of E. faecium found 95-97% sensitive to Synercid, also suggests that use of virginiamycin has not, in practice, reduced the value of streptogramins as a human therapeutic agent. Streptogramins are proving to be robust in the clinical setting and this is probably due to their dual nature. Resistance to Type B streptogramins alone has little effect on the antibiotic and, while resistance to the Type A component alone will substantially reduce its effectiveness, the mixture retains at bacteriostatic effect which may help to prevent the clonal spread of resistance.

Conclusion 10 "Streptogramin resistant E. faecium and staphylococci selected by the use of virginiamycin as a growth promoter constitute an emerging public health risk."

Comment

No data was presented on the incidence of resistance to pristinamycin or Synercid amongst strains of E faecium and staphylococci isolated from the healthy population of Denmark or from clinical samples from nosocomial infections in Danish hospitals. Without such data, to conclude that resistance to virginiamycin amongst enterococci and staphylococci found in the digestive tract of Danish pigs and poultry and contaminating meat products, represents a risk to public health is speculative and, furthermore, is not supported

by the French and American data cited above.

Conclusion

I

Having considered the evidence provided by the Danish government in support of their action taken under the safeguard clause against virginiamycin, SCAN concludes that:

1. *no new evidence has been provided to substantiate the transfer of a streptogramin or vancomycin resistance from organisms of animal origin to those resident in the human digestive tract and so compromise the future use of therapeutics in human medicine.*
2. *the development of vancomycin resistance amongst E. faecium and methicillin- resistant strains of Staphylococcus aureus, which SCAN recognises are increasingly responsible for nosocomial infections worldwide, are evidently a cause for concern. However the data provided in the Danish Report does not justify the immediate action taken by Denmark to preserve streptogramins as therapeutic agents of last resort in humans.*
3. *as survey data provided under the aegis of DANMAP and included in the Danish Report failed to detect a single case of VRE, as Denmark has amongst the lowest incidence of MRSA in Europe and North America, and as coagulase-negative staphylococci remain sensitive to vancomycin, there are no clinical reasons to require the introduction of streptogramins as human therapeutics in Denmark now or in the immediate future. Furthermore, as the Commission has elected to take the precautionary action of removing avoparcin from the antibiotics permitted for use as growth promoters to help preserve the efficacy of vancomycin in human therapy, any future need for streptogramins might be delayed further in Denmark.*

For these reasons SCAN concludes that the use of virginiamycin as a growth promoter does not constitute an immediate risk to public health in Denmark.

II

SCAN is sympathetic to the general concern highlighted by the Danish action about the hazard that a reservoir of resistance genes within the animal population poses for humans. However it is of the opinion that a full risk assessment cannot be made until quantitative evidence of the extent of transfer of antimicrobial resistance from livestock sources is obtained and the significance of this within the overall use of antimicrobials for clinical and non-clinical purposes evaluated. SCAN is also of the opinion that this is best approached by considering the totality of antimicrobial use within the countries of the European Union rather than on a case by case basis. The Scientific Steering Committee has established a multidisciplinary working group with this remit.

SCAN also notes that in countries that permit the use of streptogramins in both animal production and human medicine, notably France and the USA, the use of pristinamycin has not been compromised by the use of virginiamycin as growth promoter.

SCAN is therefore firmly of the opinion that any risk that might be posed in the future by the use of virginiamycin as a growth promoter will not materialise in the time required to make such an evaluation and most probably not for some years afterwards. In the meantime monitoring initiated by the Danish government and the EU will be able to detect any significant increases in glycopeptide and streptogramin resistance in enterococci and staphylococci should that occur.

Documents supplied to SCAN

Report from the Danish Veterinary Laboratory - The effect of virginiamycin on bacterial antimicrobial resistance. April 1998. Which included:
Technical Report 1. Association between the use of virginiamycin and the occurrence of resistance among Enterococcus faecium from broilers and pigs in Denmark, Finland and Norway.
Technical Report 2. Occurrence of the satA and vgb genes in streptogramin resistant Enterococcus faecium isolates of animal and human origin from the Netherlands.
Technical Report 3. Presence of the vatB gene encoding streptogramin resistance in staphylococci from broilers.
Technical Report 4. Transfer of the satA gene and other gene(s) encoding streptogramin resistance to a sensitive Enterococcus faecium strain.
Pfizer's Response Dossier - Danish ban on the use of virginiamycin as a feed additive. March 1998.
Pfizer's Response Dossier No 2 - Danish ban on the use of virginiamycin as a feed additive. May 1998.

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