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THE BAN OF ANTIBIOTICS AS FEED ADDITIVES: A SCIENTIFIC ISSUE? *

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INTRODUCTION

In recent years concern about the safety of foods of animal origin has heightened due to problems arising from BSE, dioxin contamination, outbreaks of foodborne bacterial infections, as well as growing concern about veterinary drug residues and microbial resistance to antibiotics.

The global livestock output grew at a rate of 2.4% in 1999, being expected to increase by over 70% in the next 30 years while world demand and consumption of livestock products is expected to nearly double in the next 20 years. This is expected to take place mostly in Asia, with increases in white meat and a decline in red meat. This has important implications because it is the poultry, pig and dairy industries that are the principal users of processed animal feeds.

The livestock sector comprises widely differing production systems ranging from subsistence livestock farming to highly intensive systems. The problems related to the quality and safety of foods of animal origin can therefore be highly variable in different regions of the world.

FEEDBORNE HAZARDS

A vast number of potential hazards are associated with animal feeds.

1) **Mycotoxins** (and their metabolites)- produced by fungi of various generations, many have been identified to be carcinogenic, oestrogenic, neurotoxic, nephrotoxic, dermonecrotic or immunosuppressive. They are regularly found in feed ingredients and difficult to be destroyed by feed processing.

2) **Agricultural and other chemicals** – include excessive residues of pesticides and fungicides or other environmental contaminants such as the polychlorinated biphenyls (PCBs), dioxins and heavy metals (e.g. mercury, lead and cadmium).

* Conferencia pronunciada en la Real Academia de Doctores el 31 de octubre de 2001.

3) **Infectious agents** – among others, these include *Salmonella enterica, Bacillus anthracis, Toxoplasma gondii, Trichinella spiralis,* and possibly the agent of BSE.

4) **Genetically modified organisms** - some 70% of the soya beans and of maize in the USA, and imported to Europe to be used as feedsuffs, are GMOs; still under debate its potential hazard.

5) **Veterinary drugs** – widely used in feeds for livestock and aquaculture. This is the area focused in this communication, with particular emphasis on antibiotics.

ANTIBIOTICS AS FEED ADDITIVES: THEIR IMPACT ON THE CONSUMER

The development of resistance to antibiotics by pathogenic bacteria is a problem of increasing concern, resulting in treatment failures and longer periods of morbidity. In particular, pathogens are increasingly multi-resistant. Resistant bacteria may emerge following exposure of bacterial populations to sublethal concentrations of antibiotics. This can occur as a result of antibiotic use in clinical and veterinary practice and in agriculture.

Antibiotics are used widely in agriculture and aquaculture for therapeutic, prophylatic and growth-promoting purposes; residual antibiotics or their residues remaining in the flesh at the time of slaughter may result in direct exposure of the consumer to these drugs. In addition, the presence of low levels of antibiotic may select for resistant bacteria in the gut microflora intended for human consumption.

There is growing evidence that the use of antibiotics during the production of food animals can lead to the exposure of consumers to antibiotic-resistant bacteria.

Control measures are being introduced in the developed world to limit the use of clinically important antibiotics as growth promoters. However, this is not yet taking place in significant developed countries (eg USA; South American countries) and in many developing countries.

With increasing globalisation of our food market, an international approach to the control of antibiotic use is essential.

However, whilst moves to ensure the prudent use of antibiotics in man and animals (including companion animals) are to be commended, the simplistic belief that the baning of antibiotic growth promoters (as implemented by the European Commission) will halt or even reverse the current trend towards antibiotic resistance must be challenged to some extent.

THE CHALLENGE OF ANTIBIOTIC RESISTANCE

It is now common to listen scientists with worrisome slogans such as **«The Antibiotic Paradox: how miracle drugs are destroying the Miracle»; «Drug resistance: The new Apocalipse»!**

Ever since antibiotics became widely available in the 1940s (Stokstad *et al,*1949), it became increasingly common to identify bacteria that defy not only single but multiple antibiotics, therefore extremely difficult to control them.

From a stockpile of more than 100 drugs, the clinician nowadays has cases considered untreatable.

It is a fact, a well known fact! The question is, however, if it is the use in animals (production animals, aquaculture and companion animals) the responsible for this worsening of the situation, or if it is the way the human clinician has been prescribing!

In the light of such a threath, we have to point that several interacting processes are at fault. Analyses of them point to a number of actions that could help reverse the trend, if individuals, businesses and governments around the world can find the will to implement them.

In the USA the majority of antibiotic use in agriculture is for growth promotion. Data of year 1998 show that 23 000 000 kg of antibiotics are produced each year, of which more than 40% are for animal use. Of these, 80% are used as growth promoters (i.e. 7 000 000 kg/year), (WHO, 1998).

The panorama is not the same in Europe, and there was a considerable reduction since the EU Commission initiated the ban of these drugs as growth promoters. In fact, just comparing data from 1998 and 2000 it can be seen a significant reduction on its use:

	1998	*2000*
Human use	52%	65%
Veterinary Therapeutic Use	33%	29%
Use as Feed Additive	15%	6%

The interesting question is to see if on the near future, through monitoring systems already implemented, one can still blame the animal use as the main factor on resistance increases.

The situation is more worrying by the lack of new antibiotics becoming available since the quinolones in the 1980s (Bridson, 1998) and few promising drugs are currently under development (van der Meer,1998) and unlikely to pass soon all technical and regulatory hurdles needed to reach the market.

The boundaries between nutritional and veterinary sciences and between disease and health are difficult, not to say impossible to define, and we recall that over 5000 chemical elements are part of the Human diet.Therefore food safety is quite complex.

Feed additives are well defined within Council Directive 70/524/CEE, with several later amendment (namelly to include the use of probiotics/micro-organisms and enzymes).

Antimicrobial substances are divided in 3 classes: 1) Antibiotics; 2) Coccidiostatics and other medicinal substances; 3) Growth Promoters (e.g. carbadox; olanquidox – already banned).

A number of bio- and chimiosynthetic substances were used on the past as antimicrobial growth promoters. Among them it is included: arsenilic acid, avoparcin, bacitracine methilene disalicilate, carbadox, chlortetracycline, avilamicine, colistine, copper sulphate, dimetridazole, ephrotomycine, enramicine, erythromicine, flavomycine, furazolidone, ipronidazole, kitamycine, lincomycine, monensin, neomycine, nitrovin, nosiheptide, olanquidox, oleandomycine, oxitetracycline, penicilline, polinactine, ronidazole, roxarsone, salinomicine, sedemycine, spiramycine, streptomycine, triopeptine, tylosine, virginiamycine and zinc bacitracin.

Presently (October 2001), in the European Union only 4 are authorised: avilamicine, flavomycine,and the ionophores monensin and salinomycine.

Although during this communication it is restricted only to antibacterial agents, it should mentioned that the worries are extended to other types of resistances such as those related with virus (e.g. *Herpes simplex* and acyclovir), fungi (e.g. *Candida* spp and flucozanol) and protozoa (e.g. *T. falciparum* and chloroquine).

MODES OF ACTION

Many authors have reviewed this subject (Bryan, 1982; Russell & Chopra, 1990; Rosen, 1999). No one is entirely sure how the drugs support growth. Clearly, though, the long-term exposure to low doses as it is used for growth promoting, is a perfect formula for selecting increasing numbers of resistant bacteria. One can summarise, without being exaustive, on the following list of some 41 modes of action in 4 categories:

MICROBIOLOGICAL		*PHYSIOLOGICAL*	
Beneficial bacteria	+	Gut food transit time	–
Adverse bacteria	–	Gut wall diameter	–
Transferable resistance	+-0	Gut wall length	–
Competition for nutrients by gut flora	–	Gut wall weight	–
Gut floral nutrient synthesis	+	Gut absorptive capacity	+
		Feed intake	+-0
Cl. perfringens	–	Faecal moisture	–
Pathogenic *E. coli*	–	Mucosal cell turnover	–
Pathogenic *streptococci*	–	Stress	–
Beneficial *lactobacilli*	+		
Beneficial *E. coli*	+		
Debilitation of pathogens	+		

NUTRITIONAL		*METABOLIC*	
Energy retention	+	Ammonia production	–
Gut energy loss	–	Toxic amine production	–
Nitrogen retention	+	Alpha-toxin production	–
Limiting amino acid suply	+	Mitochondrial fatty acid oxidation	–

NUTRITIONAL		*METABOLIC*	
Vitamin absorption	+	Bacterial cell wall synthesis	–
Trace element absorption	+	Bacterial DNA synthesis	–
Fatty acid absorption	+	Bacterial protein synthesis	–
Glucose absorption	+	Faecal fat excretion	–
Calcium absorption	+	Liver protein synthesis	+
Plasma nutrients	+	Gut alkaline phosphatase	+
		Gut urease	

(+)denotes an increase, (–) a reduction, (0) no change.

RESISTANCE

Resistance to antibiotics is a natural phenomenon! It always existed, even before they were introduced in human or veterinary medicine. It can even occur in the absence of medication or under correct use of antibiotics. Some bacterial species and strains are naturally resistant to certain antibiotics.

The great controversy relates to the acquired resistance after exposure to a certain antibiotic. However, this intrinsic form of resistance is not considered the most worrying one for the human and animal health. Most of the resistant organisms have emerged as a result of genetic modifications, acquired through mutation or transfer of genetic material during the lifespan of the micro-organisms and subsequent selection pressure.

The rapid and universal development of resistance is without doubt a subject of great concern. It is considered by many authors that the extensive use of antibiotics both in humans and animals is the main factor contributing for the selection of resistant organisms.

There is no question that to avoid with success the problem of resistance it is necessary the effective collaboration in 4 sectors: 1) Human Medicine; 2) Veterinary Medicine; 3) Animal Production (including aquaculture) and 4) Plant protection (fruit- and horticulture). Only an integrated action in this direction can give fruitfull and significant results in this sense.

Clearly, the position of the European Commission (under the pressure or not by the northern countries ...) to ban, without scientific evidence, most of the antibiotics as growth promoters, it is with no doubt a measure with high interest in guiding the livestock, poultry and fish sectors, but, for many experts seen as a attitude with small or even no impact on the overall problem of resistance development.

Some of the antibiotics were safely, and with success, used for decades, but it is nevertheless quite understandable that some politicians want to operate in a European region with no risks. Therefore, aiming at the «zero-risk» the authorities take advantage of the «Precautionary Principle» to exagerate its terms (the normal concept is the temporary ban, under exceptional circumstances, while searching for more and better scientific evidences), even when its scientific advisers have not always agreed. Evidently, such bans are unlikely to yield results in the short term (if at all) given that resistant orgaisms persist long after use of specific growth promoters has stopped (Simonsen et al, 1999).

It should be enhanced that the growth promoters were used within the prevailing CAP (Common Agriculture Policy) where quantitative criteria were greater than the qualitative ones.

The development of antibiotic resistance in a bacterial population may arise as a result of persistent exposure to sublethal concentrations of antibiotics resulting in selection of those strains carrying resistance factors.

There are 3 potential pathways by which the consumer may be exposed to antibiotic-resistant pathogens:

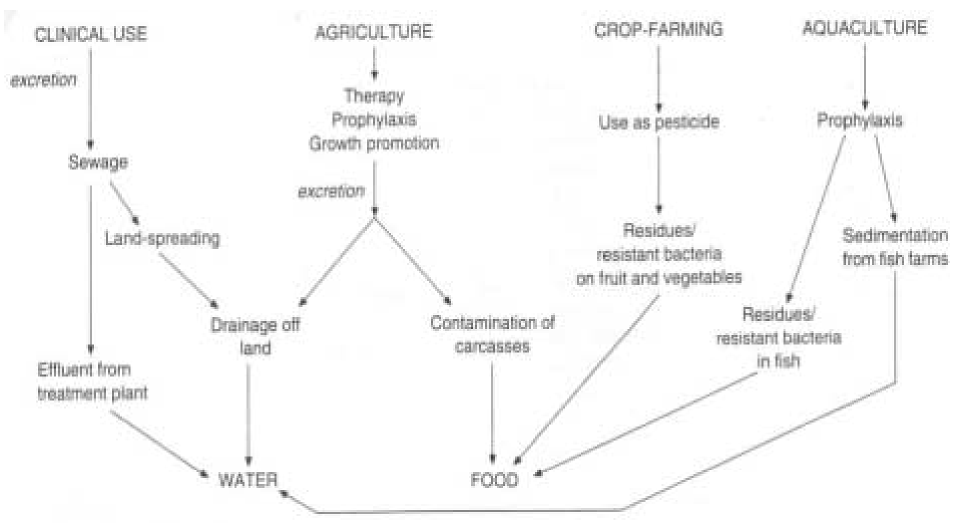
1) consumption of foods containing antibiotic residues which may directly induce the development of resistance in the gut;

2) consumption of resistant, non-pathogenic organisms may result in the transfer of resistance genes to pathogenic organisms *in vivo*;

3) antibiotic-resistant pathogenic organisms may be acquired directly by the consumption of contaminated foodstuffs.

Summary of potential routes of transmmission of antibiotic residues and resistant bacteria through the human food chain (Willis, 2000)

In many countries, withdrawal periods from antibiotic use are enforced before slaughter, to reduce the risk of residues remaining at the time of slaughter (e.g. in the UK 99% of poultry meat, 98% of eggs and almost 100% of milk were free of detectable residues in the year 1998), (Willis et al, 2000).



MUTATIONAL VS TRANSFERABLE RESISTANCE

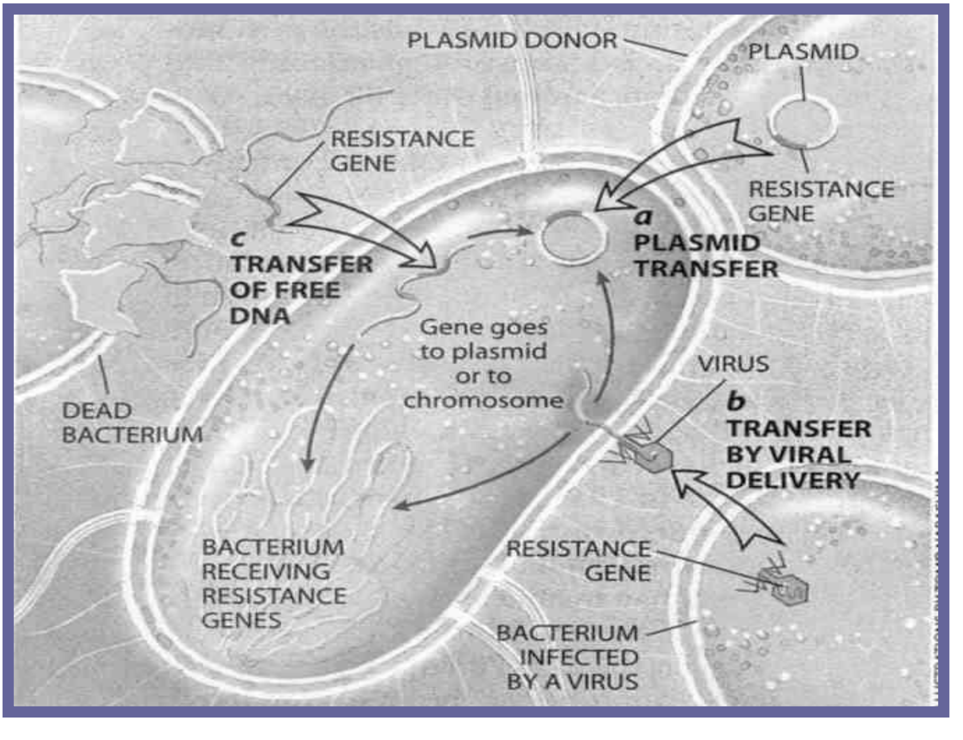
It is not the intention here to describe the detailed processes of transformation, conjugation and transduction (see Levy, 1998).

Mutational resistance develops as a spontaneous mutation in a locus of the microbial chromossome. The presence of the drug serves as the mechanism of selection to destroy susceptible micro-organisms and allow/promote therefore a greater development of the mutants. Spontaneous mutations are transmissible vertically.

The selection process is fairly straightforward. When an antibiotic attacks a group of bacteria, cells that are highly susceptible to the medicine will die. But cells that have some resistance from the start, or that acquire it later (through mutation or gene exchange), may survive, especially if too little drug is given to overwhelm the cells that are present.

Resistance can also be developed as a result of the transfer of genetic material among bacteria. Plasmids, that are small molecules of extrachromossomal DNA, transposons and integrons, that are short sequences of DNA, can be transmitted vertical or horizontally and can codify for multiresistances.

It is estimated that the great majority of acquired resistance is mediated by plasmids.



EPIDEMIOLOGY OF THE RESISTANCE

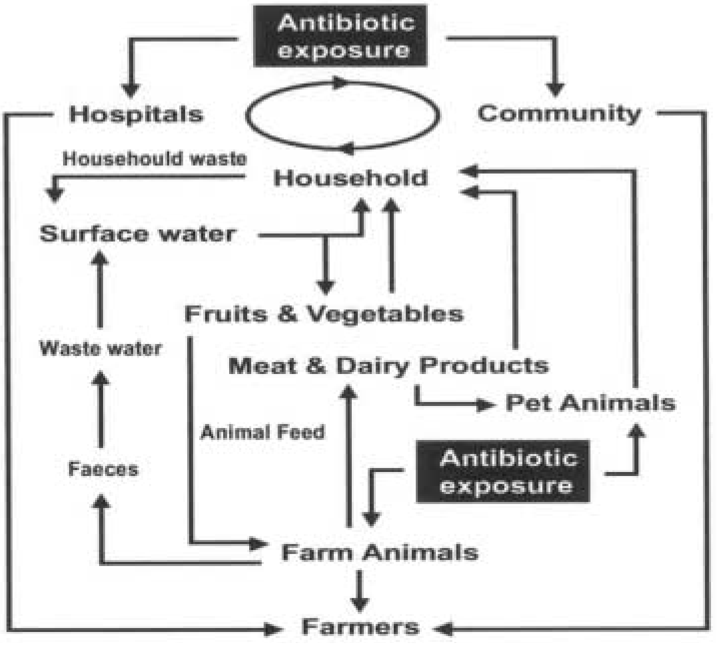
The observed resistant patterns in animals are probably afected by exposure to the antibiotic, but may also vary with other factors (Klein et al, 1998):

— the size of the microbial population;

— prevalence to pre-exposure of the resistant genes;

— competition among bacterial populations.

Main pathways of transfer of resistance



PROBLEMS ORIGINATED FROM RESISTANCES

There is no evidence that it was the use of antibiotics in animals that developed a role on the occurrence of the «super-micoorganisms» such as the MRSA (methicillin-resistant *Staphylococcus aureus*), the PRP (penicillin-resistant *pneumococci*) or the MRK (*klebesiella* multi-resistant).

The Scientific Steering Committee of the European Commission produced a Report (1999) where 14 pathogenic agents were studied. For the majority of these organisms it can be concluded that there is no connection at all between the emergence of resistance in humans and the use of antibiotics in the animal sector. For the remainder, the same Committee concludes that: «the present data are inadequate to determine which are the major contributors for the resistance problem». They summarise the data as:

Key pathogenic agents resistant in Human Medicine	
Organism	Eventual link with animal source?
MRSA	No
Mycobacterium tuberculosis	No
Streptococcus pneumoniae	No
Streptcoccus pyogenes	No
Neisseria meningitidis	No
Neisseria gonorrhoea	No
Campylobacter spp	Possible #
Salmonella spp	Possible #
Escherichia coli (urogen, 0157)	Possible ##
Vancomycin-resistant *Enterococci*	Perhaps
Pseudomonas aeruginosa	No
Klebesiella spp	No
Acinetobacter spp	No
Enterobacter spp	No
	# little evidence of the negative impact ## little evidence for lack of data

It should be enhanced that the antimicrobial substances are used in two different types of animal populations: 1) companion animals (a similar structure to the human, with small number of animals, individual lodging, veterinary intervention not dependent on economical factors, individual therapeutics – many times with no veterinary supervision); 2) animal production. It should also be stressed the variety of antibiotics used in companion animals is similar or the same used in humans, and much wider numbers than in production animals, and the phenotypical resistance of those agents is well demonstrated. What is unknown is the eventual transmission of those resistances to humans. The case complicates further when the number of species as companion animals increases in a complex way (e.g. rabbits, lizards, snakes, fish).

In addition to animal husbandry, antibiotics are frequently used as prophylatic agents in fish and shrimp farms, usually in the form of medicated feed pellets with eventual impact on the environment by sedimentation of feed particles.

The antibiotics used as growth promoters do not affect resistance in *Salmonellae*, but contribute for resistance in *campylobacter* and *enterococci.*

TRANSFER OF RESISTANT ORGANISMS TO THE CONSUMER

Food Animals can become contaminated with faecal bacteria either due to intensive farming practices or during the slaughter process. Therefore, any antibiotic-resistant bacteria which may develop in the intestine of animals exposed to antibiotics may readily contaminate meat reaching the consumer.The presence of these organisms in retail meat or dairy products suggests that, even after moderate cooking process or pasteurisation (Manie T. et al 1999), consumers may be exposed to resistant bacteria.

Antibiotic-resistant bacteria can be found on fruit and vegetables since antibiotic pesticides are used in vast quantities for therapeutic or prophylatic applications.

The use of antibiotic resistance genes as markers during genetic modification of crops has introduced a new potential route for the emergence of antibiotic-resistant bacteria. It is well known nowadays the extensive use of the maize plant containing a gene for ampicillin resistance and the potato with a gene for amikacin resistance (Jones, L. 1999). The European Commission is trying to ban this technology and their products, widely accepted in the USA and many countries.

CONTAMINATION OF THE ENVIRONMENT

Resistant organisms resulting from farming practices may be transferred into rivers and other water courses through the waste disposal system or by drainage of rainwater off farmland.

Significant quantities of antibiotic reach water courses as a result of sedimentation out of fish farms. In addition. It is estimated that 30 – 90% of an administered dose of antibiotics is lost in an active form in the urine of both humans and animals (Halling-Sorensen, B. et al,1998). Furthermore, it is possible for antibiotic-resistant bacteria to persist in water, even after treatment (e.g. chlorination) at the sewage plant (Murray G.E. et al, 1984).

CONCLUSIONS

There has been resistance to the bans on the use of growth promoters by pharmaceutical companies, livestock and poultry producers and veterinarians, who argue that insufficient evidence is available to justify these decisions. There is presently good evidence that the use of antibiotics in agriculture is contributing to the problem of antibiotic resistance amongst pathogenic bacteria. However the indiscriminate (with no prescription) use in Humans as not been the subject of enough number of trials with a similar objective.

In Europe (and to a least extent in the USA), attitudes to the use of these drugs do appear to be changing, with alternatives being sought (such as vaccination and bacteriophage therapy) and organic foods becoming increasingly popular. However, organic farming will not sort out the hunger problems of the world. Furthermore, there is very little regulation of veterinary use of antibiotics in many developing countries from which our food is imported in significant amounts and concerns are growing that cross-resistance may already exist to new drugs before they are even being used in clinical practice.

Therefore, an internationally harmonised approach to the regulation of antibiotic use appears to be essential to control the spread of antibiotic-resistant microorganisms.

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