UPDATE ON RABBIT ENTERIC DISEASES: DESPITE IMPROVED DIAGNOSTIC CAPACITY, WHERE DOES DISEASE CONTROL AND PREVENTION STAND ?

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ABSTRACT

Eight years have elapsed since the last World Rabbit Congress on rabbit farming but, since then, there has not been a radical change of knowledge on rabbit enteropathies and still little is known on the etiology of Epizootic Rabbit Enteropathy (ERE). This disease continuous to pose a severe health threat for rabbit meat production farms and it undermines their profitability. Despite improvements in the methodology to experimentally reproduce the disease, and the exclusion of several hypotheses, none of the remaining candidates for ERE etiology have yet been proven. The evolution of ERE under field conditions can be conditioned by the onset of secondary infections, by prophylactic or therapeutic interventions, as well as by management systems. Since there is no laboratory method for ERE diagnosis, as no specific etiological agent is targetable, the diagnosis is complicated further by the absence of specific clinical and anatomopathological findings. ERE outbreaks can have features that are similar to other enteric syndromes, with more or less defined signs that may be easily confused with more traditional conditioned and multifactorial enteric pathologies. Nowadays there is greater knowledge on the causes of enterotoxaemia in rabbits and on the role of *C. perfringens*, *C. difficile* and *C. spiroforme,* whereas no progress has been made on rabbit diseases due to *Enterobacteria*, especially on rabbit pathogenesis and prevention of *E. coli* enterities. Technological innovations which include a common use of biomolecular methods (in particular PCR and Real-Time PCR) enable better diagnostic results, thanks to enhanced analytical sensitivity and faster results. A lack of knowledge on the ERE etiological agent and on the pathogenesis of some rabbit enteric diseases has stalled progress to prevent and control the entire group of rabbit enterities. Even the enthusiasm for vaccines against colibacillosis seems to have come to a standstill. Differently from what happened in other animal species, broiler chickens for instance, in rabbit farms the prevention and disease control continue to rely on antimicrobials. Meanwhile the international scenario is changing and, at least in Europe, the widespread resistance to antimicrobials in bacteria is well documented and it is also influencing the policy making process. Reports published in recent years by the European Food Safety Authority (EFSA), the European Centre for Disease Prevention and Control (ECDC) and the European Medicines Agency (EMA) have agreed on the need to use antimicrobials prudently and to reduce their veterinary prescription. The requirement to moderate the use of medications works in hand with a reduction of animal diseases. Currently in Europe, policy-makers are calling on industrial farmers to improve animal welfare which helps to reduce pathologies and consequently the consumption of antimicrobials. Generally there is greater awareness that drugs and medication must be safe and must not pose a risk to the health of European citizens. This is the top priority which ranks above all other productive or commercial interests. Hence, European rabbit industrial farms will have to account for these measures, but this trend will no doubt stretch even beyond European borders. Antimicrobial resistance recognises no border and it is not solely a European problem. Disease prevention can be achieved by ensuring better conditions for farmed animals and more animal-friendly farming practices. Although it is unlikely for rabbit farms to be completely revolutionised in the upcoming years, they will nonetheless have to implement different farming systems, some of which are well known, and on promoting healthier and more hygienic farms and greater biosecurity. Animal density in farms will have to be re-examined since it affects the spreading of pathogenic agents and animal stress. Finally, drug usage will have to be limited, leaving room to alternative products like probiotics, prebiotics, essential oils or acidifiers. The aforementioned proposed actions have to to be

economically sustainable and must guarantee profit to farmers despite the current international context of rising costs of raw materials and the global economic and financial crisis. The ability to adapt and detect the changes that are taking place is of paramount importance for survival, along with the ability to come up with innovative solutions which are capable of tackling the latest rabbit farming problems.

Key words: Rabbit, enteric diseases, antimicrobial resistance.

INTRODUCTION

The 8th World Rabbit Congress held in Puebla (Mexico) in 2004, had Dr. Dominique Licois present a report on "Domestic Rabbit Enteropathies" (Licois, 2004). Eight years on his work continues to be extremely useful to anyone interested in intestinal pathologies of rabbits. So far enteropathies continue to persist in industrial rabbit farms, causing mortality, growth problems and drug-related costs which farmers must bear (Grilli *et al*., 2006; Licois, 2009; Rosell, 2003; Rosell *et al*., 2009). The latter not only affect production costs, but also favour the onset of antimicrobial resistance in microorganisms of public health importance. In Spain and Portugal, in the 1997-2007 decade, intestinal pathologies clearly prevailed over other health problems, without showing seasonal variations (Rosell *et al*., 2009). Similar reports come from France (Boucher and Laplat, 2005) and Italy (Grilli *et al.,* 2006). Feasibly, the situation is similar throughout the other producing countries.

BACTERIAL DISEASES

Colibacillosis

Colibacillosis is by far the most common intestinal disease in rabbits (Licois, 1992; Milon, 1996; Boullier and Milon, 2006; Licois, 2009). Research was mostly conducted in the 1990s and highlighted that Rabbit Enteropathogenic *E. coli* (REPEC) played a major role as enteric pathogens. Their pathogenicity is associated to the *eae* gene and adhesins or fimbriae (AF/R1, AF/R2, Ral etc.) binding to the villi of intestinal mucosa (Blanco *et al*., 1996; Fiederling *et al.,* 1997; Blanco *et al*., 1997; Boullier and Milon, 2006). REPEC strains cannot ferment rhamnose. The latter biochemical property can be used for diagnostic purposes along with the full biotyping of isolates (Okerman and Devriese, 1985; Camguilhem and Milon, 1989). In Europe, O-103 strains are the most frequently detected serotypes in colibacillosis outbreaks (Agnoletti *et al.,* 2006; Boullier and Milon, 2006; Licois, 2009). The pathogenicity of strains cannot be defined by phenotypic traits (for instance O-antigen or biotype) only or genetic traits (*eae, af/r1, af/r2, ral*) only, but it requires the combination of both sets. Despite good knowledge on the mechanisms of *E.coli* pathogenicity, rabbit colibacillosis continues to be a persistent problem and in recent years it has even expanded (Boucher and Morel Saives, 2009; Sraka and Boucher, 2009). At the same time inactivated, engineered or attenuated vaccines were being developed (Camguilhem and Milon, 1990; Milon *et al*.,1992; Stakenborg *et al*., 2001; Boullier *et al*., 2003; Bohez *et al*., 2004; Zhu *et al*., 2005), but although seemingly promising, they were not applied to farming practices. Alternative control strategies based on probiotics and on the installation of microflora barriers (Heczko *et al*., 2000; Ogawa *et al*., 2001) are however emerging.

Salmonellosis

Salmonella tiphymurium and *S.* enteritidis, and sometimes also other serovars, are etiological agents of rabbit enteropathy (Agnoletti *et al*, 2006). Unlike for other animal species (poultry and swine for instance), rabbits are normally free of *Salmonella* and, if contaminated, they develop an infection with clinical and pathologic signs such as enteritis and typhlitis that may even be necrotic (Agnoletti *et al*., 2006). Necrosis often appears during chronic infections and generally targets the liver, spleen and the intestinal tracts infiltrated by lymphatic tissue (i.e. *sacculus rotundus* and caecal appendix). *Salmonella* in rabbits is therefore sporadic (Rosell *et al*., 2009; Sraka and Boucher, 2009), and rabbitries are mostly *Salmonella* free (Agnoletti *et al.,* 1998), but those affected by *Salmonella tiphymurium* or *S. enteritidis* can be hard hit and may be severe, with a high within prevalence complicating any eradication attempt. Several factors condition the evolution of a *Salmonella*

outbreak. They include the hygienic level of rabbit farms, the frequency of farm disinfection and animal therapies, and the animals' overall health. It is unlikely that an intervention will result in the eradication of the infection, which may instead become chronic with periodic acute onsets. A study has excluded feed as being the vehicle of *Salmonella* infection (Agnoletti *et al.*, 1998) because it is made using good quality feed ingredients and the pelletted feed undergoes heat treatment. *Salmonella* most likely penetrates rabbit herds because of poor biosecurity measures, in particular a lack of rodent and insect control. Some peculiar scenarios of rabbit infection with *Salmonella* involve promiscuity with animal species that are commonly *Salmonella* carriers. Despite the low *Salmonella* prevalence in industrial farms, due to the control of this severe zoonotic agent, it should always be considered in the differential diagnosis of rabbit enteritis.

Klebsiellosis

In humans and in several animal species *Klebsiella pneumoniae* is characterized by a marked respiratory tropism (Quinn *et al.*, 1994; Koneman *et al.*, 1997), but not in rabbits whose respiratory tract is not frequently affected by *K. pneumoniae*. Enteric forms tend to be more common in rabbits (Agnoletti *et al.*, 2006) and they generally affect rabbits aged 15 to 30 days, manifesting severe hemorrhagic enteritis, congestion of small intestine and cecum. Necroscopic findings are similar to the lesions observed in rabbits affected by clostridial enteroxaemia. Violent episodes of diarrhoea with blood in the stool may also appear. Rabbits with severe klebsiellosis may show hepatic degeneration due to septicaemia (Boucher and Nouaille, 1999; Boucher and Nouaille, 2002). With few exceptions, the episodes of klebsiellosis are generally resolved spontaneously and animals grow up normally. Therapeutic intervention usually comes too late and is not very effective. Italian rabbit farms with enteric forms of *K. pneumoniae* have tackled and resolved the problem by adapting the the scheduling of farm medication protocols, especially in the maternity department, and also by reducing antibiotic consumption (Agnoletti, data not published). This result is consistent since the high resistance of *K. pneumoniae* to several antimicrobials (Boucher and Nouaille, 1999; Saggiorato *et al.*, 2008) allows this microrganism to multiply and colonize the intestinal ecosystem if under considerable antimicrobial selective pressure. Episodes of klebsiellosis can also be linked to poor hygienic conditions, especially in the management of the maternity units and of the nests. In older rabbits which have been weaned, the episodes of enteric klebsiellosis reported in Italy were always related to an intensive use of antimicrobials administered orally. The episodes are often associated to a iota enterotoxaemia due to *C. spiroforme* and are classified as dismicrobism of iatrogenic nature.

Clostridial enteropathies

The genus *Clostridium* contains bacterial species that are the etiological agents of different diseases for many animal species (Borriello, 1995; Songer, 1996). In rabbits, clostridia cause enterotoxaemia which is an enteropathy of the cecum and other intestinal tracts that is triggered by an overgrowth of clostridia in the intestinal lumen with the production of proteic exotoxins that invade the intestinal mucosa, enter the bloodstream and cause toxaemia. Similarly to other agents, the clostridial overgrowth depends on different predisposing factors, and enterotoxaemias are classified as conditioned pathologies (Percy and Barthold, 2001; Harcourt-Brown, 2002). Risk factors include feed, usually an unbalanced diet which favours a substrate (for instance starch) which enhance the multiplication of clostridia (Peeters *et al.,* 1993; Peeters *et al.,* 1995), or decreased intestinal motility which may reduce the clearance of toxins continuously produced by clostridia in the intestinal lumen even in physiological conditions, or hyperproteic diets that trigger abundant secretion of trypsin which leads to scission with activation of *C. spiroforme* binary toxins (Ellis *et al.*, 1991). Enterotoxaemia, in some instances, is a iatrogenic disease, triggered by an overuse of antibiotics which cause a disequilibrium in the cecum microbiota (Harcourt-Brown, 2002). In the conditioned pathologies described above some clostridia, *C. piliforme*, *C. difficile, C. spiroforme* and *C. perfringens* type E, play a primary pathogenic role (Harcourt-Brown F., 2002), while there is insufficient information on minor clostridia.

Clostridium piliforme

Clostridium piliforme is the causative agent of Tyzzer's disease. It used to be rather common (Peeters, 1988) but nowadays it is quite rare. In fact, Italian industrial farms have not seen an outbreak of Tyzzer's disease in years (Agnoletti, data not published). Current biomolecular methods can detect the DNA of *C. piliforme* in the liver of dead animals (Niepceron and Licois, 2010) using effective diagnostic screening procedures whereas in past, histological methods were expensive and less sensitive. *C. piliforme* may be involved in cases where the enteric disease is characterized by phenomena of miliar liver necrosis that may be associated to oedema of the caecal wall. Although rare, intensive farms with good hygiene quality may experience severe episodes of the disease (Le Normand *et al.,* 2005).

Clostridium difficile

Among the etiological agents of enteric syndromes in rabbits *C. difficile* is responsable for Antibiotic Associated Diarrhoea (AAD) which affects humans as well as other animal species, and also for a spontaneous disease in untreated animals (Perkins *et al.,* 1995). In France, 6 out of 46 clostridial strains isolated from the caecum of rabbits affected by ERE were *C. difficile* (Bouvier *et al.*, 2005). An Italian survey on a large sample of herds and animals found *C. difficile* in 5% of the farms tested (Bano *et al*., 2008). In most cases strains were toxigenic with toxin A and toxin B genes, but also the binary toxin (CDT) coding gene. Subsequent studies have highlighted that in industrial Italian farms *C. difficile* is an enteric pathogen found at low prevalence, generally correlated to high antibiotic consumption (Agnoletti, unpublished data). Further, *C. difficile* rabbit isolates from the above studies and human isolates display some common ribotypes, possibly implying interspecies circulation of *C. difficile* and a potential zoonotic role (Agnoletti, unpublished data).

Clostridium spiroforme

C. spiroforme plays a significant role in rabbit enteropathies, not only in Italy (Agnoletti *et al*., 2006) but also in France (Boucher and Nouaille, 2002; Le Guennec *et al*., 2007). The diagnosis is simply made by a bacterioscopic examination of the cecum content to detect the helically coiled morphological configuration of this microorganism (Licois, 2009). Research on *C. spiroforme* has improved as diagnostic laboratories can currently isolate the micro-organism on selective culture mediums (Agnoletti *et al*., 2004). Compared to the past, biomolecular technology has simplified the identification of *C. spiroforme* (Drigo *et al*., 2008). Although all of the above have no doubt improved its diagnosis, therapy is still not that simple. In fact, a survey on minimum inhibitory concentration on *C. spiroforme* to several antimicrobials pinpoints an overall reduced susceptibility and some intrinsic resistance to antimicrobials (Agnoletti *et al*., 2009). For this reason prevention avoiding predisposing factors is surely more effective than therapy against *C. spiroforme* iota enterotoxaemia. It is noteworthy that some outbreaks reported in past have a iatrogenic nature (Carman and Evans, 1984; Carman and Wilkins, 1991).

Clostridium perfringens

C. perfringens is often isolated in enterotoxaemia outbreaks in intensive farms, but its involvement as causative agent is questionable and has triggered a difficult ongoing debate between veterinary practitioners and veterinary researchers. On the one hand, prophylactic treatment of feed strives to limit the signs that characterize rabbit enterotoxaemia, as swelling of the cecum, episodes of diarrhoea, serosal haemorrhaging of the cecum and the intestine. On the other, the role of *C. perfringens* toxintype A, commonly isolated, even in high counts in affected rabbits, is being questioned by researchers since attempts to experimentally reproduce enterotoxaemia have failed. *C. perfringens* toxin type E produces a iota toxin whose effects are similar and were therefore confused with those of the binary toxin of *C. spiroforme* (Carman and Evans, 1984). Only toxin type E of *C. perfringens* has been proven to have a pathogenic role in the rabbit (Percy *et al*., 1993). Lately researchers have been focusing on *C. perfringens* because of its potential implication in the development of ERE (Dewrée *et al.,* 2003; Le Normand *et al.,* 2003; Richez *et al.,* 2007). A recent survey on rabbit's enteric disease has shown that 99% isolates are toxin type A whereas *C. pefringens* toxin type E is limited to less than

1% (Cocchi *et al.*, 2008). Biomolecular technology was applied to toxin type *C. perfringens* of several animal species (Songer and Meer, 1996; Meer and Songer, 1997; Yoo *et al.*, 1997; Garmory *et al.*, 2000; Baums *et al.*, 2004; Jost *et al.*, 2005). These methods, applied to rabbit isolates, have pointed out that 25% of *C. perfringens* have the beta 2 toxin coding gene (Cocchi *et al.*, 2008), of the latter figure 94% had the allelic variant *consensus (cpb2^{con})* (Cocchi *et al.*, 2009). In i*n vitro* tests on Caco-2 cells, *Cpb*2^{con} reveal to be roughly 10 times more cytotoxic compared to the *atypical* allelic variant (Fisher *et al.,* 2005). Keeping in mind that rabbit enterotoxaemias have a conditioned nature, such new insight, added to known pathological role of *C. perfringens* type A in other animal species, may lead to reconsider the role of type A *C. perfringens* in rabbits.

PARASITIC DISEASES

Parasitic diseases of rabbits are not a mainstream research topic, perhaps due to the fact that exposure to ectoparasites and helminths can be effectively controlled applying sanitation and hygiene standards in farming practices and planning prophylactic treatments. In the past decade however, coccidosis has been drawing more attention as its pathological role grew following the appearance of ERE and the development of resistance against the drugs used to normally control its diffusion (Licois, 2009). As generally known, among the 11 species of rabbit coccidia only very few are highly pathogenic (*Eimeria flavescens*, *Eimeria intestinalis*), the others are not very pathogenic or are apathogenic (*E. perforans*, *E. coecicola*) (Eckert *et al.*, 1995). Diagnosis is often based on the morphology of oocysts, and thus a certain amount of uncertainty and error due to variability in size and form of oocysts within the same coccidial species ought to be considered (Licois, 2004). Research on precocious lines (pathogenic attenuated strains) in poultry have led to the diffusion of vaccinations and a lower consumption of sulfonamides. Similar studies are now being conducted for rabbits (Niepceron *et al.*,2009a) but to date there is still no commercial vaccine available. Considering the evolution of the EU veterinary pharmaceutical legislative framework, this vaccine is highly desirable. Meanwhile PCR technologies to identify the pathogenic species are developing (Niepceron *et al.,* 2009b) and may soon be available for diagnostic purposes. Screening the farms affected by highly or moderately pathogenic coccidial species using highly sensitive and specific biomolecular methods would enable a more targeted and effective use of anticoccidials in chemoprophylaxis.

EPIZOOTIC RABBIT ENTEROPATHY (ERE)

Despite the considerable amount of research conducted in the late 1990s, when ERE first appeared in Europe (Duval, 1998; Licois *et al.*, 1998; Marlier and Vindevogel, 1998), this disease is presently a major health threat to the modern rabbit production (Dewree *et al.*, 2003). The causative agent remains unknown and veterinarians and breeders are forced to cohabit with this pathology. As generally known, ERE arises suddenly in animals that are weaned, causing high mortality, lasting normally 15-20 days. Its onset is associated with a sudden decrease in food intake and water consumption (Licois *et al.*, 2005) followed by abdominal meteorism, with alternating diarrhoea and constipation. Observable lesions include gastric meteorism, distension of the intestinal lumen, abundant liquid in the gastro-enteric tract, occasionaly associated with mucous, caecal paresis, with no particular phenomena of inflammation. The secondary bacterial contamination on ERE lesions and the unability to perform a laboratory diagnosis complicate identification of ERE affected rabbitries and make ERE an enteric syndrome with objectively blurred contours. Research conducted over the years has excluded the etiological role of animal feed containing toxic substances (Lebas, 1998; Le Gall *et al.*, 1998) and the involvement of viral agents (Licois *et al.*, 2006; Licois, 2007; Szalo *et al.*, 2007). The cause is without a doubt infectious, and experimental reproduction is potentially possible with the inocula developed in INRA (Huybens *et al.*, 2009; Duperray *et al.*, 2011). Presently, the most widely accepted etiological assumption points to the role of a toxigenic bacteria, presumably an aerotolerant anaerobe which cannot grow onto a normal agarized medium, which is capable of producing toxins. The involvement of a soluble heat sensitive toxin has been demonstrated (Licois, 2007). Finally, the role of *C.*

perfringens has presently been reconsidered and despite its direct etiological role has been excluded it may complicate the evolution of the pathology (Licois, 2009).

VIRAL ENTERITIS

Viruses also can cause rabbit enteric syndromes but normally they are never primary agents, and most often they act in association with different pathogens and different predisposing factors (Lavazza and Capucci, 2008). The most important viruses are Rotavirus and Coronavirus. Rabbit rotavirus Group A can cause light or more severe enteritis, mostly in post-weaning rabbits, often associated to bacterial or parasitic pathogens. The transmission is usually oral-faecal. Diarrhoea may be caused by a degeneration of microvilli on the intestinal mucosa with consequent malabsorption. In intensive farms rotavirus group A is widespread, and most adult rabbits test positive with serological assays (Peeters *et al.*, 1984; Di Giacomo and Thouless, 1986). In Italy the rotavirus prevalence ranges from 16% (Nieddu *et al.*, 2000; Lavazza *et al.*,2008) to 23% (Cerioli *et al.*, 2004) in post- weaning rabbits with enteric symptoms. Rabbit Coronavirus (RbCoV) can also determine enteropathy (Lapierre *et al*, 1980; Osterhaus *et al.*, 1982) and seems widespread in rabbit farms (Deeb *et al.*, 1993). In Italy, Lavazza reports a 100% RbCoV prevalence at farm level, and 3% to 40% within the farm at rabbit level. This data underscores the involvement of RbCoV in subclinical infections and its role as opportunistic pathogen (Lavazza, 2008). The significance of Rabbit Parvovirus (Matsunaga *et al.*, 1977) remains unclear, it is supposed to be associated to light enteritis (Matsunaga and Chino, 1981). Adenovirus (Bodon and Prohaszka, 1980), Picobirnavirus (Lusert *et al.*, 1995) and Vesivirus (Martin-Alonso *et al.*, 2005) are isolated from rabbit stool with variable frequency, but there are no reports on a pathogenic involvement.

OTHER CAUSES OF ENTEROPATHY

Paradoxically, antimicrobials are among the causes of enteropathy. After outbreaks of ERE spread throughout Europe, antimicrobial consumption to control rabbit enteric diseases became more diffuse both for treatment and prophylaxis. Antimicrobials can be administered orally, in feed or water, and depending on the country, they can be used in different dosages and combined with other medications. An overuse of antimicrobials can damage the cecal microbiota, and it may cause aspecific enteropathies or more often, *C. spiroforme* clostridiosis. The latter, being notoriously resistant to antibiotics (Agnoletti *et al.*, 2009; Agnoletti *et al.*, 2010), proliferates in the absence of antagonistic bacterial flora. Another point worth noting is the toxicity of beta lactams antibiotics for rabbits. This occurrence is not common but rabbit feed can be cross contaminated with beta lactams destined for other species. Even a very low dosage of beta lactams, can trigger episodes of massive mortality with clinically hyperacute diarrheal episodes. The intoxicated animals present lesions and bacteriological findings were similar to *C. spiroforme* iota enterotoxaemia (Agnoletti, unpublished data). In other cases, orally administered ampicillin may potentially cause enteric dysbiosis classified as colibacillosis (Rosell *et al.*, 2009). Presently, the relationship between poisoning by beta lactams antibiotics and the proliferation of *C. spiroforme,* which is very sensitive to this family of antibiotics, remains unclear (Agnoletti *et al.*, 2009).

DIAGNOSTIC PROGRESS

The diagnosis of rabbit enteropathy has evolved remarkably since 2004 thanks to biomolecular technology which has improved the diagnostic capacity of laboratories and reduced the cost of instruments. To diagnose colibacillosis for instance, it is quite common nowadays to resort to the characterization of bacterial isolates using PCR amplification of *eae* and adhesins Af/r1 and Af/r2 coding genes (Agnoletti *et al.*, 2006; Boucher *et al.,* 2011). Microarray analyses were employed to simultaneously determine the virulence factors of several codifying genes (Tonelli *et al.,* 2008), but due to its high costs, microarray has been limited to the research domain. PCR (Liu *et al.*, 2007; DebRoy *et al.*, 2011) and microarrays (Liu *et al.*, 2010) can serotype *E. coli* by detecting the somatic

antigen codifying gene, thus avoiding using traditional slow sero-agglutination which is rather complex and time-consuming.

Special progress was made in the domain of rabbit clostridiosis: in the past few years highly sensitive PCR can detect *C. piliforme* in rabbit organs (Niepceron and Licois, 2010), PCR identifies *C. spiroforme* (Drigo *et al*., 2008), and real-time PCR identifies *C. difficile* (Bélanger *et al*., 2003; Penders *et al*., 2005; Peterson *et al*., 2007). Moreover many laboratories can now use biomolecular methods for the genotyping *C. perfringens*, including the beta2 toxin gene, its allelic variant and the gene coding the enterotoxin (Songer and Meer, 1996; Meer and Songer, 1997; Garmory *et al.,* 2000; Baums *et al*., 2004; Jost *et al*., 2005) and *C. difficile* (Kato *et al*., 1998; Bidet *et al.,* 1999; Stubbs *et al*., 2000; Lemee *et al.,* 2004). Biomolecular sciences produce valuable data for epidemiological analysis which may for example lead to hypothesize a rabbit-humans interspecies circulation of *C. difficile*, based on the homology of ribotypes isolated from the two different species (Bano *et al*., 2008). Moreover, much more information about cecal microbiota composition is now available, while for years this research had been poor due to the limits and to the costs of traditional bacteriological methods. The turning point in diagnostic capacity occurred with the diffusion of biomolecular methods (Combes *et al.,* 2011) which initially were only qualitative and later they could quantify findings. Furthermore, these methods, and real time PCR in particular, have become easier and simpler to use research tools, thus facilitating their diffusion (Matsuki *et al*., 2004; Ott *et al*., 2004; Malinen *et al.,* 2005; Penders *et al*., 2005). Finally, the significant progress made in identifying bacteria is worth noting, in particular the diffusion of mass spectrometry instruments in the veterinary field instead of the more conventional identification systems based on the interpretation of biochemical reactions to specific substrates. These instruments, which adopt the MALDI-TOF technology (matrix laser desorption ionisation - time of flight), are quick, the testing cost per sample is low, therefore enhancing the analytical capacity of laboratories and thus responding to needs of veterinary practitioners (Carbonnelle *et al*., 2011; Neville *et al*., 2011). Moreover, MALDI-TOF mass spectrometry, while identifying bacterial species can also measure the homology of several different bacterial isolates and express results using different graphic representations. The epidemiological information obtained has the same operating advantages, rapidity and reduced costs as those mentioned above and it can be combined to the data of more complex and time-consuming biomolecular methods (Murray, 2010; Wolters *et al*., 2011).

PREVENTION AND CONTROL

The above described improvements in diagnostic technology do not match progress made to control and prevent enteropathies in industrial farms. After the ERE onset, veterinarians, technicians and breeders have managed to keep the disease under control, or rather to live with it. Nowadays, with some exceptions, the reported mortality during an ERE outbreak is by no means comparable to the high figures reported when the infection had first appeared. In France for example, from 2004 until now, the average mortality rate for weaned rabbits has been decreasing (Coutelet, 2011). The factors that have contributed the most to control ERE and rabbit enteropathies include better sanitation and hygienic conditions of the all-in all-out farming systems, the feeding rationing and overall the better environmental conditions (Licois, 2009). However the control of ERE, as well as other infectious or parasitic enteropathies is still associated with the use of drugs for prevention rather than for treatment.

Compared to other animal species raised for food production, rabbit farms have fewer vaccines available for disease prevention, perhaps due to the fact that the diseases are mostly bacterial, or often multifactorial, like rabbit enteropathies. This production system has therefore fewer tools for disease prevention. The European Union, for some time now, has been addressing veterinary drug consumption, basing itself on the most up-to-date knowledge on antimicrobial resistance (AMR) and its impact and costs for human health. In 2007 in the annual epidemiological report on communicable diseases in Europe, the European Centre for Disease Prevention and Control (ECDC) reported AMR to be one of the most serious public health risks globally, and in Europe (ECDC, 2007). The report gives an overall picture of the AMR situation in Europe, AMR trends, and provides an impact assessment of AMR on human health and on the costs to manage AMR in humans. It furthermore highlights that multidrug-resistant Gram-negative bacteria are increasing. In 2008, the European Food

Safety Authority (EFSA) published the scientific opinion of the Panel on Biological Hazards "Foodborne antimicrobial resistance as a biological hazard" (EFSA, 2008). Its conclusion is that "antimicrobial resistant (AMR) bacteria represent a biological hazard associated with increased human morbidity and mortality therefore representing a public health concern". Furthermore, special focus was dedicated to the issue of antimicrobial resistance of some pathogens able to infect humans (*Salmonella*, *Campylobacter*, VTEC, MRSA, *Shigella* and *Vibrio*). Some of these zoonotic agents are transmitted to humans through the food chain, but they are not very common in rabbits. At the same time, priorities have been set. They include preventing human transmission of AMR agents, with an emphasis on targeting resistance to quinolones, third and fourth generation cephalosporines and macrolides. It is noteworthy that quinolones and macrolides are commonly used in the therapy of rabbit enteropathies. Finally, the above mentioned report states that one of the best options to control and reduce the spread of resistant bacteria is to reduce the use of antimicrobials, which in turn leads to reducing disease burden of farmed animals. Reducing the use of antimicrobials in general, or at least certain categories of antimicrobials, will lower the selective pressure and reduce the prevalence of resistant bacteria. In 2009, ECDC and the European Medicines Agency (EMA) published a joint technical report entitled "The bacterial challenge: time to react" (ECDC/EMEA, 2009) stresses "the growing gap between the increasing frequency of infections caused by multidrug-resistant bacteria and the decline in research and development of new antibiotics" which "is now threatening to take us back to the pre-antibiotic era". In 2010 EFSA published "The Community Summary Report on antimicrobial resistance in zoonotic and indicator bacteria from animals and food in the European Union in 2008" (EFSA, 2010) presents the levels of antimicrobial resistance and confirms certain critical areas, in particular the high level resistance of *Salmonella* and *Campylobacter* to fluorquinolones. These results were confirmed in subsequent reports for the 2009 and 2010 data (EFSA, 2011; EFSA-ECDC, 2012). Because of the high human and economic burden associated with drug resistant bacteria (ECDC/EMEA, 2009), in 2011, the European Commission presented an "Action plan against antimicrobial resistance" (European Commission, 2011). The plan comprises of 12 concrete actions, five of which are directly related to veterinary practices. They include:
1. strengthening EU law on veterinary medicines and on medicated feed;
2. introducing recommendations for prudent use of antimicrobials in veterinary medicine, including follow-up reports;
3. introducing legal tools to tighten prevention and control of infections in animals in the new EU Animal Health Law;
4. promoting efforts to analyse the need for new antibiotics in veterinary medicine;
5. strengthening surveillance systems on AMR and antimicrobial consumption in animal medicines.

Clearly the ultimate goal of the action plan is to recommend the prudent use of antimicrobials and also, and most of all, to reduce the quantity of antimicrobials used in animal production. In the upcoming five years there will be a need to reduce the consumption of antimicrobials in the rabbit farming industry in Europe. At present this tendency is clear to both veterinarians and European breeders. It is therefore not surprising that the animal production sector of some countries in the European Union are adopting the national policies to achieve this goal. European rabbit production will have to acknowledge and take actions in compliance with this plan. And yet this trend cannot be overlooked by other countries since its boundaries stretch beyond European borders, making antimicrobial resistance a global problem, not only limited to Europe (Nasinyama, 2011; Park. 2011). Producers of rabbit meat should be fully aware that the current use of medicines in intensive rabbit farms will only worsen the phenomenon of antimicrobial resistance, a burden that the European risk managers and policy makers cannot accept.

Regrettably, the current industrial rabbit farming system has not been using drugs appropriately and their use is in marked contrast with the principles of prudent use of antimicrobial agents (OIE, 2011). This is especially true for the following practices:
1. mostly oral administration;
2. underdosage due to oral treatment of large groups of animals that will feasibly have some which do not reach the full recommended dose;
3. high frequency of treatment;
4. long duration of treatment.

The huge size of bacterial population which colonises the rabbit's intestine ($1x10^{12}$-$1x10^{13}$ cfu/g) together with all of the above-mentioned factors increases the likelihood of selecting antimicrobial resistant bacteria. And this is exactly what happened in the Italian industrial farms. A recent analysis conducted in Italy, involving more than 2000 strains of *E. coli* collected for diagnostic purposes, from 2004 to 2010, has pointed to high levels of resistance to many antibiotics and a reduced susceptibility trend of the isolates to apramycin, paromomycin, neomicin, sulfonamides-trimethoprim, tetracyclines and ampicillin. An issue of concern, in 2010, is the fact that more than 20% of the *E. coli* strains tested developed resistance to at least three out of six tested antimicrobials (apramycin, paromomycin, colistin, sulfonamides+trimethoprim, enrofloxacin and flumequine) (Bano *et al.*, 2011). The same study (2003-2010) tested clinical antimicrobial resistance in more than 350 strains of *K. pneumoniae*. Results showed a downward trend of susceptibility to apramycin and colistin. Tetracyclines are normally administered orally, in sub-therapeutic doses and for long periods. In the study they resulted to be completely ineffective against enterobacteria with more than 98% resistant isolates (Bano *et al.* 2011). A trend analysis on antimicrobial resistance in clostridia is not available but high MIC levels against many antimicrobials used in rabbit farms were reported (Agnoletti *et al.*, 2009; Agnoletti *et al.*, 2010). As stated in the ECDC-EMEA joint report, one of the main issues is that quinolones, the newest class of antibiotics used in veterinary medicine, were discovered 50 years ago, and since then, there has been no other antimicrobial class of molecules available for veterinary use. In short, the drugs currently used are quite old while the trend of antimicrobial resistance has been on the rise. This scenario should encourage greater commitment on the part of researchers and vaccine producers. Although no significant innovation was made in vaccines development, rabbit breeders and veterinarians are calling for preventive tools, similar to those achieved in poultry production, where vaccines for disease prophylaxis have significantly reduced disease incidence and consequent antimicrobial consumption. However it seems that veterinarians will be disappointed, at least in the short to medium period.

To be realistic, and considering state-of-the-art innovations, in the upcoming years, if the concern is to improve the overall health of rabbits we will have to focus on concepts that are already familiar, and which, whenever applied, have yielded good results. When translated into practical terms this means an all-in, all-out production system, hygiene, biosecurity health surveillance of breeders and proper feed formulation (Maertens, 2007). The limitations to medicine usage will produce a gap that can partially be filled with alternative products as probiotics, prebiotics, organic acids, plant extracts, enzymes etc. (Maertens *et al.*, 2006; Falcão-e-Cunha *et al.*, 2007; Maertens, 2007; Romero *et al.*, 2011). These alternatives do however require more research and field experience, but studies can take advantage of the latest diagnostic technologies available in microbiology laboratories. Several biomolecular methods can be used nowadays to perform an analysis of the bacterial populations in the animal's intestine (Samal *et al.*, 2011), they include real-time PCR, which is fast and easy to use. Such methods allow the identification and quantification of bacteria in the intestinal lumen enabling the evaluation of the impact that different feed (Carabaño *et al.*, 2006) or diseases (Tajima *et al.*, 2001; Matsuki *et al.*, 2004; Ott *et al.*, 2004; Malinen *et al.*, 2005; Penders *et al.*, 2005) have on the caecal microbiota are also simpler and more accurate. Biomolecular methods are also being applied to study the intestinal microbe population in rabbits (Combes *et al.*, 2011), pinpointing to the current limits of knowledge. Percentages, ranging from 80% to 90% of the bacterial sequencing from the intestinal content of rabbit are not currently available in the database, therefore resultas unknown bacterial species (Abecia *et al.*, 2005; Monteils *et al.*, 2008). More work is needed to fully take advantage of the potential that these methods can offer when applied to disease research in rabbits. In order to succeed greater synergy and an integration of the skills of pathologists, microbiologist and nutritionists is required.

CONCLUSION

Rapid changes observed in the last few years on food safety have led the European Union to develop some guidelines on antimicrobial consumption in food producing animals. These guidelines take into account the health and welfare of food producing animals on the one hand, and on the other hand the public health risk of antimicrobial resistance in food borne bacteria. The European Union policy is clearly striving to protect public health over the interests of food production, but it also true that antimicrobial resistance is a common problem worldwide.

People working in rabbit industry will know that most rabbit diseases are closely related to the farming system hence to: the type of housing (pens and shed), the animal density, the environmental and microclimatic conditions, the type and quality of feed, the reproductive rhythm. As generally acknowledged the welfare of rabbit farming needs to be improved, not only for ethical reasons, but mostly to decrease the disease burden and therefore medicine consumption. We know where we are going but we still need to understand how far European policy makers will push their regulations, for instance whether the current model of rabbit meat production in industrial farming, based on intensive breeding in cages, will survive in the future scenario. In the next few years drug consumption will no doubt have to be reduced, and it will have to be oriented more and more towards a therapeutic rather than a prophylactic use. There will be a substantial shift from medical prophylaxis, commonly used in European intensive rabbit farming, to preventing disease by improving animal welfare and ensuring better production practices. Modern rabbit farming has a notable challenge ahead. Its outcome is however uncertain, being staged in the midst of the European economic crisis, amid rising costs of energy and raw materials and considering the rapid changes of consumer behaviour. In the next future, management will probably need to promote full disinfection at the end of the fattening cycles for better farm hygiene. Animal density is also another important aspect that will have to be reconsidered in response to the citizens' opinion to convert farms into more animal-friendly places that foster animal welfare (Vanhonacker *et al.*, 2009; Vanhonhacker *et al.*, 2010) and at the same time reduce the risk of diseases. Density plays a pivotal role in spreading infectious disease among animal populations for it has direct effect on pathogen transmission, and an indirect effect by stressing animals, affecting their immune system. A lower animal density will nonetheless need to make allowance economic sustainability and farming profits, the latter being a prerequisite for the farm's survival. The redistribution of fixed costs on a smaller number of does seems to have a considerable, negative, impact on the economic performance of farming activities (Verspecht *et al.*, 2011). More research focus is needed on the potential of complementary and alternative medicine products (as probiotics, prebiotics, essential oils, acidifiers etc..), or on different rabbit breeding methods that are less industrial and more integrated with the entire animal farming. At this peculiar point in time, it is my opinion very few will have an answer on what should be done and where intensive rabbit farming is heading, especially European farming. In the next few years greater efforts are needed to integrate different skills which entail a closer cooperation between producers, institutions and researchers. As survival and evolution are intrinsically connected to the ability to detect and adapt to changes, original and innovative solutions are necessary to tackle novel problems. So the future of the modern rabbit farming is in our hands.

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