LAWSONIA BACTERIA, AN UNKNOWN PATHOGEN, NEWLY DISCOVERED IN RABBIT FARMS.

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ABSTRACT

The pathogenicity of *Lawsonia* infection in rabbit farms was never reported. This field case reports *Lawsonia* present in manure samples derived from rabbit farms**. S**ince the ban in 2006 on growth promotors as feed additives in animal feed, rabbits farms in The Netherlands suffer more with problems after weaning, because of Epizoötic Rabbit Enteropathy (ERE). This field survey shows that *Lawsonia* might be one of the pathogens causing ERE in meat rabbits. The diagnosis *Lawsonia* may give veterinarians possibilities to treat rabbits short before slaughter with registered antibiotics instead of antibiotics off label use with 28 days withdrawal time.

Key words: Lawsonia, ERE, rabbit, tylosine, tiamulin

INTRODUCTION

Lawsonia bacteria can cause Epizootic Rabbit Enteropathy (ERE). *Lawsonia* intracellularis has been detected in pigs and small rodents many times (Vannucci *et al*, 2013) (Won *et al*, 2018). In this study faecal samples of 13 Dutch rabbit farms were collected and screened for antigens of *Lawsonia* and *Brachyspira* by PCR and IFT. In 6 farms PCR appeared positive for *Lawsonia* and this may be linked to enteric problems.
After treatment with tylosinetartrate or tiamulinhydrogenfumarate (registered as veterinary antibiotics in The Netherlands for treatment of *Lawsonia*) death rate caused by enteric diseases in these weaned rabbits went down from about 25% to under 10%. In rabbit farms ERE was long time seen as a management problem concerning ingredients in rabbit feed, hygienic problems and climate inside the stables. Diarrhea and dehydration are main causes of losses and mortality after weaning in rabbits. Bowel disorders are main reasons of morbidity and mortality in meat rabbits (Marlier *et al*. 2003). It is regarded by rabbit farmers as one of the main convicted outbursts of death in the rabbits (personal communication). In The Netherlands mortality after weaning was till 2005 under10 % of weaned rabbits (age 35~81 days). At this moment mortality in Dutch rabbit farms is 8 to 15 % with outliers to 40 % after weaning (age 35~84 days) (TEA). Mortality rates increase after 2006, because prebiotics like flavomycin and flavofosfolipol were forbidden to include standard in feed for rabbits (Staatscourant). Therefore endemic infections like coccidiosis and Clostridium and till now never discovered bacteria *Lawsonia* can strike intestines of rabbits in intensive farms. Years after 2005 ERE was named as the new syndrome, fat belly syndrome or Caecal Paresis Pneumonie Syndrome (CPP). Later this disease was named ERE (Licois and Coudert 2001), because at autopsy no inflammation spots were observed in the intestinal walls (Licois *et al.* 1998).

Lawsonia is an obligate intracellular bacteria, found in animals with proliferative enteritis (PE) (Vannucci *et al*, 2013). *Lawsonia* and PE are found in different kinds of mammals and birds (Hillyer *et al*, 1997). PE is known in nearly all little laboratory animals, but natural infection is not known in the mouse (Percy *et al*, 2002). Base sequences from the 16S rRNA from isolates found in hamster, a ferret, a deer, a pig and an ostrich look very similar to each other. This suggests there is a relationship between these detected bacteria, but does not prove that they are identical (Abbott *et al.* 2007). Different kinds of bacteria are found since the use of PCR DNA techniques on samples of unborn manure of animals. Bacteria as Escherichia coli, Bacteroïdes, Clostridium perfringens, Clostridium

clostridiiforme, Ruminococcus, Peptostreptococcus, Helicobacter, and Fusobacteria have been detected. Design of new molecular tools that helps in diagnosing ERE, that helps to prevent ERE and the reduction of using antimicrobials in rabbit farms, are principal objectives of practical field research (Badiola *et al*. 2016). Therefore rabbits from 13 farms with diarrhea were tested on the presence of *Lawsonia*.

SYMPTOMS AND PATHOGENICITY

In pig farms similar symptoms of diarrhea in weaned pigs is observed, where in the intestinal contents a bacteria called *Lawsonia brachyspira* was determined. This bacteria is the cause of this porcine intestinal adenomatosis (Smith *et al*, 2014). Lawsonia is associated with intestinal crypt epithelial cell proliferation but the mechanisms responsible need to be defined (Smith *et al*, 2014). Proliferative Enteritis (PE) is best described in colonies of hamsters and pigs, because mainly these animals are kept as a group. In literature many synonyms are found for PE, e.g. proliferative ileitis, regional ileitis, terminal ileitis, enzootic intestinal adenocarcinoma, atypic ileum hyperplasia, hamster enteritis, wet tail syndrome. PE in pigs is also known as proliferative haemorragic enteropathy or intestinal adenomatose from ileum and colon. This type of enteritis has incidently been found in guinea pigs, rats, rabbits (enterocaecocolitis) and in many other kind animals, as recently found in non-human primates. (Friedman *et al*, 2008). Therefore *Lawsonia* may also pose a risk for rabbit farmers and workers during direct contact with rabbits. In rabbits PE is observed in weaned rabbits (age 5-12 weeks) and in rearing female rabbits (age 5-19 weeks). These sick rabbits show lethargic outfit, no appetite and produce slimy half fluid faeces, with crystal clear gel. The (histo)pathogenicity varies. Often it is a purulent caecocolitis, with hyperplastic glandepithelic and exsudate in the lumen of the intestines. *Lawsonia* bacteria are always excreted by faeces. Contamination between rabbits is probably by faecal oral route. Coprofagia will promote and maintain this infection. A vertical infection routing is probably not correct. There are no indications that this bacteria goes further than as inclusion in the enterocyt cells.

MATERIALS AND METHODS

In 13 intensive rabbit farms faecal samples were taken during veterinary visits from 2006 till 2008 at various sick rabbits with diarrhea from each barn. In any group rabbits with diarrhea after weaning the practitioner veterinarian took unborn manure directly out of the intestinal contents. Samples out of the content of the caecum and small intestines were collected after euthanizing and dissecting live sick rabbits. Samples were transported to the laboratory Gezondheidsdienst voor Dieren at Deventer (The Netherlands) in a cooled container (4 °C). To evaluate bacterial selection, IFT was performed on these manures to detect Brachyspira. Bacterial culturing (aerobic or anaerobic on agar) does not show this *Lawsonia* bacteria. Due to extreme difficulty of *in vitro* culture of this pathogen, molecular characterization of protein components of *Lawsonia* in unborn faeces was carried out with PCR-enhanced immunoassay (PIA). This new PCR technique in determining bacteria made it easier to find microaerofilic *Lawsonia* bacteria. Isolation and cultivation of *Lawsonia* bacteria was only achieved by using dividing cells in culture under strict microaerophilic conditions. These fastidious properties restrict opportunities to study the dynamics of *Lawsonia* in causing ERE in rabbits (Vannucci *et al*, 2012). Faecal qualitative PIA in rabbit unborn faecal samples revealed amounts of bacterial DNA associated with duration of shedding *Lawsonia* bacteria. ERE is mostly diagnosed after dissection live sick animals with typical clinical symptoms. Autopsy makes perceptible segmental thickened intestinal walls visible. *Lawsonia* bacteria are also serologic related, because a monoclonal antibody from a pig isolate of *Lawsonia* intracellularis reacted positively with the bacteria found in other animal kinds. Histology of enteric walls can prove the proliferation of enterocyts cells in combination with comma-shaped 1 to 3 micrometer bacteria in the cytoplasma of enterocyts at the top of villi. These bacteria can not be seen well in coupes, which are HE coloured. Special Warthin Starry colouring makes *Lawsonia* visible as silber coloured small shapes. A modified Ziehl Neelsen colouring, with

IFA test and IP test with antisera and under electronic microscopic view makes the diagnosis valuable. These methods take a lot of time and effort and are too costly for rabbit farmers.

RESULTS AND DISCUSSION

During summer 2007 in 6 of 13 rabbit farms in The Netherlands the bacteria *Lawsonia* was found. In all 13 farms no *Brachyspira* bacteria was found. Because of high costs for different methods to cultivate and make *Lawsonia* visible, in this study PIA was chosen to use on unborn faeces samples. Tiamulinhydrogenfumarate and tylosinetartrate are registered as veterinary antibiotics with indication *Lawsonia* treatment in pigs. In treatment of rabbits also tiamulinhydrogenfumarate and tylosinetartrate are used, but mostly after dissecting dead ill rabbits with enteric disease. Only bacterial growth on agar is done on these rabbits, with detection of many different bacteria. *Lawsonia* is never found before as primary cause of diarrhea. In this study *Lawsonia* is detected by PIA on faecal sampels collected in farms with high mortality after weaning with enteric diseases. Different antibiotics are used as therapy, and also as metaphylactic or even in preventive program and as growth promotion in rabbit farms. But the abuse of antibiotics in farm animal production can lead to multi-resistance in bacteria, which in turn causes multi-resistance against antibiotics used to treat human bacterial infections.

Before 2006 tylosinetartrate was used as a veterinary medicin in rabbit farms to prevent many secondary bacterial diseases in intestines of rabbits. It was so named as a growth promotor. Since january 2006 application of antibiotics in feed for farm animals is forbidden as well as growth promotors in feedpellets. Also preventive use of antibiotics on a regular base is forbidden since then. Extra hygiëne measures and a new management system in producting rabbits, called bande unique (ping pong system), together with artificial insemination on hybrid rabbits have given progress in technical results. New welfare rules and regulations for farms made that rabbits are kept in large numbers in a group (park) and led to an increase of infectious diseases. Prophylactic treatment with other antibiotics like Beta Lactames, Lincomycin, Clindamycin is dangerous, because they are very poisonous for rabbits, although they are indicated for the same bacteria and disease. New dutch formularium for rabbits is dedicated by the dutch government, so that only registered veterinary antibiotics are used in treatment of meat rabbits. Tiamulinhydrogenfumarate and tylosinetartrate are registered in The Netherlands with indication *Lawsonia* in pig, calve, chicken and turkey. Off label use of tiamulinhydrogenfumarate or tylosinetartrate products give permission to treat rabbits after detecting *Lawsonia*. Diagnosing by PIA makes it possible to treat legally diarrhea in rabbits by usage of these antibiotics. Off label use with these antibiotics reduces mortality from 25 % to under 10 % in rabbits after weaning (age 35-81 days). New welfare rules with rabbits in parcs with plastic floor and balcons intensifies the risk of contaminating rabbits each other.

CONCLUSIONS

Intensive direct or indirect contact between rabbits kept in groups in new welfare cages with plastic bottoms and balconies increases contact between contaminated faeces and rabbits. Antibiotic and new vaccines are now the strategy to treat and prevent this cause of diarrhea in pig farms. This way of strategy can be applied in rabbit farms, but only with registered antibiotics and vaccines for these animals and indication. By using new research techniques like PIA on diarrhea samples *Lawsonia* could be detected in rabbits. *Lawsonia* might be one of the possible cause of ERE in weaned rabbits. Further studies are necessary with cross infections to demonstrate the implication of *Lawsonia*. By regularly taken manure samples and analysis by PIA on DNA of *Lawsonia*, it coule be possible to set up a good program in treatment and prevention of ERE in rabbit farms. Besides treatment with tiamulinhydrogenfumarate or tylosinetartrate a good hygiene program and new management system of all-in all out (ping pong system) make it possible to defend endemic spreading of this bacteria in a rabbit farm.

Good vaccines against *Lawsonia* in pigs are registered and hopefully used in pig farms. At the moment no vaccine is available nor registered to prevent ERE in Rabbits. In 6 out of 13 farms in The Netherlands the bacteria *Lawsonia* is isolated by PIA after clinical signs of ERE and mortality after weaning. At routine bacterial aerobic and anaerobic culturing on unborn manure of sick rabbits, no *Lawsonia* was found. In many of these cases Clostridium perfringens bacteria were found and indicated as the cause of gastrointestinal problems.

By the embargo on feed additives like flavomycine and flavofosfolipol in rabbit feedpellets from 2005 and onward there was an increase in mortality after weaning till slaughtering in rabbitfarms. The new formularium for antibiotic use in rabbits has given rabbits farmers a tool to treat and to use antibiotic in a correct way (off label use). Other European countries where rabbits are farmed, *Lawsonia* research should be done to confirm this infectious agent as a possible cause of diarrhea in rabbits. Proper research and regularly visits by the practitioner veterinarian will lead to correct use of antibiotics. As side effect it leads to reduction of total antibiotic use in rabbit farms. All these actions give an improvement in production parameters such as lower mortality rates and higher daily weight gain in weaned rabbits.

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