OUTBREAK OF EIMERIOSIS IN AN ESTONIAN RABBIT FARM

Toivo Järvis, Erika Mägi, Brian Lassen
Department of Infectious Diseases, Institute of Veterinary Medicine and Animal Sciences
Estonian University of Life Sciences
E-mail: brian.lassen@gmail.com

Abstract. Eimeriosis in rabbits is a disease that can result in high numbers of mortalities especially at indoor rabbit farms. This study is an investigation of an outbreak in an Estonian commercial rabbit farm in 2011. Rabbits found dead in their cages were taken to the laboratory and necropsied (N=15). In addition, faecal samples were taken from cages (N=65). All autopsied young rabbits (N=14) were infected with *Eimeria*, high infection intensities were found in 53% of the intestinal contents. Higher infection intensities were observed in samples scored diarrhoeic (P=0.04). Association between higher infection intensities and increasing age of the dead rabbits was observed (P=0.02) as were days post weaning (P=0.01). Samples collected from cages were mainly light infections and *E. magna*, *E. media, E. exigua, E. perforans*, and *E. vejdovskyi* were found more frequently in the high infections. Only *E. irresidua* was found more commonly in necropsied animals with high infection intensities (P=0.004). In total 10 *Eimeria species* were identified, and samples were dominated by *E. media* and *E. magna*. The findings indicate that mortalities were likely due to eimeriosis acquired in cages post weaning and *E. irresidua,* possibly in combination with *E. magna* and *E. media*, may have been the main contributing species. Insufficient hygiene is a likely assisting factor for the outbreak.
Keywords: rabbits, *Eimeria* spp, prevalence, necropsy, eimeriosis.

EIMERIOZĖS PROTRŪKIS ESTIJOS TRIUŠIŲ FERMOJE

Toivo Järvis, Erika Mägi, Brian Lassen
Infekcinių ligų katedra, Veterinarinės medicinos ir gyvulininkystės mokslų institutas
Estijos gyvybės mokslų universitetas
el. paštas: brian.lassen@gmail.com

Santrauka. Triušių eimeriozė – tai liga, kuri uždarose fermose gali tapti masinio triušių gaišimo priežastimi. Straipsnis paremtas šios ligos protrūkio tyrimu vienoje Estijos triušių fermoje 2011 metais. Laboratorijoje buvo atliktas narvuose nugaišusių triušių skrodimas (n=15). Be to, iš narvų buvo paimti fekalijų pavyzdžiai (n=65). Skrodimas parodė, jog visi jauni (n=14) nugaišę triušiai buvo užsikrėtę eimerijomis (*Eimeria)*. Didelio intensyvumo infekcija nustatyta 53 proc. žarnyno turinio pavyzdžių. Didesnio intensyvumo infekcija taip pat nustatyta ir fekalijose, kurios klasifikuotos kaip sukeltos viduriavimo (p=0,04). Pastebėtas ryšys tarp didesnio intensyvumo infekcijos, vyresnio triušių amžiaus (p=0,02) bei dienų skaičiaus po atjunkymo (p=0,01). Pavyzdžiai, paimti iš narvų, dažniausiai buvo silpnai infekuoti sukėlėjų *E. magna*, *E. media, E. exigua, E. perforans*. *E. vejdovskyi* buvo dažnesnis didesnio intensyvumo infekcijos sukėlėjas. Nekropsija parodė, kad dažniausias didelio intensyvumo infekcijos sukėlėjas buvo *E. irresidua* (p=0,04). Iš viso nustatyta dešimt *Eimeria* rūšių. Pavyzdžiuose vyravo *E. media* ir *E. magna*. Tyrimų duomenys leidžia manyti, kad pagrindinė triušių gaišimo priežastis buvo eimeriozės infekcija, kuria jauni atjunkyti triušiai užsikrėtė perkelti į narvus. Pagrindinis sukėlėjas buvo *E. irresidua,* veikiausiai kartu su *E. magna* ir *E. media*. Galima manyti, kad prie infekcijos plitimo prisidėjo ir nepakankama higiena.
Raktažodžiai: triušiai, *Eimeria* spp., paplitimas, nekropsija, eimeriozė.

Introduction. Eimeriosis is a significant contagious infection of young rabbits caused by protozoan parasites of genus *Eimeria* (*Apicomplexa*). The main symptoms of hepatic eimeriosis, caused by *E. stiedae*, are enlargement of liver, icterus and high mortality (Pellérdy, 1974; Pakandl, 2009). The intestinal eimeriosis that is caused by other *Eimeria* species and is commonly observed to cause diarrhoea, reduces growth rate and emaciation is commonly observed (Pakandl, 2009). In total 15 *Eimeria* species have been described in rabbits (*Oryctolagus cuniculus*) (Taylor et al., 2007; Baker, 2007; Pan et al., 2008), but 11 species are currently acknowledged (Kvičerová et al., 2008; Pakandl, 2009). Mostly the rabbits are infected with several intestinal *Eimeria* species, including those with high (*E. intestinalis* and *E. flavescens*), mild to moderate (*E. media*, *E. magna*, *E. piriformis*, and *E. irresidua*) and slight pathogenicity (*E. perforans, E. exigua* and *E. vejdovskyi*), and non-pathogenic (*E. coecicola*) (Coudert et al., 1995; Taylor et al., 2007; Pakandl, 2009). *Eimeria stiedae* which occurs in the epithelium of the bile ducts is classified as moderately to highly pathogenic.

This case study examined unusual mortalities of young rabbits over the weaning period in a commercial rabbit breeding farm in the spring 2011. In addition, the general composition of species and infection intensity of rabbits in the farm was estimated using samples collected from cages other than those of the dead rabbits.

Materials and methods. The pre-industrial rabbit farm of around 300 rabbits kept the rabbits in cages constructed of wire-netting including the bottom. Cages were placed side by side without a partition wall. Rabbits

were fed with commercial granules and hay was generally absent in the ration. Only small amounts of litter were found in cages. Cages were cleaned 1–2 times per year and disinfected with Virkon S. The Metaphylactic application of anticoccidials to rabbits was not practised.

A total 14 young and one adult rabbits were found dead in their cages. The juveniles were mostly cross-breeds and were weaned and moved to new cages at an age of 36±14 standard deviation (STDV) days. Mortalities occurred 2–54 days (mean: 21±18 STDV) after weaning. Rabbits collected from the farm were 48–91 (mean: 59±12 STDV) days old when dead and brought to the laboratory for necropsy one day following the time of death. During necropsies, the rabbits were scored with presence of clinical symptoms (diarrhoea, colour of conjunctiva) and pathological findings (enteritis, colitis, gut metorism, liver enlargement, hyperaemia and dystrophy). In addition to necropsies and examination of the intestinal content (N=15) for *Eimeria* oocysts, pooled samples were collected from 65 arbitrarily picked rabbit cages out of 191. Cage samples were collected from different cages than the dead rabbits during the same period. Samples were examined using simple saline flotation liquid (Roepstorff and Nansen, 1998). *Eimeria* species were identified by oocyst morphology (Baker, 2007; Taylor et al., 2007; Kvičerová et al., 2008). The intensity of infection (II, the amount of oocysts per visual area) was estimated as follows: 1) no oocysts; 2) 1 – 5 oocysts = light II; 3) 6 – 10 oocysts = moderate II; 4) 11 – 30 oocysts = heavy II and 5) more than 30 oocysts = massive II (Fig. 1). Infections were classified containing pathogenic species according to Pakandl (2009) if one or more of the following were found: *E. intestinalis, E. media, E. magna, E. piriformis, E. irresidua,* and *E. stiedae.*

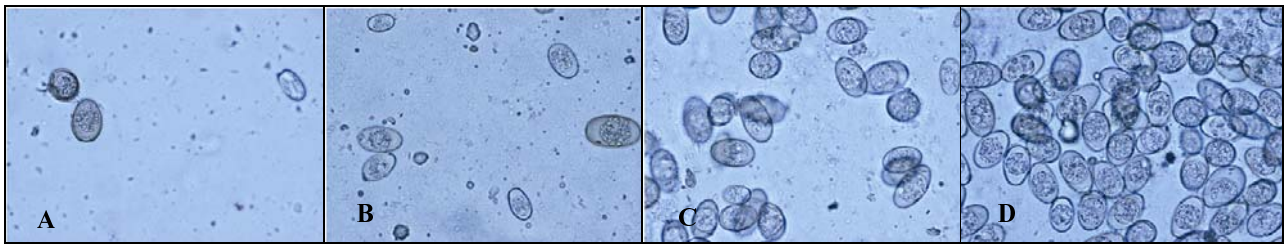

Fig. 1. ***Eimeria* oocysts in rabbit faeces**
A – light infection; B – moderate infection; C – heavy infection; D – massive infection

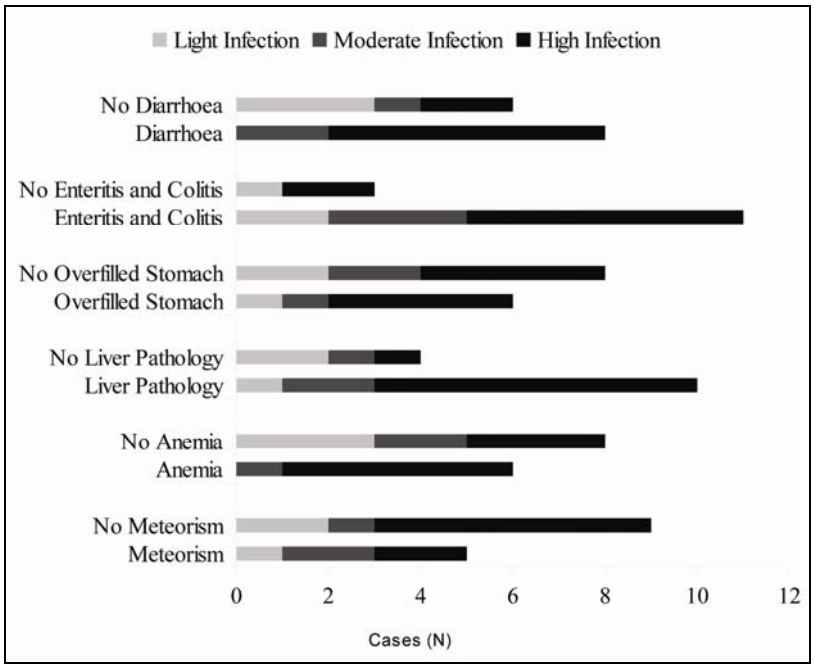

Fig. 2. **Necropsy findings in young rabbits** (N=14) **and infection intensity**
Light (1–5 oocysts per visual area (OVA)), moderate (6–10 OVA), high (>10 OVA)

Statistics were performed with R v. 2.8.0 (The R Foundation for Statistical Computing, Institute for Statistics and Mathematics, Vienna, Austria). Due to few samples classified as "heavy" infection intensity this group was combined with "massive" in the data analysis and referred henceforth as "high" infections. A generalized linear model (GLM) was used to evaluate connections between II and: presence and absence of

observations in the necropsies, and *Eimeria* sp. The age at death and days post weaning were also investigated using a GLM for the association with II.

Results. Necropsies. Enteritis, colitis, liver pathology and diarrhoea were observed in the majority of necropsied rabbits while meteorism, anemia, and overfilled stomachs were found in fewer cases (Fig. 2). Higher II (P=0.04) were found in animals that were also diarrhoeic. There were no statistical evidence that enteritis, meteorism, or overfilled stomach were associated with II, but liver pathology (P=0.09) and anemia (P=0.07) showed tendencies towards being related with heavier infections. The GLM indicated that the older the animal was at the time of death the higher II was observed (P=0.02). The same increase in II was observed for increasing numbers of days post weaning (P=0.01).

All faecal samples obtained from the intestines contained *Eimeria* oocysts. Ten species were identified and dominated by *E. media* (73%) and *E. magna* (67%). Over half (53%) of the samples had high II, and otherwise light (27%) to moderate (20%). The majority (80%) of samples contained at least one of the pathogenic species and were found in all samples with high infections of the 53%. Only findings of *E. irresidua* in the intestinal contents could be associated with higher II (Table 1).

Cage samples. *Eimeria* oocysts were found in 89% of samples collected from cages. Infections were mostly light (48%) and with only few (19%) high II. A large proportion of samples contained a least one pathogenic species (69%) and were found in all of the high infections (19%). *Eimeria magna* (48%) dominated the samples followed by *E. media* (37%). Several species were associated with higher II: *E. magna*, *E. media, E. perforans*, *E. piriformis,* and *E. exigua* (Table 1).

Table 1. **Species and infection intensities found in the intestines of dead rabbits and faecal samples collected from cages of a pre-industrial Estonian farm in 2011**

Species	Intestinal contents (N=15)			Faecal samples, cages (N=65)		
	N	% [95% CI]	P	N	% [95% CI]	P
E. coecicola	0	0 [0-18]	NA	4	6 [2-14]	0.980
E. exigua	3	20 [5-45]	0.890	21	32 [22-44]	0.050
E. intestinalis	2	13 [2-38]	0.660	4	6 [2-14]	0.090
E. irresidua	6	40 [18-66]	0.004	14	22 [13-33]	0.254
E. magna	10	67 [41-87]	0.160	31	48 [36-60]	0.000
E. media	11	73 [48-91]	0.500	24	37 [26-49]	0.000
E. perforans	5	33 [13-59]	0.700	17	26 [17-38]	0.003
E. piriformis	5	33 [13-59]	0.100	10	15 [8-26]	0.020
E. stiedae	2	13 [2-38]	0.660	12	18 [10-29]	0.290
E. vejdovskyi	1	7 [0-29]	0.140	16	25 [15-36]	0.200
Infection Intensity						
None	0	0 [0-18]		7	11 [5-20]	
Light	4	27 [9-53]		31	48 [36-60]	
Moderate	3	20 [5-45]		15	23 [14-35]	
High	8	53 [29-77]		12	19 [10-29]	
Evidence of coccidiosis						
>0 pathogenic species	14	93 [71-100]		44	68 [56-78]	
>0 pathogenic species and high infection	8	53 [29-77]		12	19 [10-29]	
>0 pathogenic species and moderate to high infection	11	73 [48-91]		26	40 [29-52]	
>0 pathogenic species, high infection, and diarrhoea	6	40 [18-66]		-	-	
>0 pathogenic species, moderate to high infection, and diarrhoea	8	53 [29-77]		-	-	

Mid-P exact 95% CI. Pathogenic species: *E. magna, E. media, E. piriformis, E. intestinalis, E irresidua, E. stiedae*. Infection intensity: Light (1-5 oocysts per visual area (OVA)), Moderate (6-10 OVA), high (>10 OVA). P-values indicate the linear fit of the presence of the *Eimeria* species with increasing infection intensity

Discussion. Necropsies were performed to provide evidence of eimeriosis being the cause of the deaths. Severity of the eimeriosis depends on age, species of *Eimeria* involved, the number of oocysts eaten by the animal, and the immune status especially around weaning (Pakandl, 2009).

All autopsied rabbits were infected with *Eimeria*. Almost all (93%) necropsied rabbits contained at least one species considered as a pathogenic species of *Eimeria* and about half (53%) of the samples were also scored as high

infections. The most commonly found species were *E. media* and *E. magna.* Animals with high infections also were found to be more frequent to have diarrhoeic contents and contain *E. irresidua* in the diverse mix of species. Signs of liver pathology and anemia also showed tendencies of being associated with increasing II, but the evidence may have been weakened by the small sample size. Hepatic eimeriosis could be ruled out in this outbreak due to the very low presence of *E. stiedae* and significance of associated liver pathology.

It has been reported that in French industrial rabbit farms only *E. magna*, *E. media*, and *E. perforans* are observed (Pakandl, 2009). The diverse combination of pathogenic species observed in the Estonian farm may indicate that several pathogenic species were present but *E. irresidua* may have been new to the farm and possibly caused more severe symptoms in the rabbit population if previously unexposed. Mainly younger rabbits found dead were examined but one adult was also submitted for investigation. This rabbit had a low II of *E. perforans* and *E. intestinalis* and may have died from different reasons than eimeriosis. These combined evidences indicate that eimeriosis is a likely cause of the mortalities in young rabbits.

The prevalence of *Eimeria* in pooled samples taken from cages of rabbits was 89% dominated by light infections (48%). Cage samples also had a majority of samples with at least one pathogenic species (68%) but few classified as high infections (19%). The species composition were more clearly dominated by the presence of *E. magna,* and *E. media* than in necropsies, but *E. magna* was more commonly found of the two species in the samples from cages. The dominating species, in addition to *E. exigua, E. perforans*, and *E. piriformis*, were also more frequently found in samples with larger II. It is thus clear that the rabbits' living environment was contaminated with a diverse combination of slight to moderately pathogenic species.

Coudert and colleagues (1995) have stated that there is no correlation between oocyst excretion and infection dose. However, the minimum dose for getting the maximum yield of oocysts shed in the faeces was estimated as: 80 for *E. magna*, 100 for *E. flavescens* and *E. irresidua*, and 200 for *E. media* and *E. perforans* (Coudert et al., 1995). Other species require >1000 oocysts. This taken into account the picture of species observed may persist even despite hygiene measures as very small infection doses would be required to spread the infections anew. The farms level of hygiene, cleaning 1–2 times a year, is insufficient to control pathogens such as *Eimeria*. Oocysts should be reduced in numbers before they are sporulated to ensure that the unavoidable infections are mild and provide the rabbits with a natural immunity.

We found a connection between increasing II and the age of the rabbits as well as the age at weaning. Rabbits under the age of 20 days is generally considered not be infected with coccidia (Coudert et al., 1991; Pakandl and Hlásková, 2007). Clinical eimeriosis mainly occurs only in young rabbits, frequently at the age of three weeks to two months, whereas adults are mostly asymptomatic (Bhat et al., 1996). Rabbits found dead were aged from 48 to 91 days and thus were beyond the critical age when normally expected to succumb to eimeriosis.

It is speculated that the main source of infection for young rabbits are infected adult rabbits excreting *Eimeria* oocysts. Though one 54 days old rabbit was found in the cage with its mother before weaning most lethal cases occurred several weeks after weaning and relocation. This indicates that the living environment may have played a larger role in infecting the weaned rabbits and was the cause of higher II in older rabbits. In the studied farm, most of the faeces would drop out the cage due to the net wiring of the bottom. Contact of faeces material containing *Eimeria* oocysts with cage parts may be sufficient contamination for eventually infecting new animals; especially if accumulating over months as in this case. Faeces may also reach rabbits through the contamination of food, water, tools used for cages, and coprophagia.

Due to anatomical peculiarities of the rabbit stomach, the main factor pushing feed masses into the intestine is forage (Barone, 1997). If the ration consists only or mostly of granules, as in this case, food does not move out of the stomach. This may explain why we observed overfilled stomachs in 43% of the young dead rabbits. Though this finding in the necropsy was not statistically significant, it is possible that it could have added an additional stress to the infected rabbits with these symptoms.

The change and implementation of the food composition and the maturation of the immune response are also important factors in young rabbits after weaning (Pakandl, 2009). The weaned rabbits can be at high risk of coccidiosis when introduced into an environment containing sporulated oocysts. One explanation for the timing of mortalities may thus be that rabbits have continued to pick up different pathogenic species of *Eimeria* in the matter of 1–3 weeks post weaning and eventually succumbing to the physical stress of the consequent infections acquired.

The farm was recommended to introduce rapid control measures against eimeriosis. Recommendations covered treatment and metaphylactic embedding of anticoccidials, big dry food content in the ration, partition walls between cages, and no less strict prophylactic measures: thorough cleaning of cages, feeding, and watering equipment using a torch and disinfectants with high anticoccidial activity.

In conclusion, we found evidence of rabbit eimeriosis in the farm. There was evidence of pathogenic species in cages examined despite wired bottoms. Dead rabbits were generally diarrhoeic with high II. *Eimeria irresidua* was the only species in dead rabbits to be associated with high II and may have played a role in the outbreaks of mortalities. Higher age and post-weaning time also were associated with higher II in dead young rabbits. The assisting factor in the outbreak is likely insufficient hygiene of cages used for the post-weaned rabbits.

Acknowledgements

The present work was carried out on the basis of job contract Nr 2 between The Institute of Veterinary Medicine and Animal Sciences (Estonian University of Life Sciences) and University of Tartu, Estonia.

References

1. Baker D. G. Flynn's Parasites of Laboratory Animals. Second Edition. Blackwell Publishing. 2007. 813 P.

2. Barone R. Anatomie comparée des mammifères domestiques. T3: Splanchologie 1: Appareil digestif. Appareil respiratoire. Paris: Vigot. 1997. 329–331.

3. Bhat T. K., Jithedran K. P., Kurade N. P. Rabbit coccidiosis and its control: a review. World Rabbit Science. 1996. 4. P. 37–41.

4. Coudert P., Licois D., Drouet-Viard F. *Eimeria* species and strains of rabbit. In: Eckert, J., Braun, R., Shirley, M. W., Coudert, P. (Eds.), COST. 89/820. Biotechnology: Guidelines on Techniques in Coccidiosis Research. Office of Official Publications of the European Communities, Luxembourg. 1995. P. 52–73.

5. Coudert P., Licois D., Provôt F., Drouet-Viard F. Mammalian coccidiosis: natural resistance of suckling rabbits. 2nd Conference COST-Action 1989, 2–5 April 1991, Münchenwiler, Switzerland.

6. Kvičerová J., Pakandl M., Hypša V. Phylogenetic relationships among *Eimeria* spp. (Apicomplexa, Eimeriidae) infecting rabbits: evolutionary significance of biological and morphological features. Parasitology. 2008. 135. P. 443–452.

7. Pakandl M. Coccidia of rabbit: a review. Folia Parasitologica. 2009. 56. P. 275–280.

8. Pakandl M., Hlásková L. The reproduction of *Eimeria flavescens* and *Eimeria intestinalis* in suckling rabbits. Parasitol. Res. 2007. 101. P. 1435–1437.

9. Pan B.L., Zhang Y.F., Suo X., Xue Y. Effect of subcutaneously administered diclazuril on the output of *Eimeria* species oocysts by experimentally infected rabbits. Vet. Rec. 2008. 162, P. 153–155.

10. Pellérdy L.P. Coccidia and Coccidiosis. Akadémiai Kaidó, Budapest. 1974. 959 PP.

11. Roepstorff A., Nansen P. Epidemiology, diagnosis and control of helminth parasites of swine. FAO Animal Health Manual. FAO, Rome. 1998. P. 38–41.

12. Taylor M. A., Coop R. L., Wall R. L. Veterinary Parasitology. Third Edition. Blackwell Publishing. 2007. 874 p.

Received 15 June 2012
Accepted 2 October 2013