CONFERENCE PROCEEDINGS
OF 4TH INTERNATIONAL
TECHNICAL CONFERENCE

EIMERIANA AVIA

Managing coccidiosis and other invasive poultry diseases – challenges for today and tomorrow!

16 – 17 February 2024 r.

Hotel Windsor Palace in Jachranka near Warsaw

In Memoriam

prof. dr hab. dr *h.c.* Michał Mazurkiewicz

2024

EIMERIANA AVIA

Conference proceedings of

4th International Technical Conference

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Department of Bird, Exotic Animal and Fish Diseases, the Department of Veterinary Pathology and Diagnostics, the Institute of Veterinary Medicine of the Warsaw University of Life Sciences

Department of Bird, Exotic, Fur and Laboratory Diseases of the Faculty of Veterinary Medicine, Wrocław University of Environmental and Life Sciences

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Foreword

Ladies and Gentlemen!

On behalf of the Organisers, the Department of Bird, Exotic Animal and Fish Diseases, the Department of Veterinary Pathology and Diagnostics, the Institute of Veterinary Medicine of the Warsaw University of Life Sciences and the Department of Bird, Exotic, Fur and Laboratory Diseases of the Faculty of Veterinary Medicine, Wrocław University of Environmental and Life Sciences, we would like to warmly welcome you - all participants of the 4th International Technical Conference *Eimeriana Avia*[@] "Challenges in the management of coccidiosis and other invasive poultry diseases - today and tomorrow!"

Our Conference was preceded by very popular specialised workshops, which were successfully held on February 15, 2024 at the campus of WULS.

On 28 February 2024, 11 years will have passed since the unexpected passing of the late Professor Michał Mazurkiewicz, a scientist highly distinguished for the development of Polish poultry pathology. In the national community of aviopathologists, the Professor is remembered not only by his worthy successors from the Wrocław institution who continue his work, not only by those who had the privilege of knowing Professor Mazurkiewicz personally, but also by the youngest generation of aviopathology specialists, to whom the profile of this outstanding researcher is introduced during specialisation classes. We also talk about the Professor to our students at all veterinary faculties. In particular, *Eimeriana Avia*[@] project from 2016 year, keeps alive the memory of Prof. Ph.D. dr *h.c.* Michał Mazurkiewicz, who was a pioneer of research on the prevention of coccidiosis in poultry in the country.

The author of the *Eimeriana Avia*[@] project is Prof. Ph.D. Piotr Szeleszczuk. This initiative aims to create a national platform for discussion on the broad issues of coccidiosis and other practically important invasive bird diseases.

The project assumes conducting cyclical Technical Conferences organised in the second half of February alternately by the Department of Bird, Exotic, Fur and Laboratory Diseases of the Faculty of Veterinary Medicine of the University of Life Sciences in Wrocław and the Department of Bird, Exotic Animal and Fish Diseases of the Institute of Veterinary Medicine of the Warsaw University of Life Sciences. The first conference of the *Eimeria Avia@* project took place on 26-27 February, 2016 in Wrocław and ended with a great substantive and organisational success. The meeting participants were excellent lecturers and the best national specialists dealing with poultry diseases.

After two years, *Eimeriana Avia@* II took place on March 2-3, 2018 in Warsaw. The coordinators of the organisational activities of this meeting were Prof. Ph.D. Piotr Szeleszczuk and Ph.D. hab. Andrzej Gaweł. The next third conference meeting of the project took place on February 20-22, 2020 in Wrocław. It also ended with great training and organisational success. According to the adopted rule, after the break resulting from the COVID pandemic, the next 4th conference, organised by the Warsaw University of Life Sciences, will take place at the **Windsor Palace hotel in Jachranka**.

The main goal of the project is to increase knowledge about coccidia invasion in birds among scientists, veterinary practitioners, zootechnical services, the broadly understood feed industry, poultry producers, pigeon and pet bird breeders.

The specific mission of the *Eimeriana Avia@* project is to promote activities aimed at reducing losses caused by coccidiosis in intensive poultry production. However, the need of the hour and the topicality of the problems caused the organisers to expand the conference topic to include issues related to other invasive bird diseases.

It is obvious that in the first article of the conference proceedings we emphasise the merits of the late Professor Michał Mazurkiewicz. Prof. Andrzej Gaweł, the professor's student, recalls his life, work and merits in his lecture.

It is with great pleasure that we also present to you our outstanding authors who gave us the honour of accepting the invitation to participate in the Conference.

A very great honour for the Organisers is the presence at the Conference of Professor Damer Blake from the Royal Veterinary College, University of London, the world's most outstanding young generation specialist in the field of research on poultry coccidia.

The group of foreign lecturers was enriched by such outstanding professionals as Laure Bignon, Jana Brabcová, Philoppos Fidiarakis, Gulgielmo Gallina, George Gould, Corrado Longoni, Luis Pantoja Millas, Jan van Spil and Monita Vereecken. This excellent line-up of lecturers was also complemented by the most competent Polish authors: dr inż. Paulina Abramowicz-Pindor, lek. wet. Agnieszka Chłodowska, mgr Żanetta Chodorowska, prof. dr hab. Andrzej Gaweł, mgr inż. Wojciech Gbiorczyk, prezes Dariusz Goszczyński, dr wet. Piotr Kwieciński, dr hab. Małgorzata Olejnik, dr Monika Roczeń-Karczmarz, lek. wet. Monika Rogala Hnatowska, dr inż. Natalia Sobczak- Zuzaniuk, dr Barbara Szczepankiewicz, prof. dr hab. Krzysztof Tomczuk, lek. wet. Michał Turek and Ph. D. hab. Bartłomiej Tykałowski.

We would like to thank everyone who participated in the organisational work of our Conference. I express my special thanks to our outstanding foreign and domestic lecturers for their efforts in preparing materials and giving presentations. The organisers hope that you will find the meeting programme interesting and useful.

We are also convinced that the Conference will be an excellent opportunity to exchange views on the practical aspects of reducing losses caused by coccidiosis, histomoniasis, invasions of mites, and intestinal worms.

We also thank the conference sponsors because without their kind support, our meeting would not be possible. We would especially like to thank the Platinum Sponsors companies: DSM Firmenich and ELANCO AH for their committed co-organisation of our meeting, and not less warmly the Diamond

Sponsors companies: Adifeed, CCPA, Hipra, Huvepharma, MSD, and Zoetis. Our donors are also the Silver Sponsors companies: Addicoo, Biopoint, Intermag, Phibro, and Ravet.

On behalf of the Organisers, we wish all Conference Participants fruitful deliberations and pleasant experiences during their stay at the hospitable Windsor Palace hotel in Jachranka. We hope that our conference will be a useful professional experience and will help you solve problems in the care of poultry flocks.

On behalf of the Organisers

Prof. dr hab. Andrzej Gaweł ***Prof. dr hab. Piotr Szeleszczuk***

Warsaw, 16-17 February 2024

Andrzej Gaweł

Zakład Chorób Ptaków, Zwierząt Egzotycznych, Futerkowych i Laboratoryjnych Wydziału Medycyny Weterynaryjnej Uniwersytetu Przyrodniczego we Wrocławiu

PROF. PH.D. DR H.C. MICHAŁ MAZURKIEWICZ (1941 – 2013) HIS LIFE AND WORK

February 29, 2024 will be the 11th anniversary of the death of the outstanding Polish aviopathologist, Professor Michał Mazurkiewicz (8). Over the 10 years since the Professor's funeral, a new generation of veterinarians who no longer remember him have begun to actively practice their profession. Professor Mazurkiewicz's name is most often associated with the coursebook "Poultry Diseases", which is fundamental for Polish poultry pathology (2,3,4). It is therefore not surprising that many young doctors do not realize what a significant impact Professor Michał Mazurkiewicz had on the development of knowledge and unification the community related to poultry pathology. The Eimeriana Avia project, created by Professor Piotr Szeleszczuk, in memory of Professor Michał Mazurkiewicz, enables the expansion of knowledge in the field of parasitic diseases and the integration of the community of veterinarians, aviopathologists, zootechnicians and people associated with the broadly understood poultry industry, just as our Master did (1 ,5,6).

Michał Mazurkiewicz was born in Łówcza, in the Podkarpackie Voivodeship, on April 10, 1941. He completed his veterinary studies at the Veterinary Faculty of WSR in Wrocław, obtaining a veterinary diploma in 1966. In 1970, he obtained a PhD in veterinary sciences based on his doctoral thesis entitled “Water and electrolyte management in chickens with

experimentally induced urate diathesis", and the academic degree of habilitated doctor in 1976 on the basis of the habilitation thesis entitled "The importance of bone blood supply in calcium metabolism in laying hens". He obtained the academic title of professor in 1983, and the position of full professor in 1991. In 1994, he was the first in the history of Polish veterinary medicine to obtain the title of specialist in poultry and ornamental bird diseases. Professor Michał Mazurkiewicz had a unique gift for winning people over and had a huge impact on uniting the poultry farming community in Poland. The professor was one of the initiators and a member of the "Wrocław poultry group" - a group of scientists that included, apart from the professor, prof. Bronisława Chełmońska (poultry breeding, reproduction), prof. Zbigniew Dobrzański (environment, zoohygiene), prof. Tadeusz Trziszka and prof. Teresa Smolińska (quality of poultry products) and prof. Dorota Jamroz (poultry nutrition). This group cooperated in the 1980s in both scientific and advisory fields, contributing to the development of Polish poultry farming.

Professor Mazurkiewicz's scientific achievements are impressive and include approximately 400 publications, including: assessment of intermediate metabolism in physiological and pathological states in poultry, optimization of poultry keeping conditions and pathogenesis, diagnosis and control of bacterial and parasitic diseases of birds, with particular emphasis on coccidiosis (7). Over the years of his scientific work, he has been the manager or main contractor of many research projects, including salmonellosis and coccidiosis. Professor Michał Mazurkiewicz spread knowledge about poultry farming, among others, by lecturing at training courses for local zootechnical and veterinary services. From 1979 to 1982, he headed the Postgraduate Study in Breeding Technology, Prevention and Control of Diseases in Large-scale Poultry Farming, and also gave lectures and supervised the completion of diploma theses. The professor

supervised 16 doctoral theses and actively participated in the specialization education of veterinarians as the National Head of the Specialization in Poultry and Ornamental Birds Diseases.

Professor M. Mazurkiewicz was continuously involved in teaching activities on poultry diseases and general epizootiology since 1966, and since 1976, in addition to classes on poultry diseases, he was also conducted lectures on this subject.

For the course, together with Professor Zenon Wachnik, he developed two editions of a textbook on poultry diseases.

In 2005, a coursebook on Poultry Diseases was published, edited by the Professor, who received the 1st degree Team Award from the Minister of the Department.

The second edition was published in 2011, and the third edition edited by Professor and prof. Alina Wieliczko – in 2019. This book is currently the basic source of current Polish knowledge in the field of poultry diseases.

Professor M. Mazurkiewicz was the Rector of the University for two terms (2002-2008), and held a number of responsible positions, including: he was the Head of the Department of Epizootiology of the University of Environmental and Life Sciences in Wrocław, Chairman of the College of Vice-Rectors of Wrocław Universities, Member of the Feed Assessment Committee, Member of the Scientific and Technical Council at the Minister of Agriculture, Forestry and Food Economy, Chairman of the Team of Veterinary Experts at the Minister of National Education, Member of the Central Commission for Degrees and Titles, Member of the Scientific Council of PIWet-PIB in Puławy, Member of the Sanitary and Epizootic Council at the Chief Veterinary Officer.

Professor M. Mazurkiewicz was a member of many professional organizations, including: World Veterinary Poultry Association, World Poultry Science Association, the Committee of Veterinary Sciences of the Polish Academy of Sciences, the Wrocław Scientific Society and the Polish Society of Veterinary Sciences PTNW. As part of the latter, from 1983 to 2003 he chaired the Poultry Pathology Committee. The result of this activity was the organization of 5 symposia and 15 scientific conferences on poultry. Moreover, Professor M. Mazurkiewicz was a co-organizer of the 10th edition of the International Congress PRO ANIMALI ET HOMINE (1994-2003) and 5 cyclical International Conferences UNA MEDICINA UNA HYGIENA (2006-2010).

The Professor's work has been appreciated many times, as evidenced by numerous distinctions and awards. Professor M. Mazurkiewicz was awarded: Srebrny Krzyż Zasługi (1975), Medal Komisji Edukacji Narodowej (1985), Krzyż Kawalerski OOP (1986), Brązowy Medal „Za Zasługi w Obronności Kraju" (1983), Badge „Zasłużony dla Środowiska Akademickiego Wrocławia" (1984), Badge „Zasłużony dla Województwa i Miasta Wrocławia" (1985), Badge „Za Zasługi dla Województwa Legnickiego" (1985), Badge Honorową „Zasłużony Pracownik Rolnictwa" (1985, 2003), Badge „Zasłużony dla Przemysłu Paszowego" (1988), Złota Honorowa Odznaka Zrzeszenia Lekarzy i Techników Weterynaryjnych (1988), Medals „Zasłużony dla Łowiectwa Wrocławskiego" (2001) and „Łowiectwa Dolnośląskiego" (2005), Honour badge „MERITUM" (2005), Medal Senatu RP (1997) and badge „Zasłużony dla PTNW" (1992), Honour badge „Pro Scientia Veterinaria Polona" (2002), and Krzyż Oficerski Orderu Odrodzenia Polski (2011).In addition, he received the Minister's Award 12 times, several awards from the Rector, a distinction from the Minister of Agriculture, Forestry and Food for scientific research, as well as 1st and 3rd degree team awards and a PTNW distinction. On May 18,

2012, he received an honorary doctorate from the Lviv National University of Veterinary Medicine and Biotechnology. Stefan Zanowicz Grzycki (former Academy of Veterinary Medicine in Lviv). Professor Michał Mazurkiewicz was an extraordinary person - committed to working for science and expanding knowledge, which he then passed on to subsequent generations of students and veterinarians, and at the same time, he was a person full of warmth and kindness for which he will be remembered by many!

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Damer P. Blake

Pathobiology and Population Sciences, Royal Veterinary College, Hawkshead Lane, North Mymms, Hertfordshire, UK

THE CHANGING COST OF COCCIDIOSIS AND THE IMPACT OF GLOBAL TRENDS

Introduction

Global production of chickens is increasing every year (FAOSTAT 2021), highlighting the importance of pathogens that can infect chickens. *Eimeria* species are parasites that can cause the enteric disease coccidiosis, most notably in chickens, where they have a huge impact on health and welfare (Chapman *et al.* 2013). Understanding the financial cost of pathogens like *Eimeria* can be helpful to inform decisions at local, national, and international levels, encouraging prioritisation of resources and permitting comparison of the relative value of different husbandry systems or approaches for control. Recently, the Williams compartmentalised model for estimating the financial cost of coccidiosis in chickens was updated to include developments such as the use of vaccination in commercial broiler chickens (Williams 1999, Blake *et al.* 2020). Using data from 2016, the global cost of coccidiosis in chickens was estimated to exceed UK£ 10.3 billion per annum (range UK£ 7.7-UK£ 13.0 billion), equivalent to UK£ 0.16 per chicken produced. Since publication of the work global markets have experienced a rapid succession of shocks including the COVID-19 pandemic, multiple wars including the conflict in Ukraine, and dramatic fluctuation in costs of oil, services such as electricity, and inflation. Under these circumstances, variation in the costs incurred by pathogens such as

Eimeria is inevitable. Here, we will explore the impact of these variables on the cost of coccidiosis in chickens. The model to estimate the cost of coccidiosis is shown in full at https://doi.org/10.1186/s13567-020-00837-2 (Blake *et al.* 2020).

Commercial trends

Chicken production in the United Kingdom of Great Britain and Northern Ireland

In 1995 the total cost of coccidiosis in chickens produced in the United Kingdom (UK) was estimated to exceed UK£ 38.5 million, primarily attributed to the effects of mortality, reduced body weight gain and compromised food conversion (Williams 1999). By 2016 this figure had increased to more than UK£ 99 million (Blake *et al.* 2020). Drivers of morbidity represented the major contribution to cost in 2016 (83.1%), including reduced body weight gain and increased food conversion ratio (FCR). Close scrutiny of costs associated with body weight gain highlights the importance of feed prices, including raw components such as wheat, maize (corn) and/or soy, varying by region. Global guide prices for wheat have fluctuated enormously in recent years, ranging from a low price of US$ 122 per metric ton in 2016 to a peak of US$ 444 during the early phase of the conflict in Ukraine (Figure 1), the latter coinciding with unusual weakness in UK sterling compared to the US dollar (US$ 1.07 per UK£ 1.00, 26th September 2022). Recalculating the cost of coccidiosis in September 2022, including the recent record peak cost of wheat and the low value of sterling against the dollar, indicated a cost of coccidiosis in excess of £123 million to the UK, 24% higher than estimated for 2016 (Figure 1).

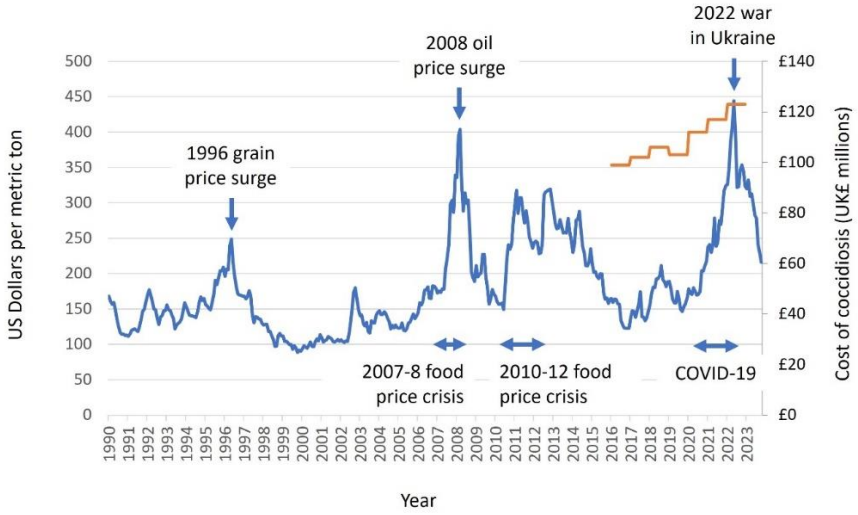

Figure 1. Global wheat price 1990-2023 (blue) and the estimated financial cost of coccidiosis in chickens in the UK 2016-2022 (orange). Wheat graph modified from www.economicshelp.org, data accessed St Louis Fed PWHEATUSDM 13th November 2023.

Other industry changes of note that were included in the updated estimate have included a trend towards heavier finishing weights in the UK, changing from an average 2.1 Kg in 2016 to 2.4 Kg in 2022 (DEFRA 2023a), and greater value of chicken meat per Kg illustrated by the UK producer price index rising from 99.3 in 2016 to 117.1 in 2022 (DEFRA 2023b). Combined, these figures indicate a higher value per chicken and a greater financial loss per individual compromised or lost due to coccidiosis.

Parasitological trends

Costs associated with utilities and raw materials required for chicken production can be clearly documented, benefitting from recent or even real time

figures, but the impact of variation in the parasite populations that cause coccidiosis can be much harder to define. For example, identification of three new *Eimeria* species that infect chickens and escape current anticoccidial vaccines indicates a risk of increased occurrence of coccidiosis (Table 1; Figure 2) (Blake *et al.* 2021). In 2016 UK producers indicated that approximately 2% of broiler flocks experience clinical coccidiosis (Blake *et al.* 2020). The appearance of *Eimeria* species that escape current vaccines would be expected to increase the occurrence of coccidiosis, as described in a case study from Australia (Morris *et al.* 2007). Currently, most broilers in the UK are raised using anticoccidial drugs, suggesting a limited impact of vaccine escape by these species, but the impact could be far greater in markets such as the US where broiler vaccination is more common or in the future should a reduction/ban on anticoccidial drug use be introduced.

Table 1. Summary of *Eimeria* species that can infect the chicken (*Gallus gallus domesticus*).

Species	Status	Gut location	Pathogenicity	Pre-patent period (h)	Oocyst size (L × W)
E. acervulina	Established	Top	++	89	18.3 × 14.6
E. brunetti	Established	Bottom	++++	120	24.6 × 18.8
E. maxima	Established	Middle	+++	120	30.5 × 20.7
E. mitis	Established	Middle	++	91	15.6 × 14.2
E. lata[1]	New	Top	+++	125-130	30.8 × 23.8
E. nagambie[2]	New	Top	+++	132	26.7 × 22.8
E. necatrix	Established	Middle*	+++++	138	20.4 × 17.2
E. praecox	Established	Top	+	84	21.3 × 17.1
E. tenella	Established	Caeca	++++	132	22.0 × 19.0
E. zaria[3]	New	Top	++	130-135	17.7 × 15.2

[1]Formerly known as OTU-X. [2]Formerly known as OTU-Y. [3]Formerly known as OTU-Z. (Cantacessi *et al.* 2008, Blake *et al.* 2021).

*Sexual stage occurs in the caeca.

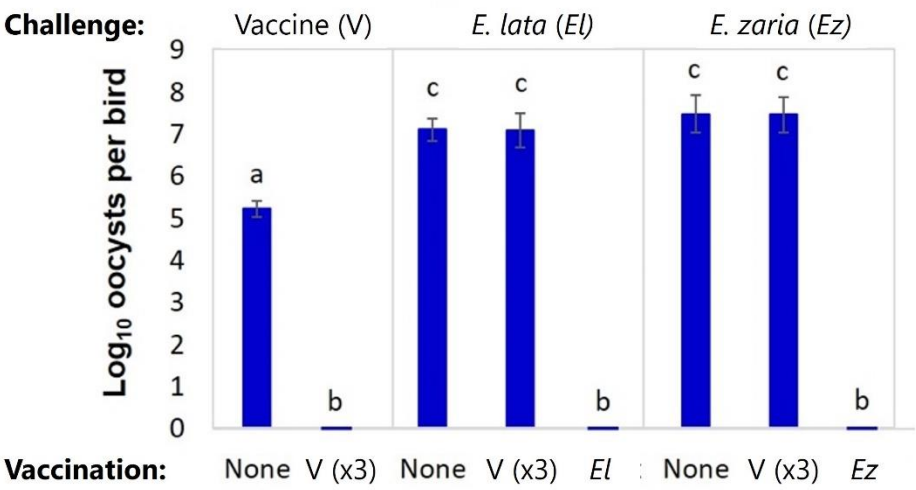

Figure 2. Current live anticoccidial vaccines do not induce immune protection against challenge by the new *Eimeria* species *E. lata* (*El*) or *E. zaria* (*Ez*). The example shown represents a European attenuated anticoccidial vaccine (V) and is comparable with results achieved using other vaccines (Blake *et al.* 2021).

Other parasitological characteristics that could influence the cost of coccidiosis include resistance to anticoccidial drugs and the introduction of new products to control infection or reduce disease, both of which would affect body weight gain and FCR during infection. Williams estimated 0.1 Kg average broiler weight loss due to coccidiosis in exposed flocks, although a conservative estimate of 0.07 Kg has been used in the model (Williams 1999, Blake *et al.* 2020). Adjusting the reduction in bodyweight gain in ± 0.01 Kg steps from 0.04 to 0.10 Kg revealed -9.0% to +6.6% changes in the cost of coccidiosis per step. Similarly, the increase in FCR due to *Eimeria* was estimated to be 0.1, with a conservative estimate of 0.05 used in the model (Williams 1999, Blake *et al.* 2020). Adjusting the change in FCR in ± 0.01 steps from 0.02 to 0.08 suggested -7.2% to +6.3% changes in the total cost of coccidiosis in the UK per 0.01

change. When scaled up to the national or international flock such changes would be worth tens of millions of pounds.

Gaps in the model

The model specifically focuses on the financial costs of coccidiosis and does not include social costs. Indirect costs including the impact of enteric dysbiosis (Macdonald *et al.* 2017), litter quality and pododermatitis, and increased colonisation and shedding of foodborne pathogens such as *Campylobacter jejuni* and *Salmonella* Typhimurium (Baba *et al.* 1985, Macdonald *et al.* 2019) are not included. Similarly, the value added by the impact of ionophores on Gram positive bacteria such as *Clostridium perfringens*, cause of necrotic enteritis, independently estimated to incur costs of US$ 6 billion per annum (Wade and Keyburn 2015), is not factored in. Calculating costs and values for many of these features will be complex, but all will influence the global cost of coccidiosis.

Conclusions

The financial cost of coccidiosis in chickens is consistently high, but can vary significantly under the influence of global commercial trends. Understanding sources of variation in the costs associated with disease can be used to improve consistency in livestock production and inform decision making.

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Piotr Kwieciński, Piotr Szeleszczuk*, Aneta Nowak**

Vet-Lab Brudzew
**Department of Diseases of Birds, Exotic Animals and Fish, Department of Veterinary Pathology and Diagnostics, Institute of Veterinary Medicine, Warsaw University of Life Sciences in Warsaw*
***5th year student at the Faculty of Veterinary Medicine, Warsaw University of Life Sciences*

THE FEDERATION OF VETERINARIANS OF EUROPE (FVE) POSITION

PAPER ON COCCIDIA CONTROL IN POULTRY

It is evident that without solving the problem of coccidiosis control, efficient large-scale poultry production could not exist. However, it has been argued for decades that some of the coccidiosis control agents (including in particular ionophore coccidiostats) used since the 1950s have had an increasingly visible effect on the development of coccidial resistance.

Considering the growing concerns among the public about the 'chemicalisation' of animal production, exactly 20 years ago, the European Union declared through Regulation 1831/2003/EC that: *'Considering of all the risks of using coccidiostats, it has been decided to ban their further addition to poultry (and other animal) feed from 1 January 2013.'* New solutions were to be developed within 10 years to make this possible. Objectively, it has to be admitted that enormous resources have been spent on the search for these solutions, but none of them have been successful enough to make the implementation of this Regulation feasible within the expected timeframe. The

European Commission's 2008 report to the Council and the European Parliament regarding the use of coccidiostats and histomonostats used as feed additives reported in accordance with Article 11 of Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrition included a very categorical statement that:

'At the present time, the use of coccidiostats as a preventive measure for the control of coccidiosis in modern poultry production is essential. This practice contributes significantly to the protection of both animal health and animal welfare by preventing a disease that is present on all farms. Production without coccidiostats in the present circumstances in Europe would be very severely economically compromised and the effect of not using coccidiostats would be to deprive EU consumers of access to poultry, turkey and rabbit meat produced according to the high EU safety and welfare standards.' On 13 November 2012, Polish Chief Veterinary Officer at that time issued an interpretation of Article 11 of Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 regarding coccidiostats and histomonostats used as feed additives in animal nutrition. This document stated that *'According to the interpretation of Article 11 of Regulation (EC) No 1831/2003 received from the Section President: Animal Nutrition of DG SANCO, the legal position resulting from Article 11 of Regulation (EC) No. 1831/2003 does not constitute a basis for the control services in Poland and other Member States to take action to eliminate these feed additives from the market and use in animal nutrition. The current wording of Article 11 of Regulation (EC) No. 1831/2003 cannot be interpreted as prohibiting the use of coccidiostats and histomonostats from 31 December 2012.'* The status quo remains unchanged to this day and, despite the declaration mentioned earlier,

after two decades coccidiostats are still a significant component of intensive poultry production management. Of course, modern strategies for the prevention of poultry coccidiosis recommend a very rational and balanced use of coccidiostats because their effectiveness is constantly decreasing.

In the EU, the production and sale of coccidiostats, premixes with coccidiostats and feed with coccidiostats are regulated by Regulation (EC) No 183/2005 of the European Parliament and of the Council of 12 January 2005 establishing requirements for feed hygiene. There are currently 11 different coccidiostats available in Europe with 28 granted authorisations for use as feed additives. It is noteworthy that, as opposed to the EU, in the United States, ionophore coccidiostats are not allowed for use in the 'No Antibiotics Ever' (NAE) and 'Raised Without Antibiotics' (RWA) programmes. US as a trendsetter in industrial poultry technology for more than 70 years does not use coccidiostats in approximately 50% of its reared broiler chicken flocks (The Poultry Site, 2019). Therefore it seems that until effective alternatives to chemoprevention of coccidiosis are developed, formal action is needed to allow the judicious use of coccidiostats as part of programmes to reduce the build-up of antimicrobial resistance. The scientific community, the associations of practising aviopathologists (Position paper of the working group anticoccidials of the PVSG concerning the phasing out of anticoccidials as mentioned in EU Regulation 1831/ lack of 2003 of 21 06 2021), as well as organisations representing the veterinary community are speaking out on this issue. Among these opinions, the Federation of Veterinarians of Europe (FVE) position is discussed particularly widely. A first position on coccidiostats was published by the FVE as long as eight years ago (FVE/15/doc/040 Adopted, Marche-en-Famenne, 3 June 2016). Based on a risk analysis of the use of coccidiostats in intensive poultry production, FVE recommends that coccidiostats should be

used as veterinary prescription products. ,This would allow the farm veterinarian to choose the best strategy to phase out the use of coccidiostats in the long term, and in the meantime prolong the lifespan of coccidiostats, minimising resistance. Moreover, feedback should be provided on any adverse effects observed, including lack of efficacy, and compliance with withdrawal periods should be ensured. The FVE recommends the inclusion of coccidiostats in the ESVAC (The European Surveillance of Veterinary Antimicrobial Consumption) monitoring system to ensure that transposition into the new legal system is carried out in a way that maintains the availability of these products on the European market.

In 2022, this position was updated (FVE/22/doc/028_adopted November 2022) based on new published scientific research and a new legal framework, as from 28 January 2022, the Regulation of the European Parliament and of the Council (EU) of 11 December 2018 is explicitly and directly applicable in all Member States of the European Union. No. 2019/6 on veterinary medicinal products and repealing Directive 2001/82/EC approved on 28 January 2019. This EU Regulation, commonly referred to as the 'new veterinary pharmaceutical law', regulates i.e. the issues of authorisation, manufacture, distribution, control, and use of medicinal products for animals.

The Federation of European Veterinarians (FVE) position paper on coccidia control in poultry, quoted in detail in our review, begins with a detailed summary: *"Coccidiosis is a parasitic disease, which is ubiquitously prevalent in all poultry production systems worldwide. Even where the sanitary and management standards are high coccidial infections can occur with a serious potential impact on animal health and welfare. Therefore, effective long-term management of coccidia is indispensable, through a combination of holistic*

flock health management, optimised stocking density, litter management, feeding and drinking regime as well as nutraceuticals, accompanied by appropriate biosecurity measures, vaccination and coccidiostats, where indicated. In European legislation, coccidiostats or anticoccidials are categorised either as feed additives or as veterinary medicinal products, depending on their pharmacologically active substance, mode of action, pharmaceutical form, target species and route of application. Challenges in coccidia control are due to parasitic and bacterial drug (cross-)resistance. Coccidiostats also interact with other veterinary medicinal products and have a secondary residual activity against gram-positive bacteria. Regular monitoring of performance and parasitic burden at flock level has been a fundamental part of developing rotational and alternative strategies which have helped to maintain the effectiveness of these medicinal products in the field. A standard procedure/ guideline for such monitoring should be developed by e.g. EFSA, to enable rapid and low-cost national and regional monitoring. Especially quantitative tests would be beneficial for ongoing surveillance and monitoring purposes. Though there is no legislative requirement for veterinary supervision of in-feed coccidiostats, FVE strongly believes that it is of paramount importance to improve veterinary oversight of coccidiostat use in poultry production to further strengthen the prudent and responsible use of coccidiostats. FVE recommends that monitoring of polyether ionophores coccidiostats sales and potentially use should be included in the ESVAC system. However, the in-feed or in-water use of coccidiostats or anticoccidial medicinal products remains for the time being a necessary option for rearing of short-living birds such as broiler chickens in the EU due to their short grow out and for turkeys due to the unavailability of an EU-licensed vaccine. Feed containing coccidiostats must always be labelled in a clear and comprehensive manner,

including for hobby farmers, to allow for immediate identification of the pharmacologically active ingredient, its concentration and withdrawal period."

"Coccidiostats use requires veterinary supervision"

FVE recommends that:

- Decisions on the most appropriate, efficacious and safest coccidiosis control options should be elaborated between the supervising veterinarian and the poultry farmer formulating a medium to long-term strategy
- based on comprehensive and continuous on-farm surveillance of excretion levels in each flock
- by using firstly all appropriate strategies in the toolbox for coccidiosis control including flock health management, appropriate biosecurity measures, vaccines, nutraceuticals, as well as coccidiostats and anticoccidials prudently and responsibly, only where indicated.
- based on veterinary examination, diagnosis and/or supervision prior to use of a feed additive by the veterinarian in charge who can check interactions with other medications and liaise - if necessary - with the feed mill prior to the supply of feeds containing coccidiostats.
- In production units where in-feed coccidiostats are the norm rather than the exception and a relevant vaccine can be provided, it is highly advisable to vaccinate against coccidiosis.

Moreover:

- The development of rapid, low-cost and especially quantitative diagnostic tests for ongoing surveillance and monitoring purposes should be promoted.
- EU-licensed anticoccidial vaccine for other poultry species than chicken, most importantly turkey, should be marketed. Monitoring of polyether ionophores coccidiostats sales should be included in the ESVAC (European Surveillance of Veterinary Antimicrobial) system.
- Feed containing coccidiostats must always be labelled in a clear and comprehensive manner, including for hobby farmers, to allow for immediate identification of the pharmacologically active ingredient, its concentration and withdrawal period.

The position begins with background information where the authors of their report recall that: *"Coccidiosis is a universally seen parasitic disease in modern livestock husbandry and without doubt the most important parasitic disease in poultry. It is also of major importance in other species such as rabbits, ruminants and pigs. The infection of the intestinal tract is caused by a family of single celled obligate intracellular parasites, and affects all livestock species as well as wildlife and companion animals. The most common genera affecting livestock are Eimeria spp., which are highly host-specific and has a specific site of development in the intestine [1,2]. E. necatrix and E. tenella are the most pathogenic in chickens, E. adenoides and E. meleagrimitis are considered most pathogenic in turkeys [3]. After ingestion of infective oocysts,*

the parasite penetrates the intestinal mucosa or epithelial cells of the host and starts to multiply within 4-7 days, during which damage develops to the (sub)mucosal tissues of the intestine. Oocysts develop and are discharged in the faeces. The extent of the intestinal damage is a consequence of the coccidial species infecting the host, the host immunity system and the level of exposure. Clinical signs of coccidiosis develop due to the intestinal damage. Clinical coccidiosis is most prevalent after ingestion of relatively large numbers of sporulated oocysts under imperfect sanitary conditions, e.g., contaminated environment, and stressors such as high stocking density [3]. In addition, mucosal damage caused by coccidia predispose to the development of necrotic enteritis in chicken. Mortality concurrently infected with Eimeria species was 25% higher than in those affected by necrotic enteritis alone [4]."

With the following paragraph succinctly discussing the diagnostic problems of this invasion, as *"The clinical signs of coccidiosis may or may not be accompanied by large numbers of oocysts being shed in the faeces. Currently the most commonly used diagnostic methods are oocyst counts and lesion scoring of freshly dead carcasses, but rapid alternative methods have been developed as well [5–7]. More rapid, low-cost and especially quantitative diagnostics tests such as rt-qPCRs would be beneficial for ongoing surveillance and monitoring purposes. Anticoccidial sensitivity testing is available, and is beneficial to monitor sensitivity levels of field and vaccine strains as well as efficacy testing of drug for regulatory purposes. It has however it limitations as it requires laborious in-vivo experimental inoculation in the target species and consequently necropsies [8–10]. Therefore, routine testing for sensitivity in field isolates has only begun in recent years [11,12]."*

While presenting the position developed by FVE experts, a review of available means for coccidiosis control was conducted.. The paper highlights that: *"Coccidiosis control is of paramount importance and based on limiting the intake of sporulated oocysts by susceptible individuals so that a subclinical infection is established to induce immunity but not clinical signs. Best feeding and watering practices and good flock health management, including temperature, light, litter, air, stocking density and disease control for immunosuppressive diseases such as Marek's, contribute to this goal. Whilst there is no specific requirement under feed additives legislation for a veterinary examination and/or oversight prior to use of a feed additive in poultry production, it is best practice for the supervising veterinarian to liaise with the poultry farmer and feed mill to develop a coccidiostat programme prior to the supply of feeds containing in-feed coccidiostats. Decisions on the most appropriate, efficacious and safest coccidiosis control strategy should be elaborated between the supervising veterinarian and the poultry farmer formulating a medium to long-term strategy based on comprehensive and continuous on-farm surveillance of excretion levels in each flock, implementing firstly all strategies in the toolbox for coccidiosis control including flock health management, appropriate biosecurity measures, vaccines, nutraceuticals, as well as prudent and responsible use of coccidiostats and anticoccidials, where indicated."*

The report highlighted that new groups of nutraceuticals and agents for the destroying the oocysts in the environment (disinvasion) have appeared on the market, such as: *"phytochemicals (e.g., plant extracts), and probiotics due to their capacity to diminish oocyst burden and improving intestinal integrity [13,14]. When applied in the proper feeding period, probiotics, natural herbal extracts with bioactive molecules (i.e., saponins, artemisin, and curcumin) and*

short chain fatty acid (SCFA) such as coated butyrate, and threonine (an essential amino acid) were shown to support chicken resilience during coccidiosis infection [15]. Results of nutritional interventions like medium chained fatty acid additives and sophorolipids were promising to decrease intestinal lesions and improve feed conversion rates (FCR) in combination with coccidiosis vaccines [16,17]. It was shown that supplementation of organic acids significantly increased body weight gain, improved feed conversion ratio (FCR), reduced lesion scores and oocyst shedding[18]. Many phytochemicals that contain natural active compounds are now commercially available to assist coccidia control [19,20]. Nonetheless, and even where hygiene and management standards are high, coccidiosis can occur with a serious potential impact on animal health and welfare and potentially high mortality rates as protozoal oocysts are highly resistant in the environment. Therefore, proper sanitation and disinfection protocols are essential to lower the oocyst burden. Ammonium hydroxide as cleaning agent and sanitizer inactivates coccidial oocysts which are resistant to most standard chemical disinfectants. Halogens as strong oxidising agents in high concentrations, ozone and halogenated phenols are efficient as well [21]."

The Federation emphasises that: *"Alternative preventive ways such as vaccination are available for some species, especially for chicken. Current commercial vaccines consist of live, sporulated oocysts of the various coccidial species administered at low doses to stimulate the development of immunity [2]. Modern anticoccidial vaccines are intended for day-old chicks and can be applied to chicks either via semi-automatic applicators which delivers coarse sprays or gel drops onto the chicks in the crate or box to ensure uniform application. Manual application as well as application via feed or drinking water are also possible but harbour a higher risk for non-uniform application*

and may result in a sub optimal immune response by the flock. An indicator (food grade dye or milk) should be added to the vaccine solution to allow for vaccine uptake monitoring and increased preening [22]. Live vaccines serve to introduce a low dose of fully susceptible oocysts and chickens are re-exposed to the vaccine strain through their excretion, further stimulating increasing their level of immunity [23]. Depending on the strain, two to three cycles of re-ingestion are necessary to achieve the best possible immunity. During this period, it is important to limit possible stressors, avoid antibiotics with a residual activity against Eimeria and any anticoccidials and feed containing anticoccidials. This highlights the importance of veterinary oversight and education in relation to implementation and monitoring of vaccination programmes. Feed containing coccidiostats must always be labelled in a clear and comprehensive manner, including for hobby farmers, to allow for immediate identification of the pharmacologically active ingredient, its concentration and withdrawal period. Monitoring the development of the immunity should be done by determining oocyst burden per gram of faeces during the first 4 weeks post-vaccination. Although anticoccidial drugs have been preferred for protection of poultry for many years, vaccination programmes are gaining popularity, especially in long-living poultry such as breeding stocks and layers and in organic farming [23,24]. Although experience from organic farming and form certain conventional farming in certain countries, i.e. Norway demonstrates the possibility to manage vaccination programmes in broiler chickens, the short grow out of broiler chickens hampers vaccine use [25]. Better administration techniques, formulations, higher concentration, and tailored choice of Eimeria strains must be considered to improve the feasibility of vaccination in broiler chickens in the future. In addition, the importance of the cell-mediated immunity against

coccidiosis has to be considered [26,27]. For example, novel in ovo vaccines delivered promising results to pass maternal antibodies to their offspring [28–30]. Though marketed in other regions in the world, there is no EU-licensed vaccine for turkey which is a major drawback. Multiepitope antigen proteins are the most recent potential vaccine candidates [31].”

The authors of the analyzed Federation's position briefly described coccidiostats acting in specific stages of the parasite's life cycle or exerting their effects at multiple stages of the developmental cycle, concluding that: *“Coccidiostats or anticoccidial drugs act at specific times during the life cycle of the parasite, or exert their effects at several phases. Coccidiostats can act on extracellular stages (sporozoites and merozoites) to prevent penetration of cells or on the intracellular stages to stop or inhibit development, and a few anticoccidials affect the sporulation of oocysts after they are excreted. All coccidiostats inhibit reproduction and do not fully eliminate the parasite from the intestine of the animal. Administration of in-feed coccidiostats is recommended when animals even under best management regimens can be predictably expected to develop clinical coccidiosis and other measures are unable to limit clinical signs but should never be the norm. Coccidiostats can be grouped into two major classes, namely polyether ionophores (i.e. monensin, lasalocid sodium, maduramicin, narasin, salinomycin, semduramicin) and the synthetic products not of an ionophoric nature (decoquinate, robenidine hydrochloride, amprolium, halofuginone, diclazuril, toltrazuril, nicarbazin and sulfonamides) as well as combinations of different classes (i.e. narasin and nicarbazin, sulfonamides with trimethoprim, ormetoprim or pyrimethamine) and act on different stages of the lifecycle. Polyether ionophores are by far the most widely used coccidiostats. They have some residual antimicrobial activity against gram-positive bacteria, and aid in controlling simultaneously*

pathogens such as Clostridium perfingens [32]. Recently, targeted studies are divided on how in-feed coccidiostat use contributes to economically sustainable animal production, particularly on the long term [12,33,34]. In Norway, narasin was gradually phased out as an in-feed coccidiostat for broilers by 2016 and various measures, such as nutraceuticals and vaccination, were successfully employed in order to prevent increased occurrence of clinical coccidiosis and necrotic enteritis (NE)[35]. They are not currently used in human medicine and therefore not classified as medically important antimicrobials by WHO [36]. Nonetheless, some pharmacologically active substances (i.e. monensin, salinomycin) are being studied such as possible bioactive molecules for future cancer therapy drugs, but to date none have been licenced for this purpose [37–39]."

As mentioned earlier, FVE published its first position on coccidiostats in 2016. In 2022, this position was updated based on new published scientific studies and the new legal framework that came into force. As the next section of the opinion indicates: *"The current legislative background for coccidiostats in the European Union (EU) considers them as feed additives for poultry (category of coccidiostats and histomonostats). The legal basis for additives for use in animal nutrition is laid down in Regulation (EC) No. 1831/2003. Several coccidiostats containing polyether ionophore antibiotics or chemical anticoccidial agents for use in chickens, turkeys and rabbits are included in the list of feed additives. Coccidiostats for poultry are usually fed via a premixture. This guarantees good mixing and homogeneity, and no over/under dosing or ´off label´ use is allowed [40]. On top of the legal requirement, almost all feed manufacturers in the EU are also certified by voluntary quality system with additional safety requirements. An immediate change in the legislative status of coccidiostats from the feed additive legislation towards the VMP Regulation*

has the danger that manufacturers would be unable or unwilling to update an existing dossier or compile a new dossier because of insufficient data and hamper their use [41]. The Regulation (EU) 2018/848 bans the use of coccidiostats in organic farming [24]."

The FVE experts have noted a significant improvement in the area of coccidiostat residues, as the mandatory withdrawal period, crucial in the treatment of all production animals, aims to prevent drug residues in products of animal origin. They concluded that: "*Historically anticoccidial residues were one of the most frequently veterinary drug residues. However, the most recent EFSA report for 2020 on the results from the monitoring of veterinary medicinal product residues and other substances in live animals and animal products, found only 0.07% of the samples analysed to be non-compliant (0.05% in 2019) of which pigs (0.01%), poultry (0.06%) and eggs (0.35%) [42]. From 2009 to 2019, an overall important decrease has been observed in the frequency of non-compliant samples for anticoccidials in poultry. This decrease is most likely the result of the awareness and the measures that followed the implementation of the Commission Directive 2009/8/EC setting up maximum levels (ML) of unavoidable carry-over of coccidiostats in non-target feed. In summary, residues are nowadays well managed,occur rarely and technical cross-contamination of animal feeds would not be expected to adversely affect the health of consumers [43]. In addition, authorisation and prerequisites for their use are defined for individual products (brand names) following review by the European Food Safety Authority (EFSA). Feed additives are subject to post-market monitoring plans, regular revised safety, efficacy and vigilance environmental risk assessments to ensure a responsible handling and low risk of adverse events."*

The next section of the document is devoted to the key issue in the use of coccidiostats, which is the growing problem of resistance to these drugs. Since the introduction of chemical coccidiostats to control the invasion of this protozoan in poultry flocks, a significant limitation has been the "*development of resistance by the coccidia to coccidiostats [44]. A number of strategies have been developed to extend the useful life of coccidiostats, while still controlling coccidiosis; such as through 'shuttle use' or 'rotational use'. Rotational use involves changing the in-feed coccidiostats used every 4–6 months with combinations of anticoccidials comprising drugs with different modes of action [45,46]. 'Shuttle use' employs two or more products most suited to each phase of the grow out, i.e. one medicine for the starter period, one for grower and another for the finisher phase [3]. However, it increases the useful life of the drug but does not fully avoid the acquisition of resistance [47,48]. Resistance to coccidiostats is generally thought to be stable, nevertheless, relaxation of selection pressure through vaccination for 2 or 3 consecutive cycles can be advantageous in rotational programmes to re-colonise broiler chicken houses with fully susceptible strains and is employed for example in Spain, France and Italy [49]. Consequently, strategies have to be employ all tools for control of coccidiosis, including vaccination, to mitigate resistance development [50]. Cross-resistance to combinations of polyether ionophore and chemical coccidiostats class was already shown more than 30 years ago and is still evident today [10,51]. The loss in sensitivity was attributed more recently to the polyether ionophore component of the combination [52]. Cross-resistance between polyether ionophores can also occur, although strain differences in response to specific polyether ionophores have been demonstrated. In general, resistance to a monovalent polyether ionophore confer some cross-resistance to other monovalent polyether ionophores (salinomycin, monensin, narasin,*

maduramicin, and semduramicin, but susceptibility to monovalent and divalent polyether ionophores (lasalocid) may be retained [53,54]."

The penultimate subsection of the European veterinarians' position paper addresses the problem of antimicrobial resistance of coccidiostats. As we read this section of the report: *"In the last decades, bacterial resistances to polyether ionophores were discovered. Aarestrup et al. found up to 6% Staphylococcus hyicus and Enterococcus spp. in Danish pigs with reduced monensin sensitivity through official monitoring 25 years ago [55]. Nilsson et al. (2012) described a reduced susceptibility in a large proportion of Enterococcus faecium from Swedish broiler chickens to the polyether ionophore narasin and discovered a plasmid-borne narasin resistance transferred together with vancomycin resistance [56,57]. However, sequencing of the plasmids has shown that the responsible genes are not located next to each other on the same plasmid and weakened the hypothesis of the narasin influence on persistence of vancomycin resistant enterococci in Swedish broiler chickens [58]. Preliminary research data showed a plasmid-borne resistance gene against salinomycin together with resistance genes towards different antibiotics in Dutch broiler chicken [59]. Currently, the prevalence of phenotypical polyether ionophore resistance is however difficult to assess since there are no clinical breakpoint values for resistance. It is acknowledged that the use of polyether ionophores still carries risks owing to the possibility of cross-resistance or co-selection as shown before for antibiotics [60,61]. More research data will be required to systematically investigate the contribution of polyether ionophores to the burden of antimicrobial resistance [50]such as the ICONIC project investigating Ionophore coccidiostats and the risk of CO-selection of antimicrobial resistance. FVE monitors the situation carefully in order to adapt recommendations when indicated in line with EMA recommendations [62]."*

The analysed study is completed by a subsection shortly describing the interactions of polyether ionophore coccidiostats with other antibiotics. This is a very important issue for each practising veterinarian, because: *"Studies have reported interactions between macrolide antibiotics and/or pleuromutilin derivative (tiamulin) administered concurrently with several compounds including polyether ionophore coccidiostats (monensin, salinomycin) which have metabolism partly or entirely dependent on the cytochrome P450 drug metabolising system of the liver [63]. Moreover, toxic interactions between polyether ionophores (mainly monensin) and sulphonamides, erythromycin, and enrofloxacin have been observed [64,65]. All these other active ingredients are already subject to prescription when used in veterinary medicine. Veterinarians prescribing and dispensing antibiotics or other veterinary medicinal products should, as part of their due diligence and judicious use of medicines, check with the farmer if any polyether ionophore coccidiostats are being administered in feed prior to the dispensing of any medication which may result in these adverse interactions. Furthermore, the requirements for feed mills to adhere to good manufacturing practice (GMP) should minimise any adverse reactions associated with inaccurate dosing or carryover in the feed mill."*

It seems that, despite the lack of formal requirements for the availability of coccidiostats, as veterinarians, aviopathologists should be involved as much as possible in the rational prevention of coccidiosis at every stage by whatever means. The authors hope that the presented FVE position statement will be helpful for the successful implementation of this requirement.

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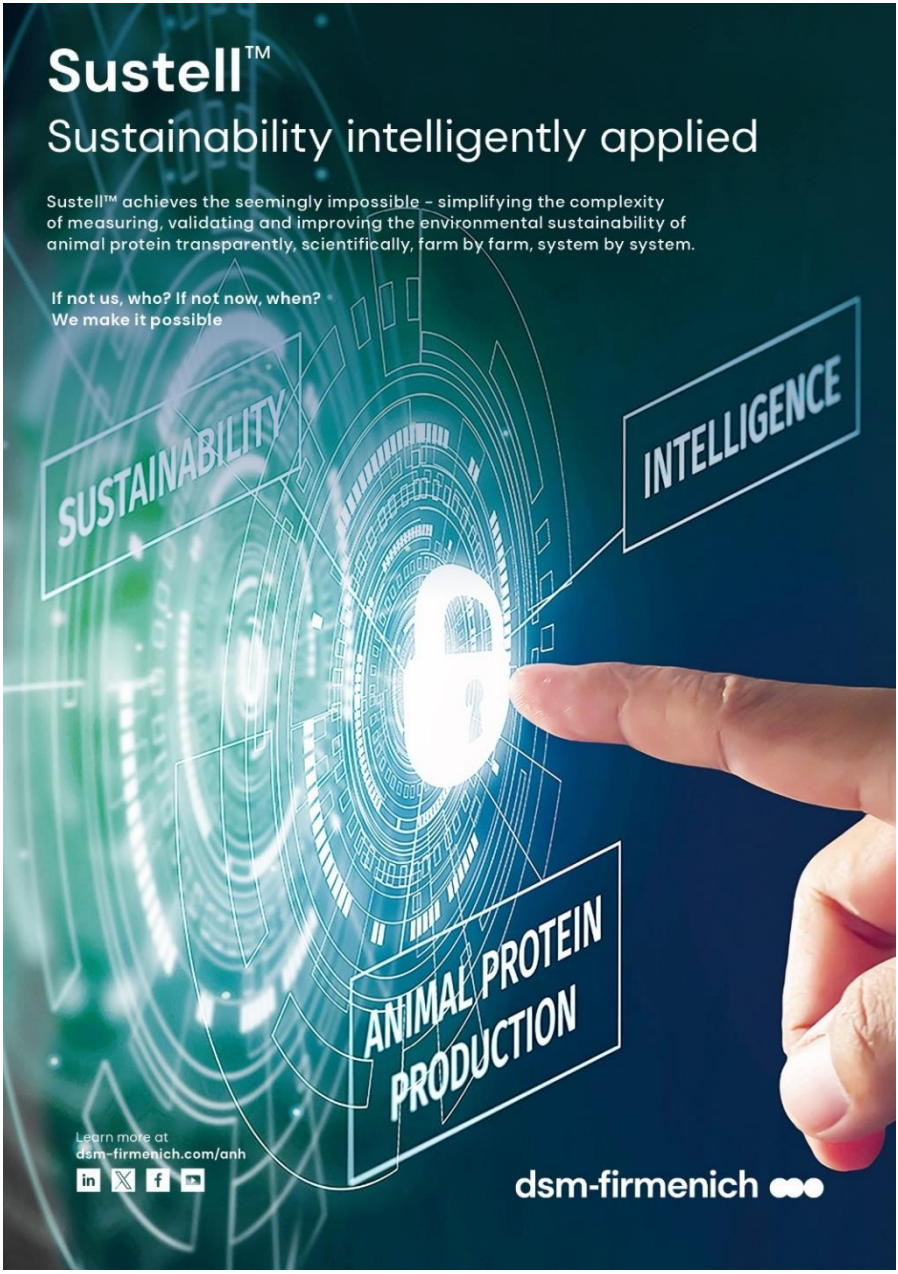
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HOW BEST WE CAN CONTROL COCCIDIOSIS IN THE FUTURE

Coccidiosis is endemic in the commercial broiler industry and capable of inflicting devastating economic losses to poultry operations. To prevent coccidiosis, an effective disease management system must be in place. A floor pen trial was conducted to investigate the efficacy of the combination of a multi-species synbiotic product and a complex glycan mixture technically defined as a Precision Biotic associated to coccidiosis vaccination on performance and coccidiosis control in male Ross 308 broilers raised to 42-day of age. The floor pen trial included 1800 broiler chicks that were assigned at day of hatch to 6 treatments with 15 replicates. Treatment included: Infected Untreated Control (IUC); Infected Vaccinated (coccidiosis vaccine) (IV); Infected Vaccinated treated with symbiotic 500g/MT + complex glycan mixture 250g/MT (IVPS); Uninfected Vaccinated control (UVC); Uninfected Untreated Control group (UUC); Infected Treated with Narasin 50 mg/kg + Nicarbazin 50 mg/kg (ITNN). On day 21, all groups (except UVC and UUC) were orally inoculated with an *Eimeria* spp. virulent strain containing *E. acervulina*, *E. maxima* and *E.tenella*. Body weight, feed consumption and mortality were recorded throughout the experiment and the overall performances of the birds were evaluated by the European Production Efficiency Factor (EPEF) calculated at day 35 and 42. On day 27 and 35 intestinal lesions were scored. On day 20, 27 and 35, fresh faecal material was collected per pen to evaluate oocyst shedding. Compared to birds of IUC group, the results of uninfected groups (UUC and

UVC) confirmed the success of the experimental coccidiosis infection. Compared to birds of IUC group, chickens immunized with coccidiosis vaccine (IV) displayed significant lower lesion score for *E tenella* at day 27, *E acervulina* at day 35, Total Intestinal Score at day 27 and 35 and significant lower oocysts shedding at day 35. Compared to birds of IUC group, chickens immunized with coccidiosis vaccine and receiving the feed supplemented with synbiotic + complex glycan mixture (IVPS) displayed significant better EPEF at day 35 and 42, significant lower lesion score for *E.maxima*, *E tenella*, Total Intestinal Score at day 27 and significant lower oocysts shedding at day 35. Compared to birds of IUC group, chickens treated with Narasin 50 mg/kg + Nicarbazin 50 mg/kg (ITNN) displayed significant better EPEF at day 35 and 42, significant lower lesion score for *E.maxima* at day 27, but no significant reduction was observed for *E. tenella*. Considering direct comparison of IVPS *vs* ITNN, the results showed that no significant differences were observed for the overall performances evaluated as EPEF (day 42: IVPS=546.2; ITNN=544.1) while, at day 27, *E. tenella* lesions were significant lower in IVPS group (IVPS=0.13; ITNN=1.89). The results of this trial indicate that supplementation of synbiotic and complex glycan mixture with coccidiosis vaccination can be considered a potential innovative strategy to control coccidiosis in broiler chickens.

George Gould

DVM, Principal Technical Advisor – Global Poultry, Elanco Animal Health

IMPORTANCE OF EFFECTIVE MONITORING FOR STABLE COCCIDIOSIS CONTROL

Coccidiosis remains one of the most significant threats to broiler production, impacting both performance, health, and welfare of flocks. As such, constant evaluation and optimization of coccidiosis control strategies should be considered a priority to deliver sustainable production.

The most successful and stable control strategies take a holistic view of the challenge and focus on addressing three key areas of control. As well as a robust and effective program, managing the environment/farm conditions as well as ensuring optimal bird immunity are critical to maintain control of the challenge. Given the nature of the parasite and the relatively short production cycle, the situation at a farm level remains dynamic and can change rapidly. As such continuous monitoring and assessment of our strategies is advisable.

This presentation examines some of the methodologies widely offered to assess program efficacy and will attempt to give a more practical view of using and interpreting these tools. Literature and laboratory results are often at odds with what is seen in the field particularly with regards ionophore anticoccidial programs leading to frequent rotations and unstable performance. Ionophore anticoccidials have represented the mainstay of control in broiler production for decades despite much of the literature and laboratory-based studies suggesting a loss of efficacy. Only by examining the mode of action of these products,

alongside the methods used to assess them, can we start to explain the dichotomy here. Selecting and understanding appropriate methods for evaluating control strategies is likely to yield far more stable control of this costly disease at a time when efficiency not only means profitability but more sustainable production. Ultimately allowing the birds in the field to tell us the story is the most valuable data we have at our disposal.

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Monita Vereecken

Huvepharma NV, Uitbreidingstraat 80, 2600 Antwerp, Belgium

AN UPDATE ON COCCIDIOSIS PREVALENCE AND CONTROL IN CHICKENS AND TURKEYS

Introduction

Coccidiosis, caused by protozoan parasites of the genus *Eimeria*, is perhaps the most widespread and difficult to manage poultry disease, resulting in considerable economic losses (Blake et al., 2020; Williams, 1999).

Birds suffering from clinical coccidiosis will typically show signs like diarrhoea, bloody droppings, increased mortality, decreased feed intake and impaired performance. Insufficient control of coccidiosis might also lead to impaired growth and feed conversion ratio, without the presence of evident clinical signs (referred to as subclinical coccidiosis). In a recent study, the global prevalence of clinical coccidiosis in broilers was estimated at 5% of the global poultry production and at 20% for subclinical coccidiosis (Kadykalo et al., 2018). From practical experience in the field, however, this estimate seems low. In turkeys, information on coccidiosis prevalence in field conditions is scarce, as lesions often go undetected and oocyst counting is rarely performed.

Prevalence of *Eimeria* in European broiler and turkey flocks

In 2 studies, prevalence of *Eimeria* in European broiler and turkey flocks was assessed by means of lesion scoring for broilers and oocyst counting followed by PCR analysis for turkeys.

Data from broilers were gathered using Aviapp®, a tool for evaluating the health status and performance of poultry flocks. The analysis comprised data gathered in Europe in 2022 in 1,079 broiler farms, 4,121 flocks and scoring results (according to the system of Johnson and Reid, 1970) of 23,495 birds. *Eimeria acervulina* proved to be the most prevalent species followed by *Eimeria maxima* and *Eimeria tenella*. The average total mean lesion score (sum of *E. acervulina*, *E. maxima* and *E. tenella* lesion scores) for 2022 was 0.88. The average peak scores for *E. acervulina*, *E. maxima* and *E. tenella* were scores of 0.66, 0.24 and 0.07 at 25, 29 and 29 days of age respectively. The average scores for the different *Eimeria* species remain quite stable over the years, demonstrating that coccidiostat programmes in place are effective (Table 3).

Table 3: Summary of age at peak and peak lesion scores for *Eimeria acervulina*, *E. maxima* and *E. tenella* in the years 2019-2022. Data were collected in Europe in Aviapp®, a tool for evaluating the health status and performance of poultry flocks.

Year		2019	2020	2021	2022
Number of flocks evaluated		2,850	2,765	3,739	4,121
E. acervulina	Age at peak	25	26	26	25
	Peak score	0.62	0.68	0.62	0.66
E. maxima	Age at peak	32	32	32	29
	Peak score	0.3	0.22	0.24	0.24
E. tenella	Age at peak	29	27	32	29
	Peak score	0.05	0.03	0.04	0.07

For turkeys, coccidiosis prevalence and identification was performed on faecal samples from European turkey flocks using microscopy and PCR techniques (qPCR) (Vereecken et al, 2023). The use of molecular techniques allows better identification of coccidiosis species than microscopy as overlapping oocysts sizes of the different species create a possibility for errors. Except for *E. subrotunda*, primers for the 6 other turkey species (*E. meleagrimitis*, *E. meleagridis*, *E. adenoeides*, *E. gallopavonis*. *E. dispersa*, *E. innocua*) have been described. In total, 289 samples collected between 2018-2022 from 111 different commercial turkey farms located in 6 different European countries, were investigated. The age of the turkeys at sampling ranged between 1 and 40 weeks, with a median age of 5 weeks. Samples were collected using a standardized protocol. First, samples were microscopically examined for identification of *Eimeria* species and determination of oocyst per gram (OPG) and a part of the samples was further investigated by qPCR analysis in the same laboratory.

E. meleagrimitis was the most prevalent species and was detected throughout all ages. As the age of the turkeys increases, co-infections with other species or infections with other species alone were detected. This shift was evident from both microscopic and qPCR investigations. *E. meleagridis*, which causes lesions in the caeca, was the 2nd most prevalent species detected by qPCR techniques, followed by *E. adenoeides* and *E. gallopavonis*. *E. dispersa*, which multiplies in the mid-intestine, the same area as *E. meleagrimitis*, had the lowest prevalence of all the species that could be detected by qPCR. *E. innocua* was not detected at all. Considering that no substantial difference in reproduction potential between the turkey *Eimeria* species has been described in literature, it can be concluded that *E. meleagrimitis* is the dominant species in European turkey flocks.

Coccidiosis control

Preventive chemotherapy by using coccidiostats continuously in the feed in order to tackle the parasites early in the life cycle is still the most common prevention tool worldwide for coccidiosis control in broilers and turkeys. Registered coccidiostat products with a claimed anticoccidial activity can be classified in 3 different categories: synthetic coccidiostats, ionophore coccidiostats and combination products. A summary of the current EU registered products (status December 2023) is given in Tables 1 and 2.

Table 1: EU Registered coccidiostats – chickens for fattening (Status December 2023)

Type	Brand Name	Compound	Company	Dose (ppm)	Withdrawal time (days)
Ionophore	Sacox	Salinomycin sodium	Huvepharma	50-70	0
	Coxidin	Monensin sodium	Huvepharma	100-125	1
	Elancoban	Monensin sodium	Elanco	100-125	1
	Monteban	Narasin	Elanco	60-70	0
	Avatec	Lasalocid A sodium	Zoetis	90	3
Combination	Monimax	Monensin/nicarbazin	Huvepharma	80-100	0
	Maxiban	Narasin/nicarbazin	Elanco	80-100	0
Synthetic	Stenorol	Halofuginone	Huvepharma	2-3	5
	Coxiril	Diclazuril	Huvepharma	0.8 -1.2	0
	Coxam	Amprolium hydrchloride	Huvepharma	125	0
	Robenz	Robenidine HCl	Zoetis	36	5
	Deccox/Avi-Deccox	Decoquinate	Zoetis	30-40	0
	Nicarbazin	Nicarbazin	Elanco (Phibro)	125	1
	Clinacox	Diclazuril	Elanco	1	0

Table 2: EU Registered coccidiostats – turkeys (Status December 2023)

Type	Brand Name	Compound	Company	Dose (ppm)	WT (days)	Maximum age (weeks)
Ionophore	Coxidin	Monensin-sodium	Huvepharma	60-100	1	16
	Elancoban	Monensin sodium	Elanco	60-100	1	16
	Avatec	Lasalocid sodium	Zoetis	75-125	5	16
Combination	Monimax	Monensin /nicarbazin	Huvepharma	80-100	0	16
Synthetic	Stenorol	Halofuginone	Huvepharma	2-3	5	12
	Coxiril	Diclazuril	Huvepharma	0.8-1.2	0	-

WT: Withdrawal time

Synthetic coccidiostats (also called chemicals) were the first to be discovered and comprise a diverse array of molecules that are absorbed into the blood stream of the host and kill developing parasites in the epithelial cells of the villi in the intestine (Chapman et al., 2016). After introduction, failures were observed regularly due to the rapid development of resistance by the parasite to the synthetic compounds that were used.

The introduction of the first ionophore coccidiostat (monensin) in the seventies proved to be critical for the development of modern poultry production (Chapman, 2014). Ionophores have a different mode of action since they are able to destroy motile stages in the *Eimeria* life cycle (sporozoites and merozoites) in the gut lumen (Smith and Strout, 1979). To be effective, the ionophore must be present in the intestinal lumen at the time that the motile stages are present. The tissue concentrations of ionophores have no effect on coccidial development. It is therefore important to avoid interrupted medication since birds kept on litter ingest oocysts continuously. The application of ionophores after discovery of clinical signs will be too late to prevent mortality

and morbidity losses. Furthermore, other birds will get infected by the oocysts which will be already excreted (Reid, 1990) and environmental contamination is inevitable. Therefore, ionophores are not suited to be used as curative products. Resistance development to ionophores is slow due to the "leakage principle" and therefore ionophores remain the most important global coccidiostat products. The usage of ionophores has significantly helped in the development of modern poultry production and increased the level of health and welfare of the animals (Report from the Commission to the Council and the European Parliament on the use of coccidiostats and histomonostats as feed additives, 2008).

The third option for coccidiosis control by coccidiostats are the **combination products**, combining more than one active ingredient (often the combination of a synthetic and an ionophore molecule). Combinations of nicarbazin with ionophores are the most common examples worldwide and the only products registered in Europe. The combination of the intracellular working mechanism of nicarbazin, with the efficacy of ionophores in the gut lumen, leads to a potentiated activity that allows lower concentrations of both products.

A survey on the coccidiostat use in Europe estimates that approximately 95% of the preventive chemotherapy programmes in place include ionophore or combination products and the remaining 5% synthetic products. Shuttle programmes starting with combination products followed by ionophores are the most popular for broilers, while for turkeys most producers use full programmes.

Vaccination of broilers with coccidiosis vaccines has gained more attention in the last years as a rotation tool in summer in some poultry markets

like Spain and Italy and for vaccination of longer living broilers or organic broilers for niche markets. The majority of the European coccidiosis vaccines contain 'precocious' attenuated lines of *Eimeria*. Restoration of sensitivity is considered due to the replacement of existing coccidiostat-resistant field strains with vaccinal strains that are sensitive to coccidiostats, the consequence of which is that in subsequent flocks, coccidiostats are better able to control *Eimeria* infections (Chapman and Jeffers, 2014). Studies assessing restoration of sensitivity to a broad range of products are scarce. In a recent study the impact of the use of a coccidiosis vaccine in a commercial situation was assessed to a broad range of coccidiostats currently available for the control of coccidiosis (Vereecken et al., 2021). The efficacy of the coccidiostats amprolium (Coxam®); clopidol (Coyden 25%®); diclazuril (Coxiril 0.2%®); monensin (Coxidin®); monensin + nicarbazin (Monimax®); narasin; narasin + nicarbazin and salinomycin (Sacox 120®) against field isolates of *E. acervulina* obtained from a commercial broiler enterprise before and after immunization with a coccidiosis vaccine was investigated. Before vaccination the field strain was resistant to all products tested, evaluated by weight gain, feed conversion, and lesion score after challenge. By contrast, after vaccination the field strain was sensitive to all tested coccidiostats, evaluated by weight gain, and to most products for feed conversion and lesion score. Control programmes, involving the alternation of chemotherapy and vaccination, may play a valuable role in the sustainable control of coccidiosis in the future.

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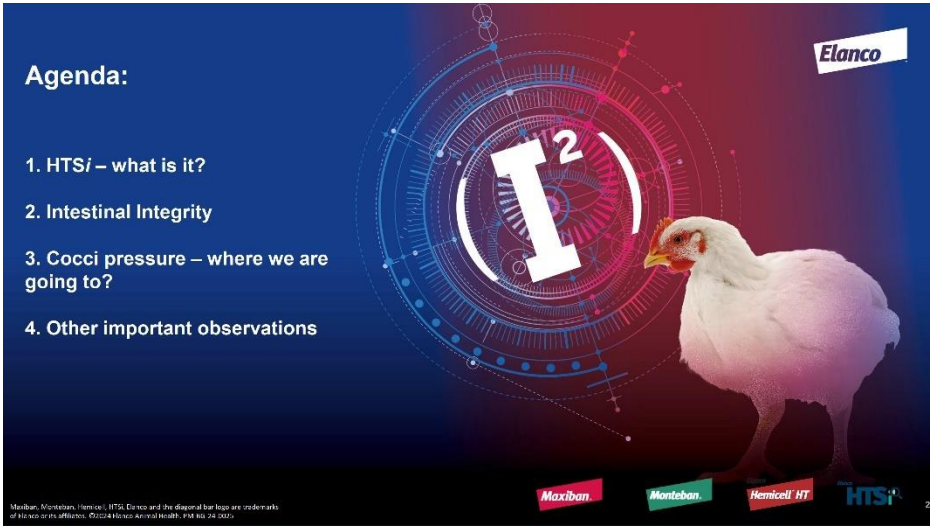
Agenda:
1. HTSi – what is it?
2. Intestinal Integrity
3. Cocci pressure – where we are going to?
4. Other important observations
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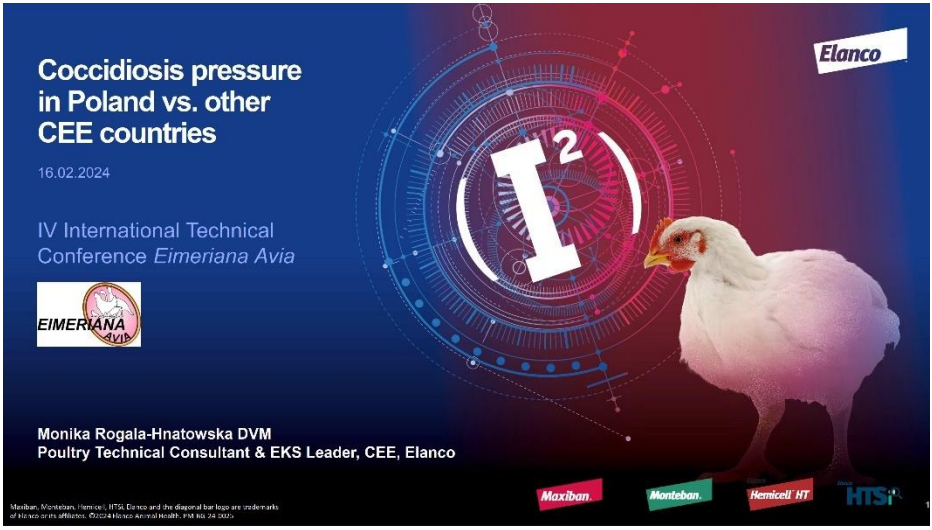
Coccidiosis pressure in Poland vs. other CEE countries
16.02.2024
IV International Technical Conference Eimeriana Avia
EIMERIANA AVIA
Monika Rogala-Hnatowska DVM
Poultry Technical Consultant & EKS Leader, CEE, Elanco
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Agenda:

1. HTS*i* – what is it?
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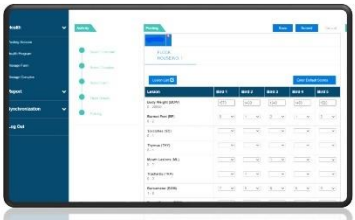
What is HTS*i*?

HTS*i* is unique computer database containing data from necropsies of chickens and turkeys from every country, Customer, farm, house.

It is the system of birds' health control, including coccidia invasions.

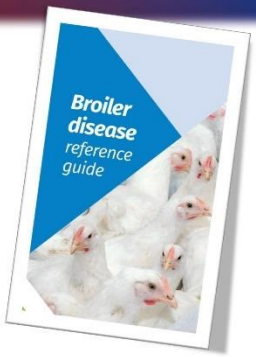
It helps millions of our Customers with coccidiosis management.

H - health
T - tracking
S - system
i - information, integration, intelligence



What does HTS*i* monitor?

- HTS*i* let us monitor and investigation:
  - Over 50 aspects of chicken's health
  - Over 60 aspects of turkey's health
- The system of scoring:
  - Coccidiosis *(Johnson&Reid methodology based)*[1]
  - Intestinal Integrity Index
    - More than 20 parameters of gastro-Intestinal tract
  - Respiratory Index
    - Register of issues related to respiratory routes
  - Gross lesions in the organs responsible for immunity
  - Environment and management related pathologies
  - Welfare signals



1. Johnson J et al Experimental Parasitilogy 1970 30

HTS*i* power – what can we do with data provided by over 15 000 birds/year[2]?

2. Elanco Data on File: CEE HTSi Database 2020-2023

Each farm visit is entered into the system, analyzed by the statistical program and documented with a report.

We can return to all data from the HTS*i* system at any time, you can perform a retrospective analysis of data from several years ago, make comparisons at the chicken house, farm, customer and country level.

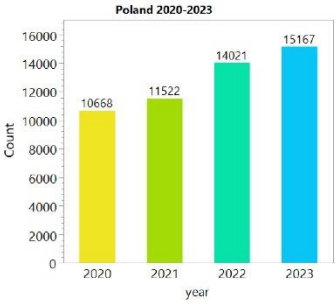
From farm to fork…

… or from farm to solution:

Elanco HTSi

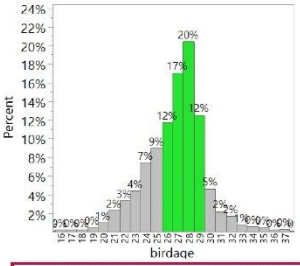
HTS*i* in numbers in CEE, 2020-2023[2]

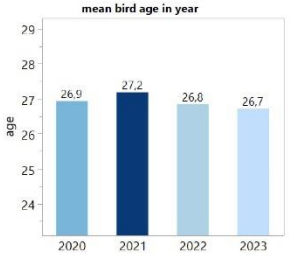
13460 flocks in CEE!



2. Elanco Data on File: CEE HTSi Database 2020-2023

HTS*i* in numbers: bird age – Poland[2]





The highest percent of necropsied chickens is between 26 and 29 days of rearing.

The age of birds for HTSi in the farm is determined by the period of the highest potential risk for broiler's health.

Last two years show decrease of the birds' age – does it mean increase of risk factor pressure?

2. Elanco Data on File: CEE HTSi Database 2020-2023

Maxiban. Monteban. Hemicell HT HTSi

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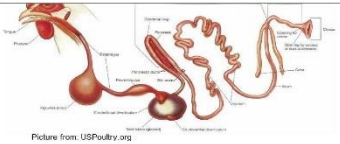
Maxiban. Monteban. Hemicell HT HTSi

Intestinal Integrity
The importance of Intestinal Integrity

1. Can be defined as The OPTIMAL FUNCTIONALITY of the INTESTINALTRACT
2. Consider the functions of the intestines - optimal functioning of these processes is

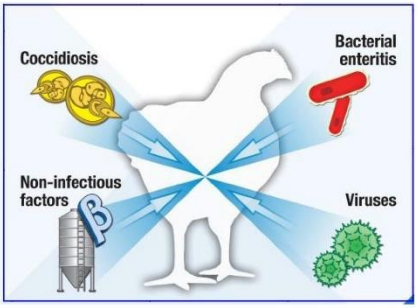
Intestinal Integrity= **Better convert nutrients to energy and growth +**
Waste less energy on maintenance and repair
PRIMARY DRIVER of BROILER PERFORMANCE


Picture from: USPoultry.org

Intestinal Integrity

Optimal functions of gastro-intestinal tract is a first line of the factors responsible for growth and development of the chicken flocks

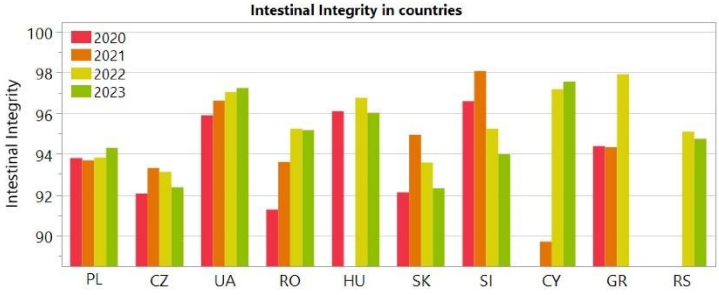
The index is the calculation of numerically assessed lesions in the gastrointestinal tract and can be as high as **100**, thus determining the physiological condition of the digestive tract under investigation.



PROTECTION OF THE INTESTINAL INTEGRITY → PROTECTION OF OVERALL HEALTH AND WELFARE → PROTECTION OF THE FOOD SAFETY

3. Bilgili SF et al Physiology Growth Yield 2006

Intestinal Integrity – Central & Easter Europe[2]



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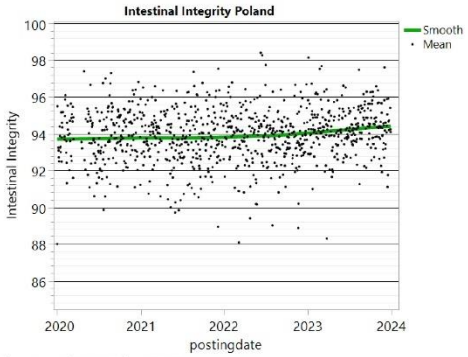
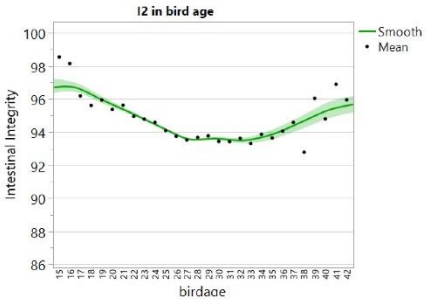
Intestinal Integrity – Poland[2]





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Agenda:

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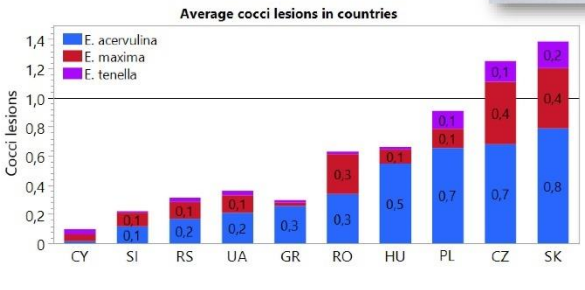
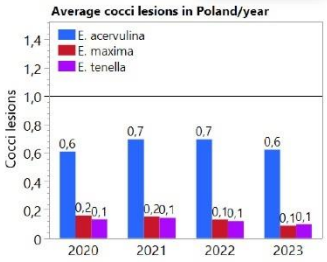
Cocci lesions in broilers – CEE/PL[2]





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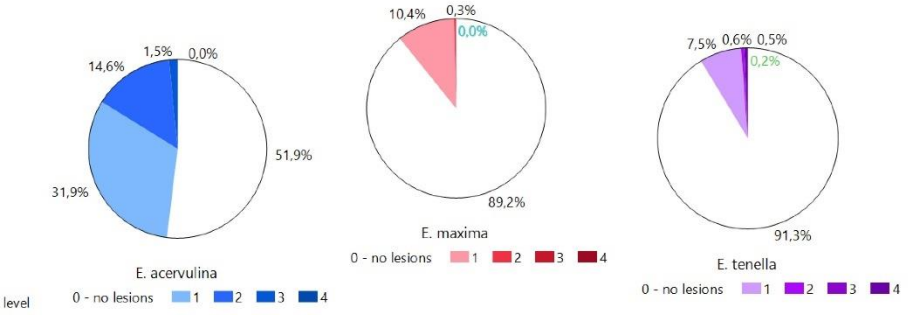
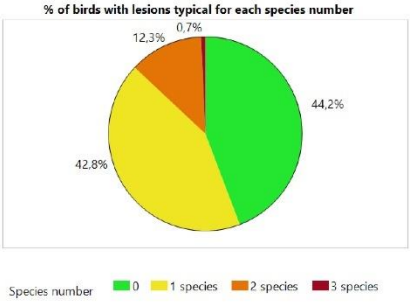
Coccidia severity – Poland[2]



2. Elanco Data on File: CEE HTSi Database 2020-2023

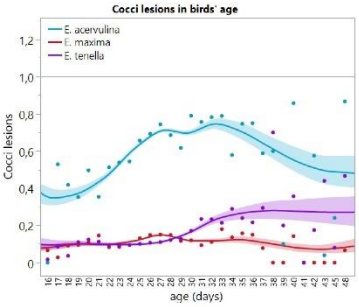
How complex is the incidence of coccidiosis in Poland?[2]



- **44,2%** of birds did not have any cocci lesions.
- **42,8%** of birds showed lesions typical for 1 species.
- **12,3%** of birds had lesions typical for 2 species of coccidia.
- **0,7%** of chickens showed lesions typical for each 3 species

2. Elanco Data on File: CEE HTSi Database 2020-2023

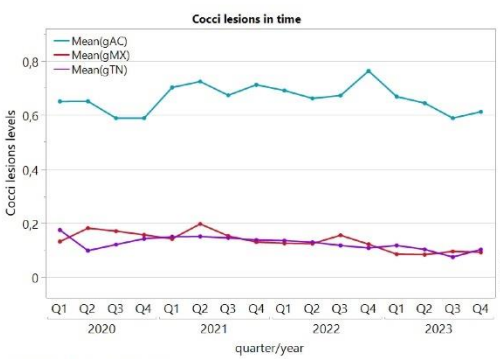
Coccidia's activity trends[2]

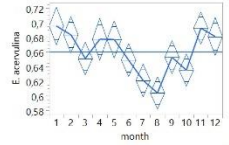


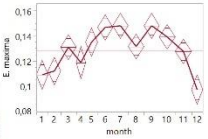
- ***E. acervulina*** – the most „popular", with typical peak around 25-35 days.
- ***E. maxima*** – less visible in the field, the potential risk in later period of rearing. Can cause serious secondary problems.
- ***E. tenella*** – the most „spectacular" one, the risk increases with the age of birds.

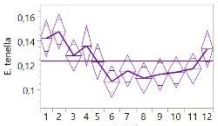
2. Elanco Data on File: CEE HTSi Database 2020-2023

Cocci lesions in seasons[2]









2. Elanco Data on File: CEE HTSi Database 2020-2023

Body weight[2]

2. Elanco Data on File: CEE HTSi Database 2020-2023

ACC programs and their effectiveness[2]

ACC basic	N
MXB+	30750
SAL	15025
other	2828
MMX+	1589
VACCINE	1186

ACC basal	Birds bumber	*E. acervulina*	*E. maxima*	*E. tenella*	Intestinal Integrity
MXB+	29820	0,61 ^{D}	**0,11** ^{D}	**0,08** ^{D}	**94,1** ^{A}
SAL	15025	0,75 ^{B}	0,15 ^{C}	0,19 ^{B}	93,7 ^{B}
MMX+	1589	0,71 ^{C}	0,14 ^{C}	0,13 ^{C}	93,9 ^{AB}
Vaccine	1186	0,86 ^{A}	0,23 ^{A}	0,24 ^{A}	92,6 ^{C}
other	2828	**0,60** ^{D}	0,19 ^{B}	0,13 ^{C}	94,0 ^{A}
SUM	**50428**	*p<0,0001*	*p<0,0001*	*p<0,0001*	*p<0,0001*

- **50428 birds included from Poland 2020-2023**
- **P = 0,95**
- **Basal ACC means anticoccidial used from day 0 to at least d18**
- **Programs with short MXB (<18d) have been excluded (950 birds)**
- **Other means: random programs, pure chemical and mixes of 3 and more anticoccidials in one program**

2. Elanco Data on File: CEE HTSi Database 2020-2023

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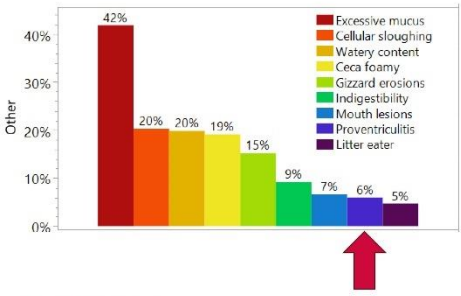
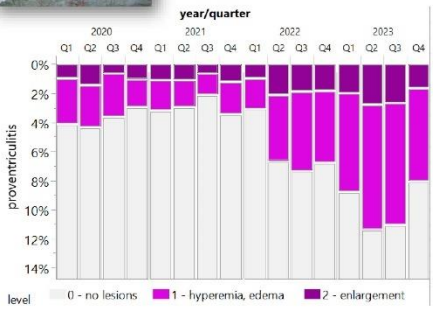
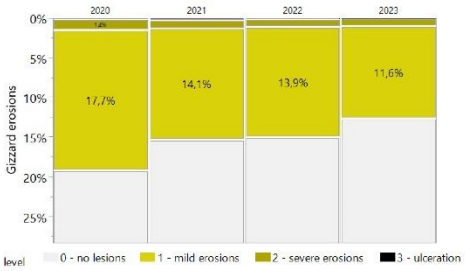
Other lesions[2]

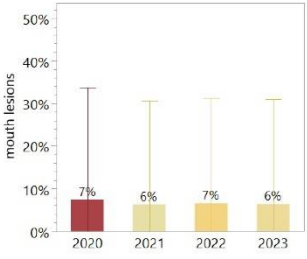




2. Elanco Data on File: CEE HTSi Database 2020-2023

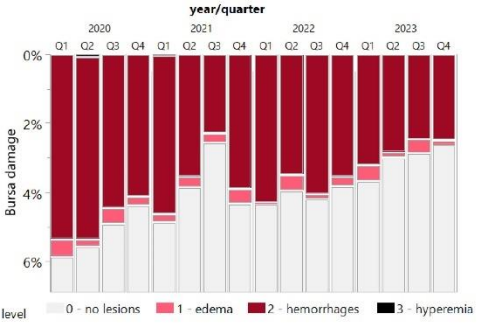
Gizzard erosions&mouth lesions[2]

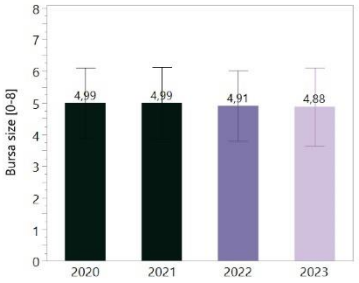




2. Elanco Data on File: CEE HTSI Database 2020-2023

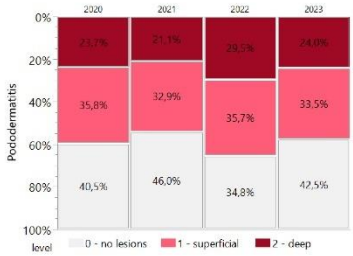
Bursa of Fabricii status[2]

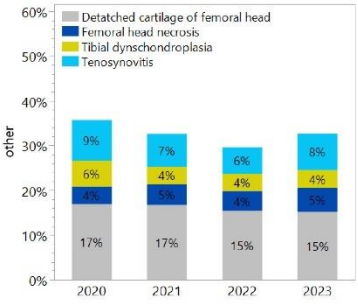




2. Elanco Data on File: CEE HTSI Database 2020-2023

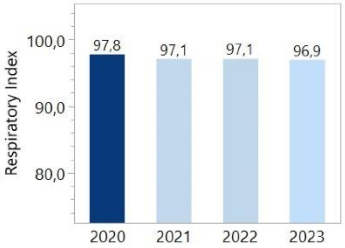
Pododermatitis&joints[2]

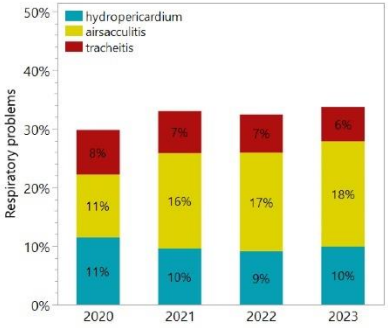




2. Elanco Data on File: CEE HTSI Database 2020-2023

Respiratory index[2]





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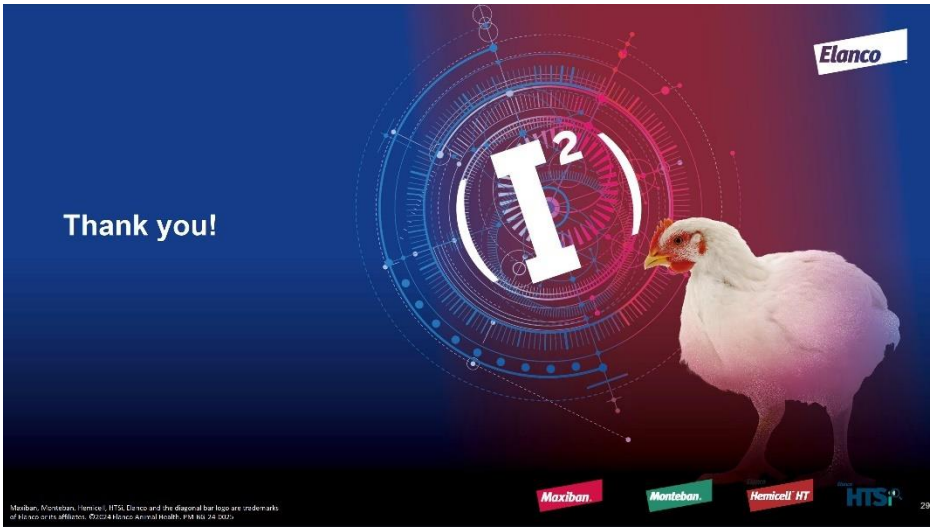

Signature Page for PM-BG-24-0025 v1.0

Regulatory Approval	Borislav Borisov Regulatory 07-Feb-2024 14:10:30 GMT+0000

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OCENA WPŁYWU DWÓCH KOKCYDIOSTATYKÓW **JONOFOROWYCHWPOŁĄCZENIUZNIKARBAZYNĄ** NA INTEGRALNOŚĆ JELIT I WYNIKI PRODUKCYJNE

Integralność Jelit = OPTYMALNA FUNKCJONALNOŚĆ PRZEWODU POKARMOWEGO. Oznacza zachowanie wszystkich struktur jelit oraz ich zdolności do realizowania funkcji metabolicznych, takich jak: trawienie, wydzielanie, wchłanianie i transport składników odżywczych. Kokcydia zwykle stanowią największe zagrożenie dla Integralności Jelit w ciągu całego życia ptaków. Są uznawane za podstawowy czynnik uszkadzający nabłonek i inne struktury jelit. Wielokrotnie otwierają drzwi dla wtargnięcia innych, wtórnych czynników, takich jak *Clostridium perfringens*[1].

Wspólnie, obydwa patogeny mogą dotkliwie uszkadzać przewód pokarmowy. Straty mogą dochodzić do 10,2 centów na ptaka[2,3].

Ochrona Integralności Jelit: kokcydiostatyki

Wiele substancji kokcydiostatycznych zostało wprowadzonych na globalny rynek drobiowy jako dodatki paszowe. Kokcydiostatyki chemiczne działają dobrze w przypadku silnych inwazji kokcydiów, jednakże długotrwałe ich używanie może prowadzić do wzrostu oporności kokcydiów na te substancje. Z drugiej strony, jonofory pozwalają na efektywne zapobieganie kokcydiozie, jednocześnie umożliwiając rozwój naturalnej odporności ptaka[4].

O badaniu: Materiały i metody[4]

Opisane doświadczenie miało na celu porównanie skuteczności dwóch jonoforów, monenzyny i narazyny, w połączeniu z nikarbazyną na skuteczność kontroli kokcydiozy oraz określenie różnic w we wpływie tych mieszanek na parametry produkcyjne oraz zdrowie jelit kurcząt.

Do badania wykorzystano 4400 kurcząt brojlerów linii Ross 308 poddanych eksperymentalnemu zarażeniu kokcydiami oraz poddano je ocenie uwzględniającej wydajność ich przyrostu oraz obecność makroskopowych zmian w narządach wewnętrznych.

Ptaki otrzymywały paszę z dodatkiem mieszanki monenzyny i nikarbazyny (MN) lub narazyny i nikarbazyny (NN) od 1. do 27. dnia odchowu, a następnie paszę z dodatkiem narazyny do końca odchowu.

25% ptaków z każdego kojca było losowo ważone w 27. oraz w końcowym, 33. dniu odchowu. Zarejestrowano spożycie paszy w każdym z kojców w 27. i 33. dniu odchowu oraz został obliczony współczynnik konwersji paszy (FCR). Scoring zmian jelitowych został przeprowadzony na 66 ptakach z każdej grupy żywieniowej (po 3 ptaki z każdego kojca).

Wyniki: Integralność Jelit[4]

Liczba ptaków, u których zaobserwowano makroskopowe zmiany w jelitach została zredukowana w obydwu badanych grupach, zarówno w grupie otrzymującej mieszankę monenzyny i nikarbazyny, jak i w grupie otrzymującej mieszankę narazyny i nikarbazyny, bez istotnych statystycznie różnic.

OCENA PUNKTOWA ZMIAN KOKCYDIOZOWYCH* W 22. DNIU ODCHOWU U BROJLERÓW OTRZYMUJĄCYCH MIESZANKĘ MN LUB NN

OCENA	Monenzyna + Nikarbazyna	Narazyna + Nikarbazyna
Zmiany typowe dla *Eimeria acervulina* (gAC)		
0	66	66
Zmiany typowe dla *Eimeria maxima* (gMX)		
0	55	55
1	8	10
2	3	1
Zmiany typowe dla *Eimeria tenella* (gTN)		
0	57	56
1	9	10

*Ocena zmian: 0 = brak, 1 = lekkie, 2 = zaawansowane (n = 66 ptaków/grupę badawczą)

FCR i masa ciała[4]

Grupa otrzymująca paszę z mieszanką narazyny i nikarbazyny uzyskała statystycznie istotne wyniki: niższy współczynnik konwersji paszy oraz wyższą masę ciała mierzoną w 27. oraz 33. dniu cyklu, w porównaniu z grupą otrzymującą mieszankę monenzyny i nikarbazyny.

WYNIKI PRODUKCYJNE PROJLERÓW OTRZYMUJĄCYCH MIESZANKĘ MN LUB NN MIERZONE W 27. DNIU ODCHOWU

Parametr	Monenzyna + Nikarbazyna	Narazyna + Nikarbazyna
FCR	1,566[a]	1,458[b]
Masa ciała (g)	1284[a]	1405[b]

[a,b] Wartości różniące się literami w indeksie górnym wykazują różnice statystycznie istotne (P<0,05).
n = 550 ptaków/grupę badawczą

WYNIKI PRODUKCYJNE BROJLERÓW OTRZYMUJĄCYCH MIESZANKĘ MN LUB NN MIERZONE W 33. DNIU ODCHOWU

Parametr	Monenzyna + Nikarbazyna	Narazyna + Nikarbazyna
FCR	1,642[a]	1,549[b]
Masa ciała (g)	2026[a]	2178[b]

[a,b] Wartości różniące się literami w indeksie górnym wykazują różnice statystycznie istotne (P<0,05).
n = 550 ptaków/grupę badawczą

Jakość tuszki[4]

Masa tuszki gotowej do spożycia oraz masa mięśnia piersiowego były istotnie wyższe w grupie otrzymującej mieszankę narazyny i nikarbazyny w porównaniu z grupą otrzymującą mieszankę monenzyny i nikarbazyny.

WYDAJNOŚĆ RZEŹNA U BROJLERÓW OTRZYMUJĄCYCH MIESZANKĘ MN LUB NN UZYSKANA W CIĄGU 33 DNI ODCHOWU

Parametr	Monenzyna + Nikarbazyna	Narazyna + Nikarbazyna
Masa żywca (g)	2032[b]	2159[a]
Masa gotowej tuszki (g)	1400[a]	1517[b]
Masa mięśnia piersiowego (g)	580[b]	656[a]

[a,b] Wartości różniące się literami w indeksie górnym wykazują różnice statystycznie istotne (P<0,05).
n = 110 ptaków/grupę badawczą

Materiał sponsorowany przez firmę Elanco

KĄCIK INTEGRALNOŚCI JELIT (I²)

Kluczowe punkty: Kombinacje jonoforów z nikarbazyną

- *Mieszanka narazyny i nikarbazyny istotnie wpłynęła na parametry produkcyjne kurcząt brojlerów linii Ross 308 poddanych kontaktowi z kokcydiami, a mianowicie na masę żywca w wieku rynkowym oraz FCR w porównaniu z mieszanką monenzyny i nikarbazyny*[4].
- *Mieszanka narazyny z nikarbazyną wpłynęła istotnie na jakość tuszki w porównaniu z grupą otrzymującą mieszankę monenzyny i nikarbazyny. Masy gotowej tuszki oraz części tuszki były istotnie wyższe w grupie otrzymującej narazynę z nikarbazyną w kontekście wagowym oraz procentowym*[4].
- *W wyborze kokcydiostatyków, połączenie narazyny z nikarbazyną jest uważane za istotnie lepszy wybór, stanowiący większą wartość dla producentów brojlerów, w porównaniu z połączeniem monenzyny z nikarbazyną*[4].

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Paulina Abramowicz-Pindor

Research and Development Department

AdiFeed® LTD, Poland

PHYTONCIDES IN COMMERCIAL CHICKEN BROILER PRODUCTION

It is clear that food serves not only as nourishment. An aware consumer is searching for nourishment at the cellular level. Hence the growing popularity of foods with an added value like organic food, nutraceutical foods, insect protein, and lately so-called "cultured meat." Today more than ever consumer needs to consider the Planet as food production must be conducted on a larger scale but in a different way so as not to put so much burden on the environment as conventional production does. Sustainable agriculture products are a solution befitting our times: not as expensive as organic foods but fitting into the trend of climate protection and conscious resource exploitation. Due to the global problem of growing antimicrobial resistance (AMR) sustainable agriculture needs to be extended to antibiotic use reduction as well as other chemotherapeutics reduction as e.g. ionophore coccidiostats show some of the antibiotic mode of action and there is a withdrawal of coccidiostats in the same legislation that has been repealed since 2012. Still, there are plenty of issues in broiler production. The quality of day-old chicks and their low immunity, overstocking, and shortages in the animal environment. Until recently these issues justified the use of antibiotic growth promoters and antimicrobials in animal production. Moreover, there is a problem of Salmonella and Campylobacter occurrence, and periodically also of avian influenza. As long as

not much can be done about a virus, the problems of industrial animal farming can be remedied, but metaphylaxis cannot be the solution. The limitations in the use of antimicrobial agents are necessary to preserve their efficacy for the future. Since antibiotic growth promotors were withdrawn in 2006 questions about alternatives are being raised now and then. Management systems at poultry farms supported with good biosecurity, vaccines, and probiotics are crucial for good prophylaxis and obtaining the desired level of health status of birds. Among others, phytobiotics can be successfully used as an alternative.

Phytoncides are secondary metabolites produced and secreted by cormophytes. They have antiprotozoal, antibacterial, antiviral, and antifungal properties. Phytoncides proved to be an effective tool in antimicrobial reduction in poultry farming in many studies. Active components of herbs can protect antimicrobials effectiveness and help to overcome many issues on a daily routine in broiler chicken farming. Anticoccidial activity has been reported in many studies concerning herbs, herbal extracts, as well as phytogenic products that could be used in effective coccidiosis prevention programs despite the geographical region. A few different studies were conducted to examine the performance of broiler chickens fed different phytobiotics in different geographical regions to prove the effectiveness of phytobiotics in coccidiosis control and as AGP alternative.

The purpose of this study was to test a versatile mixture of herbs to create AGP and coccidiostats alternative that provides high-performance results and the health status of the birds. To obtain the goal the meta-analysis of three different experiments was conducted. Two out of three concerned challenges with coccidiosis and there was also a comparison with the live vaccine and “bioshuttle” program with chemical coccidiostat being used. Each study was

held in controlled conditions at the university or independent institute unit. All treatments consisted of a three-phase dietary program using commercial feed formulation standards. All experimental diets were fed ad libitum for the duration of the study. Treatments depended on the region, where particular coccidiostats (chemical/ionophore) or antibiotic growth promoters are registered and available, were applied. Meta-analysis of all the conducted studies concerning phytogenic products proved that phytobiotics may be compared to standard coccidiosis control programs as well as to "bioshuttle" programs and herbal treatments providing an effective alternative to antibiotic growth promoters. The feeding of phytobiotics as a standalone or in a "bioshuttle" program demonstrated improvements in performance in birds. Whether it was an experiment where 200 grams of phytobiotic were put in line with Amprolium (0.0125%) where challenge 5x105 and 2x104 E. tenella at day 14th was applied or a "bioshuttle" coccidiostat control program with live vaccine and Zoelene (125 ppm) used at day 21st versus 300 grams of phytobiotics being utilized, phytoncides were found to be effective in case of performance and OPG reduction. The study comparing AGP and 100 grams of phytobiotic showed similar body weight gains and feed conversion ratios. No decrease in meat quality was detected in experimental groups compared to control treatments. As a result of meta-analysis it can be stated that phytoncides make an alternative to chemotherapeutics without compromising the economic aspects of poultry farming.

Keywords: coccidiosis, phytobiotics, sustainable agriculture, AMR, AGP

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Żanetta Chodorowska

DSM Product Manager Mycotoxin Risk Management EMEA

MYCOTOXINS - CAUSE INCREASED SUSCEPTIBILITY TO DISEASES IN BIRDS

The response of birds to the ingestion of mycotoxin-contaminated feed is not uniform, but depends on age, production performance and health status, the level of contamination, the type of mycotoxin and the interaction between them. The environment in which the animals are housed is also very important.

Depending on the situation, we may be dealing with high morbidity and increased mortality in birds, or a subclinical form that is difficult to detect, with reduced feed intake and utilisation, and reduced resistance of the animals to pathogenic microorganisms.

In practice, we are most often dealing with chronic low intakes of mycotoxins, causing a range of unnoticed metabolic, physiological and immunological disorders that are not compensated for by short production cycles.

Historically, mycotoxins in poultry have been associated with classic symptoms such as reduced feed intake, oral lesions and reduced flock productivity, but the link between mycotoxin contamination of feed and avian health remains unclear.

In vitro and ex vivo studies indicate that DON and FB1 are able to increase the permeability of the intestinal epithelial layer in birds. The main

organs exposed to the toxic effects of mycotoxins are the intestinal mucosal epithelium and the respiratory tract, together with the underlying mucosal lymphoid tissues and immune cells.

The FAO estimates the prevalence of mycotoxins above the EU I Codex limits at 25%. This figure significantly underestimates the occurrence of mycotoxins above detectable levels (60-80%). In practice, on average 60% of raw materials tested are contaminated with mycotoxins and 60-80% of finished feeds analysed are contaminated with at least one mycotoxin (Eskola et al., 2019). The high prevalence of mycotoxins can be explained by a combination of increasing sensitivity of analytical methods and the effects of climate change. DSM and formerly Biomin have been conducting mycotoxin contamination studies of feed in Europe in Poland for 20 years. Analyses in 2023 showed that 95% of finished poultry feeds were contaminated with mycotoxins, 82% of which were positive for more than one mycotoxin, meaning that the toxic effect may have been exacerbated. Mycotoxins are low molecular weight, natural secondary fungal metabolites produced mainly by the fungi *Aspergillus*, *Penicillium* and *Fusarium*. Chronic low-level contamination of feed with mycotoxins reduces animal resistance to disease and exacerbates disease, depending on the age of the birds, dose and duration of exposure. The co-occurrence of deoxynivalenol (DON) with fumonisins (FUM) and T-2 toxin is common in poultry feed. The main organs exposed to these mycotoxins are the intestinal and respiratory epithelium and the underlying mucosal lymphoid tissues. The presence of DON and FUM in feed reduces intestinal integrity and, when ingested by animals, contributes to increased intestinal permeability, leading to 'leaky gut' syndrome. This results in increased proliferation and translocation of intestinal pathogens. Impaired gut barrier integrity increases the potential for colonisation and

translocation of pathogens such as *Salmonella* spp. (Vandenbroucke et al., 2011, increased bacterial penetration), *Clostridia* (Antonissen et al., 2014; increased necrotizing enteritis lesions) and *Eimeria* (Grenier et al., 2016; increased lesions and oocyst shedding). Studies on the effect of mycotoxins on animal susceptibility to infectious diseases have mainly focused on exposure to single major mycotoxins, and there is limited information on the effect of multiple co-occurring mycotoxins and plant mycotoxin metabolites on this interaction. Girgis et al. (2008) showed that the combination of DON, 15-acetyl-DON (15-AcDON), ZEN and fumonisins altered the immune response induced by Eimeria. Interestingly, contamination of broiler feed with mycotoxins may reduce the efficacy of treatment against coccidiosis. Salmonellosis is a risk factor for inflammatory bowel disease (IBD) and *Clostridium difficile* infection, which damages the intestinal mucosa. Ingestion of low concentrations of DON renders IECs (intestinal epithelial cells) more susceptible to S. Typhimurium infection and subsequent mucosal inflammatory responses due to increased translocation of S. Typhimurium. T-2 toxin has a very detrimental effect on chick immunity to salmonellosis, which is not accompanied by marked changes in T or B cell responses to mitogenic stimulation. *Clostridium perfringens* (*C. perfringens*) is a risk factor for Necrotic enteritis in broilers. Necrotic enteritis is one of the most important intestinal diseases in poultry. Consumption of feed contaminated with DON (3000-4000 μg/kg, in vivo) at concentrations below the European recommended maximum level of 5000 μg/kg is a predisposing factor for severe disruption of the intestinal barrier and increased growth and production of C. perfringens toxins, leading to the development of NE in broiler chickens. Exposure to mycotoxins increases the likelihood of serious enteric diseases caused by *C. perfringens*, in particular necrotizing enterocolitis and

hemorrhagic or Necrotic enteritis enterotoxaemia. The inflammatory response to mycotoxins is an energy cost to the animal, resulting in a significant loss of productivity.

The presence of mycotoxins is one of the reasons for the failure of vaccination programmes.

Vaccines are used to mitigate and control viral, bacterial and protozoal diseases. Despite the widespread availability of vaccines and vaccination programmes, producers still face challenges in controlling disease outbreaks that affect the productivity of their flocks. Some of the areas where vaccines fail can be attributed to problems inherent in the vaccine itself, user or application errors and factors inherent in the organism itself, such as immunosuppression. The presence of mycotoxins reduces the efficacy of vaccines by interfering with the innate and acquired immune response. The most common mechanism of immunosuppression is the inhibition of protein synthesis. The result is a reduction in signals for the synthesis of antibodies and immunoglobulins. Studies have shown that *Fusarium* mycotoxins, such as DON, have a negative effect on antibody titres to Newcastle disease and infectious bronchitis virus in breeding flocks. The presence of DON may be the reason for a reduced immune response to the infectious bronchitis virus (IBV) viral vaccine, affecting clinical serum biochemistry and antibody titres (Ghareeb et al., 2016). Immunosuppression can result from chronic exposure to mycotoxins, even at low levels. Inadequate vaccine response, secondary bacterial infections, problems with flock homogeneity, atrophy of immune-related organs, reduced performance parameters, increased morbidity and mortality are some of the characteristics of immunosuppressed birds. Some of the most commonly studied mycotoxins that can directly interfere with vaccines are aflatoxins,

trichothecenes, fumonisins and ochratoxins. Other mycotoxins such as cyclopiazonic acid (CPA), rubratoxins and citrinin can also cause immunosuppression and vaccine failure. Vaccination programmes are expensive to implement. Therefore, the failure of a vaccine or vaccination programme has both economic and health consequences. Immunosuppression caused by mycotoxins results in reduced resistance to infection and increased susceptibility to enteric pathogens, while immunostimulation, which has also been shown to result from the presence of mycotoxins in feed, is energy costly and results in reduced productivity. Mycotoxin-induced immunomodulation can affect innate and adaptive immunity through impaired macrophage and neutrophil function, reduced T and B lymphocyte activity and antibody production, and often goes unnoticed. Without established procedures for testing feed and raw materials for mycotoxins, and without a plan of action, mycotoxins often remain an unrecognised problem. It has also been shown that mycotoxins can remain in animal/chicken products such as eggs and meat when chickens consume contaminated feed (Vlachou et al., 2022).

Given all the links between mycotoxins and disease, a mycotoxin risk management programme is essential to protect the health of poultry in any flock. It includes the monitoring of mycotoxin levels in raw materials and feed, proper feed storage, and the prophylactic use of mycotoxin deactivating products. It is essential that the product used is effective in decomposing especially mycotoxins of the Trichothecenes group such as DON and T-2 and FUM, which can only be controlled by biotransformation, which destroys the toxic part of their chemical structure. Only EFSA-registered substances from the group of compounds for the deactivation of mycotoxins in feed are safe to use, guarantee

specific de-activation of mycotoxins and guarantee the production of non-toxic and animal- and environmentally safe metabolites.

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Laure Bignon, Christophe Briens, Paul-Alexandre Guevellou,
Marina Panhéleux

CCPA Group - ZA du Bois de Teillay Quartier du Haut Bois – 35150 Janzé – FRANCE

SUPPORTING ZOOTECHNICAL PERFORMANCE IN A CONTEXT OF COCCIDIAL CHALLENGE

Introduction

Coccidiosis is a parasitic disease due to Eimeria and affects all poultry species (chicken, Turkey, Guinea fowl, Game birds…) with a high specificity for its host poultry specie and anatomic / histologic sites of infestation. Eimeria can remain infectant more than one year in the soil through its free external resistance form, the sporulated oocyst (Delaplane & Stuart, 1935). Reyna et al., (1983) suggests that the survival of oocysts is poor in litter and that the carryover from one batch to the next come from outside soil, house dust and arthropods. Though some disinfectants are registered as efficient against Eimeria (DVG methodology as indicated by ECHA 20°C - 4 hours), in real life, the best cleaning-disinfection and biosecurity procedures reduce the pressure of infection but do not eliminate it totally. And Eimeria has a huge capacity to multiply in the parasitised animal. One ingested oocyst can lead to the excretion of a 2-3 million new oocysts in 5 to 7 days, after 2 to 4 schizogonies and 1 gametogony. Consequently, coccidiosis belongs to the top five "production diseases" with an incidence of 90 to 100% which means that disease is nearly ubiquitous. The financial impact of coccidiosis represents 17 Eurocent per bird when efficiently controlled and 22 Eurocents when uncontrolled (Jones et al.,

2019). A more recent study estimated the cost of coccidiosis to 19 Eurocents per chicken placed (Blake et al., 2020). That cost includes mortality, decreased growth, degradation of feed conversion ratio, prevention, and treatment costs. Coccidiosis is often subclinical but induces inflammation of the gut and oxidant stress leading to alteration of the microbiota, with bad litter and pododermatitis, increases necrotic enteritis (Williams, 2005), higher *Campylobacter* (Macdonald et al., 2019) and *Salmonella* shedding (Baba et al., 1982).

Prevention methods are dominated by the supplementation of feed with coccidiostats divided into chemicals and ionophore. To keep good efficiency and avoid cross-resistance with some antibiotics (Peek & Landman, 2003; Nilsson et al., 2016), rotation programs of coccidiostats are scheduled. Each rotation is made of a full program (single coccidiostat along a batch) or shuttle program (several coccidiostats within a batch). A good monitoring program of coccidiosis and inflammation lesions indexing combined with batches zootechnical results is necessary to design rotation program.

Other prevention programs including live *Eimeria* oocysts vaccines, chemical coccidiostats and phytogenic products have been used in some USA broiler production without antibiotics (Cervantes & McDougald, 2023). In some free-range and organic production, rotation programs with vaccination and phytogenic products have been used for years. In fast growth broiler and turkey, phytogenic products have been used in finishing diets or during coccidiostats withdrawal period.

Now, our new generation product combines plant extracts, essential oils, and spices. Some components have been selected for their inhibiting effect on *Eimeria* sporulation in vitro, others have a protective action on gut mucosa, or promote natural defences of the bird.

The following chapters will describe how we selected the ingredients through a bibliographic study, *in vitro* oocysts sporulation screening tests and *in vivo* floor pens challenge experimentations.

Selection of active ingredients for test.

Garlic essential oil could, on chickens artificially infected with *Eimeria tenella*, significantly reduce the clinical symptoms, caecal lesions, the number of oocysts, and increase the weight of sick chickens compared with a non-medicated control (Chang et al., 2021). When inoculated with *E. acervulina*, broilers fed a feed supplemented with garlic extracts had a better growth than broilers fed a non-supplemented diet with less oocyst excretion and inhibition of *NF-kB* activation, demonstrating garlic anti-inflammatory properties (Kim et al., 2013).

After a challenge with mixed *Eimeria* species altering significantly zootechnical serum biochemistry parameters, Eugenol, main active component of Clove, decreased oocyst per gram (OPG) excretion compared to unmedicated infected control (UIC) and restored daily weight gain (DWG), daily feed intake (DFI) and feed conversion ratio (FCR) at a similar level as uninfected unmedicated control (UUC). Biochemistry of Eugenol group was similar to diclazuril medicated infected control and UUC. For authors, anticoccidial properties along with anti-inflammatory and antioxidant properties of Eugenol could explain this good result (Youssefi et al., 2023).

Turmeric (3%) lowered and delayed the peak of excretion of oocysts, and mild bloody diarrhoea like salinomycin on inoculated broilers with *E. tenella*.

Feed intake, growth, FCR were significantly improved compared with UIC and similar to salinomycin or UUC (Abbas et al., 2010).

Cinnamaldehyde was tested on broilers which experienced zootechnical alteration after vaccination with a live non attenuated *Eimeria* vaccine. Cinnamaldehyde treated birds improved their DWG and FCR and viability (0-28 days) compared with control but not at the level of unvaccinated birds. Necrotic enteritis was more prevalent (63%) in vaccinated birds than in non-vaccinated (19%). Cinnamaldehyde reduced the incidence of necrotic enteritis (31%) and *Clostridium* + *Enterococcus* counts in the caeca of treated animals (Yang et al., 2020). In another paper, Cinnamaldehyde improved circulating IgM and the relative weight of organs (spleen, thymus, Fabricius bursa) and increased Lactic acid bacteria caecal counts (Saied et al., 2022).

In vitro oocysts sporulation inhibition tests.

The disturbance of sporulation process or sporogony is a critical point for the control of the infection. In the last decades, the availability of *in vitro* tests has been implemented, providing interesting insights for the evaluation of alternative strategies for the control of avian coccidiosis by means of botanical and natural products. Different Garlic extracts have shown sporulation inhibition activity (Abd-ELrahman et al., 2022). In other in vitro model of cultured cells invasion by sporozoites, Garlic and Origano best reduced invasion efficiency (Felici et al., 2023). Olive leaf had also some in vitro destructive activity against Eimeria oocysts (Debbou-Iouknane et al., 2021).

The objective of this *in vitro* test adapted from Saratsis et al., (2012) was to investigate the efficacy of three different botanical formulations A, B, C (Allium, Origanum, Olive) on the sporulation of *E. acervulina, E. maxima* and

E. tenella. The tested concentrations were the following: A1/A2/A3: 100/1000/10000 ppm; B1/B2/B3 40/400/4000 ppm; C1/C2/C3: 300/3000/30000 ppm, a negative control PBS (NC), and a positive control Toltrazuril (PC). At Day0, the inoculum was selected, Day6, oocysts were collected and purified, Day7 the trial was set up. The *Eimeria* strain containing *E. acervulina*, *E. maxima* and *E. tenella* was suspended in PBS and poured into individual vials, each counting approximately 10,000 oocysts in 1 ml, along with 1 ml of the test material. 2 replicates were dedicated per treatment group. Vials were incubated at 29°C. Each day, for three consecutive days (i.e., 24, 48 and 72h), the sporulation rate of the *E. acervulina*, *E. maxima* and *E. tenella* was recorded in each vial and compared to the untreated control. Overall results have been obtained as a mean value of each replicate / *Eimeria* specie. Based on sporulation (S%) and sporulation inhibition (SI%) percentages, the effect of the products on sporulation dynamics was analysed. The sporulated oocysts were counted, and the inhibitory sporulation percentage was calculated from the equations as suggested by Cedric et al., (2018). Sporulation inhibition (SI) % =

[(Sporulation % of control − Sporulation % of treatment)/ Sporulation % of control] × 100

Table 1: Sporulation results of in vitro test with 3 different botanical formulations and dosages

	Sporulation % 24h	Sporulation % 48h	Sporulation % 72h	Sporulation Inhibition 72 h %
A1	7.7	19.2	26.2	69.4
A2	11.2	15.3	28.2	67.1
A3	16.2	21.5	21.3	75.1
B1	4.8	23.8	27.5	67.9

B2	11	17.5	25.7	70.0
B3	10.2	16.7	22.3	74.0
C1	17.5	30.2	37.5	56.2
C2	11.2	23.2	30.3	64.6
C3	20.7	25.3	28	67.3
PC	**0**	**0**	**0**	100.0
NC	**36.5**	**78.7**	**85.7**	

Garlic gave the best Sporulation Inhibition index at low concentration and was a good candidate for an in vivo test after that screening of botanicals in vitro (cf table 1). Based on these studies, we tested a mix of plant extracts (M) with complementary properties. Beside the anticoccidial effect of garlic, curcuma, and clove, Cinnamon can balance microbiota and enhance immune response of young birds, clove and curcuma bring anti-inflammatory and antioxidant activities.

In vivo trials with a combination of phytogenic compounds

Two trials were carried out in Mexico to test a mix of plant extracts and essential oils (M) with the active molecules described above. In each 1400 male Ross 308 at hatch, were divided in 6 treatment, ten pens of 40 animals / treatment. All feed were in mash form. Five feeds were distributed during animal life: pre-starter (0-10d), starter (11-21d), grower (22-28d), finisher (29-42d) and preslaughter (43-49d). 50 ppm of total xanthophyl were added in feed from 21d. Live weight and feed consumption were recorded at each feed transition to have per phase: mortality, weight gain, feed consumption and feed

conversion ratio. The coloration of the skin was measured on hot carcasses at slaughter (49d) in L*, a*, b* (CIELAB) system (2 animals per pen, 20 measurements/ treatments). Oral inoculation of oocysts (*E. acervulina* – 100 000 oocysts, *E tenella* – 20 000 oocysts, *E maxima* – 20 000 oocysts) was carried out at 14 d for all inoculated treatments. Oocysts per gram of feces (opg) were counted using McMaster chamber and routine procedures at d14, before inoculation (-1 D), at d18 (+4 DPI – Day Post Inoculation), d19 (+5 DPI), d20 (+6 DPI), d21 (+7 DPI), d28 (+14 DPI), d35 (+21 DPI) and d42 (+28 DPI). The lesional scoring of Johnson and Reid (Johnson & Reid, 1970) was carried out at d21 (+7 DPI), d28 (+14 DPI), d35 (+21 DPI) and d42 (+28 DPI) on 1 animal / pen, i.e. 10 / treatment. To assess the livability of oocysts, the integrity of membrane was observed from 7 DPI at each collection day. 1-way anova was performed with multiple comparisons of Tukey-Kramer or Fisher's LSD. Significant differences were considered at 5%.

a. First trial

In the first trial, the 6 treatments were as followed: C: control without coccidiosis inoculation without any coccidiostats; NC: negative control with coccidiosis inoculation without any coccidiostat; PC: NC + 110ppm of monensin; M100: NC + M at full dosage (100%) from 0 to 49d; M75: NC + M at full dosage from 0 to 28d and 75% from 28 to 49d; M50: NC + M at full dosage from 0 to 28d, 75% from 28 to 42d and 50% from 42 to 49d.

Despite coccidian challenge, mortality was not different amongst treatments. Numerically, the lowest mortalities were recorded for C, PC, M100 and the highest for NC. As expected, zootechnical performance was negatively

impacted by the coccidian challenge. Average feed consumption from 0 to 49d was not affected by any of the treatment. Weight was significantly impaired by coccidiosis infection from 28d and till the end of the trial. PC, M100 and M75 restored weight at the same level as C. M50 is not significantly different from C or any other treatments but, at 49d, was numerically lower than PC, M100 and M75 (C=3037.5g, NC=2761.2g, PC=2989.0 g, M100=3039.9g, M75=3064.3g, M50=2941.5g; $p<0.05$). At the end of the trial, FCR was not significantly impaired by treatments, however it was till 42d (data not shown). Numerically, NC had the worst FCR, all the other treatments were at least 0.10 points under it and very close to the C. In terms of coloration based on the measure of the b* (yellowness), NC impaired significantly the yellowness of the skin compared to C, M50 did not allow any improvement but b* of all the other treatments were at the same level as b* of C (C=27.13, NC=22.35, PC=27.54, M100=27.43, M75=26.36, M50=24.83, $p<0.001$) (table 2).

Table 2: Zootechnical and coloration results in trial 1

	C	NC	PC	M100	M75	M50	P
Weight (g) at 0d	43.5 ± *0.5*	43.3 ± *0.5*	43.3 ± *0.5*	43.4 ± *0.4*	43.6 ± *0.6*	43.1 ± *1.0*	0.57
Weight (g) at 21d	742 ± *20* **a**	615 ± 40 **b**	598 ± 40 **b**	608 ± 30 **b**	605 ± 60 **b**	562 ± 40 **b**	**<0.001**
Weight (g) at 28d	1213 ± *28* **a**	954 ± *56* **b**	1242 ± *65* **a**	1265 ± *65* **a**	1244 ± *77* **a**	1215 ± *60* **a**	**<0.001**
Weight (g) at 42d	2191 ± *141* **ab**	1936 ± *266* **b**	2171 ± *266* **ab**	2304 ± *184* **a**	2385 ± *209* **a**	2215 ± *271* **ab**	**<0.001**
Weight (g) at 49d	3038 ± *131* **a**	2761 ± *348* **b**	2989 ± *70* **a**	3040 ± *152* **a**	3064 ± *327* **a**	2942 ± *223* **ab**	**<0.05**
Mortality (%)	4.5	6.75	4.25	4.00	6.50	5.75	0.22
FI 0-49d (g/an/d)	121.6 ± *17.5*	119.8 ± *6.4*	121.2 ± *6.1*	122.6 ± *4.3*	120.7 ± 4.5	119.1 ± 5.2	0.95
FCR 0-49d	1.990 ± 0.264	2.159 ± *0.286*	2.017 ± *0.097*	2.003 ± *0.089*	1.959 ± *0.156*	2.012 ± *0.195*	0.17
b* at 49d	27.13 ± *2.38* **a**	22.35 ± *1.97* **b**	27.54 ± *2.08* **a**	27.43 ± *2.66* **a**	26.36 ± *1.43* **a**	24.83 ± *2.31* **b**	**<0.001**

The figures are presented mean ± SD (standard deviation). a, b, c letters indicate significant differences at p<0.05%.

The variability of oocyst excretion was very high within the treatments (CV from 65% to 259%). Four days post inoculation, animals of NC excreted oocysts in feces but no oocysts were excreted in the other groups. Excretion of oocysts began the day after without any differences between groups. At 6 DPI, the opg of feces from PC, M100, M75 and M50 were numerically above the NC. The peak of oocysts excretion appeared at 7 DPI, the opg of feces from NC was the highest, all other treatments were between C and NC without difference between PC and other treatments. After this date, no significant differences were highlighted between treatment on the number of oocysts / g of feces, but the viable rate of these oocysts was lowered by all products in feed compared to NC ($p < 0.001$ 21 and 28d after challenge) (Table 3).

Table 3: Total oocysts excretion / g of feces and viable oocyst rate in trial 1

	C	NC	PC	M100	M75	M50	P
Oocysts/ g of feces (%CV)							
- 1 D	0	0	0	0	0	0	-
+ 4 DPI	0 **a**	390 **b** *(123.4%)*	0 **a**	0 **a**	0 **a**	0 **a**	**<0.001**
+ 5 DPI	0	218 660 *(187.8%)*	134 110 *(168.8%)*	77 455 *(98.3%)*	170 620 *(65.3%)*	213 805 *(129.2%)*	0.24
+ 6 DPI	0 **a**	49 735 *(93.1%)* **ab**	83 240 *(123.1%)* **b**	68 500 *(55.7%)* **ab**	71 445 *(93.6%)* **ab**	70 420 *(85.7%)* **ab**	**<0.05**
+ 7 DPI	0 **a**	624 740 *(76.6%)* **b**	268 805 *(137.0%)* **ab**	220 735 *(96.9%)* **ab**	227 840 *(127.9%)* **ab**	398 750 *(94.0%)* **ab**	**<0.05**
+14 DPI	0	15 175 *(94.5%)*	8 440 *(130.0%)*	2 350 *(86.3%)*	40 390 *(167.6%)*	24 775 *(187.1%)*	0.09
+21 DPI	0	800 *(134.1%)*	660 *(258.9%)*	190 *(105.8%)*	70 *(139.6%)*	335 *(211.1%)*	0.25
+28 DPI	150 *(157.9%)*	840 *(145.4%)*	810 *(189.9%)*	440 *(114.9%)*	250 *(177.1%)*	1 455 *(123.9%)*	0.12
% viable oocysts							
+ 7 DPI	-	47.0	59.5	49.6	59.6	55.9	0.11
+ 14 DPI	-	23.0	18.5	21.0	18.1	18.5	0.86
+ 21 DPI	-	86.4 **a**	43.0 **b**	16.25 **b**	13.85 **b**	19.00 **b**	**<0.001**
+ 28 DPI	31.1 **b**	51.8 **a**	21.54 **b**	20.0 **b**	27.5 **b**	24.0 **b**	**<0.001**

The figures of oocysts /g of feces are presented as geometric mean of opg *(CV)*. a, b, c letters indicate significant differences at p<0.05%. DPI: days post-inoculation

The total lesion score of NC was maximum 7 days after coccidian challenge. All feed treatments allowed to limit this lesion score. At 14 days after inoculation, the three treatments with plant extracts supplemented feed had lesion score lowered compared to NC and PC, supplemented with monensin.

The decrease of the dosage of plant extracts (100% in M100 to 75% in M75 and M50) from 28 to 42d did not change anything about the restoration of intestinal integrity (Table 4).

Table 4: Evolution of intestinal lesion score of Johnson & Reid in trial 1

		C	NC	PC	M100	M75	M50
At 21 d +7 DPI	Duodenum	0.0	1.4	1.2	0.7	0.9	0.7
	Jejunum	0.0	0.6	1.0	0.7	0.5	0.7
	Ileum	0.0	0.3	0.1	0.1	0.4	0.2
	Caeca	0.0	0.8	0.5	0.8	1.1	0.9
	Total lesion score	**0.0**	**3.1**	**2.8**	**2.3**	**2.9**	**2.5**
At 28 d +14 DPI	Duodenum	0.0	1.1	0.7	0.6	0.6	0.8
	Jejunum	0.0	0.5	0.2	0.0	0.4	0.4
	Ileum	0.0	0.3	0.2	0.0	0.0	0.0
	Caeca	0.0	0.7	0.7	0.2	0.6	0.7
	Total lesion score	**0.0**	**2.6**	**1.8**	**0.8**	**1.6**	**1.9**
At 35 d +21 DPI	Duodenum	0.0	0.6	0.2	0.1	0.1	0.4
	Jejunum	0.0	0.1	0.0	0.1	0.2	0.1
	Ileum	0.0	0.2	0.0	0.0	0.0	0.0
	Caeca	0.0	0.3	0.1	0.1	0.1	0.4
	Total lesion score	**0.0**	**1.2**	**0.3**	**0.3**	**0.4**	**0.9**
At 42d +28 DPI	Duodenum	0.0	0.2	0.0	0.0	0.0	0.0
	Jejunum	0.0	0.1	0.0	0.0	0.0	0.0
	Ileum	0.0	0.0	0.0	0.0	0.0	0.0
	Caeca	0.0	0.2	0.0	0.0	0.0	0.0
	Total lesion score	**0.0**	**0.5**	**0.0**	**0.0**	**0.0**	**0.0**

b. Second trial

In the second trial, the 6 treatments were as followed: C: control without coccidiosis inoculation without any coccidiostat; NC: negative control with coccidiosis inoculation without any coccidiostat; PC: NC + 60ppm of salinomycin; M100: NC + M at full dosage (100%) from 0 to 49d; M75: NC + M at 75% from 0 to 49d; M50: NC + M at 50% from 0 to 49d.

Table 5: Zootechnical and coloration results in trial 2

	C	NC	PC	M100	M75	M50	P
Weight (g) at 0d	38.7 ± *0.6*	38.9 ± *0.3*	39.1 ± *0.4*	38.6 ± *0.6*	38.9 ± *0.3*	38.8 ± *0.4*	0.29
Weight (g) at 21d	715 ± 32 **a**	623 ± 37 **b**	638 ± 30 **b**	623 ± 34 **b**	629 ± 13 **b**	618 ± 35 **b**	**<0.001**
Weight (g) at 28d	1177 ± *58* **a**	1009 ± *30* **c**	1111 ± *40* **b**	1123 ± *25* **ab**	1109 ± *34* **b**	1058 ± *67* **bc**	**<0.001**
Weight (g) at 42d	2259 ± *94* **ab**	2185 ± *42* **b**	2311 ± *66* **a**	2309 ± *40* **a**	2303 ± *91* **a**	2239 ± *63* **ab**	**<0.001**
Weight (g) at 49d	2880 ± *131* **bc**	2806 ± *63* **c**	3033 ± *98* **a**	2997 ± *75* **ab**	2973 ± *77* **ab**	2938 ± *71* **ab**	**<0.001**
Mortality (%)	4.00	4.50	3.50	3.25	3.75	4.00	0.98
FI 0-49d (g/an/d)	123.5 ± *1* **b**	125.0 ± *2* **ab**	126.9 ± *2* **a**	125.2 ± *2* **ab**	126.1 ± *2* **a**	125.3 ± *2* **ab**	**<0.05**
FCR 0-49d	2.129 ± *0.096* **a**	2.212 ± *0.061* **b**	2.077 ± *0.048* **a**	2.073 ± *0.055* **a**	2.105 ± *0.047* **a**	2.117 ± *0.043* **a**	**<0.001**
b* at 49d	26.19 ± *2.59* **a**	23.31 ± *1.87* **b**	26.35 ± *2.13* **a**	25.88 ± *2.37* **a**	25.87 ± *1.57* **a**	25.08 ± *2.02* **ab**	**<0.001**

The figures are presented mean ± SD (standard deviation). a, b, c letters indicate significant differences at p<0.05%.

In this trial, mortality was kept below 4.5% for all treatments without any difference amongst them. At 28 days of age, coccidian challenge impaired significantly the zootechnical performance and all products in feed limited the impact on weight. M100 weight at 28d was similar to the C, on the other hand M75, M50 and PC had intermediate weights. At 42 and 49d, PC had the best weight. At the end, weights of M100, M75 and M50 are intermediate between C and PC. The lowest weight is recorded for NC (C=2880; NC=2806; PC=3033; M100=2997; M75=2973; M50=2938; p<0.001). The decrease of the dosage of phytogenic at the end of the rearing period did not affect the final weight but

there is a numerical trend to decrease final body weight by decreasing the dose of the product. In this trial, average feed intake was the lowest for C and the highest for PC and M75, all the other treatments were intermediate. The FCR0-49d was significantly highest for NC and all the other treatments were on the same level. Decrease the level of inclusion of phytogenic numerically impaired the feed conversion ratio. In terms of coloration, this trial confirmed the degradation of skin coloration with coccidiosis. PC, M100 and M75 allowed to restore it at the same level of b* than C, M50 was intermediate (Table 5).

Table 6: Total oocysts excretion / g of feces and viable oocyst rate in trial 2

	C	**NC**	**PC**	**M100**	**M75**	**M50**	**P**
Oocysts/ g of feces (%CV)							
- 1 D	122 *(255.7%)*	16 *(228.6%)*	45 (169.3%)	105 (196.3%)	60 (263.9%)	104 (194.6%)	0.98
+ 4 DPI	332 *(233.8%)* **a**	13 010 *(90.1%)* **c**	11 020 *(167.5%)* **c**	4 405 *(135.7%)* **bc**	2 235 *(74.6%)* **ab**	3 400 *(188.1%)* **ab**	**<0.05**
+ 5 DPI	1 530 *(83.9%)*	354 175 *(112.9%)*	297 045 *(135.6%)*	289 890 *(94.6%)*	327 350 *(80.5%)*	404 500 *(85.8%)*	0.08
+ 6 DPI	5 280 *(68.3%)*	197 160 *(173.7%)*	67 925 *(80.1%)*	67 600 *(60.7%)*	99 560 *(137.0%)*	80 435 *(91.9%)*	0.16
+ 7 DPI	36 950 *(78.7%)* **a**	179 335 *(57.7%)* **b**	149 110 *(114.6%)* **b**	93 270 *(43.4%)* **ab**	97 810 *(51.5%)* **ab**	129 295 *(96.6%)* **b**	**<0.05**
+14 DPI	25 305 *(242.6%)*	9 365 *(125.7%)*	16 315 *(123.4%)*	3 790 *(120.3%)*	4 660 *(116.4%)*	3 115 *(82.7%)*	0.38
+21 DPI	1 990 *(107.3%)*	970 *(153.3%)*	1 543 *(249.8%)*	845 *(201.9%)*	465 *(184.2%)*	260 *(156.2%)*	0.42
+28 DPI	1 225 *(168.8%)*	215 *(168.4%)*	50 *(216%)*	350 *(178.8%)*	1 100 *(259.8%)*	65 *(181.5%)*	0.28
% viable oocysts							
+ 7 DPI	52.1	47.9	48.3	45.3	52.7	47.5	0.17
+ 14 DPI	29.1 **ab**	53.1 **a**	35.4 **ab**	27.1 **ab**	22.0 **b**	23.1 **b**	**<0.01**
+ 21 DPI	23.5 **ab**	37.9 **a**	49.1 **a**	16.3 **b**	13.9 **b**	19.0 **b**	**<0.01**

+ 28 DPI	18.0	31.7	20.0	11.3	20.0	15.7	0.21

The figures of oocysts /g of feces are presented as geometric mean opg *(CV)*. a, b, c letters indicate significant differences at p<0.05%. DPI: days post-inoculation

Before any inoculation, coccidiosis was present in the farm and contamination was detected one day prior to infection. In this second trial, the variability of oocyst excretion was very high within the treatments (CV from 61% to 256%). The peak of oocysts excretion appeared at 5 DPI. At this date, results about treatment effects were not significant. At 4 DPI, phytogenic (M100, M75 and M50) decreased oocysts excretion compared to NC and PC. At 7 DPI, only were intermediate between C and NC, PC and M50 were not different from NC. At 14 and 21 DPI, the viable rate of oocysts was lowered by all phytogenic products compared to NC (p< 0.01 21 and 28d after challenge). The results for PC were less clear (Table 6).

The total lesion score was maximum at 14 DPI. All feed treatments allowed to limit this lesion score. At 14 DPI, the three treatments with plant extracts had lesion score lowered compared to NC and PC, supplemented with salinomycin. The decrease of the dosage of plant extracts in M75 and M50 compared to M100 did not impact the maximum lesion score reached at 14 DPI but delayed the restoration of intestinal mucosa (Table 7).

Table 7: Evolution of intestinal lesion score of Johnson & Reid in trial 2

		C	**NC**	**PC**	**M100**	**M75**	**M50**
At 21 d +7 DPI	Duodenum	0.3	2.8	2.0	2.1	2.1	2.3
	Jejunum	0.1	0.4	0.4	0.9	0.8	0.6
	Ileum	0.0	0.3	0.4	0.0	0.1	0.4
	Caeca	0.0	0.6	0.5	0.4	0.5	0.2
	Total lesion score	**0.3**	**2.8**	**2.0**	**2.1**	**2.1**	**2.3**
At 28 d	Duodenum	0.6	1.1	1.2	1.3	0.9	1.0

+14 DPI	Jejunum	0.1	1.2	0.7	0.6	0.7	1.1
	Ileum	0.2	0.7	0.8	0.2	0.2	0.5
	Caeca	0.0	0.5	0.6	0.3	0.6	0.6
	Total lesion score	**0.9**	**3.5**	**3.3**	**2.4**	**2.4**	**2.3**
At 35 d +21 DPI	Duodenum	0.5	0.8	0.6	0.4	0.4	0.9
	Jejunum	0.3	0.3	0.4	0.3	0.4	0.4
	Ileum	0.4	0.4	0.3	0.0	0.0	0.1
	Caeca	0.7	0.5	0.1	0.3	0.6	0.2
	Total lesion score	**1.9**	**2.0**	**1.4**	**1.0**	**1.4**	**1.6**
At 42d +28 DPI	Duodenum	0.2	0.5	0.5	0.2	0.2	0.3
	Jejunum	0.1	0.2	0.3	0.2	0.5	0.4
	Ileum	0.2	0.2	0.2	0.1	0.0	0.1
	Caeca	0.4	0.5	0.1	0.3	0.3	0.3
	Total lesion score	**0.9**	**1.4**	**1.1**	**0.8**	**1.0**	**1.1**

Discussion and conclusion

Thanks to an extensive and careful literature screening of active phyto-ingredients, the *in vitro* test allowed us to select a garlic-based mix of plants for a first set of *in vivo* studies.

In our *in vivo* studies, peak oocyst shedding occurred around 5 to 7 DPI, as mentioned in literature. In both trials, the phytogenic product at the highest dosage had the same effect than tested ionophore (monensin or salinomycine) on oocyst excretion. At that time, 21 days of age, despite different levels of oocyst shedding amongst treatments, all inoculated animals had lighter body weight than control and the same lesion score. This result is in line with Chasser et al. (2020) who reported the difficulty to draw conclusions on efficacy of treatment with only body weight and lesion score. He promoted the need to evaluate the kinetics of oocyst shedding in addition to performance and lesion score measurements to be able to define some active component effects.

As mentioned, lesion score did not differ at 7 DPI but a quicker recovery than with ionophores or without anything was observed with plant extract and essential oils mix, whatever the dosage. This can be explained by antioxidant and anti-inflammatory properties of garlic and eugenol (Kim et al., 2013; Youssefi et al., 2023) and impact of cinnamaldehyde on microbiota and support of intestinal integrity (Orengo et al., 2012; Yang et al., 2020). This quick recovery of intestinal functions allowed animals to catch quickly with the C weight and FCR 0-49d. This improvement of growth performance was in the same level than the ionophore one in these 2 studies.

The results also showed that a sufficient dosage of active ingredients (M100) is necessary to decrease oocyst excretion and support intestinal health. Interestingly, in the first trial, despite very good results in terms of growth with decreasing dosage during the life of animal, the coloration of the skin was impaired with lower dosage. Thus, this showed a potential negative effect on intestinal integrity and / or oxidative status of birds when the active component concentration is too low and validated the need to keep the full dosage of the tested mix from D0 to the end of the life of broilers.

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Luis Pantoja Millas

Regional Technical & Marketing Manager, HIPRA, Avda. La Selva 135, 17170, Amer (Girona), Spain

A MILESTONE IN BROILER COCCIDIOSIS VACCINATION: EVANOVO®

Trends in coccidiosis prevention

Avian coccidiosis is one of the main destabilizing agents of the digestive health of birds, with the destruction of enterocytes and affecting intestinal integrity. The lesions it causes, the inflammatory process, the reduced absorption and consequent excess of nutrients in the lumen, contribute to the proliferation of certain bacterial groups, in particular the colonization by *Salmonella* spp. (Takimoto et al., 1984), *Escherichia coli* (Nakamura et al., 1990) and, above all, *Clostridium perfringens* (Porter et al., 1998). This is why one of the most important decisions to make in broiler production in order to preserve or improve zootechnical or financial results is the design of the preventative treatment for the control of coccidiosis.

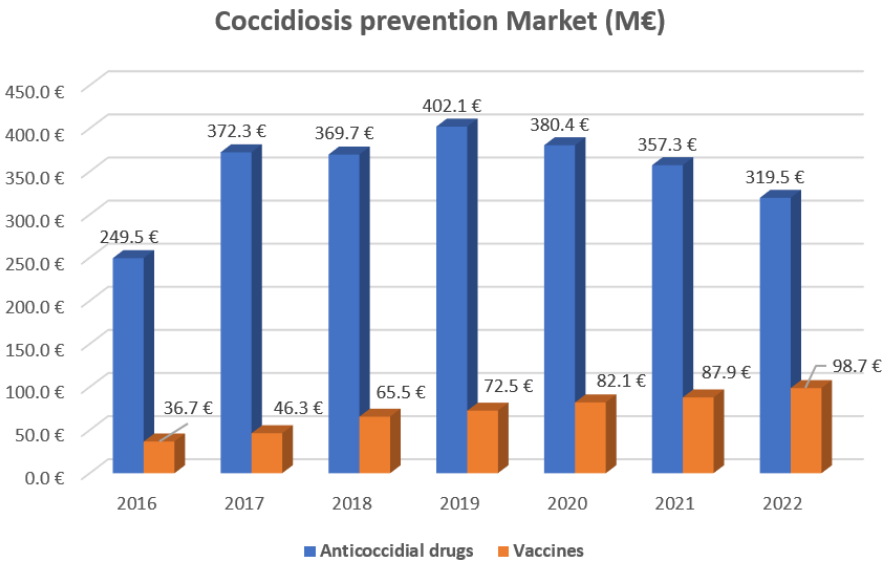
In these preventive programmes, coccidiostat drugs are generally used in the feed, which have traditionally been considered sufficient to control the coccidiosis disease. However, it has been observed that the degree of resistance of *Eimeria* spp. to coccidiostats is continuing to increase, even with the use of strategies of rotating different products during and between the production cycles.

These resistances are due to the continuous and excessive use of the same anticoccidial molecules, as no new active ingredients have been introduced in the last 30 years.

Moreover, the growing trend of consumers and authorities to demand products of animal origin produced without the use of antibiotics, anticoccidials and other drugs, together with the risk of contamination of these animal productions because of the high doses of coccidiostats that are needed, have led the poultry sector to search for alternatives to control the coccidiosis disease.

For this reason, the use of live vaccines against coccidiosis in broiler chickens is becoming the most interesting alternative to coccidiostats as a highly effective solution in the face of that increasing resistance shown by the parasites of *Eimeria* spp. and the aforementioned changes in production trends.

Fig**ure 1**. Yearly evolution of the coccidiosis prevention market (2016-2022). Sales (in €M) of anticoccidial drugs and coccidiosis vaccines. Pharmaceutical companies CEESA data



Furthermore, in-ovo vaccination is attracting increased interest from poultry producers as a method of administration of avian vaccines, as it makes the process individual, more precise and reliable, and cheaper in many cases. Due to that, the use of new vaccines against avian coccidiosis attenuated by precociousness and expressly developed for in-ovo administration are also becoming an option to control the disease.

As a veterinary laboratory specializing in prevention for animal health, HIPRA has been making an important contribution to the promotion of intestinal health in poultry for many years, bringing to the market vaccines for the prevention of coccidiosis in chickens, characterized by their high levels of efficacy and safety (**EVALON®, EVANT®, HIPRACOX®**).

As HIPRA´s next step in gut health promotion, a new coccidiosis vaccine for chickens has been launched in the market, being developed specifically to be administered in-ovo: **EVANOVO®**.

EVANOVO®: main characteristics for the success of a coccidiosis vaccine for in-ovo administration

Sensitive strains and attenuation by precociousness:

The usage of vaccinal strains sensitive to anticoccidial drugs, together with the attenuation by precociousness (Jeffers T., 1975) reduces resistance against anticoccidial drugs through recombination with field strains (Shirley et al., 2007), by the seeding of sensitive strains in the field and reducing the selective pressure induced by anticoccidial drugs (Chapman et al., 1997).

Consequently, an ideal situation is that all the *Eimeria* strains included in the vaccine are sensitive to anticoccidial drugs and attenuated by this method.

Sanitization of the vaccine solution:

Ensuring the safety of a vaccine is always crucial to develop an adequate product. Thus, it is of great importance to avoid any kind of contamination (mainly other microorganisms or organic material) of the vaccinal solution.

Sanitizing by other methods than including antimicrobials or chemicals will allow the production of a vaccine that does not interact with other live strategies used in poultry production (bacterial live vaccines, probiotics, etc.).

Wide spectrum protection:

For the prevention of coccidiosis in chickens through live vaccines, as no cross protection between species is feasible, it is essential to include in the vaccine composition the species that are needed to prevent against for every type of chicken production.

In the case of broilers, it is necessary to include the main species that cause clinical coccidiosis and have the highest prevalence: *Eimeria acervulina*, *E. maxima* and *E. tenella*. It is also very important to provide immunity against those *Eimeria* species that can cause subclinical coccidiosis and affect the broiler performance such as *E. praecox*.

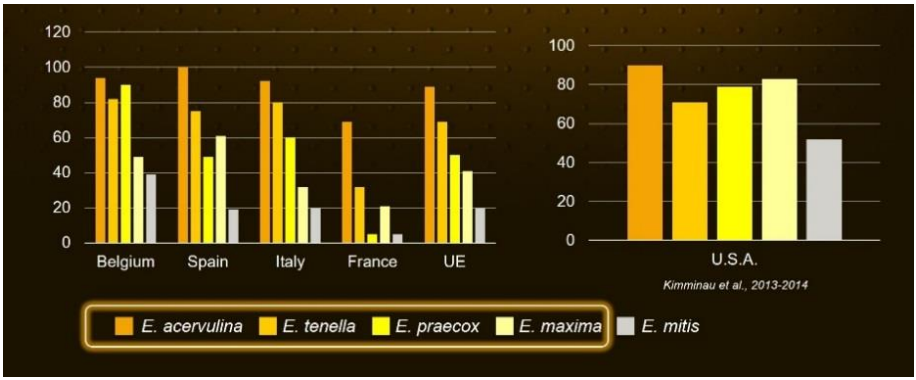


Figure 2. Prevalence of *Eimeria* spp. in broilers farms in Belgium, Spain, Italy, France and European Union.

Joint application with other in-ovo vaccines:

The current strategy of vaccination through in-ovo devices was mainly designed to administer vectorised type vaccines (covering Marek disease alone or together with Newcastle disease and/or Gumboro disease) or immune-complex vaccines against Gumboro disease, or a combination of all these.

Consequently, an in-ovo vaccine against coccidiosis in chickens should be able to be administered together with these vaccines, without affecting the stability/safety or efficacy of any of them.

See below some figures of a study comparing the results of several combinations of vaccines:

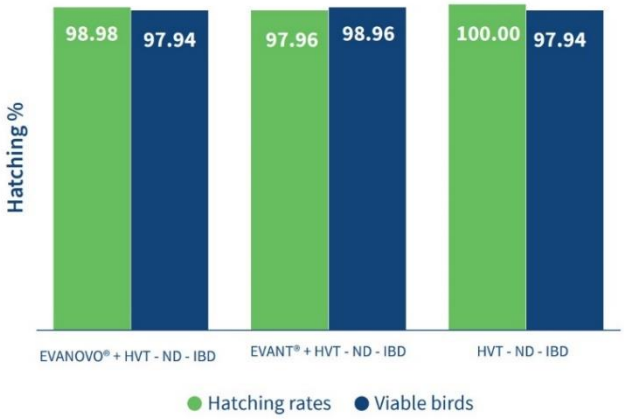


Figure 3. Hatching rates and viable birds according to the combination of vaccines.

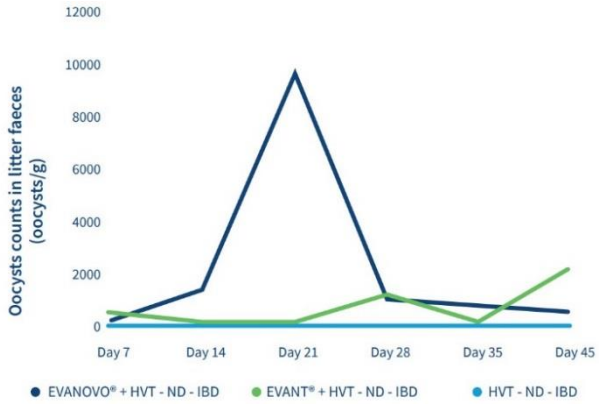


Figure 4. Average oocyst counts in litter faeces of each vaccinated group at different time points.

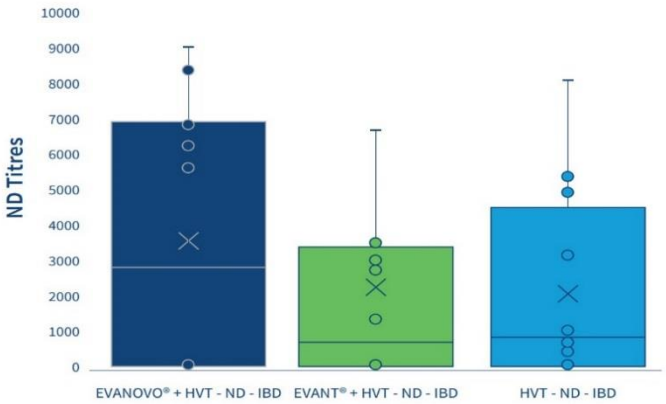


Figure 5. ND seroconversion titres at the end of the study period (day 45 post-hatching). A commercial diagnostic ELISA kit was implemented: BioCheck® NDV.

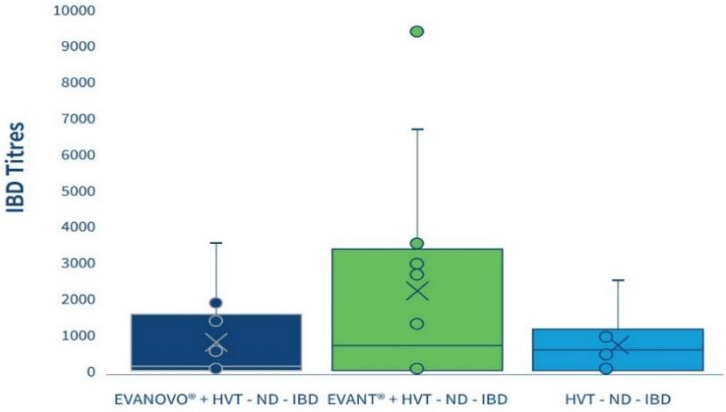


Figure 6. Titres of IBD seroconversion at the end of the study period (day 45 post-hatching). A commercial diagnostic ELISA kit was implemented: BioCheck® IBD CK113.

<u>Administration, key to ensuring the success of the process</u>

The in-ovo vaccination process is carried out in the transfer phase in the hatchery, when transferring the embryonated eggs from the incubator machines to the hatchers. This process is usually performed between days 18 and 19.5 of embryonic development.

Vaccines applied in ovo for other pathologies, such as Marek's, ND or IBD vaccines, may have adequate results when administered to the embryo.

In-ovo vaccines against coccidiosis in chickens contain live *Eimeria* oocysts, and due to its mechanism of action, they should be applied into the amniotic cavity. If this is happens, the embryo will consume the vaccine solution orally and, at hatching, the replication process of the vaccine strains will begin in the chick's intestine.

To achieve this goal, auditing the **Site of Injection (SOI)** of the embryonated eggs before the vaccination can be very helpful to optimize the vaccination procedure. With this prior evaluation, it is possible to know the current percentage of embryonated eggs that are injected into the amnion and to take the necessary measures, such as equipment calibration, to ensure that the device's needles perform the most precise vaccination possible. For this reason, any in-ovo vaccination device on the market has the possibility of using an in-ovo vaccine against coccidiosis in chickens with excellent results.

Experience on the field with EVANOVO® vaccination

The objective of this experience was to evaluate and compare the zootechnical performance of broilers after their vaccination with the in-ovo vaccine against avian coccidiosis, EVANOVO®, in comparison with another

vaccine against avian coccidiosis in broilers administered by coarse spray, widely and commonly used in the last years. The experience comparing these two products was performed in Spain on several farms being part of the same integration company.

This study was carried out in 2022, involving a total of 70 flocks and almost 1,700,000 chickens vaccinated with EVANOVO® and around 1,400,000 broilers vaccinated with the coarse spray vaccine.

The in-ovo vaccination was performed with a device for conventional use (EMBREX INOVOJECT®). An immunocomplex vaccine against IBDV vaccine was injected together with the in-ovo vaccine against avian coccidiosis.

The following graphs show a summary of the results obtained:

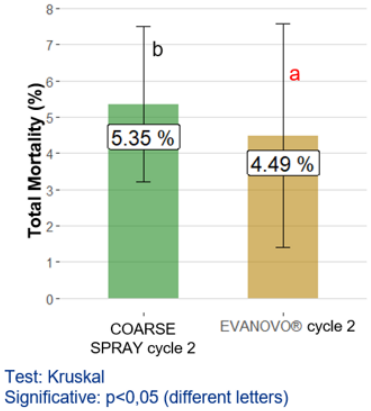


Figure 7. Mortality percentage.

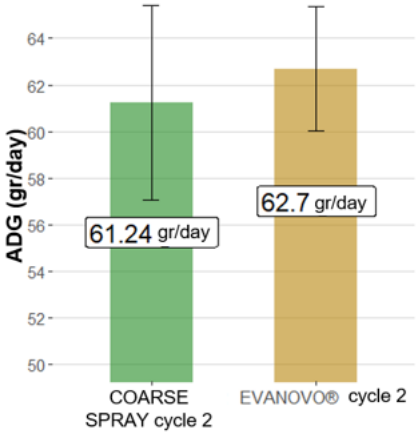


Figure 8. Average Daily Gain (ADG), in grams per day.

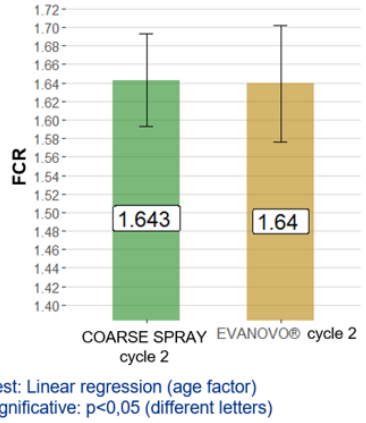


Figure 9. Feed Conversion Ratio (FCR).

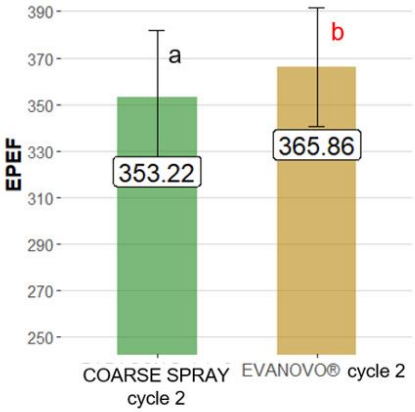


Figure 10. European Production Efficiency Factor (EPEF).

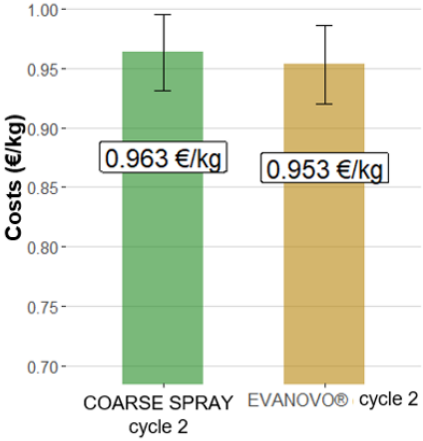


Figure 11. Cost of production, in € per kg of chicken meat.

In some of the parameters evaluated, it was a numerical difference between the broiler performance in favour of those vaccinated with

EVANOVO®. Then, the average daily gain and the feed conversion ratio were numerically better in those chickens.

There was also a statistically significant difference between the two vaccines in the mortality of the flocks, being lower in the broilers vaccinated with EVANOVO®.

Because of these differences in the performance of the chickens, the final analysis shown a statistically significant improvement in the European Production Efficiency Factor (EPEF) in the broilers vaccinated with EVANOVO®. Besides, it was calculated a reduction in the cost of production of 0.01€/kg of chicken meat.

In conclusion, these results of this experience show that this HIPRA's new in-ovo coccidiosis vaccine, EVANOVO®, is as effective for the protection of chickens as the traditional used vaccines, delivering even a better production performance of the broilers.

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Corrado Longoni

MSD Animal Health

COCCIDIOSIS MANAGEMENT – IS EVERYTHING UNDER CONTROL?

The poultry industry has experienced significant growth in production, leading to increased stress and incidence of diseases, including coccidiosis, which is caused by protozoa parasites and has various negative effects on poultry health and productivity. Coccidiosis is particularly challenging to manage in the broiler industry, causing intestinal lesions, poor weight gain and reduced feed conversion. It also increases the risk of other epidemic disorders. Coccidiosis is highly infectious and spreads through contact with infected feces, especially in warm and humid environments. It can have a synergistic effect with other infections, leading to higher mortality rates. The economic consequences of coccidiosis include drops in animal production, increased mortality, and costs associated with treatment and prevention. The annual monetary impact of coccidiosis on commercial birds worldwide has been estimated at 10.4 billion dollars. Treatment and prevention costs, including the use of coccidiostats in poultry feed, contribute to the economic burden. The cost per bird can exceed 0.16 dollars [1].

Controlling coccidiosis is challenging due to the specific characteristics of the disease and the development of coccidiostat resistance. Coccidiosis in poultry can be controlled using coccidiostats, which are drugs that inhibit the growth and reproduction of the *Eimeria* parasites responsible for the disease. These coccidiostats are commonly added to poultry feed to prevent and manage

coccidiosis. Rotation programs that alternate between vaccination and drug use in successive flocks can help sustain long-term coccidiosis control in poultry. Good management practices, such as providing ample floor space, feeders, and waterers, are important in preventing overcrowding and reducing the risk of coccidiosis.

Poultry anticoccidials are substances used to prevent and control coccidiosis; these anticoccidials are commonly added to poultry feed to maintain animal health and improve feed conversion. They work by targeting the *Eimeria* species, which are responsible for causing coccidiosis in poultry. The most used anticoccidials in poultry production include various drugs. However, improper use of anticoccidial drugs in poultry can lead to drug-resistant varieties and the presence of residues in meat products, which are not permissible for human consumption due to their toxicity. Poultry anticoccidials can be categorized into two main groups: polyether ionophores and synthetic compounds. Polyether ionophores include substances such as lasalocid, monensin, maduramicin, narasin, salinomycin, and semduramicin, which are produced by different bacteria. Synthetic compounds used as anticoccidials in poultry include decoquinate, diclazuril, halofuginone, nicarbazin, and robenidine. Some anticoccidials are used in combination, such as a mixture of a synthetic compound and an ionophore or two synthetic compounds. These categories of anticoccidials are authorized as poultry feed additives in the European Union (EU).

Ionophores are a type of poultry anticoccidials that work by disrupting the ion balance in the parasites, specifically the *Eimeria* species, which cause coccidiosis in poultry. They act by forming complexes with metal ions, such as sodium, potassium, and calcium, and transporting them across the parasite's cell

membranes This disruption of ion balance interferes with the normal functioning of the parasites' cells, leading to their death. Ionophores also have an effect on the host bird's immune response, stimulating the production of antibodies and enhancing the bird's ability to fight against the *Eimeria* parasites. By targeting the parasites and boosting the bird's immune system, ionophores help to prevent and control coccidiosis in poultry.

Ionophores can help maintain a healthy gut microflora in chickens by reducing the population of harmful pathogens, such as certain bacteria. They can promote the growth of beneficial bacteria, such as lactobacilli, which contribute to improved gut health and overall performance of the birds. Ionophores can enhance the balance of the gut microflora by reducing the competition between harmful and beneficial bacteria, leading to a more favorable microbial community. It is important to note that the impact of ionophores on the chicken gut microflora can vary depending on factors such as dosage, duration of use, and individual bird characteristics.

Overall, while ionophores can have positive effects on the chicken gut microflora by reducing harmful pathogens and promoting beneficial bacteria, their use can also disrupt the balance of the microflora, potentially leading to negative consequences.

Synthetic drugs were the first to be discovered and comprise a diverse array of molecules that are absorbed into the blood stream of the host and kill developing parasites in the epithelial cells of the villi in the intestines. One of the oldest synthetic drugs is nicarbazin (coccidiostat agent). The molecular mechanism of nicarbazin is based on inhibiting the development of the first and second generations of the schizonts stage of the parasites. There are some molecular mechanisms proposed for nicarbazin’s avian adverse effect, but no

research group to date has conducted in vivo research [2]. Nicarbazin is one of the most successful drugs and is still widely used today [3, 4]. Another coccidiostat synthetic drug with a wide range of action is amprolium and has been shown to inhibit the uptake of thiamine by second generation schizonts of *E. tenella*. Quinolone drugs inhibit cellular respiration by blocking the electron transport chain in the parasite mitochondrion thus arresting the parasite in the initial stages of development [5, 6]. Since the discovery of sulfonamide sixty-five years ago as a potent compound to control *Eimeria* infections, the development of anticoccidial drugs has continued in earnest. The use of several drugs, alone or in combination, has proven to be an effective mechanism in the struggle against avian coccidiosis. However, the emergence of drug-resistant strains, especially after prolonged uses of a drug, is a real problem [7]. To combat resistance, shuttle and rotation systems of drugs are employed. In the shuttle program, the different drugs are used during a period of juvenile growth to market size growth, whereas in the rotation program, the type of drug used is switched after one or several grow-out periods or seasonally [8]. However, even with the shuttle and rotation programs there is no method to fully prevent drug resistance. This has been observed when ionophores, such as monensin or lasalocid, are used in the field and drug resistant parasites emerge [9]. Due to the constant pressures by government agencies and consumers to ban the use of drugs in animals intended for human consumption, other alternatives to the control of coccidiosis are now available. The demand for alternative methods has constantly increased in European countries, Australia, and the US [10].

Consequently, the development and use of vaccines and other alternatives have showed a significant increase. Immunity to *Eimeria* is stimulated by the initial developing parasite stages, particularly the schizonts, and is subsequently boosted and maintained by multiple re exposures to oocysts in the litter.

Accordingly, there cycling of infection, following the administration of live oocysts, is critical for the development of protective immunity [11]. Two types of vaccines are currently used with the aim of controlling coccidiosis in a chemical-free way: unattenuated and attenuated vaccines. Their effectiveness is based on the recycling of what are initially extremely low doses of oocyst and on the gradual buildup of solid immunity [12]. The use of live unattenuated vaccines is limited due to the risk induced by the live parasites, so their use is accompanied by chemical treatments to control the inherent pathogenicity of the parasites. However, this practice is no longer required due to the improved methods of administration of the oocysts [13]. The success of live attenuated vaccines is based on the fact that there is a lower risk of disease occurring because there is a reduction in the proliferation of the parasites and as a result less damage to the intestine of the bird [10].Today, attenuation of *Eimeria* species is based on precociousness. This refers to populations of parasites that complete their life cycle up to 30h faster than parasites from the same parent strain, resulting in parasites with attenuated virulence and a significant reduction in their reproductive capacity [14, 15, 16]. Today, precocious lines are described for all species of *Eimeria* [17].

As previously mentioned, the prolonged use of anticoccidials can determine the reduction of their efficacy leading to a resistance or partial resistance of the different strains of *Eimeria*. Is possible measuring the level of resistance using the so called Anticoccidial Sensitivity Test (AST). An anticoccidial sensitivity test is a laboratory test used to determine the effectiveness of different anticoccidial drugs against *Eimeria* parasites. The test involves exposing *Eimeria* parasites to different concentrations of the anticoccidial drugs and observing their response. This helps in determining the minimum inhibitory concentration (MIC) of the drugs, which is the lowest

concentration that effectively inhibits the growth and reproduction of the parasites. The sensitivity test is important in assessing the efficacy of different anticoccidial drugs and identifying any potential resistance issues. It helps in selecting the most appropriate drug for coccidiosis control in poultry. The test can also be used to monitor the development of resistance over time and guide the development of new drugs or alternative control strategies.

A study done in broiler's farms by MSD Animal Health in ten European countries between 2020 and 2021 evaluated the Anticoccidial Sensitivity index [18] in the twenty-seven samples collected. Twenty-three out of 27 originated by farms which used coccidiostats during the previous flocks, 4 out of 27 were coming from farms that used coccidiosis vaccine in the previous cycles. AST investigated the sensitivity toa combination of nicarbazin+narasin.

Isolate origin (country)	Cocci control program	% weight gain	% lesion score reduction	AST index Mathis (2006) Method	Good, moderate or poor sensitivity
BE	Anticoccidial	84.0136	14.71	49.36	Poor
BE	Anticoccidial	66.6667	2.05	34.36	Poor
PO	Anticoccidial	87.5	13.95	50.72	Poor
PO	Anticoccidial	79.7753	11.08	45.43	Poor
SE	Anticoccidial	92.4157	52.63	72.52	Moderate
DK	Anticoccidial	97.6912	32.00	64.85	Poor
ES	Anticoccidial	97.9798	-25.00	36.49	Poor
ES	Anticoccidial	82.3954	-2.78	39.81	Poor
ES	Anticoccidial	85.2814	8.57	46.93	Poor
ES	Anticoccidial	93.6508	29.41	61.53	Poor
ES	Anticoccidial	91.6306	10.71	51.17	Poor
IE	Anticoccidial	92.9293	28.95	60.94	Poor
DE	Anticoccidial	78.9322	7.14	43.04	Poor
DE	Anticoccidial	81.5296	21.57	51.55	Poor
DE	Anticoccidial	83.3333	1.22	42.28	Poor
DE	Anticoccidial	90.3061	6.74	48.52	Poor
IT	Anticoccidial	79.3651	5.88	42.62	Poor
IT	Anticoccidial	85.1371	5.00	45.07	Poor
IT	Anticoccidial	90.0433	16.67	53.35	Poor
NL	Anticoccidial	77.4892	-18.92	29.29	Poor
UK	Anticoccidial	77.4892	-3.12	37.18	Poor
UK	Anticoccidial	86.2915	-13.33	36.48	Poor
UK	Anticoccidial	75.9019	-13.79	31.05	Poor

In the samples collected from the farms which used coccidiostats 95.7% of isolates (22 out of 23) samples had poor sensitivity index score; 4.3% of isolates (1 out of 23) samples had moderate sensitivity index score meanwhile 0% of isolates (0 out of 23) samples had good sensitivity index score. In samples collected from farms that used coccidia vaccines in the previous flocks 100% of isolates (4 out of 4) samples had good sensitivity index score, 0% of isolates (0 out of 4) samples had moderate sensitivity index score, 0% of isolates (4 out of 4) samples had poor sensitivity index score due to limited lesion score reduction.

Suboptimal control of coccidiosis may determine reduction in daily weight gain and increased feed conversion rate. Subclinical coccidiosis, even if not macroscopically detectable may be a cause of loss in performance, especially when higher OPGs are present close to age of slaughtering [20] with an impact on EPI.

A study done at Oklahoma State University and presented at Arkansas Nutrition Conference in 2010 by Robert Teeter, converted loss due to coccidiosis infection into caloric equivalent. Using calorimetric chambers, he determined that a +2 subclinical coccidiosis during the final week can make a 2.4 kg broiler fed a 3250-kcal diet perform as though it had been fed a 2700 kcal diet. Even a +1 subclinical coccidiosis can cause performance loss equivalent to feed a 2975 diet [21].

The rotation of coccidiostats with coccidia vaccines allows the restoration of sensitivity to coccidiostats after few cycles vaccinated [19].

The success of coccidiosis vaccination implies multiple actions along the productive chain. Feed mill, hatchery and farmers must be involved in the

optimization of the process. Feed must be produced without any anticoccidial, and those “blank” feed should be produced in dedicated feed-mill where no anticoccidials are used. Often this is not possible so an accurate procedure which allows the cleaning of the pipelines where the feed is needed.

Golden standard for coccidiosis vaccination is hatchery administration; vaccine is normally administered via coarse spray on healthy chickens allowing then the preening (so the vaccine will be swallowed). Dedicated and specific spray cabinet are available for this purpose, and the standard procedure is adding a colorant (red dye) to the vaccine to increase preening from the chicken. Vaccine can be diluted in tap or distilled water or can be mix with specific diluents (gel). The standard procedure requires from 21 to 28 ml of spray for one hundred chicks and the cabinet must be set to allow at least 95% of coverage of the chicken. Preening could be checked looking at chicken’s tongues to see if they are colored. Is important to keep chicken for no less than 20 minutes in a comfortable stocking area with enough light (no less than 20 lux) to allow the activity of chickens and permit a good preening. Keeping birds in the dark or in environment with blue light will decrease the ingestion of the vaccine.

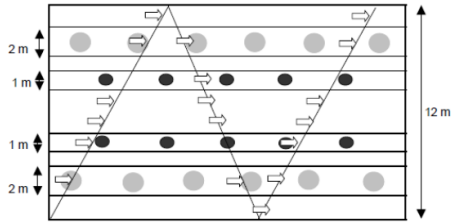
The activation of the vaccine (excystation) when swallowed, requires the combined actions of biliary salts, trypsin and mechanic activity of the gizzard. So is very important that chickens have immediate access to feed and water when they arrived at the farm. To achieve the full immunity birds, need to be exposed multiple times (3 full cycles of the vaccinal oocysts) to live oocysts and normally the full protection is gained at 21 days. The environment where the chickens are placed must allow this cycling: good amount of litter, humidity rate of the litter no lower than 25%, humidity in the environment 50.60%, good

temperature of the litter (no lower than 28°C), oxygen supply, no gas like ammonia or CO2.

Good management practices, like keeping chicks in a thermic comfort zone, with enough light (respecting recommendation about welfare), having feed availability (good feeders, open at the right step for chickens, paper with feed during the first days, proper drinkers) allows the correct development of the gut and with that allows the vaccine to work in combination with the immune system (CMI).

To allow a proper vaccine take other parameters must be under control: the use of specific antibiotic which have an activity on coccidia must be avoided. Sulfonamides, tetracycline, thiamphenicol have a detrimental impact on the live vaccine oocysts. Of course, also the feed administered must be free form antibiotics and anticoccidials.

Proper vaccine take must be evaluated: as already mentioned a uniform administration of the vaccine (in the hatchery or in the farm) is crucial, but then the farm's condition is also important for the proper cycling. To determine if good cycling is on place or not oocyst counting is the most consistent approach. Start sampling 7 days after vaccination and do it weekly until 28 days (if possible, very 3 days, to capture the rapid rise and fall of peak oocysts shedding). Take samples if possible always at same time of the day (morning is better) using a zig-zag method walk along all the barn and collect 20 samples (around 30 grams); samples must be collected in different site of the house keeping a ratio between enteric and cecal feces of 7/8 enteric and 1 cecal and avoiding "to select" feces (don't pick up feces which look pathological).



Example of sampling route

Use a flashlight inside of the house to facilitate recognizing the feces. Feces must be kept in the refrigerator and on the sample put the following information: day of sampling, farm's name, number of the house, age of the birds.

Oocysts count cannot predict always the performance impact, but they can serve to evaluate the vaccine take and, in non-vaccinated broilers, gives a map to determine when subclinical coccidiosis is appearing during the growth of the flock.

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Paracox®-5

Zawiera 5 szczepów *Eimeria*,
zapewnia szybki rozwój odporności,
redukuje presję terenową,
poprawia wyrównanie stada
oraz wyniki produkcyjne.

Paracox®-5

1. NAZWA PRODUKTU LECZNICZEGO WETERYNARYJNEGO

Paracox®-5, zawiesina do sporządzania zawiesiny doustnej dla kur. Szczepionka przeciwko kokcydiozie kurcząt.

2. SKŁAD JAKOŚCIOWY I ILOŚCIOWY

Substancje czynne:

Każda dawka 0,004 ml szczepionki zawiera następujące ilości sporulowanych oocyst uzyskanych z pięciu linii kokcydiów o skrócony rozwoju:

Eimeria acervulina HP	500-650 oocyst*
Eimeria maxima CP	200-260 oocyst*
Eimeria maxima MFP	100-130 oocyst*
Eimeria mitis HP	1000-1300 oocyst*
Eimeria tenella HP	500-650 oocyst*

*Zgodnie z procedurą liczenia *in vitro* wytwórcy w czasie mieszania oraz zwalniania.

Rozpuszczalnik do rozpylania na kurczęta
Kwas karminowy (czerwony barwnik, E120)
Guma ksantanowa (E415)
Wykaz wszystkich substancji pomocniczych patrz punkt 6.1.

3. POSTAĆ FARMACEUTYCZNA

Zawiesina do sporządzania zawiesiny doustnej.
Szczepionka: wodnista zawiesina.
Rozpuszczalnik do rozpylania na kurczęta:
pół-przeroczysty, czerwony, lepki roztwór.

4.2 Wskazania lecznicze dla poszczególnych docelowych gatunków zwierząt

Rozpylanie na paszę, rozpylanie na kurczęta bez rozpuszczalnika lub podanie z wodą do picia

Czynne uodpornianie kurcząt, w celu ograniczenia zakażenia i klinicznych objawów kokcydiozy wywoływanej przez *Eimeria acervulina, E. maxima, E. mitis i E. tenella.*
Czas powstania odporności: zaczyna rozwijać się w ciągu 14 dni od podania szczepionki.
Czas trwania odporności: co najmniej 40 dni po szczepieniu.

Rozpylanie na kurczęta z rozpuszczalnikiem

Czynne uodpornianie kurcząt przeciw kokcydiozie wywoływanej przez *Eimeria acervulina, E. maxima, E. mitis i E. tenella* w celu:

- ograniczenia wydalania oocyst *E. acervulina, E. maxima i E. tenella,*
- redukcji spadków przyrostu masy ciała w przypadku zakażenia *E. acervulina, E. mitis i E. tenella.*

Czas powstania odporności: 21 dni po szczepieniu.
Czas trwania odporności: 10 tygodni.

4.3 Przeciwwskazania

Brak

4.4 Specjalne ostrzeżenia dla każdego z docelowych gatunków zwierząt

Należy szczepić tylko zdrowe zwierzęta. Nie stosować u kurcząt pod wpływem stresu np. wyziębionych, nie pobierających pokarmu lub wody. W przypadku rozpylania na kurczęta do rozcieńczonej szczepionki należy dodać czerwony barwnik spożywczy (koszenila E120) lub szczepionkę należy rozcieńczyć z zastosowaniem zalecanego „Rozpuszczalnika do rozpylania na kurczęta". W przypadku stosowania metody rozpylania na kurczęta można obserwować znaczące obniżenie skuteczności, jeśli szczepionka zostanie rozpuszczona w wodzie wodociągowej bez dodatku czerwonego barwnika. Czystość koszenili E120 musi odpowiadać wymogom dyrektywy Komisji 95/45/EC. Kurczęta powinny być odchowywane bezpośrednio na ściółce. Paracox®-5 zawiera żywe kokcydia, nabywanie odporności zależy od rozwoju szczepionkowych linii kokcydii w organizmie szczepionych zwierząt. Powszechnie odnajduje się oocysty w przewodzie pokarmowym szczepionych ptaków od 1 do 3 lub więcej tygodni po szczepieniu. Najprawdopodobniej są to oocysty pochodzenia szczepionkowego, które krążą wewnątrz populacji ptaków za pośrednictwem ściółki. Krążenie oocyst zapewnia całemu stadu zadowalającą ochronę przeciw wszystkim patogennym gatunkom *Eimeria* zawartym w szczepionce. Należy zapewnić, że rozcieńczona szczepionka jest mieszana w regularnych odstępach czasu w trakcie szczepienia. Należy pamiętać, że dostęp do jakichkolwiek produktów leczniczych posiadających działanie przeciw kokcydiom, w jakimkolwiek czasie po szczepieniu, może skracać czas trwania skutecznej ochrony, ponieważ ochrona przeciw zakażeniom kokcydiami po zastosowaniu Paracox®-5 jest wzmacniana przez naturalne zakażenie. Jest to istotne w czasie całego życia ptaków. Aby ograniczyć prawdopodobieństwo zakażenia szczepem terenowym przed powstaniem odporności, pomiędzy cyklami produkcyjnymi należy usunąć ściółkę oraz poddać dokładnemu czyszczeniu pomieszczenia, w których utrzymywane są ptaki.

4.5. Specjalne środki ostrożności dotyczące stosowania

Specjalne środki ostrożności dotyczące stosowania u zwierząt
Należy zapewnić dokładne czyszczenie sprzętu stosowanego do szczepienia przed jego użyciem.
Specjalne środki ostrożności dla osób podających produkt leczniczy weterynaryjny zwierzętom
Podczas rozpylania szczepionki należy nosić dobrze dopasowaną maskę oraz środki ochrony oczu.

4.6 Działania niepożądane (częstotliwość i stopień nasilenia)

Łagodne zmiany związane np. z *E. acervulina i E. tenella* (stopień zmian +1 lub +2 przy zastosowaniu systemu oznaczania Johnson i Reid, 1970) stwierdzane były często u ptaków w 3 do 4 tygodni po szczepieniu w badaniach laboratoryjnych. Zmiany o takim nasileniu nie wpływają na wydajność uodpornionych kurcząt. Częstotliwość występowania działań niepożądanych przedstawia się zgodnie z poniższą regułą:

- bardzo często (więcej niż 1 na 10 leczonych zwierząt wykazujących działanie(a) niepożądane)
- często (więcej niż 1, ale mniej niż 10 na 100 leczonych zwierząt)
- niezbyt często (więcej niż 1, ale mniej niż 10 na 1000 leczonych zwierząt)
- rzadko (więcej niż 1, ale mniej niż 10 na 10000 leczonych zwierząt)
- bardzo rzadko (mniej niż 1 na 10000 leczonych zwierząt, włączając pojedyncze raporty).

4.9 Dawkowanie i droga(i) podawania

Pojedyncza dawkę Paracox®-5 należy podawać piskletom od pierwszego dnia życia rozpylając na paszę, rozpylając na kurczęta lub piskletom trzydniowym z wodą do picia.

Podawanie z paszę

Paszę typu starter w ilości wystarczającej na pierwsze 24-48 godzin życia należy podać na arkuszu papieru lub folii rozłożonej na podłodze kurnika. Nie podawać szczepionki wraz z paszą zadawaną automatycznie. Nie umieszczać paszy wraz ze szczepionką bezpośrednio pod lampami będącymi źródłem ciepła. Przed użyciem szczepionki fiolkę należy energicznie wstrząsać przez 30 sekund tak, aby nastąpiło równomierne rozproszenie oocyst w zawiesinie. Paracox®-5 rozcieńczyć w wodzie w takiej proporcji, aby 5000 dawek znajdowało się w objętości nie przekraczającej 3 litrów wody i rozpylać (gruba kropla) równomiernie nad powierzchnią paszy przy pomocy rozpylacza. Należy zapewnić kontrolowane równomierne pokrycie całej powierzchni paszy dostępnej dla kurcząt. Zbiornik rozpylacza należy wstrząsać regularnie, aby zapobiec osadzaniu się oocyst na dnie. Należy zapewnić, aby kurczęta miały dostęp tylko do paszy ze szczepionką oraz aby całkowita liczba dostarczonych wraz z paszą dawek odpowiadała liczbie ptaków w kurniku. Szczepionkę po rozcieńczeniu należy rozpylić na podawaną paszę, a kurczętom umożliwić dostęp do paszy w ciągu 2 godzin. Po spożyciu przez kurczęta całej paszy ze szczepionką można stosować rutynowe karmienie.

Podawanie z wodą do picia

Umieścić jednodniowe kurczęta w pomieszczeniu w celu adaptacji do systemu poideł smoczkowych. Kiedy kurczęta osiągną wiek 3 dni wyłączyć oświetlenie na około 7 godzin. Podnieść wszystkie linie pojenia spoza zasięgu ptaków na 2 godziny przed podaniem szczepionki. W tym samym czasie włączyć oświetlenie. Opróżnić dokładnie każdą linię pojenia. Rozpuścić szczepionkę w zimnej wodzie pitnej w taki sposób, aby 1 dawka znajdowała się w 2-4 ml. Obliczyć średnią liczbę ptaków przypadającą na linię pojenia i obliczyć objętość rozcieńczonej szczepionki wymaganej na każdą linię z uwzględnieniem 2-4 ml dla każdego ptaka. Napełnić każdą linię rozcieńczoną szczepionką, a następnie opuścić linię, aby umożliwić dostęp ptaków do poideł smoczkowych. Początkowo można zastosować (około 1 litra) wskaźnika (np. mleko) w celu uwidocznienia napełnienia linii do końca, co umożliwi jej zamknięcie bez straty szczepionki. Podczas gdy ptaki piją, należy linie utrzymywać w stanie napełnienia przez ich rezerwuary do całkowitego wykorzystania rozcieńczonej szczepionki przeznaczonej dla danej linii. Następnie wznowić normalne podawanie wody. Zaleca się, aby przed zastosowaniem szczepionki w obiekcie po raz pierwszy, upewnić się, że procedura zapewnia prawidłowe wypełnienie linii pojenia szczepionką Paracox®-5, przez pojawienie się wskaźnika w poidłach smoczkowych na końcu linii, przed umożliwieniem kurczętom pobierania wody.

Podawanie przez rozpylanie na kurczęta

W przypadku rozpylania na kurczęta do rozcieńczonej szczepionki należy dodać czerwony barwnik spożywczy (koszenila E120) lub szczepionkę należy rozcieńczyć z zastosowaniem zalecanego „Rozpuszczalnika do rozpylania na kurczęta". Rozpuszczalnik zawiera czerwony barwnik i gumę ksantanową, oba składniki wprowadzono dla lepszego pobierania szczepionki.

a) Rozpuszczalnik do rozpylania na kurczęta
Szczepionkę należy podawać w dawce o objętości pomiędzy 0,21 a 0,28 ml rozcieńczonej szczepionki na ptaka z zastosowaniem aerozolu o grubej kropli. Należy wyznaczyć wydajność urządzenia stosowanego do rozpylania w odniesieniu do objętości podawanej na 100 ptaków. Należy tę objętość pomnożyć przez 50 w celu uzyskania całkowitej objętości rozcieńczonej szczepionki wymaganej dla 5000 dawek (lub pomnożyć przez 10 dla 1000 dawek). To jest, do przygotowania 5000 dawek rozcieńczonej szczepionki wymagane jest łącznie 0,21 x 5000 = 1050 ml rozcieńczonej szczepionki, na co składa się szczepionka, rozpuszczalnik i woda, jak to podano poniżej:
1. 20 ml szczepionki Paracox®-5 (1 fiolka)
2. 500 ml rozpuszczalnika (1 butelka)
3. Uzupełnić do 1050 ml wodą wodociągową.
Woda stosowana do rozcieńczenia szczepionki powinna być świeża, chłodna i wolna od zanieczyszczeń. Do przygotowania szczepionki należy zastosować czysty pojemnik, rozpuszczalnik wprowadzić do pojemnika, dodać do pojemnika obliczoną ilość wody, następnie wody i rozpuszczalnik należy mieszać do uzyskania jednorodnego roztworu. Należy wstrząsać energicznie fiolkę 5000 dawek (lub 1000 dawek) szczepionki Paracox®-5 przez 30 sekund, tak aby nastąpiło równomierne rozproszenie oocyst w zawiesinie. Dodać całą zawartość fiolki do pojemnika z rozpuszczalnikiem i wodą, wymieszać dokładnie. Wprowadzić rozcieńczoną szczepionkę do zbiornika urządzenia stosowanego do podawania szczepionki i rozpylać równomiernie ponad ptakami z zastosowaniem aerozolu o grubej kropli. Należy zapewnić kontrolowane, równomierne pokrycie całej wewnętrznej powierzchni pudełka z piskletami. Ptaki należy pozostawić w pudełku na co najmniej 30 minut w dobrze oświetlonym miejscu, aby zapewnić piskletom czas na przeprowadzenie czyszczenia piór.

b) Czerwony barwnik spożywczy (E120)
Szczepionkę podawać w dawce o objętości od 0,21 do 0,28 ml rozcieńczonej szczepionki na ptaka z zastosowaniem aerozolu o grubej kropli. Należy oznaczyć objętość szczepionki podawaną przez urządzenie stosowane do opryskiwania na 100 ptaków. Pomnożyć tę objętość przez 50, aby osiągnąć objętość rozcieńczonej szczepionki wymaganą dla 5000 dawek (lub przez 10 dla 1000 dawek) i dodać tę objętość wody do odpowiedniego pojemnika (zazwyczaj pomiędzy 1,0 a 1,5 litra dla 5000 dawek lub 200 do 300 ml dla 1000 dawek). Szczepionka jest chętniej pobierana przez ptaki, a tym samym skuteczność szczepienia wzrasta, jeśli do rozcieńczonej szczepionki przed rozpyleniem dodany zostanie czerwony barwnik spożywczy. Dodać wystarczającą ilość czerwonego barwnika spożywczego (koszenila E120) do wody w celu uzyskania stężenia 0,1% w/v, co odpowiada 210-280 µg dla jednego ptaka. Fiolkę szczepionki Paracox®-5 zawierającą 5000 dawek (lub 1000 dawek) należy silnie wstrząsać przez 30 sekund, tak aby nastąpiło równomierne rozproszenie oocyst w zawiesinie. Dodać całą zawartość fiolki do rozcieńczalnika i dokładnie wymieszać. Wlać rozcieńczoną szczepionkę do rezerwuaru aplikatora i wykonać równomierny oprysk piskląt w komorze z zastosowaniem grubej kropli.
Zapewnić kontrolowane, równomierne pokrycie całej powierzchni wewnętrznej pudełka zawierającego piskleta. Wstrząsać rezerwuar aplikatora regularnie w czasie prowadzenia szczepienia, aby zapobiec osiadaniu oocyst. Ptaki należy pozostawić w pudełku na co najmniej 30 minut w dobrze oświetlonym miejscu aby zapewnić piskletom czas na przeprowadzenie czyszczenia piór.

4.11 Okres(-y) karencji

Zero dni

7. NAZWA I ADRES PODMIOTU ODPOWIEDZIALNEGO

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8. NUMER(-Y) POZWOLENIA NA DOPUSZCZENIE DO OBROTU

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Kategoria dostępności: Wydawany z przepisu lekarza – Rp.
Data sporządzenia: 09/09/2020
Reklama kierowana do osób uprawnionych do wystawiania recept oraz osób prowadzących obrót produktami leczniczymi.

MSD Animal Health

Wojciech Gbiorczyk

Key Account Manager Zoetis, Poland

COCCIDIOSIS IN TURKEYS IN COMMERCIAL FLOCKS - CRITICAL POINTS

Along with bacterial and viral diseases, coccidiosis is one of the main pathogens causing enteropathies of the gastrointestinal system of turkeys. With its intensive development, it leads to the damage to the villi that build the intestinal wall, leading, among others, to a deterioration in the absorption and utilization of nutrients, which negatively affects the production result. In turkeys, seven species of Eimeria are distinguished, of which: *E. meleagridis, E. meleagrimitis, E. gallopavonis, E. adenoeides* are highly pathogenic, while *E. dispersa, E. subrotunda, E. innocua* are less pathogenic. The places of multiplication coccidia and formation of intestinal lesions are the same for different species. The symptoms of subclinical coccidiosis are very similar to the lesions caused by Clostridium perfringens bacterial infections or poult enteritis mortality syndrome PEMS.

In the rearing of commercial turkey flocks, we can distinguish several critical points that have a direct impact on the onset and course of the disease. We can include: the preparation of the facility (washing, disinfection, technological break), the bedding type, the rotation of coccidiosatics and their application period, the current health state, zootechnical conditions and the day of fattening in which the turkeys are.

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CHROŃ SWOJE GOSPODARSTWO PRZED PTASIĄ GRYPĄ I ASF

Produktów biobójczych należy używać z zachowaniem środków ostrożności.
Przed każdym użyciem należy przeczytać etykietę i informacje dotyczące produktu.

Rapidal zawiera aldehyd glutarowy. **H302** Działa szkodliwie po połknięciu. **H314** Powoduje poważne oparzenia skóry i uszkodzenia oczu. **H317** Może powodować reakcję alergiczną skóry. **H330** Wdychanie grozi śmiercią. **H334** Może powodować objawy alergii lub astmy lub trudności w oddychaniu w następstwie wdychania. **H400** Działa bardzo toksycznie na organizmy wodne. **H412** Działa szkodliwie na organizmy wodne, powodując długotrwałe skutki.

Rapicid zawiera kwas siarkowy i kwas fosforowy. **H314** Powoduje poważne oparzenia skóry oraz uszkodzenia oczu. **H411** Działa toksycznie na organizmy wodne, powodując długotrwałe skutki. **H290** Może powodować korozję metali.

Rapiclean zawiera alkohol etoksyetylenowany, wodorotlenek sodu. **H315** Działa drażniąco na skórę. **H318** Powoduje poważne uszkodzenie oczu.

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Jana Brabcová

BIOPHARM, Research Institute of Biopharmacy and Veterinary Drugs, Pohoří - Chotouň 90, 254 01 Jílové u Prahy, Czech Republic

A PROPIDIUM MONOAZIDE (PMA) BASED APPROACH FOR *IN VITRO* EVALUATION OF *EIMERIA MAXIMA* OOCYST VIABILITY

Conventional methods for determining the viability of coccidial oocysts require the administration of the organisms to susceptible hosts and the monitoring of clinical signs. Several factors have been established for the purpose of testing. The measurable parameters involve the production of coccidial oocysts (OPG), the evaluation of the lesion score after challenge in the efficacy test, the levels of anti-Eimeria antibodies in the ELISA test, the feed intake (FI)/feed conversion ratio (FCR) and the body weight gain (BW) of the chickens during the study. Although these methods provide a means of assessing infection, their implementation is costly, both in terms of the animals used and the time required to complete the test. In chickens, for example, it takes 4-7 days after infection for clinical signs of disease to appear. Due to an increasing legislative pressure on the reduction of experimental animals a reliable *in vitro* system based on molecular methods to assess oocysts viability could become suitable alternative.

The aim of our study is to find the correlation between viability and *in vitro* evaluation. A propidium monoazide approach seems to be a promising tool to determine the viability of *Eimeria maxima* oocysts. Untreated and killed oocysts were incubated with PMA, a photoreactive DNA binding dye, and

analyzed by microscopy and flow cytometry. The different conditions such as the PMA concentrations and *E. maxima* oocyst's pre-treatment were evaluated to find the optimal conditions for specific discrimination of viable and dead oocysts.

The present invention provides a rapid and reliable tool in vaccine evaluations. Other applications may be in the evaluation of *Eimeria* resistance to anticoccidial drugs, particularly in the selection of appropriate anticoccidial drugs for administration to animals with clinical signs of coccidiosis to set up conditions for a convenient shuttle program. In addition, the invention may be used to evaluate the effectiveness of agents for use in disinfecting equipment and environments contaminated by coccidial protozoa. Since coccidia oocysts are highly resistant to disinfectants, a good cleaning method is required to prevent both exposure and re-infection. In the manufacture process of Livacox vaccines, the control of the success of the coccidial disinfection procedure is the crucial point for precise product preparation. Identifying an alternative *in vitro* method to assessing the viability of *Eimeria* oocysts would be both less time-consuming and labor-intensive compared to *in vivo* infection.

Results/Methods

Oocyst production

Eimeria maxima oocysts of virulent strain were produced in 10 chickens Valo, hatched from SPF eggs. The animals were housed in isolators for chickens. The infection doses (10.000 oocysts/bird) were administered by the gavage with pipette to the crops. The faeces from 10 chickens were collected in interval 144 – 192 hpi. The pooled suspensions of freshly isolated non-sporulated oocysts were sporulated in 2.5% $K_2Cr_2O_7$. For comparison between

experiments, oocysts of the same batch were used. Prior to experiments, oocysts were washed three times in sterile distilled water (dH_2O) to remove $K_2Cr_2O_7$ and subsequently purified by saccharose gradient.

Killing procedure

Experiment 1: NaClO treatment was prepared by adding NaClO to the pellet oocysts obtained after sucrose gradient (1:1) followed by incubation on ice for 30 minutes. Then the NaClO-treated oocysts were washed three times and resuspended in PBS. Then their concentration was calibrated by counting oocysts McMaster chamber.

Experiment 2: Heat-killed oocysts were obtained following incubation of the oocysts obtained after sucrose gradient diluted PBS in autoclave at 134 °C for 20 min. The effect of temperature on oocysts surviving was studied previously (data not shown).

The absence of viable oocysts following killing procedure was evaluated with PMA-qPCR.

PMA

Untreated or killed oocysts were incubated with 50 to 150 µM of PMA (Biotium Inc., Hayward, WI, USA) for 30 min in dark at room temperature (mean temperature 22 °C), 37 °C, or 45 °C, with vortexing every 5 min. PMA was applied at 100 µM for 30 min at 22 °C. For each set of qPCR experiments, untreated and heat-killed oocysts were submitted in parallel to the same incubation conditions but in the absence of PMA. Then, samples were exposed to a light-emitting diode (LED) source for 30 min using the GelLogic 212 PRO

(Carestream) (λ = 464–476 nm, 60W). After exposure, oocysts were washed three times with PBS (5000×g, 5 min). The supernatant was discarded, and the pellet was resuspended in PBS.

Microscopy

Untreated or killed oocysts were incubated with 100 µM PMA for 30 min at 22 °C and processed as described above. Pelleted oocysts were resuspended in PBS and 10 µl was used for confocal microscopy analyses. Oocysts' blue autofluorescence and PMA red fluorescence were observed under the appropriate excitation wavelength/filter sets, using a LSM 710 NLO confocal microscope (Zeiss, Germany) linked to a Chameleon infrared biphoton laser (Coherent, USA) and piloted by ZEN software (Zeiss, Germany).

Flow cytometry

Untreated or heat-killed oocysts (105) were stained with 150 µM PMA for 30 min at 22 °C and processed as described above. Pelleted oocysts were resuspended in 200 µl PBS. Data acquisition was performed using BD Accuri™ C6 (BD Biosciences, San Jose, USA) equipped with 375 and 488 nm excitation lasers and set to acquire forward scatter (FSC), side scatter (SSC), and fluorescence. The oocyst autofluorescence and PMA fluorescence were collected on the fluorescence detector at 427 ± 20 nm and at 670 nm LP, respectively. Data were analyzed with the FlowJo LLC software (Oregon, USA).

Flow cytometry analyses revealed that 100% of NaClO+heat-killed virulent *E. maxima* oocysts were permeable to PMA. This result can suggest

that killing-procedure lead to efficient oocyst wall permeabilization and PMA penetration.

Conclusion

PMA–qPCR assays shoved a decline in the viability of virulent *E. maxima* oocysts exposed to NaClO in combination with heat treatment. The efficacy of PMA-based assay was directly linked to the biological test on SPF chickens Valo. The production in group of chickens vaccinated with inactivated oocysts (NacClO+heat-killed) showed zero yield confirming the loss of viability.

In conclusion, under the conditions tested in this study, PMA–qPCR is a reliable tool to evaluate the reduction of virulent *E. maxima* oocyst viability following killing-procedure. Further works are required to evaluate the potential of this technique following the relevance of PMA–qPCR assays to characterize inactivation efficacy of industrial processes. We developed a laboratory-accessible method for discriminating viable/infective from virulent *E. maxima* oocysts.

Barbara Szczepankiewicz, Karolina Roczniak*, Kamila Bobrek, Andrzej Gaweł

Department of Epizootiology and Clinic for Birds and Exotic Animals, Faculty of Veterinary Medicine, Wroclaw University of Environmental and Life Sciences, pl. Grunwaldzki 45, 50-366 Wrocław
**6th year of Veterinary Medicine student*

MICROSPORIDIA – OPPORTUNISTIC PARASITES. IS THERE VERTICAL TRANSMISSION OF *ENTEROCYTOZOON BIENEUSI*?

Introduction

Microsporidia are opportunistic parasites with a predilection for epithelial cells of the gastrointestinal tract and respiratory systems but also the cause of disseminated microsporidiosis. Its spores are detected in almost all internal organs of mammals, birds, reptiles, amphibians, fish, and invertebrates (1). Microsporidia have been shown to infect all groups of organisms, from protists to humans. Most microsporidia species cause large economic losses in the insects, fish, and fur animals production (2). Nearly 1,300 species from over 200 genera of microsporidia have been described so far(1).

The systematics of microsporidia has been a controversial topic from the beginning of their identification. Initially, microsporidia were considered protozoa. They were classified based on several features, such as the presence of 70S ribosomes and the lack of centrioles (designating, among others, the division plane and respiratory organelles (including mitochondria). However, based on comparisons of molecular markers, gradually microsporidia were classified as highly reduced eukaryotes closely related to fungi, specifically the

oldest of the currently known groups of fungi – Cryptomycota (the name from Latin means "hidden fungi"). Based on microscopic studies and genetic material, it was shown that a characteristic feature of these unique fungi is the lack of a cell wall composed of chitin microfibrils, previously considered a feature of fungi.

Several species of microsporidia are highly important in human medicine, the most frequently described species of the genus *Encephalitozoon* (*Encephalitozoon cuniculi*, *Encephalitozoon intestinalis*, and *Encephalitozoon hellem*) and *Enterocytozoon* (*Enterocytozoon bieneusi*).

Morphology and life cycle

The invasive forms are small oval spores, 1-4 μm in size, surrounded by a complex cellular membrane composed of two layers: an outer glycoprotein layer and an inner chitin-protein layer. A special polar thread apparatus allows active penetration into the host cell. Microsporidia have developed a special invasion mechanism, which involves the release of an anchoring disk mounted on the polar strand and the contents of the spore entering the host cell. When the polar thread unwinds, the invasive sporoplasm actively enters the cytoplasm of the host cell. Subsequent stages involve asexual reproduction (merogony), resulting in the formation of up to several hundred meronts. In the next phase, meronts transform into sporonts, which next become sporoblasts. Through sporogony, sporoblasts develop into mature spores. When the host cell is completely filled with spores, it ruptures, releasing spores with a chitinous coating that provides protection against adverse external conditions. This complex developmental cycle and mechanism of active penetration into the host

cell underscore the adaptive abilities of microsporidia as invasive, parasitic fungi (1-6).

Routes of transmission

1. Horizontal Transmission

Unicellular spores are excreted in various secretions and excretions of infected hosts (including faeces, urine, sputum, semen, and milk). Infection most often starts via the faecal-oral route, through consumption of water or food contaminated with spores, and less frequently through inhalation of spores along with dust. Spores floating in dust from bird faeces suggest airborne transmission (7). Respiratory infections have been reported with *Encephalitozoon* and *E. bieneusi* (6).

2. Vertical Transmission

Recent scientific reports confirm the transovarial transmission of microsporidiosis in the silkworm (which involves the penetration of pathogenic fungi into the interior of the developing oocyte without disturbing the normal development of the arthropod embryo) (5). To date, vertical transmission has not been demonstrated in humans, although studies are ongoing examining placentas collected during childbirth. Reports of suspected transplacental transmission of microsporidiosis that were published several decades ago are based on circumstantial evidence, such as serological diagnosis (6). However, monitoring the level of antibodies in newborn rabbits does not provide clear evidence of their transfer through the placenta because maternal antibodies can be transmitted to offspring through mothers' milk (6).

Occurrence and pathogenicity

Until now, it was believed that the pathogenicity of microsporidia affected people with reduced immunity, mainly with HIV or people after internal organ transplantation, treated with immunosuppressive drugs. Later, microsporidiosis was documented in elderly people and in tourists returning from long journeys, especially from countries with low hygiene standards. However, we know now that microsporidiosis is also reported in immunocompetent people without any symptoms of the disease. These findings clearly demonstrate that exposure to microsporidia is common and that chronic microsporidiosis is not associated with any clinical symptoms in the healthy population. Moreover, the results indicate a much higher incidence of microsporidiosis among an apparently healthy population than previously reported (3). People who work with animals and animal-related material or are exposed to contact with contaminated soil and water have been demonstrated to have an increased risk for microsporidiosis (4). However, research conducted by Australian scientists showed a much higher prevalence of microsporidiosis in children under 3 years of age (2.5%) than in adults (0.3%) (6). The most common symptoms of microsporidiosis in adults include acute or chronic diarrhoea, conjunctivitis, nephritis, and hepatitis, as well as fever, coughing, and joint pain (1-7).

Avian microsporidiosis

Birds are a group of vertebrates in which microsporidia are commonly identified. Research carried out in recent years using molecular methods, has shown that poultry, as well as ornamental and free-living birds, contribute to the spread of zoonotic species of microsporidia. The incidence of microsporidiosis

in birds was 7.8%. The overall prevalence rates of *E. bieneusi*, *E. cuniculi*, *E. hellem*, and *E. intestinalis* were 13.9% (411 positive results out of 2961 stool samples), 4.4% (69 positive results out of 1662 samples), and 7, respectively. 7% (166 positive results out of 2628 samples) and 2.9% (68 positive results out of 1992 samples) (6).

E. bieneusi is a common opportunistic pathogen causing diarrhoea in humans and animals. However, epidemiological data on *E. bieneusi* infections in birds worldwide are relatively sparse.

Purpose of research

The aim of the study was to assess the occurrence of *E. bieneusi* (microsporidiosis) in Pekin duck embryos, which would confirm the vertical transmission of this parasite in poultry.

Materials and methods

The material for the occurrence of microsporidiosis research consisted of 36 Pekin duck embryos, which died on the 13th–27th day of incubation. Internal organs (liver, spleen, bursa Fabricius, kidneys, and heart) were collected from the embryos.

Diagnostics were based on molecular tests using the PCR technique with specific primers. The first PCR reaction was performed using primers 5′GGTCATAGGGATGAAGAG3′ and 5′TTCGAGTTTCTTTCGCGCTC3′. In the second reaction, primers 5′GCTCTGAATATCTATGGCT'3 and 5′ATCGCCGACGGATCCAAGTG3′ were used, and a fragment of 390 bp was amplified (7).

Results

Molecular testing did not confirm the presence of *E. bieneusi* genetic material in the internal organs of Pekin duck embryos.

Conclusions

Despite speculations about the vertical transmission of microsporidia, this thesis has not yet been proven. The presented preliminary research did not reveal the presence of *E. bieneusi* genetic material. Vertical transmission of the pathogen in Pekin ducks cannot be clearly ruled out. It is planned to continue the research, extending it to other species of microsporidia, in particular those belonging to the genus *Encephalitozoon*.

Discussion:

Microsporidia are a separate clade of parasites that have unique characteristics, such as reduced genomes and the lack of typical mitochondria, that distinguish them from Apicomplexa (which include Coccidia and *Cryptosporidium*).

Treatment of clinical disease, especially disseminated microsporidiosis, is challenging. So far, no treatment protocol has been demonstrated for poultry. In humans, specific antiparasitic medications are used to treat certain forms of microsporidiosis for approximately one month or longer. Commonly used drugs in human medicine include albendazole and fumagillin. Albendazole, belonging to the benzimidazole group, is mainly used for intestinal infections caused by *Encephalitozoon*. This drug has variable effectiveness against *E. bieneusi*, so fumagillin is usually used in this case. Research shows that despite the reduction

of clinical symptoms, antiparasitic drugs do not always eliminate microsporidiosis; therefore, in some cases, two or even three antibiotics are used, including metronidazole, azithromycin, atovaquone, nifedipine, furazolidone, and sulfadiazine. It should be added that some drugs are not registered in veterinary medicine or are even prohibited for use in poultry in the European Union (such as metronidazole, a nitroimidazole).

Due to the fact that there is still no effective method of treating microsporidiosis, attention should be paid to biosecurity and proper flocks management.

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Małgorzata Olejnik

Nicolaus Copernicus University
Faculty of Biological and Veterinary Sciences, Department of Basic and Preclinical Sciences
Lwowska St., 87-100 Torun

THE OTHER SIDE OF THE COIN – TOXICITY OF IONOPHORE COCCIDIOSTATS FOR POULTRY

The use of coccidiostats has remained one of the main methods of coccidiosis control for years. In most cases, they are given to animals as feed additives; in this form they are allowed for use in poultry and rabbits. Statistics conducted in the European Union indicate a large scale of use of feed additives containing coccidiostats. They are used in 86% of starter/grower feeds for broiler chickens, 97% of similar feeds for turkeys and 45% of feeds for rabbits. The Commission's report to the Council and the European Parliament on the use of coccidiostats as feed additives states that there is currently no alternative to their use that provides a comparable degree of protection against coccidiosis [European Commission 2008]. The list of coccidiostats approved for use in poultry is presented in Table 1.

Table 1. Coccidiostats approved to use as feed additives in poultry [European Register of Feed Additives 2023]

Coccidiostat	**Species or category of animal**	**Content in feed, mg/kg**	**Withdrawal time**
Amprolium (Coxam)	Chickens for fattening	125-125	-
	Chickens reared for laying	125-125	-

Decoquinate (Deccox, Avi-Deccox)	Chickens for fattening	30-40	-
Diclazuril (Clinacox)	Chickens for fattening Turkeys up to 16 weeks Guinea fowls Chickens reared for laying up to 16 weeks	1-1 1-1 1-1 1-1	- - - -
Diclazuril (Coxiril)	Chickens for fattening, turkeys, guinea fowl Pheasants Chickens reared for laying up to 12 weeks	0.8-1.2 1-1.2 0.8-1.2	- - -
Halofuginone (Stenorol)	Chickens for fattening	2-3	5 days
Lasalocid (Avatec)	Chickens for fattening Turkeys up to 16 weeks Pheasants, guinea fowls, quails and partridges	90-90 75-125 75-125	3 days 5 days 5 days
Monensin (Coxidin)	Chickens for fattening Turkeys up to 16 weeks Chickens reared for laying up 16 weeks	100-125 60-100 100-125	1 day 1 day 1 day
Monensin (Elancoban)	Chickens for fattening Turkeys up to 16 weeks Chickens reared for laying up to 16 weeks	100-125 60-100 100-120	3 days 3 days -
Monensin + Nicarbazin (Monimax)	Chickens for fattening Turkeys up to 16 weeks Chickens reared for laying up to 12 weeks	40-50/40-50 40-50/40-50 40-50/40-50	- - -
Narasin (Monteban)	Chickens for fattening	60-70	-
Narasin + Nicarbazin(Maxiban)	Chickens for fattening	40-50/40-50	-
Nicarbazyn	Chickens for fattening	125-125	1 day
Robenidine (Cycostat)	Chickens for fattening	36-36	5 days

Salinomycin (Sacox)	Chickens for fattening Chickens reared for laying up to 12 weeks	50-70 50-50	-
Semduramicin (Aviax)	Chickens for fattening	20-25	5 days

Currently, 11 coccidiostats are registered as feed additives in EU countries. They can be divided into two main groups: ionophore coccidiostats, which include six substances produced by fermentation (monensin sodium, lasalocid sodium, narasin, salinomycin sodium, semduramicin) and synthetic chemical coccidiostats (amprolium, decoquinate, diclazuril, robenidine hydrochloride, halofuginone hydrobromide and nicarbazin). The authorization to use coccidiostats as feed additives is granted temporarily for a period of 10 years and each time European Food Safety Authority (EFSA) reassesses the effectiveness and safety of the feed additive. Due to doubts in this regard, in recent years the authorization for the use of maduramicin was withdrawn and the scope of use of robenidine was changed.

Ionophore coccidiostats constitute a coherent group in terms of chemical structure and pharmacological properties (Fig. 1). They are widely used due to the slow development of resistance to their action and their small impact on the development of animals' immunity to coccidiosis. On the other hand, these are compounds characterized with high toxicity and a low therapeutic index.

Different sensitivity of individual animal species to their toxic effects is characteristic feature of ionophore coccidiostats (Table 2). Doses that are safe for chickens (one of the most resistant species) may turn out to be toxic or even fatal for turkeys or horses. However, even in chickens, exceeding the

recommended dose three times causes reduced productivity and toxic effects, and in some cases even increased mortality [Fowler 1995].

Table 2. Median lethal dose values, LD_{50} [mg/kg b.w.] for selected animal species

Species	**Lasalocid**	**Monensin**	**Narasin**	**Salinomycin**
Bovine	50-150	20-80		
Chicken	**72**	**200-231**	**52-67**	**45**
Horse	21,5	2-3		0.6
Mouse	146	61-125	15.8	57
Pig		16,7	6-12.2	
Guinea fowl		**84-106**		
Quail		**88**		
Rabbit	40	42		21
Rat	122	34	21.1	50
Turkeys		**347-416**	**Not tested**	**0.6**

The reasons for differences in species susceptibility to the toxic effects of ionophore coccidiostats have not been fully understood, but they are believed to be related to different degrees and/or routes of ionophore biotransformation. Monensin has been most extensively studied in terms of both toxicity and metabolism. The results of these studies indicate that metabolism is similar in all species in qualitative but not quantitative terms [Donoho 1984]. During *in vitro* experiments, a relationship between the total degree of metabolism and toxicity has been observed [Nebbia et al. 2001]. On the other hand, similar studies conducted for salinomycin showed that the different degree of metabolism does not explain the differences in the observed toxic effects

[Radko and Olejnik 2018]. Moreover, this mechanism has not yet been proven in *vivo* [Ekinci et al. 2023].

Since the toxicity of ionophore coccidiostats is related to the degree of their biotransformation, it may be enhanced by many drugs affecting the activity of cytochrome P450 enzymes. The most thoroughly studied are the pharmacokinetic interactions with tiamulin, an antibiotic used to treat infections in animals [Mazurkiewicz et al. 1989, Weisman et al. 1980]. This interaction is dose dependent. When salinomycin and tiamulin were administered to chickens simultaneously, a slight reduction in productivity was observed only at the highest dose of tiamulin (50 mg/kg feed) [Stipkovits et al. 1992]. Optimal breeding effects were achieved when salinomycin (60 mg/kg feed) was combined with tiamulin at a concentration of 20 mg/kg feed [Islam 2008]. However, the above doses of tiamulin are subtherapeutic doses, and the studies were conducted at a time when the use of the so-called antibiotic growth promoters. Currently, the use of feed with ionophore coccidiostats is an absolute contraindication to the administration of tiamulin.

The problem of toxicity of coccidiostats for some animal species has an important practical impact because feed intended for animals that should not receive coccidiostats (so called non-target feed) may be unintentionally contaminated with coccidiostats during the production, storage and transport of feed. It is believed that cross-contamination of feed is impossible to avoid, taking into account technological and economic aspects. However, it is important to ensure that the phenomenon of transfer of coccidiostats to non-target feed does not cause any negative effects on humans or animals. For this reason, the European Commission introduced the so-called maximum levels

(ML) of coccidiostats in non-target feed and food originating from animals consuming such feed [Comm. Reg. (EU) No 574/2011].

The maximum content of coccidiostats in non-target feed (i.e. feed intended for species and categories of animals other than those listed in the authorization documents) is set at 1% or 3% of the highest permitted target concentration. The two different limits represent different levels of risk associated with the consumption of coccidiostats by different categories of animals. In the case of animals particularly susceptible to the toxic effects of some coccidiostats (including salinomycin narasin by turkeys, Table 3), the acceptable level of contamination is 1% of the maximum permitted concentration in the target feed. In Poland, more than 5% of non-target feeds are still contaminated with coccidiostats, mainly ionophores, although the situation has been systematically improving for several years. From a practical point of view, the greatest importance is the high toxicity of salinomycin and narasin for turkeys - animals that often receive feed produced in feed mixing plants that also produce feed for broiler chickens.

Table 3. Maximum levels (MLs) for narasin and salinomycin in non-target feeds [Comm. Reg. (UE) No 574/2011]

Coccidiostat	**Compound feed for:**	**ML [mg/kg]**
Narasin	**turkeys**, rabbits, equine species, laying birds and chickens reared for laying (> 16 weeks),	0,7
	other animal species.	2,1
Salinomycin	equine species, **turkeys**, laying birds and chickens reared for laying (> 12 weeks)	0.7
	chickens for fattening, chickens reared for laying (< 12 weeks) and rabbits for	0.7

	fattening for the period before slaughter in which the use of salinomycin sodium is prohibited (withdrawal feed)	
	other animal species	2.1

Descriptions of field poisoning cases provide important and interesting data on the toxicity of ionophore coccidiostats (Table 4). Unfortunately, they do not show a direct relationship between the dose of coccidiostat and bird mortality, and the experimentally established relationships are not always confirmed. For example, mass deaths of several-day-old turkey chickens, theoretically the most resistant to the toxicity of ionic coccidiostats, have been associated with feed contamination with salinomycin and narasin [Szymanek-Bany et al. 2014].

One of the factors that influences the scale of harmful effects in a previously unknown way is the simultaneous exposure of birds to several coccidiostats present in low concentrations in feed. For obvious reasons, the experiments conducted by companies producing feed additives did not take into account such a scenario. On the other hand, in practice it is possible for feed to be contaminated with more than one coccidiostat; contamination of feed containing lasalocid or monensin with salinomycin or narasin is also possible. It is not known whether the mechanism of joint action of ionophore coccidiostats is additive or synergistic. For example, possible potentiation of the toxic effect of narasin by salinomycin and monensin could have contributed to higher mortality in case described in 2015 by Polish authors (Table 4) [Szymanek-Bany et al. 2015].

Table 4. Selected cases of poultry poisoning with coccidiostats

Toxic agent(s)	Mortality	Reference
Turkeys		
Salinomycin 34-89 mg/kg	47.0% (F) 30.6% (M)	Jopek et al. 1988
Monensin 280 mg/kg	76% (flock 1) 18% (flock 2)	Ficken et al.1989
Salinomycin 15.5 mg/kg	18.5%	Neufeld 1992
Salinomycin 13.4-18.4 mg/kg	21.7%	Andreasen & Schieifer 1995
Narasin 70 mg/kg	31.9% (F) 14.1% (M)	Gaweł & Mazurkiewicz 2004
Salinomycin 60 mg/kg	34.5%	Van Assen 2006
Salinomycin 29.8-94.4 mg/kg	88%	Koutoulis et al. 2013
Salinomycin 0.98 mg/kg	96%	Ševčíková & Modrá 2014
Narasin 26 mg/kg Monensin 0.85 mg/kg Salinomycin 1.19 mg/kg	85%	Szymanek-Bany et al. 2015
Lasalocid 21.9 mg/kg Narasin 82.3 mg/kg Salinomycin 8.93 mg/kg	83%	Szarek et al. 2019
Chicken		
Monensin 740 mg/kg	13.7% (F) 70.9% (M)	Zavala et al. 2011

Salinomycin 64.6-124 mg/kg	100% (euthanized)	Koutoulis et al. 2013
Salinomycin 60 mg/kg Tiamulin 225 mg/kg	22%	Hosseini Aliabad & Aryanezhad 2018
Ostriches		
Monensin 215-224 mg/kg	40.3%	Baird et al. 1997

The previously mentioned interaction with tiamulin may also be an important factor. The European Food Safety Authority's risk assessment mentioned this interaction but did not include it in the final assessment of the impact of contaminated feed consumption on animal health [EFSA 2008]. Although there is no confirmed scientific data, it appears that tiamulin may be harmful when administered to turkeys fed with feed cross-contaminated with coccidiostatic ionophores. Before administering antibiotics, it is therefore recommended to analyze the feed in this direction.

Despite common knowledge about the toxicity of ionophore coccidiostats for some animal species, it is difficult to determine the actual scale of the problem of animal poisoning with these molecules. Descriptions of individual cases of poisoning often appear in the scientific literature (Table 4), but this is certainly not a complete source of knowledge on this subject. In very few countries, data on animal poisoning is collected systematically; in Poland, to my knowledge, such a database does not exist. There is no central authority or reference laboratory to which such cases should be reported.

Diagnosis of poisoning with ionophore coccidiostats is difficult because the clinical symptoms and histopathological picture are not specific enough.

Therefore, the most common evidence of poisoning is the presence of a coccidiostat in the feed given to animals [Nicpoń and Czerw 1995]. Due to the difficulty of proving that the birds consumed this particular feed, stomach contents and internal organs are often also collected for testing [Szymanek-Bany et al. 2014, 2015]. This approach, however, may pose some difficulties because the stomachs of poisoned birds are often empty (anorexia is one of the consequences of poisoning), and ionophore coccidiostats are rarely detected in internal organs. Some authors postulate the recognition of biochemical tests as one of the diagnostic tools in cases of poisoning with ionophore coccidiostats [Neufeld 1992, Nicpoń and Czerw 1995]. Nicpoń and Czerw [1995] observed an increase in the activity of aspartate transaminase and alanine transaminase as well as metabolic alkalosis in acute horse poisoning, but the research of Neufeld [1992] does not confirm these results and indicates creatine kinase as a more selective marker of muscle damage.

Probably most cases of turkey poisoning with coccidiostats in Poland are diagnosed by the National Veterinary Research Institute. Its experiences do not exhaust the topic, but they prove that the problem of poisoning turkeys with ionophore coccidiostats is still important. In the years 2009-2014, PIWet-PIB received samples from 25 cases of suspected poisoning with ionophore coccidiostats [Szymanek-Bany et al. 2014]. In nine cases, the presence of salinomycin or narasin was found in the gastrointestinal tract, which may be considered evidence of poisoning or at least exposure to a toxic agent. As part of the diagnosis of suspected poisoning with ionophore coccidiostats in 2017-2018, the presence of narasin in feed was found in only three cases, salinomycin - in two. However, a different pattern was observed - in six out of nine feed samples which, according to the leaflet, contained monensin, its content exceeded the legally defined concentration ranges [Olejnik 2020].

The presented above diagnostic approach based on laboratory analysis of feed samples and, depending on the availability of material, samples of digestive contents of poisoned animals, seems to be the most substantively justified and effective, but it will not always provide evidence that can be used in possible court disputes. The situation is complicated by the inability to attribute estimated losses to coccidiostat concentrations in feed. As previously presented (Table 4), too many factors influence the occurrence of poisoning symptoms and it is impossible to clearly determine whether a given concentration of coccidiostat in the feed could have caused specific symptoms.

In conclusion, it is worth noting that the economic significance of the toxicity of ionophore coccidiostats to turkeys is probably underestimated. The first symptoms of poisoning, often unnoticed or unrelated to feed quality, include reduced feed intake and decreased animal weight gain. Taking into account the scale of feed contamination in Poland, this phenomenon may affect domestic turkey production.

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Vasil Stanev[1], Brecht Maertens[2], Luis Gomez[3], Sandra Bonaspetti[4], Georgina Inwood[5].

1 Phibro Animal Health SA, 1300 Wavre, Belgium; vasil.stanev@pahc.com
2 Poulpharm BVBA, 8870 Izegem, Belgium; brecht.maertens@poulpharm.be
3 Phibro Animal Health Corporation, Teaneck, NJ 07666, United States; luis.gomez@pahc.com
4 Phibro Animal Health SA, Campinas, SP 13025-170, Brazil; sandra.bonaspetti@pahc.com
5 Phibro Animal Health PTY, Bella Vista, NSW, 2153, Australia; georgina.inwood@pahc.com

POSITIVE EFFECT OF *AUILLAJA SAPONARIA* AND *YUCCA SCHIDIGERA* BLEND IN COCCIDIOSIS CHALLENGE MODEL AND POTENTIAL MODE OF ACTION

Summary

Numerous scientific publications and commercial use data indicate the positive effect of *Quillaja saponaria* and *Yucca schidigera* combination product (QY) on broiler performance when exposed to an *Eimeria* spp. challenge. This has created the perception of an anticoccidial effect of such a combination. To assess this hypothetical effect and identify the specific mode of action of QY in coccidia challenged broilers a series of *in vivo* and *in vitro* trials have been carried out. In the *in vitro* study QY did not exhibit a direct anticoccidial effect, assessed by the reduction of sporozoite viability during *in vitro* incubation at physiologically relevant concentrations in comparison to registered anticoccidial products such as salinomycin and toltrazuril. However, QY

demonstrated numerous beneficial effects when used alone or in combination with either a coccidiosis vaccine or an in-feed anticoccidial when birds were exposed to coccidiosis challenge. QY had a positive effect on performance prior to the challenge (d 0-14) on oocyst shedding expressed as oocyst per gram feces (OPG) and performance during the recovery phase (d 28-35), but not during the acute phase (d 14-28). This suggests that the positive effect of QY under coccidiosis challenge is due to improved immunity development, reduced inflammation and tissue damage and faster recovery, rather than direct anticoccidial effect.

I. INTRODUCTION

Natural triterpenoid saponins from *Quillaja saponaria* such as QS 21, QS 17, QS 18 and QS7 are known to support specific immune response towards different pathogens (Lacaille- Dubois, 2019; Marciani et al., 2000). As well, natural polyphenols from both *Q. saponaria* and *Yucca schidigera* such as piscidic acid, vanillic acid, ferulic acid, p-coumaric acid, resveratrol and yuccaols are known to have antioxidant and anti-inflammatory effects (Maier et al., 2015; Piacente et al., 2005). Furthermore, numerous scientific publications (Bafundo et al., 2020, Bafundo et al., 2022) and commercial field experience indicate positive effects of the *Q. saponaria* and *Y. schidigera* combination product (QY) (Magni-Phi®), containing a minimum of 3.5% triterpenoid (Quillaja) saponins and typically 0.8-1.0% of total polyphenols expressed as gallic acid equivalent on broiler performance when exposed to an *Eimeria* spp. challenge. This has created a perception of an anticoccidial effect of this combination. The current study aims to assess this effect and helps to identify the specific mode of action of QY in coccidia challenged broilers.

II. METHOD

***In vivo* study**. A total of 1 848 day old male Ross 308 broilers were allocated into 7 treatments: T1 - uninfected untreated control (UUC); T2 - infected untreated control (IUC); and five infected treatments: T3 - supplemented with QY; T4 - supplemented with in-feed anticoccidial: narasin+nicarbazin (100 ppm 0-21 d), followed by salinomycin (60 ppm 22-35 d) (ACC); T5 - same coccidiostat treatment supplemented with QY (ACC+QY); T6 - birds vaccinated for coccidiosis at day 0 with a commercial attenuated vaccine Evant® (VAC); and T7 - the same vaccine treatment supplemented with QY (VAC+QY). In treatments 3, 5 and 7 QY (Magni-Phi®) was used at 250 g/t from 0 to 35 d. Each treatment had 8 replicates, 35 birds each, in floor pens on fresh wood shavings. All treatments, except UUC, were challenged on day 14 by spray on feed and litter with a mix of *E. acervulina, E. maxima, E. mitis* and *E. tenella* isolates at a total of 199,000 oocysts/bird, to mimic natural field infection causing subclinical infection.

Body weight (BW) and daily weight gain (DWG) were measured at day 14, 28 and 35 and feed conversion ratio (FCR) was calculated respectively. Fecal samples for oocyst count per gram (OPG) were collected per pen and pooled per treatment at day 6, 7, 8 and 14 to confirm the status of the birds prior to the challenge. In addition, fecal samples were collected and counted per pen after the challenge for OPG at day 21, 22, 28 and 35. Total mean lesion score (TMLS), as a sum of individual *E. acervulina, E. maxima* and *E. tenella* lesion scores, were recorded according to Johnson and Reid (1970) at day 21, 22 and 28 based on four birds per pen each time. Statistical analysis using Fisher LSD test was applied; paired QY (+) and (–) treatments were compared with paired T test at $P \leq 0.05$.

***In vitro* study**. In the *in-vitro* study *E. tenella* sporozoites were incubated for 72 h in 20% *Quillaja* extract (QE) and QY solutions with concentration respectively 8.75; 17.5 and 35.0 µl/l; 50, 100 and 200 mg/l mimicking *Quillaja* saponin intestinal concentration corresponding to 250 g/t, 500 g/t, and 1000 g/t in-feed application of QY, taking into account respective dry matter of feed and intestinal content. The effect of the QE and QY on sporozoites was assessed based on sporozoites counts at 24, 48 and 72 hours of incubation and compared to sporozoites count in negative controls (solvent only – PBS and dimethyl sulfoxide – DMS) and positive controls – salinomycin at 9 and 12 mg/l mimicking intestinal concentration respective to in-feed application of 45 and 60 ppm and toltrazuril at the in-water therapeutic dose of 25.0 mg/l.

III. RESULTS

***In vivo* study.** Overview of parasitological results is provided in Table 1. Prior to the challenge at day 14 oocysts shedding was identified only in VAC and VAC+QY confirming the vaccine cycling and the coccidia-free status of all other treatments. A successful natural *Eimeria* challenge was obtained, evidenced by significantly higher TMLS and OPG at both 21-22 and 28 days in IUC compared to UUC. At day 21 and 22 only ACC+QY provided OPGs significantly lower than IUC. At day 35, QY and VAC+QY provided OPG significantly lower compared to IUC. Only ACC and ACC+QY provided significant reduction of macroscopic coccidia lesions at day 21-22 in comparison to IUC. There was no significant difference in any of the parasitological parameters between VAC and VAC+QY indicating no QY interference with coccidiosis vaccination.

Oocysts shedding in the UUC appeared at day 28, indicating contamination of the UUC group from neighboring pens. It caused coccidiosis cycling manifested by increased OPGs at day 35, at a level significantly higher than IUC and all treatments.

Mortality was not significantly different from the UUC for any of the infected groups including IUC. Overview of zootechnical performance is provided in Table 2. The highest BW and lowest FCR for the overall 0-35 day period were achieved in the ACC and ACC+QY treatments and were significantly better compared to all other groups. VAC had significantly higher FCR than all other infected treatments. QY significantly improved FCR compared pairwise for IUC vs QY and VAC vs VAC+QY with a P value of 0.024 and 0.048 respectively.

Table 1 - Overview of parasitological parameters during the different study periods per treatment group

Treatment	Total OPG d 14	Total OPG d 21	Total OPG d 22	Total OPG d 28	Total OPG d 35	TMLS d 21-22	TMLS d 28
UUC	0.0	67^{a}	47^{a}	32022^{a}	91503^{d}	0.9^{e}	1.5^{c}
IUC	0.0	54451^{b}	56809^{c}	213835^{bc}	10381^{c}	1.9^{ab}	2.1^{bc}
QY	0.0	57203^{b}	87653^{c}	228213^{bc}	1916^{a}	2.2^{a}	3.2^{c}
ACC	0.0	19347^{b}	14649^{bc}	475283^{c}	6935^{bc}	1.0^{de}	2.7^{ab}
ACC+QY	0.0	6082^{b}	3921^{b}	183419^{b}	6588^{bc}	1.4^{cd}	2.9^{a}
VAC	3600	13100^{b}	14686^{bc}	336172^{bc}	3538^{abc}	1.6^{bc}	2.8^{ab}
VAC+QY	16000	31513^{b}	25519^{bc}	163397^{b}	2865^{ab}	1.7^{bc}	2.6^{ab}

OPG – total oocyct count per gram feces; TMLS – total mean lesion score as a sum of individual *E. acervulina*, *E. maxima* and *E. tenella* lesion scores, according to Johnson and Reid (1970). Means with different superscripts are significantly different at $p < 0.05$ (LSD Fisher test)

In the period before the challenge (0-14 d) ACC and ACC+QY had significantly highest BW and lowest FCR. QY improved FCR significantly when used alone compared to non-treated groups and provided statistically the same FCR as the coccidiostat groups. VAC had significantly highest FCR and lowest BW. QY helped to partly alleviate the negative effects of the coccidiosis vaccination: VAC+QY had significantly higher BW compared to VAC.

In the acute period after the challenge (14-28 d) the IUC had significantly lower DWG and higher FCR compared to UUC, demonstrating the success of the challenge model and the impact of subclinical coccidiosis on performance. Among different infected treatments, significant improvement over IUC was recorded only in ACC and ACC+QY, showing significantly highest BW and lowest FCR. All other treatments were not different from IUC and QY did not provide significant improvement when compared pairwise to the respective non QY group.

In the recovery period (28-35 d), a deterioration of performance was noticed in the UUC caused by late coccidia cycling in this treatment evident also in the OPG counts mentioned above. The only treatment that outperformed the IUC was ACC+QY having significantly higher BWG and lower FCR. QY when added on top of the vaccine or the anticoccidial brought positive effect, statistically significant or tendency, with a P value of 0.05 and 0.09 respectively.

Table 2 - Overview of zootechnical performance during the different study periods per treatment group

Treatment	BW 14d	BW 28d	BW 35d	DWG 0-14d *	DWG 14-28d	DWG 28-35d **	DWG 0-35d	FCR 0-14d	FCR 14-28d	FCR 28-35d ***	FCR 0-35d ****
UUC	436^{cd}	1494^{b}	2202^{bc}	27.8^{cd}	75.3^{a}	99.2^{b}	55.3^{bc}	1.26^{a}	1.49^{c}	1.63^{ab}	1.47^{c}
IUC	450^{bc}	1470^{b}	2191^{bc}	28.8^{bc}	61.4^{cd}	101.6^{b}	55.0^{bc}	1.27^{a}	1.84^{a}	1.68^{a}	1.52^{b}

QY	453[b]	1499[b]	2216[b]	29.0[b]	62.6[c]	103.2[ab]	55.6[b]	1.18[b]	1.80[a]	1.67[ab]	1.48[bc]
ACC	471[a]	1616[a]	2334[a]	30.3[a]	68.5[b]	103.1[ab]	58.2[a]	1.17[b]	1.67[b]	1.68[ab]	1.43[d]
ACC+QY	470[a]	1585[a]	2362[a]	30.3[a]	67.0[b]	109.8[a]	58.8[a]	1.18[b]	1.69[b]	1.58[b]	1.42[d]
VAC	417[e]	1391[d]	2098[c]	26.6[e]	58.9[cd]	97.7[b]	51.4[d]	1.29[a]	1.87[a]	1.65[ab]	1.56[a]
VAC+QY	433[d]	1404[cd]	2133[bc]	27.6d[e]	58.3[d]	103.0[ab]	52.8[cd]	1.24[ab]	1.87[a]	1.60[ab]	1.51[b]

BW – body weight in grams; DWG – daily weight gain in grams; FCR – feed conversion ratio

Means with different letters are significantly different at $p < 0.05$ (LSD Fisher test)
*Difference between VAC and VAC+QY is close to significant showing tendency P = 0.083 (paired T test)

**Difference between VAC and VAC+QY and ACC and ACC+QY is close to significant showing tendency P = 0.165 and P = 0.170 respectively (paired T test)

***Difference between VAC and VAC+QY and ACC and ACC+QY is close to significant showing tendency P = 0.052 and P = 0.091 respectively (paired T test)

**** Differences between QY and IUC and VAC and VAC+QY is significant P = 0.024 and P = 0.048 respectively (paired T test)

***In vitro* study.** Toltrazuril significantly reduced sporozoites counts in comparison to the negative control at 24, 48 and 72 hours of incubation. Salinomycin, at concentrations mimicking 45 and 60 ppm in-feed application, significantly reduced the sporozoites counts at 48 and 72 hours of incubation while neither QE nor QY provided significant reduction at any of the tested timepoints or concentrations (Fig. 1).

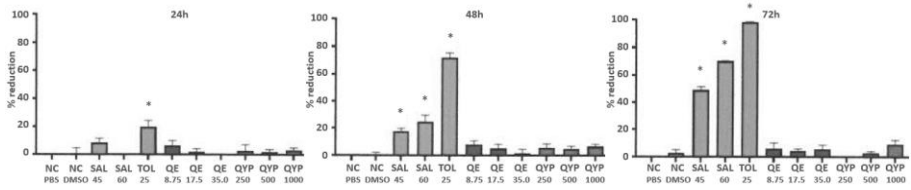

Figure 1 – Reduction (%) of sporozoites counts of different treatments compared to non-treated control after 24, 48 and 72 hours of incubation. Significant difference (P <

0.05) based on Kruskal-Wallis test with Dunn's numerous comparison with control is indicated with *.

NC PBS – negative control phosphate buffered saline; NC DMSO – negative control dimethyl sulfoxide; SAL 45 and SAL 60 – positive control salinomycin mimicking intestinal concentration respective to in-feed application of 45 and 60 ppm; TOL 25 toltrazuril 25.0 mg/l; QE 8.75; QE 17.5 and QE 35.0 Quillaja extract solution with saponin concentration of 8.75, 17.5 and 35.0 µl/l; QYP 250; QYP 500 and QYP 1000 Quillaja and Yucca product mimicking intestinal concentration respective to in-feed application of 250, 500 and 1000 g/t.

IV. DISCUSSION

The *in vitro* study did not show a direct anticoccidial effect of the QE or QY on sporozoite viability at physiologically relevant concentrations. Furthermore, the *in vivo* study demonstrated that QY did not affect vaccine cycling before the challenge, but improved performance of the coccidia vaccinated group, demonstrating compatibility with the coccidiosis vaccine. Although QY did not exhibit direct anticoccidial effect on sporozoites the *in vivo* coccidiosis challenge model confirmed the positive effect of QY on birds infected with coccidia as demonstrated in previous studies with *Eimeria spp.* challenged birds. Thus, the QY positive effect on performance prior to the challenge (d 0-14), on OPG and performance during the recovery phase (d 28-35), but not during the acute phase (d 14-28), suggest that the positive effect of Quillaja and Yucca under coccidiosis challenge is due to improved immunity development, reduced inflammation and tissue damage, and faster recovery, rather than direct anticoccidial effect. This is in line with previously reported data of improved cell mediated immunity related to *Q. saponaria* saponins and reduced inflammation and anti-oxidative effect related to *Y. schidigera* and *Q. saponaria* polyphenol fractions.

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Chłodowska A., Bogucka J., Olszewska-Tomczyk M., Wieczorkiewicz M., Olejnik M. - The effect of low doses of salinomycin on histopathological features in selected turkey organs

Chłodowska A. 1, Bogucka J. 2, Olszewska-Tomczyk M. 1,
Wieczorkiewicz M. 1, Olejnik M. 1,

[1]Nicolaus Copernicus University in Torun, Faculty of Biological and Veterinary Sciences,
Institute of Veterinary Medicine, 11 Gagarina St, 87-100 Toruń; [2]Stanlab LLC, Nakło n. Notecią

THE EFFECT OF LOW DOSES OF SALINOMYCIN ON HISTOPATHOLOGICAL FEATURES IN SELECTED TURKEY ORGANS

Salinomycin is an ionophore coccidiostat used widely in poultry. It is a common cause of intoxication in non-target animals. However, the mechanisms behind salinomycin toxicity are not yet fully understood. Significant differences in species susceptibility have been observed throughout the years. For example: the recommended dose for broiler chickens is toxic for turkeys.

For two weeks, turkeys at the age of 13 weeks were given feed containing salinomycin. The birds were divided into five groups, receiving feed containing 0; 0.7, 2.1, 7.0, and 21 mg salinomycin per kg, respectively. Immediately after the birds' euthanasia, tissue samples were collected for histopathological examination. Samples of skeletal muscles (leg and breast), cardiac muscle, liver, spleen, and sciatic nerve were fixed in 10% neutral buffered formalin, routinely processed, embedded in paraffin, and sectioned on a microtome (3.5 μm). The sections were stained with hematoxylin and eosin and analyzed by light

microscopy. The slides were evaluated qualitatively and quantitatively. The statistical analysis was performed.

Necrotic lesions in the liver were identified. Degeneration of hepatocytes and lymphocyte infiltration were visible. In some of the birds, hepatic steatosis was seen. Normal hepatic architecture was distorted by numerous round cytoplasmic vacuoles. The nuclei of the distended hepatocytes were displaced to the periphery of the cells. In the skeletal muscles, myofiber necrosis was pronounced. Many hypertrophied myofibers were also present in the skeletal muscles. As a result, in some sections, fiber splitting was noted. In cardiac muscle sections, cardiomyocytes vacuolation and loss of cross striations were pronounced. Cardiomyocytes fragmentation and necrosis were observed in some of the specimens. Additionally, some progressive changes were seen: connective tissue hyperplasia and cardiomyocyte nuclei proliferation. In the evaluation of spleen samples, irregular lymphocytes, and lymphocyte aggregation were observed. Additionally, haemorrhages, apoptotic cells and lymphocyte depletion were noted. Considering skeletal muscles, cardiac muscle, and spleen, statistically significant ($p<0.05$) results were observed in the group, with feed containing salinomycin in the concentration of 2.1 mg per kg. For the liver, statistically significant results ($p<0.05$) were observed in the group, with the salinomycin concentration of 0.7 mg per kg feed. In cardiac muscle samples, a dose-response relationship was observed.

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Kamila Bobrek, Andrzej Gaweł

Department of Epizootiology and Clinic for Birds and Exotic Animals, Faculty of Veterinary Medicine, Wroclaw University of Environmental and Life Sciences, Poland

DO *HETERAKIS* SPP. INFECTIONS PREDISPOSE TO OTHER POULTRY DISEASES? RETROSPECTIVE CASE ANALYSIS

The *Heterakis* infection is one of the most frequently reported parasitic infections in poultry. In large-scale poultry production, heterakidosis is reported in commercial and reproductive laying hens and geese. Hens (*Gallus gallus*) are infected with the nematode *Heterakis gallinarum*, while in geese flocks dominate *Heterakis dispar* (1,2). Among the *Heterakis* species, in gallinaceous birds *H. isolonche* has been described, occurring, among others, in pheasants (3,4), however, the authors of the study did not observe this species in large-scale poultry production in Poland.

Nematodes of the *Heterakis* genus are small (4-23 mm) white parasites living in the cecum of birds, and they are generally considered to have little impact on the host (3,5,6). An exception is the case when the nematodes *Heterakis gallinarum* are infected with the protozoan *Histomonas meleagridis*, which is the etiological factor of histomoniasis – a disease causing high mortality due to necrotic lesions in the liver in gallinaceous birds (7,8,9). In the literature, there are few descriptions of *Heterakis* spp. mono-invasion cases, causing pathological changes and even increasing bird mortality (10,11). However, the descriptions available in the literature concern pathological changes in gallinaceous birds, but there is no data on the impact of *Heterakis*

invasion in geese. Based on necropsy data collected in years 2015–2022, an analysis of the correlation between deaths in reproductive flocks of geese and the occurrence of Heterakis infection was carried out.

Materials and methods

The analysis used data from necropsies of dead reproductive geese carried out in 2015–2023. Data from necropsy, microbiological and histopathological examinations (if viral infection was suspected) were taken into account. Among the dead geese, 143 were found to be infected with nematodes and the data were used for further analysis.

Results

In geese with the presence of *Heterakis* spp. in the cecum, the bacterial infections were dominating causes of death, but parasitic co-infections and cancer were also recorded. The peritonitis caused by *E.coli* was diagnosed in 84 animals (58.7%) and it was the most common cause of death of birds in the group infected with *Heterakis*. Much less generalized bacterial infections (septicemia) caused by *Pasteurella multocida* (15.4%) and *Erysipelothrix rhusiopathiae* (8.4%) were observed. Co-infection with *Heterakis dispar* and *Teteratrichomonas gallinarum* (4.2%) was observed in 6 animals, of which 1 bird had additional changes typical of cannibalism. Also in 6 animals there were changes in the joints caused by infection with *Staphylococcus* spp. (5 geese) and *Streptococcus* spp. (1 bird). Necrotic enteritis was observed in 4 birds (2.8%) – in 2 birds both the small intestines and cecum, and in 2 birds only the cecum. In three dead animals infected with Heterakis, no lesions typical of other disease were found. In the remaining six birds, the cause of death was cancerous lesions

in the liver, which were histopathologically characterized by inflammatory infiltrates typical of Marek's disease.

Discussion

In the analyzed cases of *Heterakis* infections in geese, the cause of death in most birds were bacterial diseases (89.5%). In the author's opinion, *Heterakis* invasion may indirectly contribute to the more frequent occurrence of bacterial infections due to the development of larval forms in the cecal wall. *Heterakis* has a direct life cycle, in which an invasive egg eaten by the host hatches into a larva (the cycle may involve a paratenic host – an earthworm, but this is usually not the case in large-scale production). The larva is released in the duodenum, and is carried to ceca within 8-9 hours (12). The larva burrows into the epithelium near the crypts of the cecum, where it molts, and literature data indicate that it may remain associated with the mucosa until the 10th day of development (5). Damage to the epithelium promotes the penetration of bacteria naturally found in the digestive tract of birds.

Studies on the composition of the cecal microflora of geese showed the presence of bacteria from the *Clostridium* group (58.7%), *Bacteroidetes* (26.9%) and *Erysipelotrichi* (11.2%) in the cecal content, and in samples from the cecal mucosa, microorganisms from the *Gammaproteobacteria* class predominated which include *E.coli* (59.6%) and *Clostridia* (20.1%) (13). Some of the diagnosed goose diseases associated with *Heterakis* infections may therefore be of autogenic origin. It is possible that some cases of peritonitis caused by *E.coli*, erysipelas and necrotic enteritis are infections related to damage to the intestinal epithelium by *Heterakis* larvae. In the case of *Heterakis* infections in hens, the authors also observed the co-occurrence of necrotic

enteritis and peritonitis, but the number of cases examined is insufficient to draw conclusions.

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Barbara Szczepankiewicz[1], Maciej Kuczkowski[1], Jolanta Piekarska[2], Martin Kváč[3], Nikola Holubová[3], Bohumil Sak[3],Kamila Bobrek[1], Andrzej Gaweł[1]

[1] Department of Epizootiology and Clinic for Birds and Exotic Animals, Faculty of Veterinary Medicine, Wroclaw University of Environmental and Life Sciences, pl. Grunwaldzki 45, 50-366 Wrocław
[2] Division of Parasitology, Faculty of Veterinary Medicine Wroclaw University of Environmental and Life Sciences, C.K. Norwida 31, 50-375 Wroclaw, Poland
[3] Institute of Parasitology, Biology Centre, Czech Academy of Science, Branišovská 1160/31, České Budějovice, Czech Republic

AVIAN CRYPTOSPORIDIOSIS – A NEW HEALTH PROBLEM IN POULTRY?

Introduction

Cryptosporidium spp. are opportunistic parasitic protozoa that preferentially infect the epithelial cells of the digestive and respiratory systems in most vertebrates and invertebrates. To date, several species and genotypes of *Cryptosporidium* have been identified, including *C. baileyi, C. meleagridis, C. avium, C. proventriculii, C. ornithophilus, C. muris,* and *C. parvum*, as well as genotypes I–V in over 30 bird species worldwide. Clinical symptoms associated with *Cryptosporidium* invasion in birds are increasingly reported, but cryptosporidiosis in commercial flocks remains poorly recognized, partly due to its often subclinical nature or nonspecific symptoms. Moreover, cryptosporidiosis is not routinely diagnosed in veterinary laboratories, hence the epizootic data on its impact on bird health, including the clinical course of the disease and economic losses, are not fully investigated.

Over twenty species of *Cryptosporidium*, most commonly causing acute or chronic diarrhoea, have been identified in humans. The most severe cases occur in individuals with immune system disorders, where chronic cryptosporidiosis develops, and without treatment, in extreme cases, it can also lead to death. Groups at increased risk include people infected with the HIV virus and children under 2 years of age, as well as patients with pharmacologically induced immunosuppression. Epidemiological data indicate that *Cryptosporidium* is a parasite widespread worldwide, and cases of cryptosporidiosis have been documented in people in over 100 countries on all continents. The disease thus remains a serious threat not only to people with immune system disorders but also to children living in developing countries. To emphasise the importance of this disease and its impact on the population, the World Health Organization (WHO) has classified cryptosporidiosis as a neglected disease since 2004, highlighting its global impact on public health.

In humans, the two most commonly recognized species of clinical significance are *C. hominis* and *C. parvum*. Interestingly, *C. meleagridis* has been identified not only in poultry but also in the tumor tissue of an immunocompetent patient with colorectal cancer. Meanwhile, in a group of patients with various lung diseases, the first case of respiratory infection caused by *C. baileyi*, previously known only from bird infections, was identified. These findings highlight the potential risk of zoonotic and/or environmental transmissions.

Morphological characteristics and life cycle of *Cryptosporidium* spp.

Cryptosporidium are unicellular, opportunistic parasites of animals and humans, belonging to the type Apicomplexa, class Conoidasida, order

Eucoccidiorida, and family Cryptosporidiidae. Historically, *Cryptosporidium* was classified within the class Coccidia due to shared biological and morphological features. However, advancements in medical sciences have demonstrated that *Cryptosporidium* possesses unique traits distinguishing it from other intestinal parasites of the class Conoidasida, further emphasizing its uniqueness within Apicomplexa. Notably, the endogenous developmental stages of *Cryptosporidium* are limited to the surface of the host's epithelial cells, manifesting as an intracellular but extracytoplasmic location. Secondly, *Cryptosporidium* oocysts are relatively small and lack morphological structures such as sporocysts, micropyles, or polar granules. Thirdly, it is characterised by the presence of a so-called feeding organelle, which is formed through the parasite's adhesion to the host cell. Fourthly, *Cryptosporidium* produces two types of oocysts: thick-walled and thin-walled. Lastly, it's worth noting the occurrence of an extracellular gamont-like cell stage.

Over 40 species of *Cryptosporidium* have been described, most of which occur in a narrow range of hosts. However, some species exhibit low host specificity, including *C. parvum*, which is also known for its high zoonotic potential. These protozoans are characterised by a unique life cycle and the ability to infect epithelial cells of the digestive and respiratory systems. There, they develop and multiply, causing a full-symptom disease known as cryptosporidiosis

The life cycle of *Cryptosporidium* is monoxenous, meaning it occurs within a single host, combining both asexual and sexual reproduction stages, leading to the formation of invasive oocysts. Upon ingestion (or inhalation) of sporulated oocysts by the host, sporozoites are released. They move along the surface of the intestinal (or respiratory) cells and, by releasing the contents of

the apical complex, develop into trophozoites. Through merogony, they form first-type meronts with eight merozoites. The released merozoites initiate another round of merogony, followed by gametogony, resulting in the formation of micro- and macrogametes and the zygote, which transforms into thick-walled and thin-walled oocysts. *Cryptosporidium* oocysts are key to the parasite's life cycle, being round or slightly oval, with dimensions around 3-8 μm. Each oocyst contains four sporozoites after sporulation. Thick-walled oocysts excreted in faeces are responsible for spreading the infection in the environment, while thin-walled ones, which excyst in the host's body, are a source of autoendoinvasion (responsible for chronic cryptosporidiosis cases). Due to their high resistance to environmental conditions, *Cryptosporidium* oocysts can survive in water and on surfaces for extended periods, posing a significant public health risk and highlighting the need for effective methods to prevent their spread.

Avian cryptosporidiosis

Avian cryptosporidiosis is a disease caused by *Cryptosporidium*, with diarrhoea being one of the most common clinical symptoms. Birds exhibit frequent, watery stools, reluctance to drink, leading to dehydration. The protozoa causes atrophy of intestinal villi, reducing the absorptive surface area. Diarrhoea is a result of secretion disorders and impaired nutrient absorption. Inflammation and other clinical symptoms are associated with the production of inflammatory mediators like prostaglandins and tumour necrosis factor.

Based on the existing body of medical knowledge, it has been determined that:

- *C. meleagridis* is often noted for causing diarrhoea in poultry.

- *C. meleagridis* invasion in turkeys can be subclinical or manifest as intestinal inflammation and diarrhoea. Post-mortem examinations often show gas in the intestines and an abundance of mucus.
- Infections in chickens often remain subclinical or are associated with various other pathogens.
- In quails, *Cryptosporidium* invasion can lead to intestinal inflammation, diarrhoea, villi atrophy, and co-invasion with reoviruses increases mortality.
- In partridges, infections with both *C. meleagridis* and *C. baileyi* have been observed, manifesting as diarrhoea and cough with high morbidity and mortality.
- Additionally, infections with *C. galli, C. muris,* and avian genotype III can affect disease changes in the foregut of various bird species. *C. galli* infections can be subclinical or lead to apathy, diarrhoea, weight loss, and occasionally mortality.
- *C. galli* is characterised by chronic oocyst excretion, similar to *C. serpentis* in snakes, and may predispose birds to secondary infections.
- Avian genotype III causes chronic stomach disease in some birds with symptoms such as vomiting and weight loss.

The negative impact of *Cryptosporidium* on birds' overall health can lead to reduced nutrient absorption, general weakness, appetite loss, decreased egg production, and impacts on eggshell quality and laying. Respiratory symptoms associated with C. *baileyi* invasion include sneezing, coughing, mucus discharge from the nose and eyes, sinus swelling, and breathing difficulties. If the infection spreads to the lower respiratory tract, it can lead to pneumonia, with additional symptoms like wheezing and panting. High mortality is

particularly noted with bacterial (mainly *E. coli*) or viral infections (such as infectious bronchitis).

Birds with *Cryptosporidium* infection also exhibit conjunctivitis and middle ear inflammation. Additionally, immunosuppression associated with damage to the bursa of Fabricius, increasing the occurrence of other coexisting viral, bacterial, and fungal diseases, is observed. Post-mortem examination of the bursa of Fabricius often shows increased mucus and congestion.

The infection course can also be subclinical, however, asymptomatic carriers can still shed *Cryptosporidium* oocysts, contributing to the spread of the infection in the flock. The high zoonotic potential of *Cryptosporidium* is confirmed by clinical cases of human infections from poultry. Poultry farmers and veterinarians should be aware of the risk of infection with these opportunistic pathogens.

Objective

The research aimed to assess the frequency of *Cryptosporidium* invasion in birds in the Lower Silesian distinct and identify the species/genotype of these parasites.

Stage I

The study on the prevalence of *Cryptosporidium spp.* was conducted in two stages. In the first stage, the material for the study consisted of 116 stool samples collected from:

- 11 Ross 308 broiler chickens, 4 weeks old, in the poultry flocks

- 42 White Kołuda geese, 4 weeks old, in the poultry flocks
- 25 green-legged partridge chickens, aged from 8 weeks to 4 years, from a backyard farm
- 14 racing pigeons aged 1-3 years, from the vivarium of the University of Environmental and Life Sciences in Wrocław
- 24 budgerigars aged from 1 to 5 years, from the vivarium of the University of Environmental and Life Sciences in Wrocław

Microscopic evaluation of methylene blue-stained preparations and molecular studies using PCR technique and sequencing were used for diagnosis. The material was also subjected to routine flotation examination to detect possible coinvasions.

Results for Stage I:

Molecular examination detected *Cryptosporidium* in 4.52% of samples from broiler chickens and 23.81% of samples from geese. Sequencing of PCR products identified *C. baileyi* in all positive samples.

Stage II

After obtaining the results, in the second stage of the study, stool samples were collected from the infected flock of geese 4 weeks after the first examination:

- 12 White Kołuda geese, 8 weeks old in large flocks

Results for Stage II

Molecular examination detected the presence of *Cryptosporidium* material in 25% of the samples collected from geese.

Case description of clinical occurrence of *Cryptosporidiosis* in the goose flocks

The monitored flock of geese was under constant control of a veterinarian. As a preventative measure, on the first day of life, vaccination against Derzsy's disease was administered using the Deparvax vaccine. During the 112-day fattening period, the geese were treated three times:

- On the third day of life, due to navel and yolk sac inflammation, amoxicillin with clavulanic acid was used.
- In the 4th week of life, in response to death (10 birds per day for 3 days) and post-mortem changes suggesting colibacillosis, antibiotic therapy according to the antibiogram was recommended.
- In the 8th week of life, due to diarrhoea and death (10 birds per day for 3 days), post-mortem changes indicated pasteurellosis, which was treated according to the antibiogram.

The diarrhoea had a moderate response to treatment. Flotation tests and PCR stool examinations were performed twice in the 4th and 8th weeks of life and confirmed the invasion of *C. baileyi*.

This case illustrates the impact of *C. baileyi* infection on the occurrence of diarrhoea, which did not respond to standard antibiotic therapy doses recommended by the manufacturer. The prevalence of *C. baileyi* remained at about 25%.

Discussion

Cryptosporidium species are widely prevalent protozoa of significance in both veterinary and human medicine. They are opportunistic pathogens, and infections can be asymptomatic, but in certain situations, they proceed with severe clinical symptoms.

Cryptosporidium parasites have become the subject of many studies aimed at better understanding their taxonomy, biology, and epidemiology. Despite intensive research, cryptosporidiosis still poses a clinical and diagnostic problem. Diagnostic and therapeutic difficulties arise from the lack of fully effective treatment schemes and the need for additional, often more advanced diagnostic methods, as standard laboratory tests, including flotation, do not allow for the diagnosis of these organisms.

In the treatment of cryptosporidiosis, many drugs have been tested, but only nitazoxanide has been approved by the U.S. Food and Drug Administration (FDA) for use in humans. Other drugs, like halofuginone, have shown variable efficacy, and an effective treatment for animal cryptosporidiosis has not yet been developed. *Cryptosporidium* oocysts are resistant to environmental conditions and many disinfectants, making it difficult to control this parasite in poultry flocks.

These challenges highlight the need for further research to better understand the epidemiology of *Cryptosporidium* and to develop more effective diagnostic and therapeutic methods. Prevention and control of avian cryptosporidiosis are based on biosecurity and proper flock management through appropriate nutrition, hygiene, and prevention of coexisting diseases.

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Monika Roczeń-Karczmarz, Leszek Guz, Marta Demkowska-Kutrzepa, Krzysztof Puk, Maria Studzińska, Krzysztof Tomczuk

University of Life Sciences in Lublin, Faculty of Veterinary Medicine, Department of Parasitology and Fish Diseases

DERMANYSSUS GALLINAE - ALTERNATIVE CONTROL METHODS.

Dermanyssus gallinae is one of the most significant parasites prevalent in avian breeding worldwide. Known to infest chickens, ducks, pigeons, and wild birds (Cencek et al. 2000, Haitlinger 1987), it can also target other animal species (Gibasiewicz 1987) and even humans (Barlaam et al. 2022) in the absence of suitable hosts. This parasite poses a substantial economic threat to the poultry industry, impacting the laying rate of hens and the quality of eggs, and fostering cannibalism among birds. Red mite infestations result in various detrimental effects, including anaemia, feather loss, weight reduction, decreased fertility and hatchability, as well as disruptions to the avian immune system. Mass infestations may lead to the death of birds, especially young ones, due to excessive blood loss (Cencek et al. 2000, Fiddes et al. 2005). Females of *D. gallinae* lay eggs in cracks in floors, walls, and around nests. These red mites inhabit crevices, nests, feeders, waste disposal devices, and egg transportation devices (Sokół and Romaniuk 2007). The pearly white, 400×270 μm red mite eggs are laid in locations inaccessible to spraying, making their elimination from the environment challenging. Under optimal conditions of temperature and humidity (20-25˚C), the red mite population can double in just one week. The prerequisite for egg laying after fertilisation is the prior collection of blood from

the host. Females lay eggs within 24 hours, and larvae hatch within 70 hours. These larvae do not draw blood and transform into protonymphs and deutonymphs over the next 48 hours (Kirkwood 1963, Cencek et al. 2000). Red mites primarily feed at night, but due to lighting, temperature, and high relative humidity in poultry houses, they may feed on hens throughout most of the day. They only descend and hide in wall and floor cracks to lay eggs (Sokół and Romaniuk 2006). Additionally, the lengthy production cycle in poultry houses, lasting 80–90 weeks (Roy et al. 2010), contributes to the proliferation of the red mite population. Considering the life cycle of the red mite, which shortens as the ambient temperature increases, the rate of proliferation, along with its ability to survive for 9 months in hiding without food in temperatures of 5–25°C (Nordenfors et al. 1999) controlling red mite infestations proves to be an extremely challenging task.

Depending on the hens' housing system, strategies for red mite control vary, offering poultry farmers a range of options, including chemical, mechanical, and thermal methods. Chemical control methods typically involve the disinfection of entire rooms rather than individual birds and encompass the use of acaricides, among other treatments. The most popular and effective approach to controlling *Dermanyssus gallinae* is through the use of chemical acaricides. These chemicals should be applied to all red mite gathering places and clusters, such as walls, ceilings, windows, doors, cages, nests, and nesting boxes. Commonly used chemical compounds globally include organochlorines, organophosphates, pyrethroids, carbamates, amitrazes, and endectocides (Beugnet et al. 1997) showing variable levels of performance (Beugnet et al. 1997, Chauve 1998). Furthermore, restrictions on the use of carbamates and organophosphates in many countries have resulted in a reduction in the availability of products for red mite control (Sparagano et al. 2014). The use of

pesticides in poultry houses is also problematic due to extended withholding periods after spraying, residues of the product in meat, bird eggs, and the environment (Carnea et al. 2006, Kim et al. 2007, Marangi et al. 2012) as well as treatment restrictions during laying (Roy et al. 2009). In the case of most substances used, direct contact with the mite is crucial for their effectiveness. When the product comes into direct contact with the ectoparasite, the active substances effectively interact with the mite's respiratory and nervous systems, typically resulting in effective control. This direct contact allows for the concentrated action of the product at the site of infestation, which is crucial for effectively controlling mite populations and achieving the desired results in pest control (Sokół et al. 2020). Due to the excessive use of acaricides, red mites often develop resistance, which leads to diminishing the effectiveness of these treatments. Moreover, the substances used can accumulate in organs, tissues, bird eggs, and the environment (Marangi et al. 2012, Jahanabadi et al. 2023). This not only heightens the risk of resistance in red mites but also poses potential threats to bird health and the environment as these substances build up in the ecosystem. An alarming surge in red mite resistance to acaricides has been observed in many European countries. This issue has been extensively studied and documented, presenting a significant challenge to the efficacy of existing protective measures (Beugnet et al. 1997, Nordenfors et al. 2001, Fiddes et al. 2005, Marangi et al. 2009).

Alterations in abiotic conditions within infested poultry houses may provide an opportunity to reduce mite populations that struggle to thrive at low relative humidity (11%) and extreme temperatures over 45°C and below -20°C. (Maurer and Baumgartner 1992, Nordensfors et al. 1999). While a temperature of 35°C has been found to adversely affect mite development, no significant differences have been observed in the pre-laying period between temperatures

of 20–35°C, indicating that temperature does not impact this stage of the life cycle (Tucci et al. 2008). The Thermo-Kill method relies on maintaining elevated temperatures in an empty poultry house for several days to effectively control ectoparasites. The process initiates with a gradual increase in room temperature to at least 45°C, as temperatures above 45°C have a lethal effect on various forms of *D. gallinae* (Nordensfors et al. 1999). This elevated temperature is maintained for the first two days, ensuring continuous high-temperature conditions. Subsequently, there is a gradual temperature reduction, allowing a controlled return to standard environmental conditions. This thermal cycle is designed to eliminate all developmental forms of red mites. The concentrated temperature increase during the initial days is crucial for effectively neutralising pests and pathogens, while the gradual temperature decrease enables a controlled return to normal growing conditions, minimising the risk of stress (Van Emous 2005). Among various natural methods to control infestations, altering light intensity in poultry houses has shown promise. Introducing short, intermittent light/dark periods in poultry houses could potentially reduce the infestation of *Dermanyssus gallinae* by disrupting its normal nocturnal feeding cycle, according to some authors (Stafford et al. 2006). However, research by Sokół et al. in 2008 indicates that changes in the light and dark cycle in the poultry house may stimulate parasites to constant movement and promote reproduction. In conducted studies, more parasites entered traps during the dark phase than in the light phase, resulting in a higher number of parasites attacking hens during the light phase (Sokół et al. 2008). An additional measure to control red mite infestations involves the use of special traps where mites gather after feeding on blood to lay eggs and undergo subsequent developmental stages (Sokół and Romaniuk 2006). Effectively removing and incinerating mites caught in traps can help reduce the red mite

population. A red mite monitoring system (Sokół 2010, Sokół 2020) has been established to estimate population size, select appropriate control agents, and assess the effectiveness of applied treatments. Moreover, determining the sensitivity of a red mite population to acaricides is crucial. In 2011, Zdybel et al. developed a method that allows the assessment of sensitivity in a manner most resembling natural conditions.

Alternatives to chemical compounds include synthetic compounds, biopesticides, and plant compounds, such as plant oils and extracts. Acaricides that belong to the phoxim group are promising synthetic compounds that show an efficiency of 94–99% (Keita et al. 2006; Meyer-Kuhling et al. 2007). However, the study by Zdybel et al. revealed regional resistance to these compounds, with efficacy dropping to 77% in one of the central provinces (Zdybel et al. 2011). Diatomaceous earth and compounds containing it offer a natural alternative for red mite control (Abo-Taka 1990; Fletcher and Axtell 1991; Nordenfors and Höglund 2000). The characteristic properties of diatoms' frustules cause mechanical damage to the shells of red mites, leading to dehydration and, ultimately, the death of the ectoparasite. In a study by Mullens et al. (2012), diatomaceous earth (12% w/w in water) significantly reduced the number of mites only when applied for two consecutive weeks, and its effect lasted for less than two weeks (Mullens et al. 2012).

Spinosad, produced through the fermentation of the bacterium *Saccharopolyspora spinosa*, is a biopesticide with a fairly high efficacy of 95–97% (George et al. 2010, Roczeń-Karczmarz et al. 2022). In vivo studies conducted by George et al. (2010) confirmed that Spinosad, aside from its pesticidal properties, does not affect the body weight of chickens or egg production parameters, providing an additional benefit of its use. The effective

insecticidal properties of a substance derived from the bacterium *Bacillus thuringiensis* have long been recognised and successfully employed in agriculture to control crop pests. The bacteria produce a toxin known as thuringiensin, which exhibits toxicity, especially to insects with complete metamorphosis (Van der Geest et al. 2000). However, thuringiensin is toxic to vertebrates (Chapman et al. 1991), which means its use in poultry is discouraged (Abdel-Ghaffar et al. 2009).

Previous studies have demonstrated that numerous essential oils and plant extracts possess strong mite-controlling potential (Kim et al. 2004, Kim et al. 2007, George et al. 2008, Maurer et al. 2009, George et al. 2009, Magdaş et al. 2010, Martinez-Velazques et al. 2011, Nechita et al. 2015, Immediato et al. 2016, Rajabpour et al. 2018, Tabari et al. 2017, Roczeń-Karczmarz et al. 2022). Most of the research to date has concentrated on the analysis of oils primarily effective in the gas phase, presenting a potential method for the application of aerosols that target mites. However, it is worth noting that this form of application can be associated with significant inconveniences. Certain plant-based products, such as Red Mite Avian, which are based on extracts of thyme, burdock, and tanacetum and administered through drinking water for hens (a product of Bugico SA, Switzerland), cause mites that typically feed on birds to abstain from drawing blood from the host. This repellent effect makes the host's blood unpleasant and impossible for the mites to consume (Sparagano et al. 2014). Additionally, a garlic-based pesticide (a 10% solution of garlic juice in water) has proven effective against *Ornithonyssus sylviarum* (NFM) in hens (Birrenkott et al. 2000). Garlic acts as an effective insecticide, affecting both adults and larvae (Amonkar and Banerji 1971, Yazwinski et al. 2005). Azadirachtin, derived from the neem tree, at a concentration of 0.06%, has reduced but has not eliminated the total number of *O. sylviarum* mites (Mullens

et al. 2012). However, higher sulphur concentrations (≥5.3%) and even lower concentrations (0.9%) essentially eliminated mites (Mullens et al. 2012).

Control of *D. gallinae* is based on adhering to hygiene rules within the poultry house and preventing the entry of parasites from outside. A single spraying session does not guarantee the eradication of all developmental stages of the red mite, so repetition is essential. Thorough disinfection of poultry houses after each production cycle is crucial. Developing an Integrated Pest Management (IPM) system that combines various methods of eliminating *D. gallinae*, as described in previous studies (Axtel 1999, Fiddes et al. 2005), is a recommended approach. 2005).

Testing the efficacy of selected plant extracts against *Dermanyssus gallinae*.

The primary objective of the conducted research was to identify additional natural compounds with potential efficacy against *Dermanyssus gallinae*. The effects of ten alcohol extracts on adult mites in vitro have been analysed. The study has assessed the effectiveness of the extracts of the following plants: *Allium sativum*, *Eclipta alba*, *Dysphania ambrosioides*, *Artemisia absinthium*, *Cichorium intybus*, *Cannabis sativa*, *Arctium lappa*, *Cichorium intybus*, *Coptidis chinensis*, *Cichorium intybus*. The Scientific names and the parts of the plants used in the study are detailed in Table 1.

Table 1. Plant parts used in the study.

Item	Plant scientific name	Plant part
1	*Allium sativum*	Garlic bulb
2	*Eclipta alba*	Herb
3	*Dysphania ambrosioides*	Herb
4	*Artemisia absinthium*	Herb
5	Sennae sp.	Leaf
6	Arctium lappa	Root
7	Cichorium intybus	Herb
8	Cichorium intybus	Root
9	Cannabis sativa	Herb with inflorescence
10	Coptidis chinensis	Rhizome

For the toxicity test, 99% alcohol extracts were prepared. Spinosad by Elanco (Poland) at a concentration of 30 ml/3.5 l of water served as a positive control, while ethyl alcohol served as a negative control.

Colonies of *Dermanyssus gallinae* were obtained from free-ranging laying hens in South East Poland. The farm was naturally infested with parasites, and no mite treatments had been applied for six months prior to the collection. The control efficacy of the extracts was evaluated using the modified method by Zdybel et al. 2011. For each plate containing a veneer disc soaked in extracts, mite mortality was calculated with a correction to account for mortality in the control group (Abbott's formula correction). The mean was the final count from four repetitions.

As a result of the study on the effect of alcoholic extracts against *Dermanyssus gallinae*, it was found that *Cassia sp.* herb extract exhibited the highest effectiveness at 53.7%. Additionally, *C. sativa* extract showed an effectiveness level of 52.8%, while *E. alba* extract achieved an effectiveness

level of 46.10%. These three extracts proved to be the most effective in reducing red mite survival rates. It is noteworthy that the other tested extracts showed a minimal reduction in the survival rate of the red mites. However, the extracts used did not meet expectations as substances that control *D. gallinae*. There is a need to find a compound that is neutral, retains its control properties in the environment for an extended period, and is safe for animals. In addition to coccidiosis, *D. gallinae* is considered one of the primary problems in laying hens, making their control essential for maintaining poultry welfare and productivity. Therefore, a balanced approach to the use of acaricides is crucial to protecting bird health, preventing resistance, and minimising negative impacts on the environment.

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Krzysztof Tomczuk, Maria Studzińska, Klaudiusz Szczepaniak, Marta Demkowska-Kutrzepa, Monika Roczeń-Karczmarz

University of Life Sciences in Lublin, Faculty of Veterinary Medicine, Department of Parasitology and Fish Diseases Lublin, Akademicka 12 , 20-950 Lublin

CURRENT CHALLENGES IN ADDRESSING INVASIVE DISEASES IN DOMESTIC PIGEONS: INSIGHTS FROM OUR RESEARCH.

Introduction.

The exponential growth of pigeon breeding has led to an increased focus on parasitological testing within veterinary practices and diagnostic laboratories. Pigeon breeding primarily encompasses three directions, with racing pigeons (homing pigeons) and fancy pigeons being the main two, while the third direction, meat pigeons, remains less popular. Residing in flocks, pigeons face a heightened susceptibility to infectious and invasive diseases, significantly influencing their health and overall breeding success (Fafiński 1999, Piasecki 2006, Romaniuk 2000, Stenzel and Koncicki 2007). Beyond the inherent density of these avian groups, additional risk factors emerge, particularly in racing pigeons exposed to birds from other flocks while racing. Similar threats arise from introducing new birds into a native flock without prior examination and adherence to quarantine conditions. Another challenge is the possibility of asymptomatic carriers harbouring multiple pathogens, alongside the difficulty of diagnosing invasive diseases during prepatent periods. The contamination of the birds' habitats by invasive parasite forms or the presence of infected intermediate hosts (annelids, arthropods, molluscs) in the

environment also constitutes a critical, yet underestimated factor. These circumstances underscore the necessity of frequent parasitological examinations, at the same time validating their significance. Early detection of infestations provides a crucial opportunity to slow down their spread within the flock and minimise environmental contamination. Unfortunately, preventive parasitological examinations, crucial for bird health, remain relatively infrequent. Typically, bird owners initiate diagnostic measures only when clinical symptoms manifest, allowing invasive pathogens to persist in the flock and contribute to recurring invasions through environmental contamination. A distinct challenge in the management of parasitic diseases in pigeons lies in the proper utilisation of antiparasitic agents, especially antihelminthics. The limited variety of pharmaceutical options available in the pigeon market contributes to the repetitive use of the same active substances (Dolka and Szeleszczuk 2010, Ledwoń and Szeleszczuk 2016), often at underestimated doses. This trend is fostering the development of drug-resistant parasite strains, leading to prolonged infestations in flocks. Moreover, the practice of "deworming " without prior diagnosis poses the risk of ineffective control of parasites, which requires targeted chemotherapeutics and a tailored approach to treatment. The current study aims to highlight the ongoing challenges in addressing invasive diseases within pigeon flocks in the Lublin region.

Material and methods

The research was conducted at private veterinary practices and the parasitology laboratory of the Department of Parasitology and Fish Diseases at the University of Life Sciences in Lublin from January to November 2023. The study material comprised bird faecal samples from 69 flocks (39 fancy pigeon

flocks and 30 racing pigeon flocks) with a total estimated bird count of 7,490, as declared by the owners. Testing was carried out in response to ongoing diseases in 48 flocks (29 fancy pigeon flocks and 19 racing pigeon flocks) or as part of preventive flock inspections (9 fancy pigeon flocks and 12 racing pigeon flocks). Owners selected 3 to 6 faecal samples from each flock, resulting in a total of 332 samples studied. Some of the tests involved samples directly obtained from birds delivered to the veterinary practice (204 samples from 41 flocks), which were also screened for trichomoniasis.

The faeces were examined using the flotation method according to Willis (1921) (Gundłach and Sadzikowski 1992), employing a saturated solution of NaCl and sucrose with a specific gravity of 1.25 g/ml. Each time, 3 grams of faecal samples were tested, and the study considered the presence of parasite forms as well as data on sample characteristics indicating the presence or absence of diarrhoeas. The trichomoniasis test involved taking a mucus sample from the pigeons' crop using a glass rod, suspending it in a few drops of warm saline on a glass slide with a cavity, and covering it with a cover glass. The sample was then scrutinised under a biological microscope at 100x and 400x magnifications to detect the characteristic twitching motion of trichomonas.

Results and discussion

Parasite forms were confirmed in samples from 56 flocks, indicating a prevalence of 81.2% across the study. (87.2% in fancy pigeon flocks and 55% in racing pigeon flocks). The identified parasites included protozoa of the genus *Eimeria* (49.3%), roundworms *Ascarida columbae* (28.9%), nematodes

Capillaria obsignata (40.6%), *Ornithostrongylus quadriradiatus* (10.2%), *Syngamus trachea* (10.2%), and eggs of tapeworms (8.7%) and *Trichomonas gallinae*. (48.8%). Individual flocks exhibited single or "multi-taxon" infestations (mixed infestations involving different families and types of parasites). Mono-infestations prevailed in the flocks of racing pigeons. In the flocks of fancy pigeons, a preponderance of mixed infestations was recorded. Mono-infestations occurred in 26.1% of flocks, (fancy pigeons: 20.5%, racing pigeons: 33.3%). Dual infestations were found in 23.2% of flocks (racing pigeons: 20%, fancy pigeons: 25.6% of flocks). Mixed infestations with three types of parasites were found in 23.2% of flocks (racing pigeons: 23.3%, fancy pigeons: 23.1%). Infestations with four types of parasites were observed only in fancy pigeons in 10.3% of flocks (overall prevalence of 5.8%), whereas infestations with five types of parasites – in 5.1% of fancy pigeon flocks (overall prevalence of 2.9%). Detailed results illustrating the incidence of infestations in different bird groups are provided in Table 1.

Table 1. Prevalence of parasites in pigeon flocks

A feature of the herd Number of flocks	Percentage of herds with positive tests														
	Diarrhea symptoms	Overall prevalence	Ascaridia columbae	*Ornithostrongylus quadriradiatus*	*Capillaria obsignata*	*Syngamus trachea*	*Eimeria* spp.	Tapeworms	*Trichomonas gallinae*	Mono-invasions	Mixed invasions II	Mixed invasions III	Mixed invasions IV	Mixed invasions V	Free from invasion
Together N=69	52,2	81,2	28,9	10,1	40,6	10,1	49,3	8,7	48.8 N=41	26,1	23,2	23,2	5,8	2,9	18,8
fancy pigeons N = 39	61,5	87,2	41,0	15,4	48,7	10,3	51,3	12,8	47,8 , N=23	20,5	25,6	23,1	10,3	5,1	12,8
racing pigeons N = 30	40,0	73,3	13,3	3,3	30,0	10,0	46,7	3,3	50,0 N=18	33,3	20.0	23,3	0,0	0,0	26,7
with diarrhea N = 36	100	94,4	41,7	13,8	61,1	19,4	58,3	11,1	25.0	25,0	22,2	36,1	5,5	5,5	5,5
with the disease process N = 48	75,0	91,7	37,5	14,6	54,2	14,6	56,3	12,5	33,3	22,9	25,0	31,3	8,4	4,2	8,4
sick racing pigeons n=19	68,4	84,2	21,1	10,5	52,6	15,8	47,4	5,3	36,8	35,6	15,8	31,6	0.0	5,7	15,8
sick fancy pigeons n=29	79,3	96,6	48,3	17,2	55,2	13,8	62,1	17,2	31,0	17,2	31,0	31,0	13,8	3,4	3,4
asymptomatic N = 21	0,0	57,1	9,5	0,0	9,5	0,0	33,3	0,0	19,0	33,0	19.0	4,8	0.0	0,0	42,9
asymptomatic fancy pigeons N = 9	0,0	55,5	11,1	0,0	22,2	0,0	11,1	0,0	11,1	33,3	11,1	0,0	0,0	0,0	44,5
asymptomatic racing pigeons N = 12	0,0	58,3	8,3	0,0	0,0	0,0	50,0	0,0	25,0	33,3 ,	25,0	08,3	0,0	0,0	41,7

Comparing two types of breeding (racing pigeons and fancy pigeons), fancy pigeon flocks were more susceptible to parasitic infestations. These observations are consistent with the works of Raś-Noryńska et al., Stenzel and Koncicki. Fancy pigeons were nearly three times more likely to be infected with nematodes of the genus *Ascaridia*. A similar relationship, albeit in slightly smaller proportions, was observed for the nematode Capillaria, the most frequently found nematode in the studied flocks. Additionally, other parasites

such as nematodes *Ornithostrongylus quadriradiatus*, protozoa *Eimeria* spp., and tapeworms were more frequently found in fancy pigeon flocks.

The analysis revealed that coccidiosis was the predominant infestation in both racing and fancy pigeon flocks. This aligns with findings in various studies by multiple authors (Balicka-Ramisz and Pilarczyk 2014, Balicka-Ramisz et al. 2020, Bartosik et al. 2020, Bobrek et al. 2012, Dovč et al. 2004, Kaleta and Bolte 2000, Piasecki 2006,. Roy 2011, Sari et al. 2008, Stenzel and Koncicki 2007. Tomczuk et al. 2017).). Infestations of lower intensity may be asymptomatic, serving as a natural vaccine agent. Understanding the invasive disease status within the flock and appropriately interpreting it enables the utilisation of natural immunological phenomena, which concurrently reduces the risk of drug-resistant strains (Schnieder 2006). The results of the presented research additionally highlight the concerning phenomenon of the frequent occurrence of nematode infestations, particularly those of the genus Capillaria. This invasion has emerged as dominant, especially in recent years, a trend confirmed by the findings of various authors (Balicka-Ramisz et al. 2020, Bartosik et al. 2020, Bobrek 2012, Dovč 2004, Piasecki 2006, Romaniuk 2000, Sari 2008, Scullion 2013, Stenzel and Koncicki 2007, Tomczuk et al. 2017). The nematode Capillaria obsignata appears to play a significant role in inducing pathological changes in the gastrointestinal tract, leading to manifestations such as diarrhoea. The remarkably high prevalence of Capillaria infestations can be attributed to several factors. Mistakes in dehelminthization procedures, including treatment without prior recognition of the infestation, the use of substances inappropriate for the specific parasite, or incorrect dosages, contribute to ineffective control of capillariasis. The low susceptibility of these nematodes to commonly used anthelmintics perpetuates their presence in flocks. Additionally, the extended survival period of invasive forms in the environment

poses challenges for effective control. (Ledwoń and Szeleszczuk 2016, Scullion 2013).

The birds included in the study exhibited diverse clinical statuses, with 52.2% of the samples showing signs of diarrhoeal faeces (61.5% in fancy pigeons, 40.0% in racing pigeons). This symptom was particularly observed in infections with nematodes of the genus Capillaria, Ascaridia, and protozoa Eimeria spp., indicating a significant contribution of these parasites to the pathogenesis of pigeon gastrointestinal disorders. Flocks of fancy pigeons displayed exceptionally diverse parasites, with mixed invasions involving up to five types of parasites being repeatedly found. Parasites were also present in flocks where no clinical signs were observed, including infestations of roundworms, Capillaria, coccidia, and protozoa of the genus Trichomonas, with mono-infestations being the most common. The asymptomatic course of infestation was generally associated with low infestation intensity or good nutritional status and other factors contributing to a relatively high level of immunity. The racing pigeon group exhibited a higher prevalence of asymptomatic protozoan infections of the genus Eimeria and Trichomonas, while the fancy pigeon group demonstrated this trait for nematodes of the genus Capillaria and Ascaridia. This condition in racing pigeons may be attributed to higher exposure to protozoan infestations during racing. Conversely, persistent nematode infestations in flocks of fancy pigeons may indicate environmental contamination with invasive forms of nematodes characterised by extended survival rates. The demonstration of these infestations provides essential information for flock owners, emphasising the need for preventive measures, including environmental hygiene and chemoprevention. Similar findings are presented by other authors describing various types of parasites in this group of birds (Piasecki 2006, Shinde 2008, Tomczuk et al. 2017). These facts

underscore that only awareness of the risks and systematic preventive treatments can safeguard flocks from the spread of many dangerous diseases.

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Aleksandra Ledwoń, Izabella Dolka, Piotr Szeleszczuk

Department of Pathology and Veterinary Diagnostics of the Institute of Veterinary Medicine, Warsaw University of Life Sciences, Nowoursynowska 159c, 02-776 Warsaw

CASES OF ATOXOPLASMOSIS IN FRINGILLIDAE

Introduction

Atoxoplasmosis is a parasitic disease affecting mainly passerine birds, especially the Oscines, although the most frequently reported cases were in Fringillidae and Sturnidae. Bali mynas (*Leucopsar rothschildi*) are particularly sensitive to this parasite (Sandmeier, 2006). According to some American authors, *Atoxoplasma* is such a common problem in aviaries of small passerine birds that it occurs in all outdoor aviaries, as well as in birds kept indoor with poor hygiene (Norton, 2003).

The causative agent of atoxoplasmosis in *Serinus* spp. is *Atoxoplasma serini* also known as *Isospora serini*. The life cycle of the parasite begins with the ingestion of oocysts by birds. In the intestines, the oocysts release sporozoites that penetrate the intestinal wall, enter lymphocytes and macrophages and spread to organs such as the liver, spleen, lungs, pancreas and pericardium. In these organs, asexual schizogony and merozoite formation occurs. Merozoites migrate back to the intestinal mucosa, where gametogony (sexual cycle) of oocyst-producing merozoites occurs. The oocysts are excreted in the faeces (Sandmeier, 2006).

In canaries, the disease typically affects birds aged 2-9 months. Sick birds show signs of lethargy, feather ruffling and inappetence. There is diarrhoea, redness and swelling of the vent, liver enlargement visible through the abdominal integuments, rarely neurological and/or respiratory signs. Mortality can be up to 80%. Necropsy shows enlargement of the spleen, liver and dilated intestines (especially the duodenum). On cytological examination, the presence of inclusions in the cytoplasm of mononuclear cells is observed (Sandmeier, 2006).

Material and Methods

Birds with a diagnosis of atoxoplasmosis examined in 2010- 2021 were analysed. The examined birds were provided for necropsy by the breeders. At necropsy, cytologic preparations were made from the liver, spleen, lungs and intestines. Preparations were fixed and stained with Hemacolor® kit and microscopic evaluation was performed under 1000x magnification. For histopathological examination, material was collected from one canary (liver, spleen, kidney, intestines, brain) and grey-crowned European goldfinch (liver, brain). Tissues for histopathological examination were fixed in 10% formalin, routinely processed and stained with haematoxylin and eosin (H&E).

Results

Nine cases of atoxoplasmosis were diagnosed by cytological examination in canaries (*Serinus canaria*), two in fire-fronted serin (*Serinus pusillus*) and one in grey-crowned European goldfinch (*C. carduelis caniceps*). Numerous parasites were found in the liver, spleen and lungs. Clinical signs observed in the canaries were diarrhoea and lethargy, and in one canary also neurological signs. In two fire-fronted serins, a grey-crowned European goldfinch and two

canaries, in additionally numerous *Macrorhabdus ornithogaster* were found in the digestive tract. The post-mortem lesions observed were mainly varying degrees of emaciation, enlargement of the liver and spleen and, in five birds, also haemorrhagic content in the intestines. In one fire-fronted serin and one canary the enlargement of the spleen and liver was weakly expressed compared to the other birds, despite the copious number of parasites in the organs examined. In one fire-fronted serin and three canaries the liver was significantly enlarged and spotted and in six birds only enlarged. Significant enlargement of the spleen was observed in seven birds and in the others this organ was slightly or moderately enlarged. In the direct faecal preparation, quite numerous unsporulated oocysts of coccidia were also found in some birds.

Histopathological examination was performed in two birds: a canary and a goldfinch. In both of these birds, the liver showed diffuse and perivascular inflammatory infiltrates, consisting mainly of mononuclear cells, congestion, haemorrhages, parenchymal dimpling and hepatocyte atrophy. Brown hemosiderin-like deposits were found in the macrophages. In the spleen of the canary shows a moderate degree of reticular cell proliferation and macrophages. In the kidney, parenchymal darkening and necrosis of renal tubular epithelial cells were observed. In the intestines in the canary, a diffuse infiltration of mononuclear cells in the mucosa, connective tissue proliferation, glandular atrophy and congestion were observed. In the brains of both birds, congestion, oedema, glial proliferation, neuronophagy and focal features of neuronal degeneration were found, while in the goldfinch, haemorrhages were additionally visible.

Discussion

All birds studied were already feathered juveniles, which is consistent with with previous findings by other authors regarding age predilection (Sandmeier, 2006). Hemorrhagic lesions in the intestines were found in five canaries, which may be associated with coccidiosis, but also with the lack of food intake of the affected birds. Liver and spleen enlargement were the predominant signs in the postmortem picture, although not in all cases.

Our study confirmed the observations of other authors that *Atoxoplasma* is not visible on histopathological examination (Rae et al., 2006). In both birds studied, only hemosiderin-like deposits were observed in the macrophages.

The cytological examination in the cases we presented was conclusive in the microscopic diagnosis of atoxoplasmosis, although it is not a very sensitive test, especially in relation to carrier stages, and the provision of frozen birds for necropsy makes the diagnosis very difficult or even impossible. There is therefore a need to develop sensitive and specific molecular tests for the diagnosis of this disease. Admittedly, such tests have been performed (Mohr et al. 2017, Oliveira et al., 2018), but they were not genus- and species-specific, so accurate diagnosis requires sequencing of the PCR reaction product.

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Artur Żbikowski[1], Karol Pawłowski[1], Krzysztof Adamczyk[1], Joanna Turniak[1], Sophie Le Bouquin[2], Rozenn Souillard[2], Virginie Allain[2], Piotr Szeleszczuk[1]

[1]*Department of Pathology and Veterinary Diagnostics, Institute of Veterinary Medicine, Warsaw University of Life Sciences, Warsaw, Poland*

[2]*ANSES - French Agency for Food, Environmental and Occupational Health & Safety, Ploufragan, France*

BIOSECURITY IN MINIMIZING THE RISK OF *EIMERIA* SPP INVASSION ON POULTRY FARMS

Current methods of controlling Eimeria spp. invasion on poultry farms includes the use of coccidiostats, vaccines, and natural products (e.g. phytoncides). These methods are not 100% effective but are intended to keep the number of oocysts low and allow the birds to develop immunity naturally. Disinvasion aimed at reducing the number of coccidia oocysts in the poultry house environment is an essential element in controlling this invasion. When used before coccidiostats or immunoprophylaxis programs, it significantly improves their effectiveness.

In recent years, there have been increasing concerns about the effectiveness of vaccines, drug resistance, and residues in poultry products. Therefore, an essential element in protecting poultry against coccidiosis is appropriate management of the flock and implementation of an effective biosecurity program, dedicated to the farm, that includes disinvasion procedures during downtime for the destruction of oocysts and parasite eggs in the environment of the house. Biosecurity in poultry production is considered an appropriate response to preventing the spread of diseases, but compliance with

recommended practices is still not optimal. Assessing biosecurity on every farm must be the first step to developing an effective strategy and control against pathogens. Acquiring the knowledge of biosecurity practices used on poultry farms at the national level and identifying gaps will allow the development of appropriate support measures for their implementation. Currently, many projects in the EU focus on biosecurity in production as the Netpoulsafe project "Networking European poultry actors for enhancing the compliance of biosecurity measures for a sustainable production". This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No.101000728. The consortium includes 15 institutions from 7 European Union countries with high poultry production (Belgium, France, Hungary, Italy, the Netherlands, Poland, and Spain). The leader is ITAVI - the French Institute of Applied Research and Development, which serves professionals from the poultry, rabbit and aquaculture sectors. The aim of the project is to stimulate the knowledge exchange between relevant stakeholders from the poultry industry in EU in the field of biosecurity. The Polish Partner of the project is the Division of Avian, Exotic Animals and Fish Diseases, Department of Pathology and Veterinary Diagnostics, Institute of Veterinary Medicine, Warsaw University of Life Sciences.

A qualitative interview study was conducted in the 7 countries involving 192 farmers and 157 advisors. The questionnaires were developed by ANSES and WULS in collaboration with the Partners of the project, in order to collect data both on the implementation of biosecurity measures and on the support measures. The results of the interviews conducted in Poland are here presented.

In the frame of the Netpoulsafe project, the qualitative assessment of biosecurity compliance in poultry production in Poland was performed. The 26

farmers and 23 advisors from different production types: enclosed broilers, turkeys, breeders, and layers were interviewed with a semi-closed questionnaire with a focus on 38 specific biosecurity measures (table 1). The frequency of implementation of each measure, in response to the question: "Is this practice used on the farm?" (“always”, “sometimes”, “never”) and the reasons for non-compliance were assessed. Among the 38 biosecurity questioned measures as “always” implemented in the farms only 12 practices were mentioned by 100% and 7 practices by more than 90% of farmers. Advisors indicated only 4 practices in 100% and 8 by more than 90% as “always” implemented in the farms. Interestingly, some differences between the opinions for measures of farmers and advisors were observed. “Washing of the hands before entering the house by personnel” in the advisor's opinion was ”always” performed in about 22% of farms while in farmer's opinion in 73% of farms. Among "the least always used measures" in farmers' and advisors' opinions were “showering before entering the house” – “by visitors” (11% and 0% respectively) and “showering before entering the house - by personnel” (19% and 0% respectively).

What seems most disturbing, however, are the "sometimes" or "never" responses, which confirm that some of the important biosecurity practices listed below are not "always" applied on farms:

- animal production on the site: "all-in/all-out" poultry production on the site; no backyard on the site; if other animal productions on the site (cattle, pigs) sanitary barriers with poultry (personal, material …),
- structure and circulation on the site: delimitation with a barrier or closure of a professional secured area with only necessary vehicles to the poultry house (feed, chicks, poultry or eggs transport vehicles);

wheel dips for disinfection of the vehicles or pulverization before entering on the site,

- personnel, visitors or teams: specific shoes and clothes before entering in the house; washing of the hands before entering in the house; showering before entering in the house,
- visitors or teams: register for visitors and teams; specific shoes and clothes before entering in the house; washing of the hands before entering in the house; showering before entering in the house,
- the poultry at the arrival: register for the flock (origin, number of poultry, …); if the chicks deliverer enters in the house: specific clothes and shoes,
- feed and drinking water of the poultry: feed storage protection; drinking water analysis end line each year,
- biological vectors control: rodents control (deratting or other measures); wild birds control (protection of the ventilation circuit or other measures); no domestic animals on the site (pets, dogs or cats),
- management of the poultry manure: manure stored in a specific isolated area outside of the secured professional area (or if no secured area : away from the house),
- management of dead animals: removal of the carcasses at least twice a day; presence of a closed and protected rendering tank; rendering tank located outside of the secured area (or if no secured area : away from the house) allowing the passage of the truck away from the house,
- structure and circulation in the poultry house: concrete surrounds around the house; hygiene lock with 2 separated zones (clean and dirty area),

- management of the material or litter in the poultry house: recognizable separate material only for the poultry house; protection of the litter (in a closed shed or other protection, from birds or vermin …),
- cleaning and disinfection of the house and material: cleaning and disinfection of the drinking water pipeline between each flock; cleaning and disinfection of the feed silo between each flock; bacterial autocontrol of the cleaning and disinfection of the house between each flock; period of the sanitary break > 15 days between each flock.

According to the unanimous opinion of Producers and Advisors, the main reasons for non-compliance with the rules were: "not enough trained", "not enough advice", "it takes too much time", "too expensive", and "not knowing risks/advantages". To fulfill most of these gaps and needs the implementation of supporting measures such as biosecurity trainings, educational programs, support by biosecurity advisors, etc., may help to improve the compliance of biosecurity on the farms by increasing the knowledge and skills of farmers and advisors.

Unfortunately, there is no database with information on the use of targeted disinvasion against coccidia in polish poultry production. The authors' own observations indicate that such activities are not a permanent element of biosecurity programs and are most often introduced after the occurrence of a clinical form of coccidiosis, especially caused by strains resistant to coccidiostats.

The Netpoulsafe project (https://www.netpoulsafe.eu/) produces films, podcasts (https://www.youtube.com/@netpoulsafeproject/videos), e-learning courses (MOOC: https://www .futurelearn.com/courses/netpoulsafepl/1), best practice guides and much more that is already being made available to help

those in the field improve and adhere to biosecurity principles throughout the poultry production chain. This is particularly important not only in terms of protecting flocks against coccidia invasions, but also in terms of the constant threat of Avian Influenza and Newcastle disease in Poland.

Table 1. Biosecurity practices evaluated in the field questionnaires (Farmer and Advisors) in the Netpoulsafe project.

Animal production on the site	• "all-in/all-out" poultry production on the site • no backyard on the site • if other animal productions on the site (cattle, pigs) sanitary barriers with poultry (personal, material …)
Structure and circulation on the site	• delimitation with a barrier or closure of a professional secured area with only necessary vehicles to the poultry house (feed, chicks, poultry or eggs transport vehicles) • wheel dips for disinfection of the vehicles or pulverization before entering on the site
Personnel, visitors or teams	**A. Personnel** • specific clothes before entering in the house • specific shoes before entering in the house • washing of the hands before entering in the house • showering before entering in the house **B. Visitors or teams** • register for visitors and teams • specific clothes before entering in the house • specific shoes before entering in the house • washing of the hands before entering in the house • showering before entering in the house
The poultry at the arrival	• register for the flock (origin, number of poultry, …) • if the chicks deliverer enters in the house: specific clothes and shoes

Feed and drinking water of the poultry	• feed storage protection • drinking water analysis end line each year
Biological vectors control	• rodents control (deratting or other measures) • wild birds control (protection of the ventilation circuit or other measures) • no domestic animals on the site (pets, dogs or cats)
Management of the poultry manure	• manure stored in a specific isolated area outside of the secured professional area (or if no secured area : away from the house)
Management of dead animals	• removal of the carcasses at least twice a day • presence of a closed and protected rendering tank • rendering tank located outside of the secured area (or if no secured area : away from the house) allowing the passage of the truck away from the house • cleaning and disinfection of the rendering tank after each collection
Structure and circulation in the poultry house	• concrete surrounds around the house • hygiene lock with 2 separated zones (clean and dirty area)
Management of the material or litter in the poultry house	• recognizable separate material only for the poultry house • protection of the litter (in a closed shed or other protection, from birds or vermin …)
Cleaning and disinfection of the house and material	• cleaning and disinfection of the house between each flock • cleaning and disinfection of the material between each flock (feeders, drinkers, nests, material for the management of eggs, …) • cleaning and disinfection of the drinking water pipeline between each flock • cleaning and disinfection of the feed silo between each flock

	• bacterial autocontrol of the cleaning and disinfection of the house between each flock • period of the sanitary break > 15 days between each flock
Management of the poultry	• vaccination protocol of each poultry flock • daily surveillance with clinical alert criteria (water and feed consummation, mortality, eggs production)

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