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The prevalence and clinical signs caused by coccidia species in fancy and racing pigeons in Germany

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2018

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1. Introduction

Traditionally the veterinarians were responsible for the health of meat producing animals like pigs, poultry and cattle, however, during the last few decades many of them have been specialising on hobby animals like cats, dogs and horses. Finding a veterinarian who is trained and comfortable in diagnosing and treating poultry, let alone specifically pigeons is quite rare. Those veterinarians that are specialised in pigeons are mainly self-taught through years of experience and personal interest.

Diseases of racing and fancy pigeons are marginal in the veterinary medicine curriculum or are briefly discussed as part of the general poultry knowledge. Most knowledge and information about this field are acquired after the university degree. Coccidiosis of pigeons did not have the same focus for research in the past. Even the taxonomy of certain species is not clear and some *Eimeria* species appear to be synonyms of each other or in fact are two separate species. The correct interpretation and diagnosis of such a common diseases as coccidiosis is of utmost importance, as pigeon owners are reporting substantial and unnecessary losses of squabs and also reduced flight performance in racing pigeons.

The German Association of Homing Pigeon Breeders estimates around 40,000 racing pigeon breeders in Germany and roughly the same amount of fancy pigeon breeders. Coccidian parasites are one of the many parasitic pathogens that a breeder has to overcome in pigeons rearing. Many factors such as stress and poor hygiene are contributing causes of excessive oocyst shedding and an accumulation of infective oocysts in the loft. Damage caused by these parasites in the lining of the intestine during the reproduction cycle, causes interference with electrolyte and nutrient uptake and blood and protein loss. The bird is effectively weakened and can show signs of watery, greenish diarrhoea, poor growth and also leave the bird immunosuppressed and vulnerable to other infectious diseases. These clinical signs can lead to unsuccessful breeding, bad fledglings, poor flight times and faster tiring as well as poor feathering and plumage. For the hobby breeders, this parasite can be extremely frustrating but for the racing industry it has more of an economical consequence. Due to the clinical signs listed above, pigeon breeders are often forced to have a shorter racing season and have a longer recovery from training and breeding.

The aim of this thesis is to gain better understanding of the coccidian parasite in racing and fancy pigeons in Germany in order to be able to make appropriate treatment plans by increasing the knowledge of the species, clinical signs and prevalence of *Eimeria* species.

2. Literature Review

2.1. Coccidia

Coccidia is a single celled, spore forming, apicomplexan protozoa of the family *Eimeriidae*. The coccidian parasite in pigeons can cause a gastrointestinal disease showing clinical signs known as coccidiosis. These protozoa can reside in the digestive tract and among other organs such as liver, kidneys and lungs of vertebrates causing damage to the epithelial cells during its reproduction. Coccidia found in birds belong to two genera, *Eimeria* and *Isospora. Eimeria* species are homoxenous, meaning they tend to be host specific. Exception to this rule can be because of pseudoparasitism between prey and predator. In which case the oocysts are taken up by a paratenic host and become dormant until the appropriate predator ingests the host (Ghimire, 2010). *Eimeria* species of pigeon however, are species specific. Nonetheless, one bird that does share some *Eimeria* species with the pigeon is the ostrich. A study carried out in Botswana by Mushi et al. (1998) found subspherical oocysts matching the description of *E. tropicalis* in ostrich chicks. In the intestinal tract of the ostrich, protozoa including *Eimeria columbae*, *E. columbarum, E. labbeana and E. tropicalis* are all found (Mushi et al., 1998). Hence, through possible coprophagia of the pigeon faeces, as pigeons are the true host of these *Eimeria* species, could be the possible source of infection. In literature written by Varghese (1978a), *Eimeria labbeana* was reported in hosts of two different host genus, *Columbae livia* and *Streptopelia orientalis* and *Eimeria waiganiensis* is reported in both *Columbae indica* and *Otidiphaps nobles. Eimeria curvata* was found to parasite both *Columbae talpacoti* and *Scardafella squammata.* These findings suggest that *Eimeria* might not be species specific so to say but more specific at a family level in Columbiformes (Adriano et al., 2000).

Birds, such as pigeons, kept and raised in captivity are normally kept in a loft or aviary in high amounts creating almost perfect breeding ground for coccidia as the parasite needs oxygen, humidity and temperature to sporulate (sporogony). This can be one of the reasons why coccidia are associated with captive birds rather than their free ranging counterparts (Friend and Franson, 1999). Oocysts are excreted from the infected bird into the environment and are taken up by other birds through water, feed or litter (Szeleszczuk,

1995; Khan et al., 2006). In the environment it is not pathogenic until all three conditions are met and then the parasite is able to sporulate and becomes infective, usually within 48 hours. The original single celled sporont becomes four sporocysts each containing two sporozoites (Figure 1). Unlike *Isospora* species in which the sporont forms two sporocysts each containing four infective sporozoites (Matsubaraa et al., 2017). Upon digestion the sporocysts are released from the oocysts within the gizzard of the bird.

The sporozoites then escape from the sporocysts in the duodenum, they migrate and undergo asexually proliferation to form first generation meronts in the epithelial cells of the intestines. These meronts produce many first generation merozoites in a single cell. The merozoites break out of the original cell to infiltrate new epithelial cells and another developmental phase will occur again, this time producing larger second generation merozoites. All of this takes place within 5 days of ingesting the infective oocyst. Depending on the species this cycle can continue and produce third generation merozoites but second generation can also enter a new cell and instead begin gametogony. Micro- (male), and macrogametes, (female) are formed from the second generation merozoites. The macrogamont is fertilised by the microgamete and a new oocyst is formed. This oocyst enters into the intestinal canal from the epithelial cell and is passed via the faeces to the environment. This cycle can take up to 7 days to complete (Friend and Franson, 1999). Due to all of the stages of reproduction, entering and leaving the epithelial cells, microscopic lesions and damage are formed in the lining of the intestine during this time.

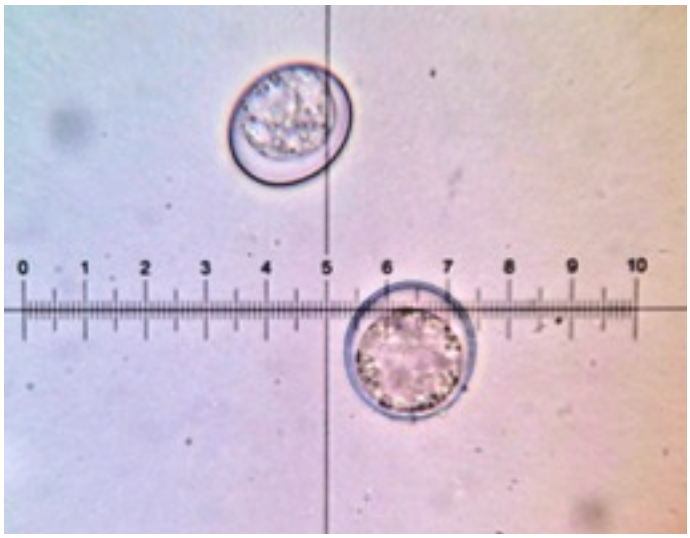


Figure 1. an unsporulated oocyst (bottom right) and a sporulated oocyst (top left)

This leads to electrolyte imbalance and can lead to immunosuppression, leaving the bird susceptible to infectious diseases, (Balicka-Ramisz and Bogumiła, 2014; Zigo et al., 2017).

Coccidia is not only an intestinal parasite but has been found in extra intestinal epithelial cells, such as renal, liver and lung. *Eimeria* species causing renal coccidiosis are less known and discussed than the intestinal counterpart. According to the study by Friend and Franson (1999) about renal coccidiosis, sexual reproduction takes place only in the kidneys and junction of ureters, however, clinical signs were mostly asymptomatic or very generalised signs like emaciation. Systemic isosporiosis or atoxoplasmosis is a disease with systemic manifestation of the coccidian parasite *Isospora*, through an extra intestinal reproductive cycle. It proliferates sexually in the intestines and undergoes an asexual cycle in mononuclear cells such as natural killer cells, macrophages and lymphocytes, (Oliveira et al., 2018). These cells are carried around the body via the bloodstream to different organs, leading to an infiltration of inflammatory cells mainly effecting the liver, spleen, kidneys and in some cases the lungs may also be damaged (Cushing et al., 2011).

The disease coccidiosis can vary from an acute to a chronic or severe disease. The acute form of the disease can result in diarrhoea which can also lead to death in squabs and chronic or severe coccidiosis which can be lethal, debilitating and resulting in the mortality of young birds (Wallis, 1991). Susceptibility to such a disease is influenced by a multitude of factors including the number of cells destroyed in the host, the size of the infecting dose of oocysts and the location of the parasites in the host's body (Brown et al., 2010).

2.2. Coccidia species of pigeons

The literature written describes numerous different species of *Eimeria* found from the family Columbidae, 16 species are discussed in detail (Jamriška and Modrý, 2012), some of which are of recent discovery, e.g. *E. lyoni* (Yabsley et al., 2015), *E. palumbi and E. zenaidae (*Adriano et al., 2003), *E. labbeanna-like* (Yang et al., 2016) or *E. columbapalumbi* (Jamriška and Modrý, 2012).

Levine (1961) calculated that there could be at least 2,654,736 different oocysts in the coccidian genus *Eimeria*. Later, Duszynski et al. (1999) advanced that the genus might be composed of approx.. 35,000 species, instead of the 1,300. Duszynski et al. (2000) has

stated that it was more likely that only two species occur in pigeons, *E. labbeanna* and *E. columbarum.*

When first released from the bird in the faeces all coccidia spores are similar in structure, consisting of round parasites of protoplasm. It is not until the sporulation is complete and characteristic changes have appeared that species differentiation is applicable (Boughton, 1937).

Eimeria columbae

First described by Mitra and Das Gupta (1937)

Host: *Columba livia intermedia,* (Indian pigeon)

Location: India

Infective site: intestines

Sporulation time: 96-120 hours

Description

A subspherical shaped oocyst with a mean oocyst size of 16 x 14 µm. Micropyle and polar granule are absent, according to the literature by Yang et al. (2016) and 2 polar granules are present according to literature by Varghese (1980) and Adriano et al. (2000). The oocyst wall is composed of two layers. The sporocysts are a mean size of 7.2x4.8. Smaller oocyst than *E. labbeana* and *E. columbarium* but similar in size to *E. curvata.* Details about the lifecycle and pathogenicity of *E. columbae* are lacking.

Eimeria columbarum

First described by Nieschulz (1935)

Host: *Columba livia,* (Rock dove or domestic pigeon)

Location: worldwide

Infective site: intestines

Sporulation time: 34-38 hours

Description

Oocysts of *E. columbarum* are spherical to ovoid and average oocyst size of 19(21) X 17.5(20) µm in size. Their double- layered wall is smooth and colourless. Oocyst residuum

and micropyle are both absent. The size of a sporocyte range is 8(11) x 5(6) μm containing sporocyst residuum. The oocyst shape index is 0.92-0.95.

Eimeria columbipalumbi

The species was described by Jamriška and Modrý (2012).

Host: *Columba palumbus*, (Common Wood Pigeon)

Location: found in Easter European countries; Slovak Republic

Infective site: Located mainly in the jejunum (endogenous stage)

Sporulation time: within 6 days

Description

The sporulated oocysts are ellipsoidal with a mean size of 17(24) × 16.9(20) μm. Oocyst wall is bilayered with the outer layer being thicker, light brownish in colour and slightly pitted. Micropyle and oocyst residuum are absent. It consists of two irregularly shaped polar granules. The oocyst shape index is 1-1.44. Sporocysts are elongate ovoidal, 13.5(11) × 6.5(7) μm. Substiedal body is absent but the oocyst does contain a stieda body. Anterior refractile body is spherical and the posterior one is elongate (Jamriška and Modrý, 2012).

Eimeria curvata

Described by Adriano et al. (2000)

Host: *Columbina talpacoti,* (Ruddy ground dove) and *Scardafella squammata,* (Scaly dove)

Location: São Paulo, Brazil

Infective site: unknown

Sporulation time: unknown

Description

The sporulated oocysts are ovoid-ellipsoid in shape with a average size range of 17(19) x 15(17). The wall of the oocyst is colourless, smooth and bilayered. A polar granule id present but is lacking a micropyle and oocyst residuum. The oocyst shape index is 1.1-1.3. The sporocysts are elongated and curved anteriorly with a mean size of 11.5(13) x 5.5(6). The stieda body is ‘nipple-like’ and the substieda body is absent. Sporocysts residuum are granular and positioned in the middle of the sporocyst.

The only other species of *Eimeria* to be comparable to *E. curvata* is *E. columbae*. They are similar in size but *E. columbae* does not have a polar granule and the sporocyst in *E. curvata* has a curved anterior portion, hence the name (Adriano et al., 2000).

Eimeria duculai

Depicted by Varghese (1980)

Host: *Ducula spilorrhoa*, (Torresian imperial pigeon)

Location: Papa New Guinea

Infective site: unknown

Sporulation time: 48-72 hours

Description

An ovoid shaped oocyst, sized at 26(21) x 23(27) µm. Bilayered oocyst wall with a light green outer wall. Present in the oocyst is a inconspicuous micropyle and a polar granule but an oocyst residuum is absent. The oocyst shape index is 1.1. Elongate sporocysts are 14(16) x 6.5(8) µm in size and contain granular residuum and a prominent stieda body; substieda body is absent.

Eimeria gourai

Described by Varghese (1980).

Host: *Goura victoria,* (Victoria crowned pigeon)

Location: Papua New Guinea

Infective site: unknown

Sporulation time: 96-120 hours

Description

Spherical to sub spherical shaped oocyst, sized at 19(22) x 18(21) µm. The oocyst has a smooth, double layered wall with the inner layer being light green in colour and the outer being darker. Both micropyle and oocyst residuum are absent but a polar granule is present in the sporulated oocyst. The oocyst shape index is 1.0. Sporocysts are 10(13) x 4(6) µm and contain dark and lighter smaller granules, stieda body is present; substieda body is absent. ‘Comma like’ sporozoites lie within the sporocysts.

Eimeria choudari

Described by Bhatia et al. (1973)

Host: *Streptopelia decaocto,* (Eurasian collared dove)

Location: India

Infective site: unknown

Sporulation time: unknown

Description

Subspherical and ellipsoidal shaped sporulated oocyst with a size of 16.9(22.1) x13 (18.2) µm. Micropyle and oocyst residuum are both absent but contains a large polar granule. The sporocyst is on average 13.6 x 7.2 µm. The sporocyst has a stieda body present but substieda body and residuum are both absent.

Eimeria turturi

Described by Golemansky (1976)

Host: *Streptopelia turtur,* (Turtle dove)

Location: Bulgaria

Infective site: large intestine

Sporulation time: 48 hours

Description

Ellipsoidal and oval shaped, colourless oocysts with a size range of 22.8(29.2) x 17.8(25.4) µm. Oocyst residuum, micropyle and polar granule are absent from the sporulated oocyst. The sporocyst is an elongated, ellipsoidal shape that tapers at one end with a dispersed residuum. Stieda and substieda body are absent.

In the genus *Streptopelia*, only the species *E. choudari* and *E. turturi* have so far been described. Besides the size and shape difference, *E. choudari* possesses a polar granule and stieda body which is not observed in *E. turturi* (Golemansky, 1976).

Eimeria janovyi

Described by Bandyopadhyay et al. (2006)

Host: *Columba livia,* (Blue Rock Pigeon)

Location:India

Infective site: unknown

Sporulation time: 48 hours

Description

An ellipsoidal shaped oocyst measuring at 24.3 x 19.8 μm. Double layered wall of the oocyst is of equal thickness. Oocyst residuum and micropyle are missing but a singular polar granule is present. The oocyst shape index is 1.2. Sporocyst are pyriform in shape and 12 x 10.1μm in size containing banana shaped sporozoites. A granular sporocyst residuum and stieda body is also present; substieda body is absent.

Eimeria kapotei

Described by Chatterjee and Ray (1969)

Host: *Columba livia intermedia* (Rock pigeon)

Location: unknown

Infective site: unknown

Sporulation time: 48-60 hours

Description

A ovoid shaped oocyst ranging in size of 24(30) x 21.6(26.4) μm. It has a bilayered oocyst wall and has a micropyle present. Oocyst residuum and polar granule are both absent. The sporocyst consists of scattered residuum and and a stieda body; Substieda body is absent.

Eimeria labbeanna

First described by Pinto (1928) in Rock doves and in domestic pigeons according to Krautwald-Junghanns et al. (2009)

Synonyms are *E. columbarum*, *Coccidium pfeifferi and Eimeria pfeifferi.*

Host: *Columba domestica*, (domestic pigeon), *Columba livia,* (ring dove), *Streptopelia turtur*, (turtle dove)

Oriental turtle dove (*S. orientalis*).

Location: found today worldwide

Infective site: both the small and large intestines (jejunum and ileum)

Sporulation time: 50 hours (depending on room temperature fluctuation).

Description

The sporulated oocyst is spherical or sub-spherical in shape with a oocyst size of 20(21) x 16(18) μm. The wall is bilayered according to Pinto (1928) but more recent studies have revealed that the oocyst wall trilayered (Krautwald-Junghanns et al., 2009), the inner being darker. Micropyle and oocyst residuum are absent. One polar granule is present. Elongated ovoidal sporocysts with a Stieda body and a mean size of 12.4 x 6.4 μm. "A sporocyst residuum is present. The sporozoites lie lengthwise, slightly crescent-shaped and have a vacuole at each end. The nucleus near the middle", (Levine, 1961)

There has been much discussion for years about the comparison of *E. labbeanna* and *E. columbarum*. One researcher Nieschulz (1935), previously separated the species into two, *E. labbeanna* being the smaller of the two (Krautwald-Junghanns et al., 2009). Duncan (1959a), carried out a study of more than 300 pigeons. He found that there was a broad range in the size of the oocysts, with an overall mean of 19 x 17 μm, with smaller oocysts appearing at the beginning of infection then reaching average size by the end of the patent period. Therefore meaning that *E. columbarum* was in fact a synonym of *E. labbeanna* (Levine, 1961).

Others, even to this day still distinguish them as separate species (Balicka-Ramisz and Bogumiła, 2014; Stenzel and Koncicki, 2007).

Eimeria labbeanna-like

Ongoing study according to Yang et al. (2016)

Host: domestic pigeons, C. domestica

Location: Perth, Western Australia

Infective site: unknown

Sporulation time: 72-96 hours

Description

Subspherical shaped oocyst with a smooth double layered wall and an average oocyst size of 22(18.9) x 15.7(18.9) μm. Micropyle is absent but a oocyst residuum as well as a single polar granule are present in the sporulated oocyst. The oocyst shape index is 1.38. Sporocyst are elongate-ovoid in a size range of 14.5(12.5) x 5.5(7) μm and contain granular sporocyst residuum. There are similarities in the size of the oocyst to *E.*

columbapalumbi, E. labbeana, E. livialis and *E. chodari* but the difference is in the morphological features. *E. labbeana-like* and E. livialis have an oocyst residuum that, *E. columbapalumbi, E. labbeana* and *E. choudari* do not possess. But *E. livialis* does not contain a polar granule unlike *E. labbeana-like* (Yang et al., 2016).

Eimeria livialis

Described by Alyousif et al. (2009)

Host: *Columba livia domestica,* (domestic pigeon)

Location: Saudi Arabia

Infective site: intestine

Sporulation time: unknown

Description

Sporulated oocysts are elongate ellipsoidal shape with an average size of 19.5(23.2) x 14.3 (16.5) µm, with a smooth, greenish-yellow bilayered wall. Micropyle and polar granule are absent; an oocyst residuum is present. The oocyst shape index is 1.35-1.49. The sporocysts are ellipsoid averaging 9.5 (11.7) x 6.2 (8.1) µm in size and have a small 'nipple-like' Stieda body, but no substieda body. The sporocyst residuum composed of scattered granules. The sporozoites are elongate, each with a small anterior and a large posterior refractile body.

Eimaria livialis is similar in size to *E. columapalumbi. E. livialis* however possesses an oocyst residuum but has no polar granule unlike *E.columbapalumbi,* which may even contain 2 (Jamriška and Modrý, 2012).

Eimeria palumbi

First described by McQuistion, (1991)

Host: *Zenaida galapagoensis,* (Galapagos Dove)

Location: Galapagos islands

Infective site: unknown

Sporulation time: unknown

Description

Oocysts are ovoid to ellipsoidal in shape, size ranging from 27(22) x 24(19) μm. The oocyst wall is colourless, double layered and smooth. Micropyle and polar granules are absent, but an oocyst residuum is present. The oocyst shape index is 1.05-1.21. Sporocysts are ellipsoid in shape and range in size of 17(15) x 8(8.5) μm with a 'nipple-like' stieda body and no substieda body. The sporocyst residuum is composed of scattered granules covering the midregion of the sporocyst.

Eimeria sphenocercae

Described by Ray (1952).

Host: S*phenocercus sphenurus,* (Wedge tailed green pigeon)

Location: South East Asia

Infective site: unknown

Sporulation time:unknown

Description

The sporulated oocyst is sized at 17.5 (25) x 12.5(15) μm. It possesses a micropyle but the oocyst residuum is absent. The sporocyst is measured at 17.5(18.7) x 12.5(13.7) μm and lacking a residuum.

Eimeria mauritiensis

First described by Ball et al. (2012)

Host: *Nesoenas mayeri,* (Pink pigeon)

Location: Mauritius

Infective site:unknown

Sporulation time: < 4weeks

Description

Oocysts are sub-spherical and 16(19) × 18 (22) μm in size. The oocyst wall is bilayered and smooth. Micropyle, oocyst residuum and polar granules are all absent. Sporocysts are 6(7) × 8(14) μm with both stieda and substieda bodies.

Eimeria lyoni

First described by Yabsley et al. (2015)

Host: *Zenaida macroura carolinensis,* (Mourning dove)

Location: USA

Infective site: small intestine

Sporulation time: <2weeks

Description

Spherical to ovoid in shape, with an average size of 23 (25) x 20(21) µm. Bilayered oocyst wall smooth, and colorless. Micropyle and oocyst residuum are absent. More than one polar granules may be present. Ovoid sporocysts with mean size of 12(14) x 6.9(8) µm and a 'knob like' stieda body extending into the oocyst wall and a substieda body. Sporocyst residuum are granular and uniform.

E. lyoni and *E. zenaidae* are found in the eared dove and *E. palumbi* are in the Galapagos dove but they are similar in size and some features overlap (Adriano et al., 2003). *E. lyoni* is also similar in size to *E. waganiensis* (Varghese, 1978a) and *E. turturi* (Golemansky, 1976) too. The morphological features that can distinguish one oocyst form the other are; the oocyst residuum, which is present in *E. palumbi* but are lacking in *E. lyoni*. *E. lyoni* can have more than one polar granule which is absent in *E. palumbi*. *E. zenaidae* and *E. plumbi* are lacking a substieda body that is found in *E. lyoni*. *E. lyoni* may overlap in size with other oocyst of *Eimeria* species described from members of the Columbidae family, but they are are always simple morphological differences to prove that *E. lyoni* is not similar to any of the other *Eimeria* species.

Eimeria tropicalis

Described by Malhotra and Ray (1961)

Host: *Columba livia intermedia,* (domestic pigeon)

Location: Worldwide

Infective site: intestine

Sporulation time: 40-48 hours

Description

A spherical to sub spherical shaped oocyst, sizing at 19(24) x18(23) µm. The oocyst wall is bilayered. The polar granules and oocyst residuum are both present but micropyle is

absent. The sporocyst are 10 x 6(7) μm in size and contain sporocyst residuum and stieda body.

Eimeria waiganiensis

Described by Varghese (1987a)

Host: *Chalcophaps indica Linnaeus*, (Green-winged ground dove) and *Otidiphaps nobilis Gould,* (Magnificent ground pigeon)

Location: Papua New Guinea

Infective site: unknown

Sporulation time: 72 hours

Description

The sporulated oocyst is ovoid with diameters of 22(25) x 19(23) μm. The oocyst wall has a single layer. The Micropyle is present and up to 4 polar granules may be present but the oocyst residuum is absent. The oocyst shape index is 1.08-1.2. The ovoid sporocysts measure 9 x 6 μm and possesses a granular sporocyst residuum, a conical stieda body and substieda body.

Eimeria zenaidae

First described by Adriano et al. (2003)

Host: *Zenaida auriculata*, (Eared Dove)

Location: Brazil

Infective site: unknown

Sporulation time: 38 hours

Description

Oocysts are spherical to subspherical with a measurement of 22.1(26.4) x 19.2(22.1) μm. The oocyst has a bilayered wall with a pitted outer layer, and a darker inner layer. Polar granule is present, but micropyle and oocyst residuum are absent. The oocyst shape index is 1.2. Sporocysts are 12.0(14.4) x 7.2(7.7) μm with scattered residuum. A large stieda body is present, but sub-Stieda body is absent.

The other *Eimeria* species to parasitise Zenaidae, a member of the Columbidea family are *E. palumbi* and an unknown "*Eimeria* species reported by Conti and Forrester

(1981) infecting *Zenaida asiatica* and *Zenaida macroura* in Florida, USA" (Adriano et al., 2003). *E. zenaidae* and *E. palumbi* are very similar in size but the oocyst of E. zenaidae lack a oocyst residuum but contain a singular polar granule and also have a more pitted appearance.

Isospora gallicolumbae

First described by Varghese (1978b)

Host: *Gallicolumba beccarii Salvadori,* (Beccari's ground dove)

Location: Papua New Guinea

Infective site: unknown

Sporulation time: 24 hours

Description

Ellipsoidal to spherical in shape and 20 x16 μm in size. The *Isospora* has a single layered, green wall and the presence of polar granules. Micropyle and oocyst residuum are absent. The sporocysts are 12 x 8 μm in size and contain a spherical mass of sporocyst residuum and a conical stieda body; substieda body are absent.

***Isospora* of domestic pigeons**

The species were describes by Matsubara et al, 2017

Host: *Columbus livia,* (domestic pigeon)

Location: Worldwide

Infective site: unknown

Sporulation time: 5 days

Description

The sporulated oocyst are spherical with a average size of 24(27.2) × 23.4(26.0) μm. The oocyst was had a single layer and appears light pink in colour. Micropyles, polar granules and oocyst residuum are all absent. The mean size of the sporocysts are 18.5(20.5) x 10.2(12.1) μm. Stieda body was 'nipple-like' and sub-Stieda bodies are also observed. Sporocyst residuum are present in clustered granules.

I.gallicolumbae is significantly smaller than the *Isospora* of domestic pigeons and *I.gallicolumbae* also lack a substieda body unlike the *Isospora* of domestic pigeons. As

both of the *Isospora* were found in different genres of the Columbidae family, it suggests a strong host specifity of isosporoids (Matsubara et al, 2017).

2.3 Pathogenesis of pigeon coccidiosis

Coccidiosis can be a mild to severe disease in birds depending on age, bird species, *Eimeria* species strain and health status of the birds in the loft. Most pigeons can carry a smaller amount of coccidia with no apparent illness. Pathogenicity of *Eimeria* infection will depend on the amount of oocysts ingested, the amount of host cells destroyed by the oocysts and the location of the infection in the host's intestines (Levine, 1961). The parasite reproduced in the epithelial and sub epithelial cells of the intestine and caeca causing enteritis and even internal haemorrhages, resulting in anaemia (Boughton, 1937). According to Krautwald-Junghanns et al. (2009) deaths in 1-4 month old pigeons can be between 5-70%, particularly in the 3 and 4 month old birds. The older adult birds are less effected by the pathogenicity of the disease coccidiosis and are mainly carriers of the parasite. However a severe infection of *Eimeria* or a resistance break caused by an increase in metabolic efforts can effect the flying performance of pigeons, primarily racing pigeons. This can happen before and after training and racing. Transportation in confined crates, sharing water systems for drinking and the tiring aspect of racing can leave the birds in the lofts very susceptible to the manifestation of coccidia (Balicka-Ramisz and Bogumiła, 2014).

The heaviest losses and those affected by coccidiosis are the young pigeons and squabs in the nest. Damage caused to the intestine of the bird, through the reproduction of the parasite, can result in interrupted feeding and the disruption of nutrient and electrolyte balance and uptake. All of which can lead to dehydration, emaciation, anaemia, increased susceptibility to other infectious diseases and death. The mechanics behind these clinical signs is the intestinal villi becoming atrophied due to the invasion of oocysts and gametocytes in the tips of the villi leading to expansion and rupture (Long et al., 1976). Those that recover from this disease are often left stunted, with poor plumage and a weakened frame. Immunity can also occur in pigeons but it does not prevent reinfection and depends always on the number of oocysts ingested by the bird (Krautwald-Junghanns

et al., 2009). Immunity is caused by the endogenous defence mechanisms being stimulated. Constant ingestion of a small amount or low levels of oocysts can be actually beneficial to the bird as it will create an equilibrium between bird and parasite (Mohammed et al., 2017)

Coccidiosis in pigeons is predominantly caused by *Eimeria* and to a lesser extent *Isospora* spp. Other species of *Eimeria*, *E. curvata* and *E. lyoni* for *example,* are said to be asymptomatic with the birds appearing healthy. Infections tend not to be caused by a single species of *Eimeria* but instead are as a result of a mixture of *Eimeria* species. This is due to the parasite developing in different areas of the intestinal canal and the amount of the oocyst ingested by the bird. Among the species of *Eimeria* observed in pigeons, *E. labbeana* is the species that appears to be the most pathogenic. *Isospora* infection of domestic pigeons*,* according to Matsubara et al. (2017) is no or rarely pathogenic.

2.4 Treatment and control of coccidiosis in pigeons

Coccidiosis is a self limiting disease, which means that a mild infection will promote immunity. Accordingly a complete absence of coccidia would not be a desired scenario as an infection of a naive loft could potentially exhibit worse clinical signs (Krautwald-Junghanns et al., 2009).

Historically, a lot of drugs like maduramycin, nicarbazin, halofuginone or other polyether antibiotics and synthetic anticoccidial drugs have been used in the poultry sector for prevention (Haberkorn, 1996). For pigeons however, a more narrow range of anticoccidials plays an important role.

Pharmaceutical drugs containing amprolium have traditionally been used a lot in carrier pigeons (Haberkorn, 1996). Amprolium is a thiamine analogue which means it blocks the thiamine transport and inhibits carbohydrate synthesis of coccidia. It is still in use today as it shows little resistance build-up but the use is rather sporadical as the efficacy is only moderate. The most frequent combination of amprolium is with sulfonamide.

Sulfonamides are still frequently used, nonetheless resistance is encountered regularly and the use of antibiotics in mild cases of coccidiosis is being questioned in developed countries, predominately in the EU. The mechanism of action is an inhibition of the dehydrofolate synthesis through a structural resemblance of paminobenzoic acid.

The most recently and frequently used drugs are clazuril, toltrazuril and diclazuril. These belong to the group of benzene-acetonitriles and they have a coccidiocidal effect (Krautwald-Junghanns et al., 2009). They act through the inhibition of enzyme systems in protozoa and/or decreasing pyrimidine synthesis. Some feather damage have been observed after the treatment of coccidia infections with benzene-acetonitrile based drugs (Krautwald-Junghanns et al., 2009). This is especially important for fancy pigeon keepers.

In recent years plants with anticoccidial properties have gained the focus of pigeon keepers and some veterinarians. For example fats like docosahexaenoic acid, eicosapen- taenoic acid and linolenic acid have shown an effect on some *Eimeria* species (Quiroz-Castañeda and Dantán-González, 2015). Curcumin was found to lower the severity of infections in some *Eimeria* species of chickens, thus suggesting an effect in pigeons could be likely (Quiroz-Castañeda and Dantán-González, 2015). Plants like *Tulbaghia violacea*, *Vitis vinifera* and *Artemisia afra* have an antioxidant effect and have shown similar results to toltrazuril. (Naidoo et al., 2008). Essential oils from artemisia, thyme, tea tree and clove have even shown destruction of oocysts in vitro at an $LC_{50} < 1$ mg/mL for oocysts (Remmal et al., 2011). The mechanism of actions has not been explained yet. Another promising extract from *A. sieberi* had a decreased number of oocysts per gram of faeces and had improved growth performance parameters such as feed intake and weight gain, among others, when compared with the effects observed with monensin treatment in a study by (Kheirabadi et al., 2014). The extract could be an alternative therapeutic agent against avian coccidiosis under field conditions.

Disinfection of the surrounding area, especially the flooring is of utmost importance as the most infective stage of coccidia is the sporulated oocyst, which is commonly on day 1-2 after shedding (Krautwald-Junghanns et al., 2009). A lot of disinfection substances are ineffective against coccidia. The most effective disinfection has so far been cresols.

A proper treatment and prevention plan no matter if chemotherapeutics or plant extracts are being used, must always include both disinfection of the surrounding and substances that are administered orally to the pigeons.

3. Methods and Materials

3.1. Pigeons sampled

A total of 301 faecal samples from pigeon breeders were gathered from across Germany between the months of May until August of 2016 (Figure 2). The samples were collected from adult racing pigeons and a mixed age range of squabs to adult fancy pigeons. The samples were screened for coccidia and 130 out of the 301 samples were positive. Out of 130, samples representing 50 breeders of 4,822 pigeons were sporulated as these samples were infected with only cocccidia. One pooled loft sample was received from each breeder. Out of the 50 samples, 33 were from fancy pigeon breeders, representing 3,333 pigeons and 17 were from racing pigeon breeders, representing 1,488 racing pigeons. The fresh samples from each pigeon breeder were collected from the ground in a loft. Each sample was accompanied by the flock's anamnesis, information regarding the health status of the loft, number of birds in the loft sampled, any previous treatment undertaken, number of deaths in the loft and any clinical signs. Previous coccidia infection history was taken for each sample along with any anti-coccidials being used or any treatments used before hand. This history taking is vital as any treatments used for any of the pigeon lofts in the 10 days before the collection will have misleading results (Wallis, 1991).

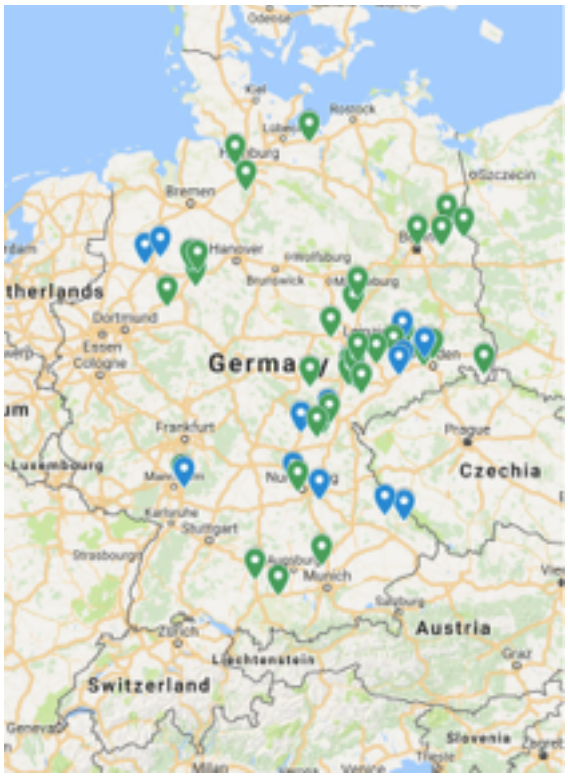


Figure 2. Locations of faecal sampling in Germany

3.2. Parasitological methods used to detect oocysts

All samples were examined macroscopically texture and colour of the faeces recorded. The flotation method is an easy and convenient tool used to diagnose parasitic infections of pigeons. Faecal flotation was conducted using a saturated sodium chloride and 50% sucrose (w/v) solution. The intensity of coccidial infection was graded on a scale of (+) to +++, (+) <20 oocysts, + 21-40 oocysts, ++ 41-80 oocysts and + + + above 80 oocysts present in the sample.

One gram from each sample was placed directly into 2-2.5% aqueous potassium dichromate (K_2Cr2O_7), leaving room between the sample and the top for aerobic condition to be met, as this is one of the important factors affecting sporulation. The potassium dichromate inhibits the growth of bacteria allowing the oocyst to sporulate. The vials of samples were kept at room temperature (23^{o}C) for average of 6 days (Long et al., 1976; Duszynski and Gardner, 1991). Each sample collected was marked as racing or fancy pigeons and their location was recorded into the database. After sporulation the samples were kept at 4^{o}C until examined.

The sporulated oocysts were separated with floatation from each dichromate solution containing faecal sample using modified sheather's solution (Sheather, 1923). A coverslip was placed on the top of each floated sample. After 15 minutes the coverslip was removed and placed on a microscope slide and examined using a light microscope at 100-400x magnification. All measurements were taken using a calibrated ocular micrometer. Once the sight of a single oocyst on the cover slip was confirmed, photographs were taken for morphological identification.

3.3. Identification of sporulated oocysts

Phenotypic characters are the conventional and most common way of classification of *Eimeria* and *Isospora* species. According to Ghimire (2010), certain phenotypical characters are examined when identifying species of *Eimeria* and *Isospora.* Measuring the structure of sporulated oocysts should always be done only under an oil immersion objective to increase the resolving power of the image. Although some species of coccidia can be identified from their unsporulated oocysts, the sporulated oocysts is often more desirable, as the position of the sporocysts, i.e lying parallel to axis, can determine the oocysts measurements (Long et al., 1976).

The morphology typical of *Eimeria* species consists of a double layered or in some species a triple layered oocyst wall e.g. *E. labbeana* (Krautwald-Junghanns et al., 2009), which may be lined with a membrane (Levine, 1985). A micropyle can be present at one end of the oocyst covered by a micropylar cap. There may be a single or numerous polar granules e.g. *E. waiganiensis* (Varghese, 1987a) present in the oocyst. Within the sporulated oocyst of *Eimeria* are 4 sporocysts each contains 2 sporozoites. The oocyst and sporocyst both contain residuums. The residuum in the sporocyst can be dispersed among the sporozoite e.g. *E. turturi* (Golemansky, 1976), scattered, granular clusters or compact. Residuum are the residues from the formation of the sporocyst and sporozoites. A stieda body and substieda body may be present at one end of the oocyst. The stieda body can be 'nipple' e.g *E. palumbi* (McQuistion, 1991) or 'knob' shaped e.g *E. lyoni* (Yabsley et al., 2015) protrusion. Sporozoites are coma or sausage shaped containing 1-2 globules and nucleus, (Duszynski and Wilber, 1997).

The morphology of typical *Isospora* species is very similar to that of *Eimeria* except within the sporulated oocysts the 2 sporocysts each containing 4 sporozoites.

Furthermore, oocyst length (ol), oocyst width (ow) together with features of the outer oocyst wall have been taken into account e.g. rough (r) or smooth (s), projections and approximate thickness (es). Sporocyst length (sl), sporocyst width (sw), oocyst and sporocyst length: width (L:W) ratios.

4. Results

Gross examination of the faecal samples showed some changes in colour and consistency of faeces. Some samples were, greenish to whitish diarrheic faeces, mucus mixed and blood tinged droppings. Examination of faecal samples by floatation method revealed the presence of unsporulated and sporulated oocysts of coccidia with which the pigeon loft was infected.

Amoung the 301 pigeon faecal samples screened for coccidia, 130 samples were positive. The prevalence of coccidia infection was 43.2%. In 50 out of the 130 samples collected from pigeons in Germany only 3 *Eimeria* and one *Isospora* species were identified. Prevalence of *E. labbeana* (Figure 3), *E. columbarum E. columbae* and *Isospora gallicolumbae* (Figure 4) was 41%, 39% 18*%* and 2*%,* respectively (Figure 5). The other 80 sample out of 130 were positive with other bacteriological, parasitological and chlamdiophidia disease, mostly calpillarim, ascarid and Salmonella.

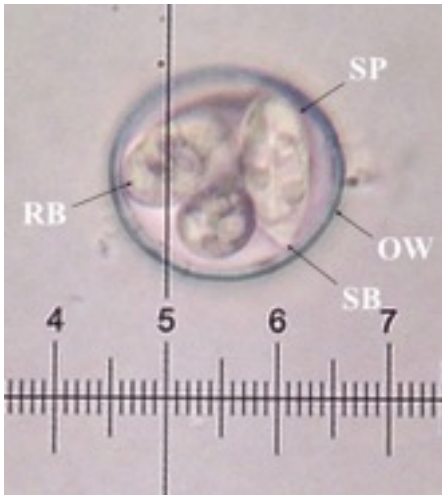


Figure. 3 *Eimeria labbeana*

Oocyst: 23x20µm

Sporocyst: 15x6µm

OW, outer wall

RB, refractile body

SB, stieda body

SP, sporocyst

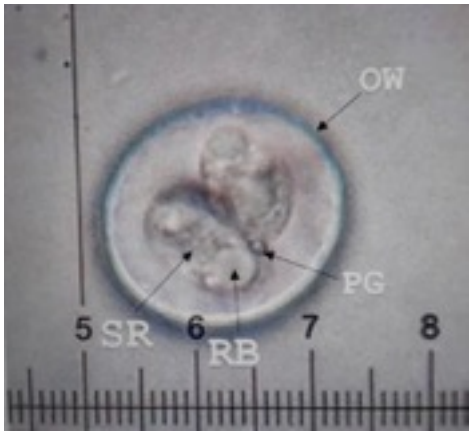


Figure. 4 *Isospora gallicolumbae*

Oocyst: 20x16µm

Sporocyst: 12x7µm

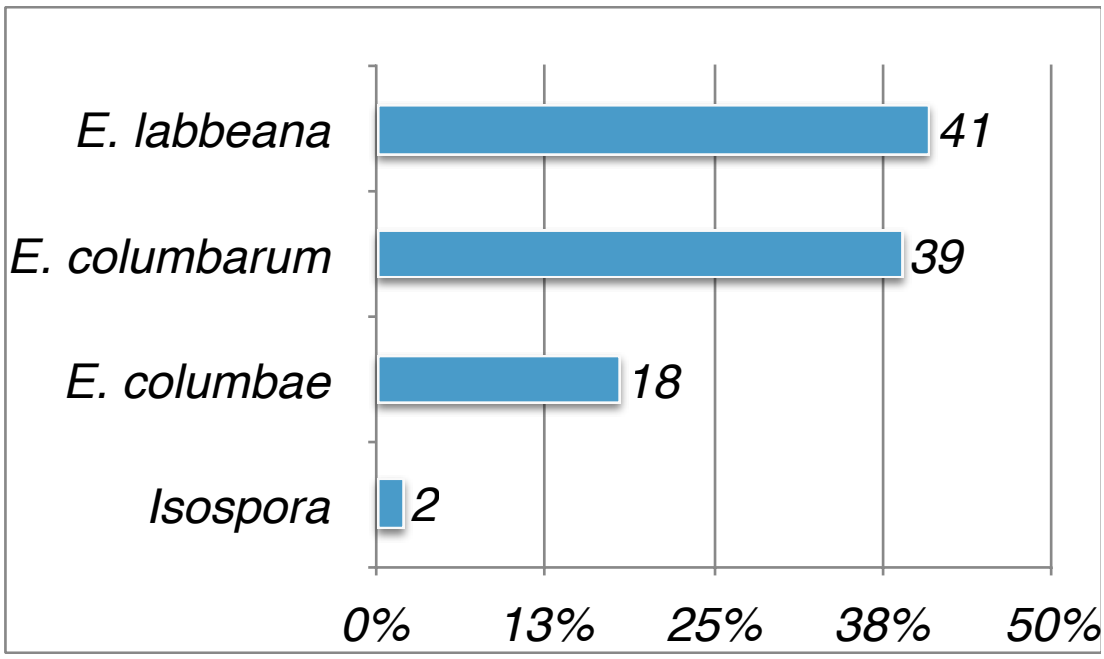
OW, outer wall

PG, polar granule

RB, refractive body

SR, sporocyst residuum

Figure 5. Percentage of coccidian species found in the samples.



Out of the 50 pooled samples, 44 were +++ (>80 oocysts), 4 were ++ (41-80 oocysts) and 2 were + (21-40 oocysts). After incubation in potassium dichromate, the number of sporulated oocysts were counted and an average of 70-85% of oocysts were observed to be sporulated in each sample. The species of the sporulated oocysts was identified.

The clinical signs recorded by the breeders from all the pigeon lofts together ranged from weight loss and watery green diarrhoea to death in birds between 10 days to 30 days of age. In some lofts no clinical signs were observed.

Amoung the 50 samples used, 17 were from racing pigeon breeders, representing 34% of the total amount of samples. Clinical signs of bad flight, unit and tiring fast of birds were observed in 4 out of the 17 (23.5&) racing pigeon lofts. In two samples from these lofts mixed infection of *E. labbeana* and *E. columbarum* occured. Based on the results *E. labbeana* and *Isopora gallicolumbae* were in one sample. One sample contained only oocysts of *Eimeria labbeana*. In the case of the racing pigeon sampled lofts that were negative for clinical signs, according to the breeders, Thirteen samples were only infected with one *Eimeria* species, *E. columbae, E. labbeana* and *E. columbae* were found in 6, 4 and 3 samples, respectively,

The remaining 33 lofts out of the 50 ones, were from fancy pigeon breeders. Out of the 33 fancy pigeon loft, 26 breeders observed clinical signs. Through the reading of the history of each loft the most prevalent clinical sign complained about about soft, watery and greenish faeces of birds. This symptom was noticed in 11/26 (42.3%) lofts, 9/26 (34.6%) of the fancy pigeons died, 4/26 (15.4%) suffered from weight loss and 2/26 (7.7%) were in bad condition with rough plumage. The pigeons of the other 7/33 (21.2%) lofts showed no clinical signs. In samples (18/26) that showed clinical signs, a mixed infection of *E. labbeana* and *E. columbarum* were observed. The rest of the lofts with history of clinical signs were positive for a single *Eimeria* species, *E. columbarum* in 5/26, *E. columbae* in 2/26 and *E. labbeana* was seen alone in 1/26 samples. The 7/33 sampled lofts that were negative for clinical signs, according to the breeders, had two samples had mixed infection of *E. columbae* and *E. columbarum*. The other (5/7) samples contained only one *Eimeria* species.

5. Discussion

Coccidiosis is a parasitic disease that can affect the health status of a bird and have drastic effects on a pigeon's racing results.Younger birds are believed to be more susceptible to the parasitic disease, showing clinical signs such as poor growth, diarrhoea and emaciation. Older birds, although more resistant can remain asymptomatic while continually shedding oocysts (Raś-Noryńska et al., 2011). Stressors, such as transport, high population density and long races can lower the immunity of a bird and increase the number of oocysts shed into the environment. Increase shedding will maximise the birds vulnerability to coccidian disease as well as other infections.

Amongst the studies carried out, *E. labbeana* and *E. columbarum* are globally the most prevalent coccidian species found in the family Columbidae (Nieschulz, 1935; Raś-Noryńska et al., 2011; Zigo et al., 2017). *Eimeria columbae* is reported in India (Mitra and Das-Gupta, 1937; Saikia et al., 2017) and in Poland (Balicka-Ramisz and Bogumiła, 2014). Nonetheless, all these species have been found in the current study in Germany.

In this recent study, it is indicative that an infection with a single species of *Eimeria* is less likely to cause an infection with obvious clinical signs than a mixed infection, which correlates with the literature by Levine (1961). The observed clinical signs in the pigeons are the resultant of the combined actions of a particular mixture of different species of coccidia. This is contraindicated in the fancy pigeons that showed no clinical signs. Within the fancy pigeon group, 21.2% (7/33) of the sampled lofts didn't show any clinical signs although two of these samples were positive for a mixed infection of *E. columbae* and *E. columbarum*. This can be due to a number of reasons; the infection could have occurred in that pigeons that were infected by these *Eimeria* species previously in the loft, creating an immunity against these species, the infected loft may have contained only adults, the clinical signs might have gone unnoticed by the breeder or because a mixed infection of *E. columbarum* and *E. columbae* is less pathogenic than other mixed infections with the species *E. labbeana*. The death rate in the fancy pigeon lofts was 34.6%. This rate was observed in birds of the affected lofts with an age average as early as 10 days and not older than 30 days. As coccidia was the only infection to be identified in this loft at the time and younger birds are more susceptible for coccidia, it might be suggestive that the young died

due to a coccidian infection. This however does not correlate with previous study by Krautwald-Junghanns (2009) in which was stated that highest occurrence of death was in 3-4 months old pigeons.

The *Isospora* oocysts found in the faeces of racing pigeon is suspecting of being *Isospora gallicolumbae*. The bird showed clinical signs of diminished flight performance. As the samples were taken between the months of May and August, which is the adult pigeon racing season (>1year old), this might be suggestive that *Isospora* species affects the flight performance of adult birds. However, because *Isospora* was found in combination with *E. labbeana,* it might be indicative that *E. labbeana* could have been responsible for the clinical signs as it is the most pathogenic *Eimeria* species in pigeons. According to Varghese (1978b), *Isospora gallicolumbae* is found primarily in Beccari's ground doves (*Gallicolumba beccarii Salvadori*), in Papua New Guinea which is a different genus in the Columbidae family to the domestic pigeon (*Columbus livia*). Further studies on a larger scale would be needed to confirm whether *Isospora gallicolumbae* can cause clinical disease in pigeons or not. Futhermore, pathological examination may reveal systemic isosporiosis which was not investigated during this current study but would be of utmost importance to clarify the reasons behind the adult pigeons showing clinical signs.

6. Conclusion

In conclusion, our findings indicate that coccidia infection in racing and fancy pigeons is prevalent in Germany. Mixed infection with different Eimeria species can be more pathogenic. Susceptibility to coccidia has been shown in both young and adult pigeons but the highest pathogenicity has been more detrimental to the younger pigeons. Constant routine veterinary surveillance is required for not only the therapy of the infected pigeons but also for routine diagnostics as a prophylactic measurement in the pigeon industry. The results of this study provide a basic insight into the prevalence and clinical signs associated with coccidia in pigeons. An expansion on the fundamental understanding of pigeon coccidia through pathological and histopathological changes that different species of coccidia cause in the pigeon is essential.

7. Acknowledgements

Foremost, I would like to express my sincere gratitude to my thesis advisor Prof. Farkas, Robert for his continuous support, patience and guidance during this thesis. The many clients of the clinic, Tierärztliche Praxis am Weinberg in Germany whom supplied us with numerous samples must be given a special mention. I would also like to thank Anne Brettschneider, the laboratory supervisor at Tierärztliche Praxis am Weinberg in Germany. Finally I would like to thank Dr. Martin Pfützner for his unfailing support and continuous encouragement.

8. Abstract

The German Association of Homing Pigeon Breeders estimates around 40,000 racing pigeon breeders in Germany and roughly the same amount of fancy pigeon breeders. Coccidian parasites are one of the many parasitic pathogens that burden a breeder during pigeons rearing. Damage caused by these parasites in the lining of the intestine during the reproduction cycle, causes interference with electrolyte and nutrient uptake and blood and protein loss. The bird is effectively weakened and can show signs of watery, greenish diarrhoea, poor growth and also leave the bird immunosuppressed and vulnerable to other infectious diseases.

The aim of this study was to examine the prevalence and clinical signs caused by coccidia species in both fancy and racing pigeons throughout Germany. A total of 301 pigeon lofts were screened for the presence of coccidia. Faecel samples pooled from every breeder positive for coccidia were processed by macroscopic examination, flotation technique and sporulation using 2-2.5% aqueous potassium dichromate. Overall 43,2% of pigeon lofts were positive for coccidia. 50 samples were sporulated overall representing 4,822 pigeons. Out of the 50 pooled samples, 33 were from fancy pigeon breeders, representing 3,333 pigeons and 17 were from racing pigeon breeders, representing 1,488 racing pigeons. Phenotypic characterisation was used to differentiate species of coccidia. Overall 3 *Eimeria* (*Eimeria labbeana*, *E. columarum* and *E. columbae)* and one *Isospora* species (*Isospora gallicolumbae)* were identified.

In the current study, it is suggestive that a infection with a single species of *Eimeria* or *Isospora* is less indicative to cause an infection showing obvious clinical signs than a mixed infection of more than one *Eimeria* and/or *Isospora* species. The observed clinical signs in the pigeons are the result of the combined actions of a particular mixture of different species of coccidia.

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