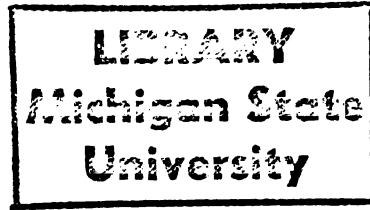This is to certify that the

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Coccidiosis in Pheasants

Reared in Confinement

presented by

Julie Ann Smith

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Martha Thomas

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COCCIDIOSIS IN PHEASANTS REARED IN CONFINEMENT

By

Julie Ann Smith

A THESIS

Submitted to
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ABSTRACT

COCCIDIOSIS IN PHEASANTS REARED IN CONFINEMENT

By

Julie Ann Smith

The epidemiology and pathology of coccidiosis in game farm reared pheasants were studied over a nine-month period. Three hatches, each reared during a different season (spring, summer and fall), were divided into two groups, one receiving a coccidiostat (control group) and one without (study group). Every week four birds from each group were randomly selected, killed, weighed, and examined for coccidial species identification. Four species were identified: _Eimeria duodenalis_, _E. phasiani_, _E. pacifica_, and _E. colchici_. _Eimeria duodenalis_ was seen first following winter but _E. phasiani_ appeared most frequently and was often found with _E. pacifica_. _Eimeria colchici_, the most pathogenic species, was seen only on occasion, primarily in the study group. Although severe coccidiosis did occur, increases in mortality tended to be stress-related, i.e. during movement to a new unit. Susceptibility to disease was more apparent in younger and non-medicated birds.

ACKNOWLEDGEMENTS

honesty have greatly contributed to the value of my graduate school experience.

I would like to express a very special thanks to my parents for once again lovingly offering their support as I pursued an educational goal. A special thanks also goes to Chester P. Callahan who by caring provided me the final impetus and strength for completion of my program.

Without the willingness of my daughters, Charlotte J. Smith and Janeen L. Smith, to change their lifestyle and sacrifice the security they had known so that I could return to college, my graduate degree would be only a dream. It is with love for them and appreciation for their patience and support that I acknowledge my deepest debt of gratitude.

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INTRODUCTION

Each year several thousand pheasants are hatched, reared and released by the Michigan Department of Natural Resources (DNR) as part of a program known as "put and take". This project was initiated as a means of providing sportsmen with an adequate supply of pheasants following a significant decline in the wild pheasant population. Changes in land-use patterns in southern Michigan during the 1960's reduced available feed, dried up marshes required for winter protection and eliminated many nesting areas through early cutting of alfalfa fields. Because of a lack of living space for the pheasants, stocking the countryside with eggs and chicks would have done little to increase the numbers of birds surviving to maturity. Thus a decision was made to breed and raise the birds in captivity and release them at 18-22 weeks of age. In 1972, breeder pheasants were placed at the Mason Game Farm and by 1973 the first pen-reared birds were released at selected Put-Take Hunting Areas around southern Michigan.

The facilities for rearing the pheasants are presently located in Dansville, Michigan. They include two buildings with special brooder units where the birds are kept from one day until 12-weeks of age and range pens for adaptation to outdoor living (Figure 1). These fenced outdoor paddocks are

Figure 1:   Sketch of DNR Pheasant Rearing Facilities at
            Dansville, Michigan

RANGE
PENS

RANGE
PENS

South | North

D
UNIT

C
UNIT

A | B
UNIT

Sketch of DNR
Pheasant-Rearing
Facilities at
Dansville, Michigan

N
W ─┼─ E
S
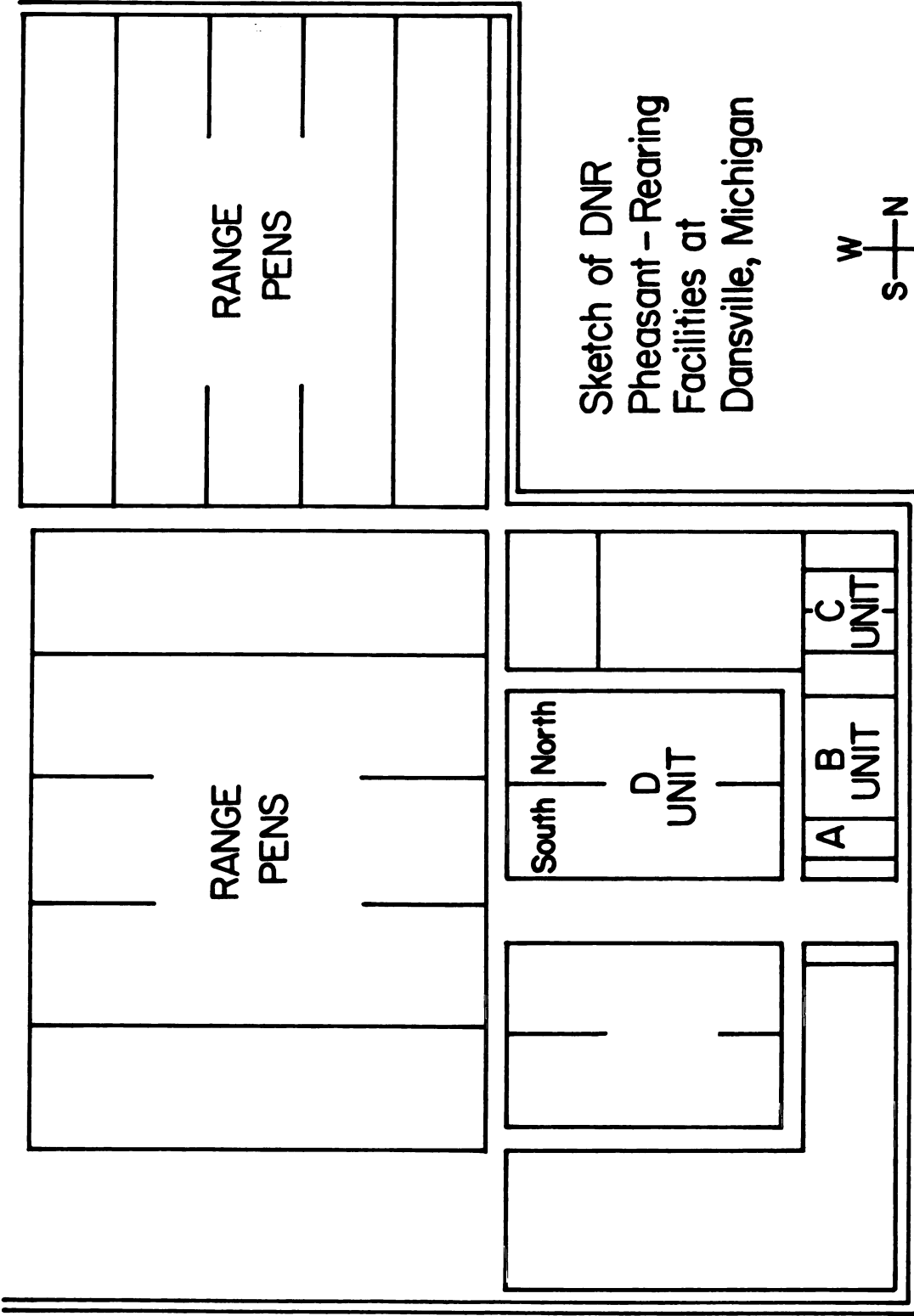
situated on a slope that begins its incline a short distance from the rear of the indoor facilities. During rainy periods water flows down the slope collecting in those pens closest to the buildings where pheasants 11-13 weeks old are kept while adjusting to the outdoor environment.

Many of these younger pheasants die when the rains are severe, accounting for a loss of approximately 10% of the total flock. On the basis of clinical symptoms, macroscopic lesions and the observation of oocysts, DNR veterinarians have diagnosed coccidiosis as the cause of death. Coccidiosis is a parasitic disease in which tissue cells are invaded and destroyed by specific protozoan organisms called coccidia.

Although pheasants reared exclusively in the wild are also susceptible to coccidiosis (Norton, 1967b), the potential for a lethal infection increases among those birds reared in a close environment (Fayer, 1980). This is due to a higher concentration of infective parasites per unit of area and subjection of the birds to stress-producing situations which, if severe, may exacerbate a preexisting coccidial infection.

Pheasant mortality and weight loss can usually be minimized with the use of appropriate anti-coccidial drugs and the application of good management techniques such as those used at the Dansville facilities (see section on Material and Methods and the appendix). However, even the

most rigid adherence to existing management policies may not prevent serious outbreaks of the disease if conditions develop which are beyond the control of the management program.

The pheasant mortality rate at Dansville attributable to coccidiosis is an economic burden of concern to the DNR. The purpose of this investigation is to help in the development of a new management program which will effectively control the disease thereby reducing the coccidial-related deaths.

The specific objectives include determining (1) the identity of those coccidial species involved and the pheasant age group affected by each, (2) the species affecting the animals during transfer to outside paddocks and (3) the influence and severity of infection level at various ages and the effect on the performance of the birds.

LITERATURE REVIEW

Introduction

Because poultry are of significant economic value, scientists and poultry breeders have, for many years, had an intense interest in any factors that affect the numbers and quality of birds and eggs produced for market. Poultry, when reared in close confinement, are at risk to a variety of disease conditions that can (1) increase the mortality rate, (2) reduce the weight gain, (3) alter the bird's general appearance, and (4) affect the quantity and quality of eggs laid. Coccidiosis is one of the more serious and common of these diseases.

Research on coccidiosis in chickens has yielded a vast amount of information on the parasite, its relationship with a host, the influence of environmental factors and modes of controlling the disease (see reviews by Horton-Smith, 1949; Farr and Wehr, 1949; Edgar, 1955; Horton-Smith and Long, 1959; Ikeda, 1959; Rose and Long, 1962; Krassner, 1963; Long and Horton-Smith, 1968; Vetterling, 1976). Much of this informational data on coccidiosis in chickens can be applied towards a general understanding of the disease in pheasants. Information relating specifically to the epidemiology and pathogenicity of coccidiosis in the pheasant is scant.

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Instead the focus has been primarily on identification of species and description of the oocysts.

Life Cycle

The coccidia included in the genus Eimeria have a life cycle divided into an endogenous phase and an exogenous phase (see Figure 2). The endogenous phase includes reproduction by asexual (schizogany) and sexual (gametogony) processes, a characteristic of most sporozoa. Sporozoites, the infective form of these coccidia, penetrate a host cell, round up to form a mononuclear schizont and begin nuclear division. As the number of nuclei increases so does the cytoplasmic mass until a mature schizont with many nuclei is produced. The cytoplasm envelops each nucleus then breaks off, yielding small, elongated mononuclear formations called merozoites. The number of merozoites is equal to the number of nuclei within the mature schizont. Their release from the host cell into the intestinal lumen represents the completion of one asexual generation. The total number of asexual generations to be completed is a characteristic of the species, but most often with those species specific for the pheasant, the number is three. Free merozoites penetrate intact host cells and then proceed to develop in one of two ways: (1) they begin another asexual phase, with nuclear division and subsequent formation of mature schizonts or (2) they begin the sexual phase by maturing into multinucleated microgametocytes and mononucleate macrogametes. Each nucleus

Figure 2: Life Cycle of the Coccidia, _Eimeria_ sp.

LIFE CYCLE OF THE COCCIDIA

Asexual Reproduction

ENDOGENOUS PHASE

Excystation

Sexual Reproduction

Sporulation

EXOGENOUS PHASE
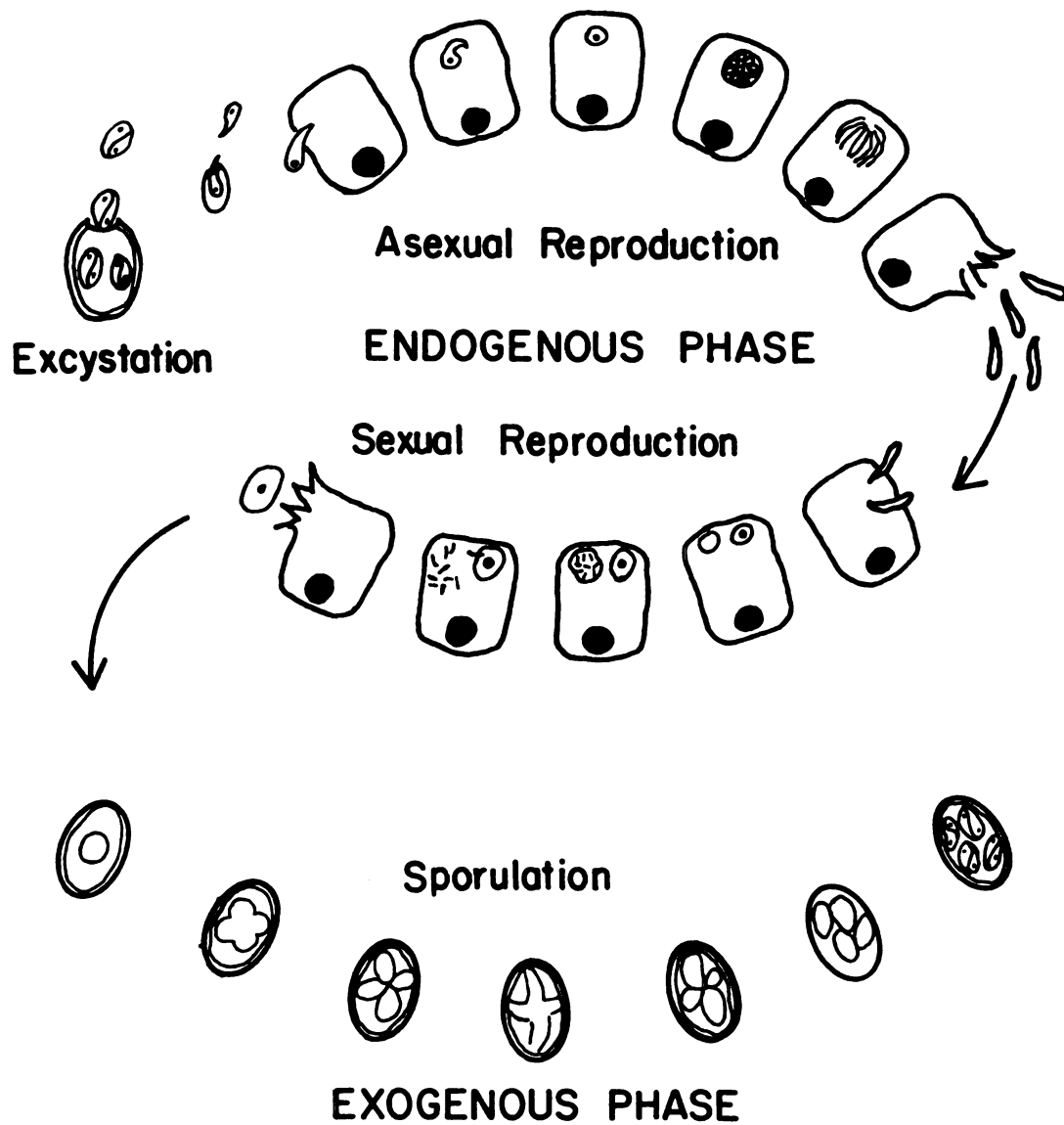
of the microgametocyte yields one motile microgamete which,
upon release, may fertilize a macrogamete. After
fertilization, a wall is built around the zygote from
granules within the macrogamete and the structure thus formed
is the noninfective oocyst. Before an oocyst becomes
infective it must leave the host and enter the external
environment where the exogenous phase commences. Division of
the zygote nucleus is followed by the formation of
protrusions on the zygote surface. These grow and separate
into sporoblasts which go through morphological and
biochemical changes producing sporocysts encased by a
delicate wall. Division of the sporocyst nucleus and
cytoplasm forms sporozoites within each sporocyst. Coccidia
of the genus Eimeria produce four sporocysts each with two
sporozoites. Once the sporozoites have formed, the oocyst is
infective, requiring ingestion and excystation for
continuation of the cycle (Kheysin, 1972).

The Parasite in the External Environment

The ability of Eimeria oocysts to sporulate and survive
within their environment outside of the host is dependent on
certain conditions: (1) free access to oxygen, (2) a
suitable temperature and (3) sufficient moisture (Kheysin,
1972).

Oxygen is a requirement for the initial and final phases
of sporulation. In its presence amylopectin, a starch stored
in granules, is converted to the energy necessary for nuclear

division. Development between these phases can continue under anaerobic conditions, but sporozoites will never be formed. An oocyst can survive an oxygen deficit for a period of time that differs for each species. If it is not returned to the aerated environment within that time, the oocyst will suffer irreversible damage. In the field, oocysts are most apt to be subjected to a lack of oxygen when contained in material heavily contaminated with bacteria which rapidly utilize available oxygen for their own respiration.

Oocysts exposed to temperatures in the range of 10-30°C will sporulate normally and survive for periods of time that potentially could span several months. Deviations from this range in either direction will begin to have deleterious effects on cell division (Marquardt, 1960). The exact temperature required before death of the parasite occurs is determined by the degree of resistance inherent within the species. Most oocysts are better able to withstand low temperature extremes than high. Farr and Wehr (1949) showed that some of the oocysts of E. tenella, E. maxima and E. acervulina, all coccidia of the chicken, could remain viable during the winter when exposed to temperatures as low as- 19°C while Marquardt (1960) found that temperatures of 37°C and 39°C caused permanent damage to both unsporulated and sporulating oocysts.

Adequate moisture is required by the oocyst in order to maintain the integrity of the cell wall. As water is lost

the wall will collapse pressing against the zygote and inhibiting its development. A humidity level of 90% or higher is considered adequate (Horton-Smith, 1947). Below this level deformation of the oocyst is also influenced by temperature. As the humidity decreases and the temperature increases, the percent of deformed oocysts increases (Ellis, 1938). Deformation does not necessarily indicate loss of viability. If exposure to drying has been of a short duration sporogony may continue once sufficient moisture is provided. However, long term dehydration is highly lethal to the oocysts. In terms of field and pen conditions, coccidia tend to sporulate rapidly and survive for long periods of time around water troughs, in moist soil and in litter with a high water content (Hawkins, 1950).

Oocysts have proven extremely resistant to a variety of chemicals. This resistance is provided by the inner layer of the cell wall which is composed primarily of proteins and a mixture of waxes (Ryley, 1973). The outer layer serves to safeguard the oocyst from mechanical damage. Removal of this external layer by sodium hypochlorite or distortion of the cell with hypertonic salt solutions does not impair viability. Chemicals lethal to the oocyst must be able to penetrate the waxes by dissolution or alteration of conformation. Among those capable of doing this are ammonia (Horton-Smith, et al, 1940), mercuric chloride (Marquardt, et al, 1960) carbon dioxide, methyl alcohol, various organic

reagents and highly concentrated bactericidal agents, e.g. formalin (Kheysin, 1972).

The Host-Parasite Relationship

Both initiation and continuation of coccidiosis, which is a self-limiting disease, require ingestion of sporulated oocysts followed by excystation, a two-phase process yielding free sporozoites. Hibbert and Hammond (1968) suggested that each species of coccidia has its own pattern of excystation which has contributed to the adaption of the parasite to its particular host. The purpose of each phase is to alter the permeability of the oocyst or sporocyst wall. The first phase is stimulated by (1) carbon dioxide, (2) a mechanical action, or (3) enzymes, while a trypsin-bile mixture is essential for the second phase (Nyberg and Hammond, 1964). Among different hosts, the sequence of sporocyst and sporozoite release varies. In gallinaceous birds, sporocysts are released first followed by liberation of the sporozoites. The two phases usually occur in different locations along the alimentary canal.

Most coccidia will parasitize the digestive tract of either a vertebrate or invertebrate host. A few, though, are known to carry out the endogenous stage of their life cycle in other organs, e.g. _E_. _stieda_ in the liver of the rabbit and _E_. _truncata_ in the kidney of geese. Free sporozoites, of pheasant and other gallinaceous bird coccidia, are genetically directed to invade a particular portion of the digestive

tract. This predilection is based on the nutritional requirements of the parasite, the physiological status of the cell and the host's immune response (Vetterling, 1976; Long, 1971). A very delicate balance exists between parasite and host, wherein the degree of cellular damage by the coccidia and the defensive activity of the host are in equilibrium. Any change in the host's nutrition, its protective mechanism or demands of the parasite will upset the balance resulting in either increased damage to the host or interference with development of the parasite.

Certain of the coccidia's nutritional requirements, such as proteins, nucleic acids, glucose or vitamin B_{12}, are synthesized by the host or its natural intestinal flora, while others are acquired via the host's diet. Researchers have determined that <u>Eimeria</u> species require the dietary vitamins thiamine, riboflavin, biotin, nicotinic acid, pyridoxine and para Amino benzoic acid (pAB). Each is essential for a specific stage of coccidial development, e.g. Warren (1968) found that biotin is required for the first schizogeny and nicotinic acid for second schizogeny by <u>E. tenella</u>. Removal of any of these vitamins whether by antagonism, inhibition or frank absence will arrest development of a coccidium at that stage for which the vitamin is a requirement.

Immunity is the host's most obvious protective mechanism. Both antibodies and cellular elements constitute

the immune response. The presence of humoral antibodies is an established fact, but research has indicated that coproantibodies may also play an important immunological role. These antibodies are of the IgA class formed in the digestive mucosa. They represent a local defense and work in conjunction with serum antibodies, which are brought into direct contact with the parasites by an increase in gut permeability (Rose and Long, 1969), immobilizing or destroying either the sporozoites or one of the other stages of development. At one time it was assumed that immunity was exclusively local in nature rather than humoral, with the tissue cell itself imparting a resistance to invasion by sporozoites or merozoites. The cellular elements now referred to when discussing immunity include macophages, lymphocytes and granulocytes. The precise role of each in coccidial immunity has not been definitely determined, but it has been suggested that their modes of action are similar to that in humans.

Within 24-48 hours following ingestion of a coccidial dose by an immune host, irreversible damage has been inflicted on the developing parasite (Leathem and Burns, 1967). Degree of immunity (total or partial) as well as the nature of the coccidial species may influence which developmental stage is affected. Rose (1968) suggests that total immunity may inhibit the formation of oocysts while partial immunity reduces the total number of oocysts

produced. Immunity is not always stimulated in the host at the first introduction to the parasite. Assuming a moderate number of oocysts per dose, the number of immunizing doses required before the host is protected against a challenge dose depends on the particular species of coccidia, e.g. E. maxima requires only a single dose in order to elicit a response whereas E. alabamensis requires four doses. This characteristic, referred to as immunogenicity, is not only a function of the number of oocysts per dose but also the location of the coccidia within the tissue. Sporozoites often localize in the epithelial cells lining either the distal or proximal half of the intestinal villi, but they may travel the lamina propria, possibly via macrophages, to settle at the base of the villi or in the glands. According to Tyzzer (1929) those species that develop superficially in the intestinal epithelium require more immunizing doses than those that develop deeper in the mucosa.

Immunity is usually specific for that species which initiated it. Although cross-reactivity rarely occurs in poultry, a host may be resistant to more than one species provided a sufficiently large dose of oocysts from each species has been ingested. Duration of immunity is dependent on such factors as the mode of immunization, age of host and the time interval between an immunizing dose and a challenge dose (Rose, 1973). In birds not subject to reimmunization, duration varies with the coccidial species. An immunity

stimulated by E. maxima is short-lived but one stimulated by E. tenella may last several months (Long, 1962; Leathem and Burns, 1967). Continual ingestion of infective oocysts may provide the host with sufficient stimulus to maintain indefinitely a competent immune status.

Coccidial survival can be enhanced by suppression of the host's immune system. Preexisting infection with bacteria, viruses or other parasites may render a host incapable of responding immunologically to invasion by the coccidia while stress-producing situations, metabolic changes or production of interferon may potentially result in reduced effectiveness or actual loss of a previously developed immunity. On the other hand, immunity to non-coccidial antigens may be suppressed by an established coccidial infection. Kaye, et al (1965) superimposed a Salmonella typhimurium infection on mice already infected with Plasmodium berghei. These animals were compared with mice infected with only one of the two microorganisms. Higher mortality occurred in mice with the superimposed infection which the author attributed to salmonellosis rather than the parasitic disease. Coccidial infected hosts, although presenting a severe immunosuppression against unrelated antigens, display high levels of specific serum immunoglobulins (Krettli and Bereira, 1981). Orjih and Nussenzweig (1979) demonstrated that reinfection by the original organism did not abolish an established immunity. Animals repeatedly infected with blood parasites

and immunized with irradiated sporozoites had unaltered antibody titers despite high levels of the parasite.

The influence of host age on coccidial survival differs with the conditions under which the bird was reared. An older host that has been housed in a coccidia-free environment will be more susceptible to both parasite reproduction and pathogenic effects than a young host reared similarly. Long and Horton-Smith (1968) and Krassner (1961) have suggested that this may be a result within the older bird of better parasite excystation, increased numbers of intestinal cells and physiological conditions more conducive to sporozoite penetration. However, young birds are far more vulnerable to disease than older birds when both age groups have been reared under conditions that allow for the development of immunity (Horton-Smith, 1947). Thus, the coccidia are better able to survive in young chicks and non-immunized older birds.

Investigators have shown that the size of an ingested dose is directly related to the effects produced within the host (Long and Horton-Smith, 1968), but inversely related to the yield per infecting oocyst (Krassner, 1963). The exception to this occurs with what is known as the "crowding factor" in which both pathogenic effects and yield are diminished per infective unit when an extremely large dose of oocysts is ingested. A few possible explanations for the occurrence of this phenomenon have been proposed: (1) very

rapid development of an immune response (Fayer, 1980), (2) loss of healthy tissue for continued reproduction (Krassner, 1963), (3) possible production of interferon or an interferon-like substance which inhibits normal replication (Long and Milne, 1971) and (4) competition for nutrients.

Pathology

Evaluation of a bird's performance and general health status may reveal symptoms that indicate disease. The clinical signs suggestive of coccidiosis include myasthenia, lethargy, weight loss, poor feather growth and diarrhea. These are related to physiological and biochemical changes such as reduced muscular glycogen stores (Long, 1973), blood loss, anorexia, vitamin deficiency induced by poor absorption of nutrients (Briggs, 1946; Preston-Mafham and Sykes, 1970) and malabsorption due to loss of intestinal epithelium (Preston-Mafhan and Sykes, 1970). The absence of clinical symptoms, however, does not exclude the presence of coccidiosis within the birds. The disease may be subclinical and undiagnosable until the intestines are examined macroscopically and microscopically.

Lesions characteristic of diseases or conditions that may have clinical signs similar to those of coccidiosis, e.g. vitamin deficiency and wood-chip blockage, may be revealed by examination of the mouth and crop. Wood-chip blockage is common among very young chicks 2-3 days old. Litter is ingested in quantities which pack the crop. The sensation of

fullness in the stomach which ensues decreases the intake of food while the blockage in the crop prevents what little food is ingested from reaching the gut. The chick subsequently dies from starvation.

Macroscopic examination of the gut will reveal lesions produced by physiological changes within tissues infected with the coccidia. Blood vessels become enlarged following an increase in blood flow (hyperemia) induced by the immune response to deliver granulocytic leukocytes and humoral antibodies to the area of infection. Leakage of protein into the mucosa with the subsequent development of edema (Long, 1973) produces a thickening of the intestinal wall. The proteins are lost due to changes in the mucosal blood capillaries. Proteins are also leaked into the intestinal lumen where fluids increase to dilute the proteins and mucus accumulates from destroyed glandular cells. Together they produce watery intestinal contents. Hemorrhage will add to the fluidity of the material within the intestines. It is observed on gross examination by redness of the serosal surface or areas of petechiae.

According to Long (1973) a change in body weight of an animal is considered by many workers to be the only indicator of subacute coccidiosis. Weight-gain reduction begins on the 4th day of an infection and reaches its maximum by day 5 when both tissue and oocysts are being sloughed. Preston-Mafham and Sykes (1970) related weight loss to severe depression in

the absorption of nutrients caused at least in part by destruction of intestinal epithelia and to anorexia.

Eimeria sp. of the Pheasant

Ten Eimeria species have been described in pheasants: Eimeria dispersa (Tyzzer, 1929), E. phasiani (Tyzzer, 1929), E. langeroni (Yakimoff and Matschoulsky, 1937), E. pacifica (Ormsbee, 1939), E. megalostomata (Ormsbee, 1939), E. gennaeuscus (Ray and Hiregaudar, 1959), E. picta (Bhatia, 1968), E. duodenalis (Norton, 1969a), E. colchici (Norton, 1969b) and E. tetartooimia (Wacha, 1973). Only six of these species have been found and confirmed in the ringneck pheasant, Phasianus colchicus torquatus, the most common pheasant in the United States. Three of the remaining four species have been found in pheasants other than the ringneck and the fourth species, E. dispersa, is regarded by Pellerdy (1974) as species incerta since it has been reported in a variety of gallinaceous birds.

Identification of the coccidia is based on (1) characteristics of the oocysts, (2) location of the different developmental stages within the intestine and tissue, (3) length of the prepatent and patent periods and (4) immunological specificity (Joyner and Long, 1974). The use of more than one criterion is particularly essential when the observed oocysts exhibit characteristics similar to those of another species. A mixed infection within a single host may require the isolation of individual species for innoculation

of "clean" hosts. Table 1 describes the oocysts of six of the Eimeria species found in the ringneck pheasant.

Pathology of Coccidiosis in Pheasants

Gross or macroscopic lesions due to infections with E. pacifica and E. megalostomata are rarely detectable (Ormsbee, 1939). Eimeria colchici, E. phasiani and E. duodenalis, however, are capable of producing severe lesions as reported by Norton (1967b, 1967a) and Trigg (1967b), respectively.

Attention to the location of these parasites both within the cell and the tissue is of importance because of the relationship between pathogenicity and site of development. Tyzzer (1929) reported that the deeper the parasite penetrates the tissue, the more severe are the effects on the host. It has been suggested that this is due to a stronger immune response. Parasites that locate above the nucleus of epithelial cells lining the superficial villi usually do not invade other areas of the tissue. Lesions are then confined to destruction of surface epithelial cells, which are readily replaced, and a mild to moderate cellular response. Coccidial localization below the nucleus of an epithelial cell is potentially followed by transversing of the basement membrane and subsequent invasion of the lamina propria, tunica propria, glands and submucosa. Disruption of capillaries will cause some hemorrhage while invasion of progenitor cells in the crypts of Lieberkuhn inhibit their division which results in villus atrophy and subsequent

Table 1

Description of Eimeria Oocysts

	Eimeria Phasiani	Eimeria Colchici	Eimeria Duodenalis	Eimeria Pacifica	Eimeria Megalostomata	Eimeria Tetartooimia
Oocyst length (um)	24.7 (20.1-30.9)	27.4 (19.0-33.5)	21.2 (18.0-24.0)	22.0 (17.0-26.0)	24.0 (21.0-29.0)	18.6 (17.0-20.4)
width (um)	16.7 (14.1-20.5)	18.6 (13.0-21.0)	18.0 (15.4-21.4)	19.0 (14.0-20.0)	16.5 (16.0-22.0)	(15.0-18.4)
Shape Index (length/width)	1.44	1.64	1.14	1.22	1.26	1.10
Shape	ellipsoidal	ellipsoidal	subspherical	oval	oval	oval
Thickness of wall (um)	1.60	1.3	1.5	1.8	2.0	2.2
Polar Inclusion	1-3	1-3 (may be more)	small, scattered	2-3	1 dark, irregular	1
Micropyle	no	inconspicuous	no	no	yes	no
Oocyst Residuum	no	no	no	no	no	
Sporocyst length (um)	14.3 (12.9-15.9)	14.6 (11.5-16.5)	12.8 (9.9-15.4)			
width (um)	6.7 (5.6-7.4)	6.6 (6.0-7.5)	7.3 (6.0-8.6)			
Sporocyst Residuum	no	yes (prominent)	yes	yes (as granules)		
Stieda Body	yes	yes	yes (prominent)	yes		
Prepatent Period	25 days	6 days	4 days			
Sporulation Time	50% of Total in 52 h at 20°C	96% of Total in 48 h at 26°C	92% of Total in 44 h at 27°C			
Ref.	Trigg, 1967	Norton, 1967	Norton, 1967	Ormsbee, 1939	Ormsbee, 1939	Wacha, 1973

reduction of surface area available for absorption. Invasion of these different areas of the tissue by the coccidia stimulates an influx of inflammatory cells and erythrocytes. Lymphocytes appear late in an infection. In moderate to severe infections, necrosis and fibrosis will develop, usually following release of most of the oocysts into the lumen.

Eimeria colchici is the most pathogenic of the pheasant coccidial species, responsible for caecal coccidiosis in young chicks 2-4 weeks old. First and second generation schizonts locate in the glands and lamina propria at the base of the villi, usually below the nucleus (towards the basement membrane) of the infected epithelial cell. The third generation schizont is found in the caecal glands while the gametocytes infect the epithelial cells lining the caecal mucosa. A mortality of 100% occurs in birds dosed with 320,000 oocysts. Soft white cores of necrotic debris, hyperemia throughout the intestines and a mucoid exudate are produced with this number of ingested oocysts. Fewer oocysts produce less severe lesions.

Eimeria phasiani is a moderately pathogenic species producing 100% mortality with a dose of 500,000 oocysts. It is most often a parasite of young pheasant poults. Location of the first asexual generation is in the glands and at the base of the villi of the upper one-third of the small intestine and the second is in the epithelial cells lining

the villi of the upper half to two-thirds of the small intestine. The third generation schozonts and gametocytes are distributed throughout the small intestine and the proximal half of the caeca. They too infect epithelial cells lining the villi. Position within the epithelial cell is usually below the nucleus. An edema, thickening of the intestinal wall, hyperemia, some hemorrhage, infiltration of many eosinophils into the tissues, and "ballooning" of epithelial cells characterizes disease with this species (Trigg, 1967a).

A dose of 5,000,000 oocysts is required for 100% mortality of 3-week old chicks infected with E. duodenalis, a mildly pathogenic species. Norton (1967a) has suggested that those deaths related to infection with E. duodenalis may, in part, be also due to a bacterial infection enhanced by the stress of coccidiosis. Although 3-week old chicks were used for pathogenicity studies, disease from this species is not necessarily found predominantly in younger birds. E. duodenalis locates in the epithelial cells lining the villi of the duodenum and upper small intestine. Location within the cell may be below the nucleus but usually is above (towards the mucosal surface). A pinkish mucoid exudate covering the duodenum and upper small intestine, caeca distended with a yellow foamy fluid and edema are characteristics lesions of more severe infections with this species.

Oocyst Counts

A diagnosis of coccidiosis cannot be based solely on the observation of oocysts in the feces (Davies, et al, 1963). The presence of coccidial developmental stages within the intestinal tissue (Norcross and Washko, 1970) and the occurrence of characteristic gross lesions are more diagnostic since non-infective oocysts or oocysts infective for another host may be ingested and directly eliminated. Conversely, oocysts may be absent in the feces of hosts with an infection severe enough to cause death (Davies, et al, 1963). A lethal oocyst dose of a species whose most destructive developmental stage is a part of the asexual cycle may cause death to the host before oocysts have been produced (Horton-Smith, 1947). Death may also follow the completion of the endogenous phase if the intestinal epithelia are so completely destroyed a malabsorption-type syndrome develops. The value of the oocyst count lies then with its use as a monitor of the progress and severity of an established infection when only one species of coccidia is involved and reinfection is prevented. The endogenous phase of coccidial infection consists of two periods: (1) the prepatent period, which begins when the first sporozoite penetrates a host cell and which ends when the first oocyst is eliminated into the external environment and (2) the patent period, which extends from passage of the first oocyst to passage of the final oocyst (Kheysin, 1972). Both periods

last several days, the exact number dependent on the infecting species. As the patent period progresses, the number of oocysts present in the feces will increase until a peak is attained after which the numbers will decrease. The size of an ingested dose will influence both the severity of the disease and the number of oocysts eliminated. Thus the oocyst count, if performed on a daily basis, can provide meaningful data for evaluation of the disease. Under conditions of intense rearing, however, it is impossible to prevent reinfection and often more than one coccidial species is present. The information garnered from the oocyst count when mixed infections, reinfections and sequential infections are present is limited. Its value is confined strictly to numbers which, if significantly elevated, will indicate a serious infection. Examination of the tissue will be required for establishment of the disease progress.

Preventative Therapy

Anticoccidial drugs are applied to hosts in environments where the immune system is unable by itself to provide adequate protection against the development of coccidiosis. Most act by interference with enzyme activity at one or more of the developmental stages. Amprolium, for instance, acts reversibly to inhibit thiamine (Cuckler, et al, 1960) affecting the first generation schizonts of E. adenoeides in turkeys (Warren and Ball, 1963) and second generation schizonts of E. acervulina in chickens (Warren, 1968).

Monensin, an ionophore, inhibits the transport of cations while arprinocid blocks hypoxanthine (Braunius, 1982). Proper selection of and treatment with a particular drug allows for development of immunity with no negative effect on weight gain. Continued use of the drug may induce the development of resistant coccidial strains. However, drug tolerance, which Braunius (1982), describes as "a state of insensitivity to a drug which ordinarily causes growth inhibition or death of a cell," is more likely to occur with long-time usage. Both resistance and tolerance are dependent on the _Eimeria_ species involved and the number of passages the species has undergone through a host. Switching to a new drug may relieve problems incurred with resistance while upping the dose may override the reduced response by a host that has developed a tolerance.

Coccidiosis in pheasants has been controlled by the addition of anticoccidial drugs to the feed or drinking water. Among those used in pheasants have been sulfaquinoxaline, amprolium, zoalene and amprolium hydrochloride/sulfaquinoxaline/ethopabate (ASE). No drugs have been cleared by the Federal regulating agency for use in small game such as pheasants. The cost of such a procedure is not economically merited at this time. However, the efficacy of drugs in pheasants has been tested through drug trials by a few researchers (Norton, 1967a and 1967b; Trigg, 1967b; Jurkovic, et al, 1982). Results obtained in the

1960's demonstrated the efficacy of amprolium against E. colchici and sulfaquinoxaline against E. duodenalis and E. phasiani. Zoalene was also found to be effective against the latter two species but not to the same degree as the sulfaquinoxaline. Because of the frequency with which mixed infections are found in birds reared in confinement, a drug such as ASE was used to provide a broad-spectrum type of protection. Recent surveys on the prevalence of coccidiosis in pheasants (Norton, 1981) indicate that these drugs have not been exerting the degree of control required to maintain a low mortality rate. This would be expected with their continued usage over a long period of time. Braunius (1982) emphasized that a direct relationship occurred between time of usage and number of Eimeria oocysts found. Norton (1981) proceeded to test the efficacy in pheasants of several drugs previously developed for use with chickens as well as two drugs traditionally used with the pheasant. These drugs included arprinocid, clopidol/methyl benzoquate, clopidol, robenidine hydrochloride, monensin, methyl benzoquate, halofuginone, ASE and sulfaquinoxaline. The results showed that three of the new drugs, halofuginone, clopidol and arpinocid, are more effective in reducing mortality, maintaining weight gain and reducing oocyst output than ASE and sulfaquinoxaline. Halofuginone (3 ppm) was able to effectively control E. phasiani and E. duodenalis while

clopidol (125 ppm) and arprinocid (65 ppm) were most effective against E. colchici.

Epidemiology

The distribution and severity of coccidiosis under both field and pen conditions are dependent on oocyst survival in the external environment and a host's capacity to protect itself. Oocysts are able to sporulate and remain viable in the presence of moisture, warm temperatures and good oxygenation. In outdoor pens these conditions exist primarily around watering troughs, in shaded areas such as under trees (Farr and Wehr, 1949) and during the warm periods of the year. Maintenance of well-drained, treeless fields could reduce the number of infective oocysts in this type of environment. Bejsovec (1973) noted a marked decrease in the incidence of coccidiosis when pheasants were moved to large, extensively drained, continuous field units. Changing of the litter used in buildings keeps moisture and the number of infective organisms at a desirable level. Regulation of personnel movement within and between buildings reduces the potential for introduction and spread of oocysts. Hosts that are well-nurtured, free of pre-existing non-coccidial infections and receiving an appropriate anti-coccidial drug while exposed to a moderate number of oocysts are best able to develop an immunity which will maintain the coccidia at the infection level, resisting progression to the disease state. This is critical to the survival of the host since

those reared under conditions of strict sanitation have little or no resistance (Horton-Smith, 1954). Introduction of a non-immune host to out-of-door units may well result in acute coccidiosis (Horton-Smith, 1947).

Gallinaceous birds such as chickens, turkeys and most particularly pheasants are very sensitive to alterations in their environment. Movement of a flock, spexing, crowding, abrupt change in lighting conditions and exposure to hard rains may serve as environmental stressors producing a "fear-like" response called the General Adaptation Syndrome (GAS) (Hill, 1983). The physiological response in humans to physical stress is a marked increase in the secretion of corticosteroids, most particularly cortisone, initiated by nerve impulses in the hypothalmus. A similar response in birds to stress, i.e. elevated serum cortisone levels, has been substantiated by researchers such as Monjan (1977). The consequence of increased cortisone levels in both humans and birds is suppression of the immune response. In humans this temporary loss is sometimes followed by fulminating spread of infectious disease in the body (Guyton, 1963) and could potentially be the same in birds.

The coccidia appear to be most susceptible to climatic conditions that reduce available moisture. Farr and Wehr (1949) showed that oocysts on bare soil exposed to direct sunlight did not survive more than 25-30 weeks, while those with some protection from the drying effects of the sun

remained infective nearly twice as long. Exposure to temperature extremes when moisture content is high has little adverse effect on their viability. Bejsovec (1973) reported that in Czechoslovakian range pens the highest incidence of pheasant coccidiosis occurred during the winter months. Chang (1937) found that unsporulated oocysts of E. tenella could survive in a water bath of 46°C for 245 minutes while Pellerdy (1965) found that E. tenella oocysts were able to survive no longer than 60 minutes at 25°C in an incubator. Survival of the coccidial oocysts is at a peak during those times of the year when the environmental moisture content is high, such as during rainy periods.

MATERIALS AND METHODS

General Considerations

The pheasant-rearing facilities at Dansville include two buildings for indoor confinement and several wired padlocks for outdoor maintenance. Each building is partitioned into six brooder units, three of which will house a single hatch during its 12-week indoor period. These rooms, labeled A-C, are of increasing size to allow for growth of the birds (Table 2). Room temperature is initially maintained at 95°F then decreased weekly by 5°F until week 5 or 6 after which no heat is added. The floor of each unit is covered with a 6-inch layer of wood chips. Areas of the litter that become wet are removed but otherwise no change is made until the hatch has been moved to the next unit. At that time the unit is emptied of equipment and litter, washed thoroughly, disinfected with an ortho-phenol (Chematrol) or a quaternary ammonium compound (Microphene) then allowed to remain empty for one week.

Pheasants are moved at 12 weeks to initial outdoor pens containing water troughs and feeder units of roughly the same number as found indoors. These pens were created for 1-2 week adjustment to the new environment. The birds are then moved to range pens which are larger and contain fewer water

troughs and feeder units. The pheasants remain in these paddocks until released at age 18-22 weeks.

Table 2

Dimensions of Indoor Units and Special Pens

| | Dimensions (ft.) | |
Unit	Main Room	Pen
A	40 X 50	4 X 6
B	40 X 150	8 X 10
C	40 X 200	10 X 12

Experimental Pens

For experimental purposes, a special pen was built inside each of the three brooder units of one building. Dimensions of the pens were chosen in such a way that the same stocking density as in the main area was maintained. Litter from the main area of a unit was added 2-3 times a week to that in the special pen in order to ensure common exposure to any parasites or other microorganisms that might be present in the main flock.

Selected Hatches

A single hatch totalled approximately 6,000 birds. Twenty-six hatches were reared in a period beginning the middle of March, 1981 and continuing until the middle of December, 1981. Three separate hatches, one from each season the "put and take" program was in operation, were selected

for this study including hatch 2 (March 26-July 16), hatch 10 (May 21-August 27) and hatch 22 (August 13-December 4). Each hatch was separated into two groups. The first consisted of 80 randomly selected, left-wing banded birds that were isolated in the special pen (study group). This number was maintained throughout the 18-22 week period by replacement of dead or removed pheasants with birds randomly selected from the main body of the hatch and banded on the right wing. The remainder of the hatch (control group) was housed in the main area of a unit. All birds in both groups were fed a mash containing 28% protein, 7% crude fiber and 2% crude fat. The coccidiostat Amprolium[+] (see Appendix) was added to the feed of the pheasants in the control group at a concentration of 125 ppm for the first 8 weeks and 40 ppm thereafter until one week before their release at which time the drug was withdrawn. This was done in keeping with the standard management program at the farm. No coccidiostat was added to the feed of birds in the study group. In all other ways the two groups were treated similarly. Every week four birds from each group were randomly selected, killed and individually weighed. A Mettler balance was used for birds up through 6 weeks of age, while birds 7 weeks and older were weighed in a Chatillon scale. Necropsy was performed using the method described by Stubbs (1954). Once the intestines were removed from the body cavity, they were examined for gross lesions; representative pieces of tissue were then

selected for subsequent study. One section from each of five areas of the gut (distal half of the duodenum, jejunum, upper ileum, caeca and lower ileum) was fixed in 10% neutral-buffered formalin for histopathological examination while a second section was taken for parasitological examination of its contents. Feces from all four birds were pooled for an oocyst count.

Histopathology

The formalin-fixed specimens were treated with alcohol-xylene for 12 hours then embedded in paraffin blocks, each of which contained a sample of the same gut area from all four birds in a group. Tissue sections of 6 um in thickness were fixed to a slide then stained with Ehrlich's hematoxylin and eosin.

Microscopic observation with the 10X objective was used to assess the condition of the tissue, note possible invasive cells and detect gamonts and oocysts. Detection of smaller parasites, differentiation of developmental stages and determination of the parasite's position within the cell was done with the 40X objective.

Parasitology

The intestinal contents of each area of the gut from the four birds in a group were pooled. Microscopic examination of the contents was made on direct smears and following sugar flotation. Dimensions of a minimum of 10 oocysts for each

species observed were measured using an eyepiece scale that had been calibrated with a stage micrometer. Other oocyst characteristics such as shape, presence or absence of a micropyle, polar granules and cell wall features were noted for species identification.

Sporulation studies utilized a pool of all intestinal contents from the four birds within a group. The material was washed three times with tap water then placed in a petri dish. Potassium dichromate (2.5%) was added to a depth of one-quarter inch. At specified time intervals (24, 48 and 72 hours) a sample of the material was transferred to a centrifuge tube for sugar flotation and subsequent microscopic examination. The percentage of sporulated oocysts for each species was determined by counting a total of 100 oocysts or by counting the total number on the slide if fewer than 100 oocysts were present. Sporocyst characteristics, e.g. stieda body, granules, shape and size, were noted at this time.

Oocyst counts were performed on feces from the following pheasants: study group: four live birds selected weekly and all dead birds; control group: four live birds selected weekly, any dead birds requested for special studies and any live birds requested for special studies. An adaptation of the McMaster's method (Technical Bulletin, 1971) was utilized. Saturated zinc sulfate (33%) was added to the feces in a ratio of 14:1. The well-mixed sample was filtered through a sieve covered with a double layer of cheese cloth.

A McMaster's counting chamber was filled with the filtered sample then allowed to stand for 5-10 minutes while the oocysts rose to the surface. The total number of oocysts in both sides of the chamber was multiplied by a factor of 50 in order to obtain the number of oocysts per gram of feces (see Appendix).

Epidemiology

Dansville farm personnel maintained a daily record of unit conditions and number of mortalities while the pheasants were confined indoors. Those conditions noted included room and floor temperatures, brooder stove settings, variable fan settings, type of feed and outside temperature and weather conditions, e.g. cloudy, humid, hard rains. Causes of death were recorded when due to accident, starvation, drowning or an animal kill. Those deaths that appeared to be disease-related were diagnosed by DNR veterinarians following necropsy of a representative few dead pheasants. Mortality rates were based on the total number of birds originally present within a group. Records during outdoor confinement included type of feed and the number of deaths with comments regarding cause if known.

Records of daily high and low temperatures and rainfall for the months of March, 1981 through December, 1981 were obtained from the National Weather Bureau located at the Lansing Airport, Lansing, Michigan.

RESULTS

Coccidial Species

Four species of coccidia were identified: *Eimeria duodenalis*, *E. pacifica*, *E. phasiani* and *E. colchici*. Identification was based on oocyst dimensions (Table 3), oocyst morphology (Table 1) and location within the intestine when possible. The size of the oocysts fell within the reported values for each of the species.

Table 3

Mean Dimensions of Oocysts

Coccidial Species	Observed Measurements (MSU/DNR Experimental)			Reported Measurements			
	Length (um)	Width (um)	Shape Index	Length (um)	Width (um)	Shape Index	Reference
E. duodenalis	22.6	20.0	1.13	20.3	18.1	1.10	Norton, 1967a
E. phasiani	25.3	18.1	1.40	24.7	17.1	1.45	Trigg, 1967a
E. pacifica	23.7	19.4	1.22	21.1	17.5	1.20	Ormsbee, 1939
E. colchici	29.8	18.6	1.60	28.7	17.3	1.64	Norton, 1967b

Oocyst Counts

The number of oocysts per gram of feces was determined for both groups of hatches 10 and 22 beginning at week 7 when the birds were large enough to provide sufficient fecal

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material. An oocyst count was also done on fecal material
from any dead birds within the study group that were received
from the Dansville farm. Elevated oocyst counts paralleled
movement of the pheasants to the C unit and to the North-D
pen (see Figure 3). This was true with both the study and
control groups although the increase in the oocyst count was
not as conspicuous in the latter group. The same correlation
could not be seen with morphology except when the birds were
in the outdoor unit. Most of the increased or high oocyst
counts indicated a severe infection which was substantiated
by evaluation of tissue sections. Oocyst counts from birds
in hatch 22 were usually greater in number and increased more
frequently than in hatch 10.

General Performance

Live pheasants delivered to the Animal Health Diagnostic
Laboratory from the Dansville facilities generally arrived in
satisfactory condition. They appeared alert with good
feather growth and no lesions other than those caused by
injury or cannabalism. On two separate occasions live birds
were selected from the control group for special studies.
Both times the birds were lethargic, small in size and had
diarrhea. Most cases of coccidiosis in this investigation
though were subclinical, diagnosed only following necropsy
and examination of the tissues.

Each bird was weighed following euthanization and the
weekly mean weight per group calculated. No weight

Figure 3: % Mortality and Oocyst Count for Each Week

of a Hatch, Both Groups
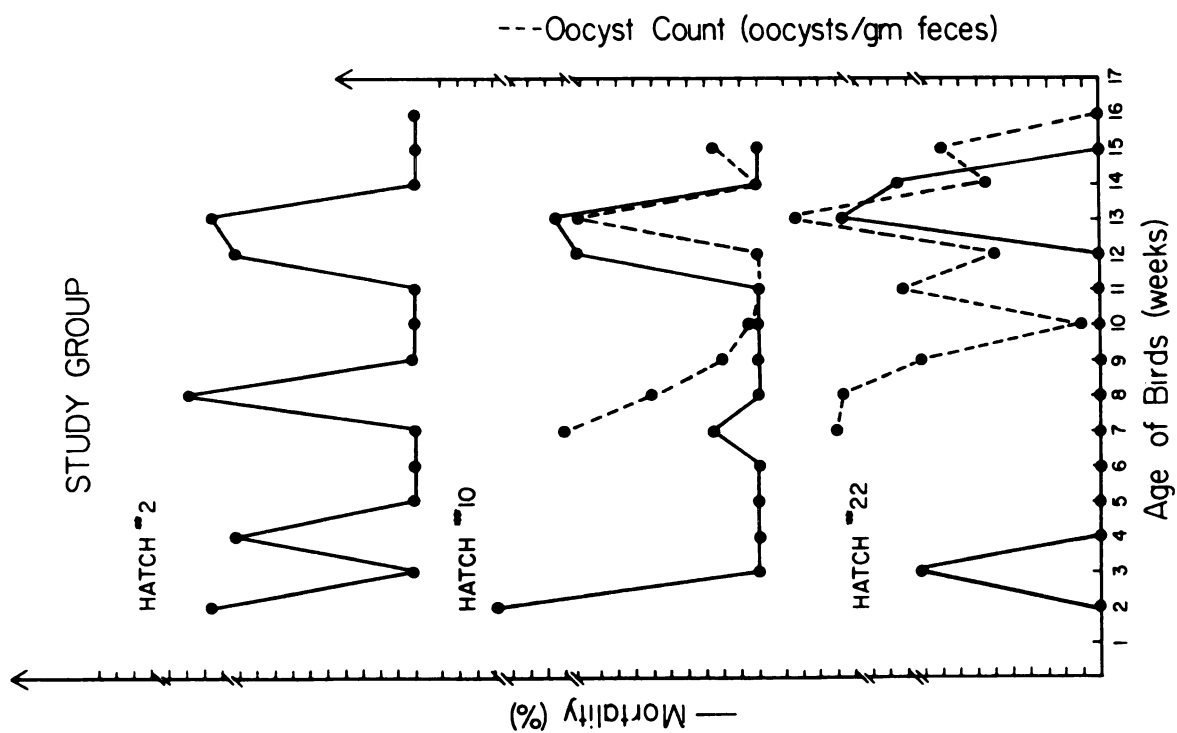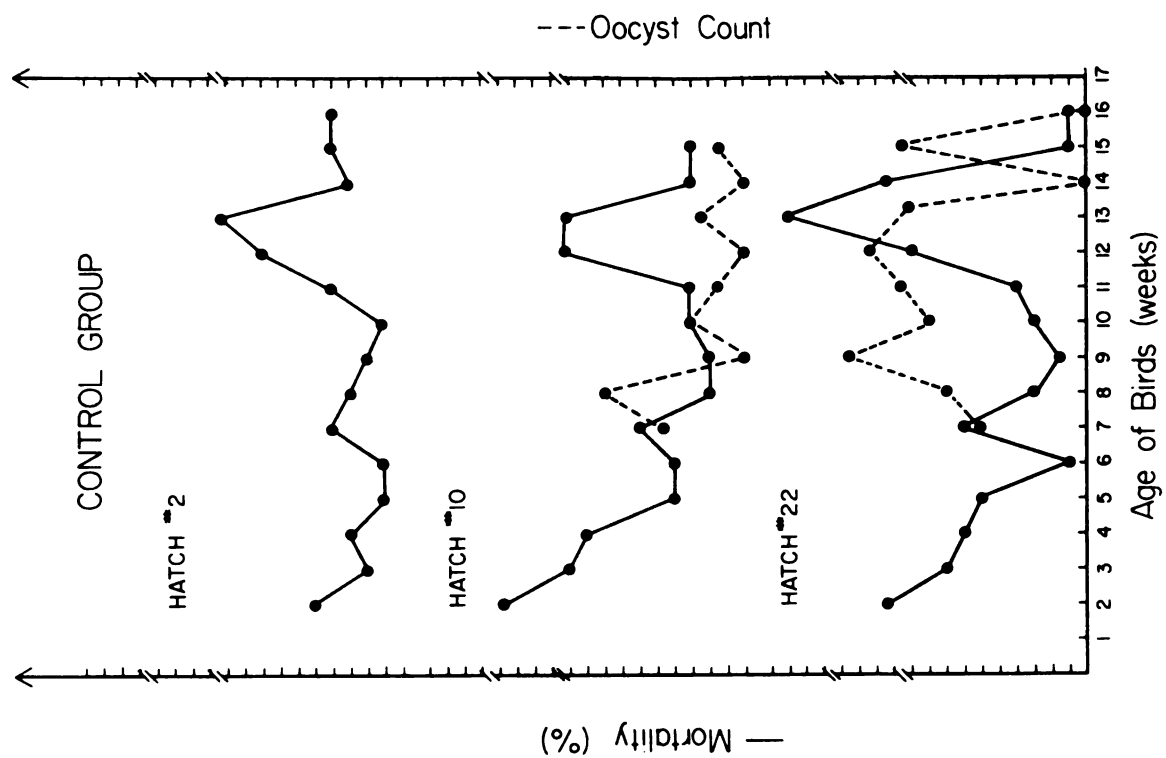
differentiation by sex was made. A frank decrease in the mean weight was seen between weeks 11 and 14 for both groups in all hatches (Figure 4). Within the study groups of hatches 10 and 22, the weight loss corresponded with an increase in mortality and in the number of oocysts present in the feces. A decrease in weight gain and an increase in mortality were observed in all hatches, both groups, with the exception of the study group hatch 22, when the birds were moved into the C unit and the North-D pen.

Gross Lesions

No lesions of the mouth were seen nor were any of the crops packed with wood chips. Macroscopic lesions in the intestines and caeca consisted of enlargement of the blood vessels, thickening of the intestinal wall, mucous covering on the outer surface, watery intestinal contents and/or hemorrhage and a white pasty material in the caeca. The lesions were usually mild in degree and were seen infrequently in hatches 2 and 10 while appearing consistently following week 3 in hatch 22. An increase in the intensity of the lesions, i.e. involvement of more areas of the gut, rather than a change in type, accompanied the few severe infections. However, birds that died at the Dansville farm often showed areas of the upper intestines in which the intestinal wall was very thin and heavily covered with mucus. These areas appeared black in color due to a granular material resembling coarse sand which was visible through the

Figure 4:   Mean Weight of Birds (grams) as Measured per
            Week of Hatch.

            Solid Line = Control Group
            Broken Line = Study Group

HATCH #2

HATCH #10

HATCH #22

Mean Weight of Birds (grams)

Age of Birds (weeks)
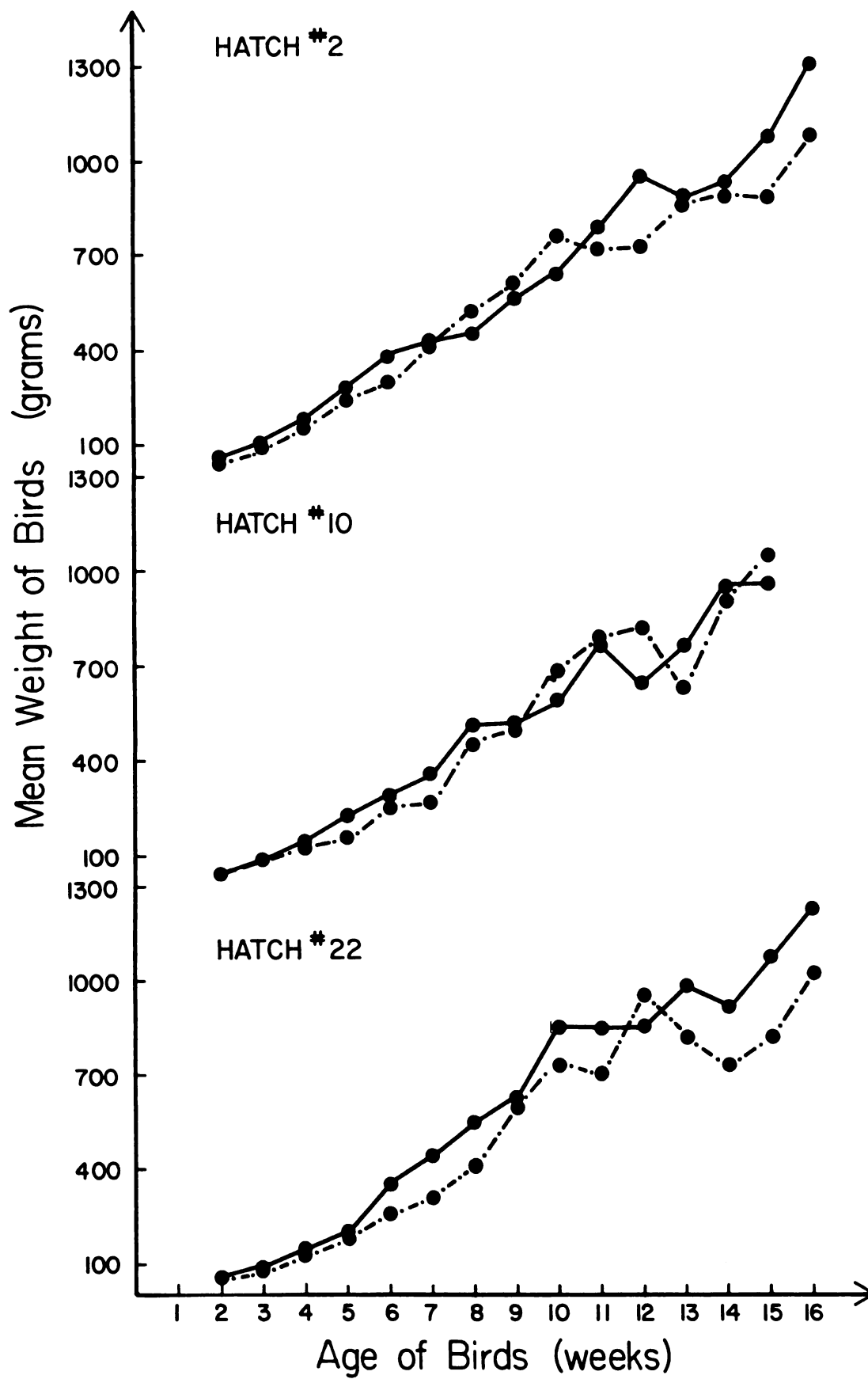
thin wall. Similar involvement of the caeca occurred on occasion. In the rectum, a thick, white, occasionally blood-tinged material was present.

Histopathology

Microscopic examination of the tissues showed a mild increase of red blood cells within the capillaries, infiltration of granulocytes and slight damage to individual epithelial cells in light coccidial infections of 10 or less parasitic forms per field with 10X objective (low power). Moderate infections of 10-30 parasitic forms per lpf (low power field) included a moderate increase in red blood cells between the villi, occasional red blood cells within the tissue and a marked increase in mononuclear and granulocytic leukocytes. Eosinophils were prominent in _E. phasiani_ infections, heterophiles in moderate infections with all species and lymphocytes in infections with more mature forms (gamonts and oocysts) of all species. Heavy infections with schizonts, gamonts and oocysts (greater than 40 parasitic forms per lpf) produced a marked increase in all blood cells with some loss of villi architecture and shredding of villi tips. Clubbing of the villi was occasionally noted. Some segments of the intestinal tissue from birds that had died of coccidiosis at the Dansville farm were fragile, necrotic and contained no observable parasitic forms. Fibrosis was observed occasionally in younger birds but more frequently in those 8 weeks and older.

Eimeria phasiani was identified in infections involving
the whole tip of a villus (Plate 1a) or those in which the
epithelial cells lining the villus were infected all the way
to the base (Plate 1b). All forms of the coccidium including
schizonts, gametocytes and oocysts were present. Infiltra-
tion of the tissues with eosinophils aided in the
confirmation. In fact, the presence of this granulocyte, in
the absence of coccidial forms, was considered suggestive
that E. phasiani had recently infected a bird or would be
found in another section of the gut. A finding of the
oocysts in the intestinal contents and/or the presence of
parasitic forms in another area of the gut substantiated this
conclusion.

Eimeria colchici was identified with certainty when
schizonts were present in the caecal glands and cautiously
when in the glands of the jejunum and ileum. In heavy
infections, E. phasiani can potentially invade this deep.
However, schizonts were observed infrequently in the glands
of the small intestines and the numbers were few. Concomi-
tant infections of the caecal glands (Plates 1c and 1d) led
us to conclude that they were the schizonts of E. colchici.
The white pasty material occasionally found in the caeca was
considered almost diagnostic of an ongoing infection or one
near its end.

Coccidial forms confined to the epithelial cells lining
the proximal or upper portion of the villi could have been

Plate 1a: Photomicrograph of a transverse section of the upper ileum showing *Eimeria phasiani* in various stages of development within the tip of a villus. 10X

Plate 1b: Photomicrograph of a transverse section of the jejunum showing *Eimeria phasiani* lining the sides of a villus to the base, various stages. 40X
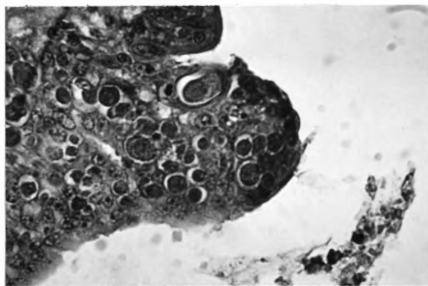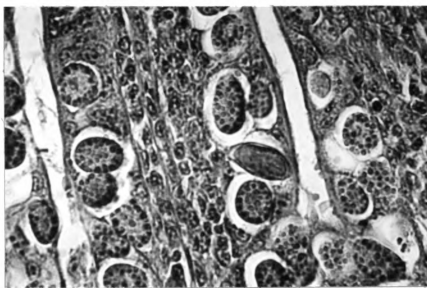
Plate 1a

Plate 1b

Plate 1c: Photomicrograph of a transverse section of the
caecum showing _Eimeria colchici_ in the glands. 10X

Plate 1d: Photomicrograph of a transverse section of the
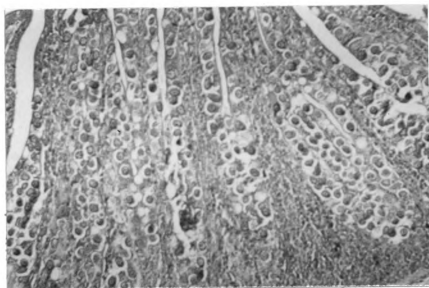caecum showing _Eimeria colchici_ in a gland. 40X

Plate 1c
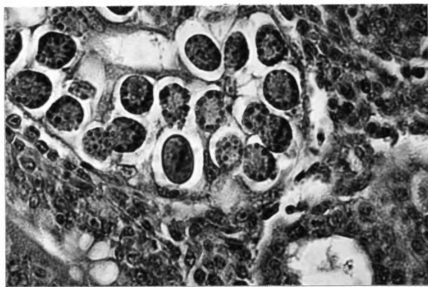
Plate 1d

often in mixed infections so that identification of each had to be made from the sporulated oocysts. Week 6 of hatch 2 was the only time that E. duodenalis was observed by itself. All coccidial forms were present in a position above the nucleus of the epithelial cells lining the upper half of the villi in the duodenum and the jejunum. It was concluded that these could very well be stages of E. duodenalis.

Epidemiology

Records of each coccidial species observed on a weekly basis show that E. duodenalis was present in both the study and control groups of each hatch for the same number of weeks (4, 5 and 3 weeks, respectively). The infections appeared no earlier than 5 weeks and were seen sporadically over the 18 weeks each hatch was studied. Eimeria duodenalis was the first species observed, which was in late April. Eimeria phasiani was seen most frequently. It affected birds of all ages, both groups, beginning at week 7 of hatch 2 and week 3 of hatches 10 and 22. It appeared with somewhat greater frequency in the study group than the control group. Another species frequently seen was E. pacifica. It appeared in hatch 2 at week 8 for both groups but in the ensuing hatches was present by week 3. It affected birds of all ages. On a weekly basis it was seen less often than E. phasiani, most notably in hatch 2, and somewhat less frequently in the control group than the study group. Eimeria colchici, the most pathogenic species for young pheasants, was seen rarely

in the control group of all hatches (0, 2 and 2 weeks, respectively) and seldom in the study group (1, 5 and 4 weeks, respectively). It was not until hatch 22 that younger birds were affected and then only at week 4, both groups, following movement into the 3B unit. Infections with this species were confined to birds housed indoors. It was not observed in either group previous to movement nor following movement into the North-D pen.

During the week previous to movement of the pheasants into the outdoors frequency and intensity of coccidial infections was usually low. _Eimeria_ _phasiani_ was in all infections and _E._ _pacifica_ was observed once. The week of movement, _E._ _phasiani_ infected the study group birds of all three hatches and the control group birds of hatches 2 and 22. _Eimeria_ _pacifica_ was not seen at all in hatch 10 but was present in the study group of hatch 2 and both groups of hatch 22. One week following transfer to the outdoor pen, both species were observed in all hatches, both groups and _Eimeria_ _duodenalis_ was present in both groups of hatches 10 and 22 as well.

Overall weight gain was greater in the control group of hatches 2 and 22 than in the study groups of the same hatches. In hatch 10, however, the weight gain for birds in the control group was lower. Individual weight gain losses and frank losses on a week-to-week basis tended to be associated with movement of older birds into a new environment (unit C and the North-D pen) and/or multiple infections,

usually of three species or more. An actual weight loss occurred in the control groups of hatches 2 and 10 and in the study group of hatch 22 at the time they were moved to the outdoors. The infections in the control birds were mild but those in the study group of hatch 22 were more severe and mixed.

Mortality records for control group birds indicate cause of death only when many died in the same period, e.g. 400 deaths in hatch 10 during weeks 1-3 due to wood-chip blockage and 95 deaths in hatch 22 during weeks 11 and 12 due to starvation. Cause of death while in the North-D pen was not reported, however, constant rains were recorded for each hatch during this period. The number of deaths were as follows: hatch 2 (128), hatch 10 (106) and hatch 22 (1428). Outdoor temperatures at the Dansville farm were not monitored but climatological data from the National Weather Service at the Lansing, Michigan Airport shows average ^{o}F temperatures of 69, 72 and 50, respectively, for the same periods. Maximum and minimum temperatures are shown in Table 4.

Table 4

Mean Temperatures During Confinement
to North-D Pen

Hatch	Maximum	Average	Minimum
2	79.8	69.2	57.8
10	83.2	71.9	60.2
22	60.2	49.5	38.6

Cause of mortality within the study group was determined by examination of all birds that had died at the farm. Table 5 shows the percentage of total deaths due to coccidiosis in birds 8 weeks or less and the percentage of the original 80 birds that died from coccidiosis. The remaining deaths were due to wood-chip blockage, starvation or bacterial infection.

Table 5

Frequency of Coccidial Infections in Birds
8 Weeks or Less

	Batch 2		Batch 10		Batch 22	
	Study	Control	Study	Control	Study	Control
Total # of Birds Examined	64	64	60	60	64	64
Total # of Birds with Parasite in Tissue	33	24	41	33	38	25
% of Total	52	38	68	55	59	39
# of Birds 8 Weeks or Less with Parasite in Tissue	12	11	21	19	23	15
% of Total	19	17	35	32	36	23

DISCUSSION

Coccidial Species

Identification of the four _Eimeria_ species was based primarily on oocyst dimensions and morphology. Usually one or more features of the sporulated oocysts were quite distinctive and the species thus readily identified. Similarities between two species in shape, size or cell wall characteristics necessitated the use of other criteria such as location within the gut, tissue and/or epithelial cell.

Microscopic examination of unsporulated oocysts made differentiation of _E_. _duodenalis_ and _E_. _pacifica_ difficult unless the material in which the oocysts were found was taken from the caeca. _E_. _duodenalis_ has not been reported in this area of the gut (Norton, 1967a) whereas _E_. _pacifica_ has (Ormsbee, 1939). Observation of sporulated oocysts revealed features distinctive for each species. The wall of _E_. _pacifica_ was thicker than that of _E_. _duodenalis_ and on occasion was striated. This feature was first reported by Wacha (1973) having been missed by Ormsbee (1939). Wacha found that these striations were not consistently present on all _E_. _pacifica_ oocysts which would explain findings of only a few striated oocyst walls. A light thinning of the inner wall near one end of the _E_. _pacifica_ oocysts gave the

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impression that a micropyle or operculum was present although in fact this species has neither. The wall of E. duodenalis oocysts was of an even thickness throughout. Although the sporocysts of both species has a "stieda" body that of the E. duodenalis sporocysts was very well defined. Wacha (1973) reports that this species also has a "substieda" body, which may aid in its distinctive appearance. Differentiation of these two species in any area of the gut other than the caeca was not possible by this investigator.

Eimeria phasiani and E. colchici oocysts, although similar in appearance, have characteristics individualistic enough to allow for differentiation. Our observations showed that both were elliptical in shape, but that E. colchici was usually longer and narrower. Its cell wall was thinner and the flattening on one side, an oocyst feature of both species, was more distinct. The sporocysts of E. colchici appeared more tapered even though both species have a well-defined "stieda" body.

Differentiation within the tissue was based on the general rule that E. colchici is a glandular parasite (Norton, 1967b) whereas E. phasiani is confined more to the epithelial cells covering the villi, often the tips (Trigg, 1967a). Two exceptions to this rule tend to complicate separate identification of the two species by an inexperienced investigator: (1) the large first-generation schizonts of E. phasiani locate in the glands of the upper

half of the small intestine where first and second generation schizonts of E. colchici may also be found and (2) the gametocytes and oocysts of E. colchici are found in the epithelial cells and lamina propria of the caecal villi where they may be mistaken for E. phasiani. Schizonts and gametocytes in both the lamina propria and epithelial cells lining the villi of portions of the gut other than the caeca were considered confirmation of an infection with E. phasiani. No stage of E. duodenalis invades the lamina propria and schizonts of E. colchici are found deeper in the tissue. All stages of E. pacifica have been reported (Ormsbee, 1939) to infect only the epithelial cells lining the villi throughout the small intestine and proximal half of the caeca. Identification of E. colchici was substantiated by the presence of schizonts in the caecal glands on six separate occasions: hatch 2 (week 11), hatch 10 (weeks 6, 7, 8 and 9) and hatch 22 (weeks 4 and 11). The oocysts were present in the intestinal contents without observable tissue involvement three times: hatch 10 (week 10) and hatch 22 (week 9 and 13). It was inferred that at these times the prepatent period had been concluded. The few infections seen were primarily in the study group (10:4).

Effects of Infection on the Pheasants

The infections seen for the most part were subclinical and mild. Damage to the intestinal tract occurred, but only on occasion were the resultant lesions life-threatening. The

numbers of parasites within the tissues were generally higher among young birds who tend to be more susceptible to infection than immune older birds (Long and Horton-Smith, 1968). Mortality among this age group, however, was relatively low except at week 2, an age when the birds are apt to incur a wood-chip blockage and/or become infected with E. colchici. This organism was not observed in the intestinal contents or tissues of the 2-week old birds we examined. None of the chicks appeared lethargic nor did they fail to gain weight. This does not rule out though the possibility that E. colchici was a cause of death since none of the dead birds at this age were made available to us for examination. It had been assumed they had died of wood-chip blockage.

An increase in mortality often occurred following spexing and movement into the C unit (week 7) and movement into the North-D pen (weeks 12 and 13). Usually an increase in the oocyst count paralleled the increased mortality suggesting that the deaths were related to coccidiosis, which was confirmed by parasitological and histopathological studies. Movement is stressful to the birds and may stimulate physiological changes within the pheasant which allow a pre-existing infection to intensify (see below).

All birds within the control group were receiving the anti-coccidial drug amprolium[+]. Trials have shown that amprolium is effective in the control of E. colchici

coccidiosis (Norton, 1967b). Amprolium[+], because of the synergistic effect of attentuating ethopabate with amprolium, could have a mode of action and efficacy different than that of amprolium alone. The drug did appear to be effective, though, against E. colchici a pathogen of 2-4 week old chicks. This organism was able to cause death in 100% of unmedicated birds with a dose of 80,000 oocysts (Norton, 1981) yet it was implicated in very few of the infections found in our control group birds and only once in chicks 2-4 weeks old (hatch 22, week 4). This may be an indication that the coccidiostat was able to prevent the parasite from developing to the oocyst stage thus reducing the number of oocysts in the environment and affording little opportunity for reinfection. A moderate number of infections caused by E. colchici were observed among our study group birds of hatches 10 and 22 while housed primarily in unit C. Only once was the parasite seen in hatch 2 which was one week previous to being moved outdoors from the same unit. It could be inferred that the parasite had been introduced into the building from an outside source while the pheasants of hatch 2 were housed in the C unit or that the coccidia had survived the winter. Although infections were often severe among the non-medicated birds, none were lethal indicating a good immunity had been established in these older birds.

Eimeria phasiani, a pathogen of young pheasants (Trigg, 1967b), was observed frequently throughout these studies.

Its first appearance in hatch 2 occurred while the birds were housed in the C unit but in hatches 10 and 22 it was first observed while the pheasants were in the A unit. The earlier appearance of the parasite in the later hatches may relate once again to management procedures. If the C unit was not disinfected as carefully as those units housing the younger pheasants, then oocysts may have survived the winter. Introduction of oocysts into units A and B by workers, once the rearing had begun, would then account for the appearance of _E. phasiani_, and possibly other species in young chicks of the summer and fall hatches. The appearance of _E. phasiani_ in pheasants of all ages is indicative of the mediocre protection afforded by the coccidiostat against this species. Since the mortality rate among both groups was relatively low while the pheasants were housed in the A and B units, it seems likely that the coccidiostat played no more than a minor role in controlling the disease. A low to moderate number of available oocysts was probably a more important factor (Long, 1973) a condition that could be related to immunity as the birds aged.

No drug trials with _E. pacifica_ have been reported in the literature. This species was also seen frequently which would suggest that amprolium[+] may not be the drug of choice. Infections in which _E. duodenalis_ was one of the causative agents were few to moderate in number. They occurred in study group birds and control group birds with nearly the

same frequency, which once again suggests better control through immunity than the coccidiostat.

Several investigators have studied the effect of host age on the reproductive and pathogenic potential of _Eimeria_ species (Rose, 1973; Krassner, 1961; Tyzzer, 1929; and Horton-Smith, 1947). Conflicting results indicated greater susceptibility in both younger and older birds. Long and Horton-Smith (1968) provided some clarification of the problem by suggesting that susceptibility to clinical disease was greater in young birds while susceptibility to oocyst reproduction was greater in older birds. Our data indicates greater number of developing coccidia in the tissue, lower mortality and less weight-gain reduction in birds 2-6 weeks of age. The number of infected birds increased between hatches 2 and 10 which suggests that the number of oocysts within the unit was increasing. This could be due to contamination from another source, increased number of shed oocysts and/or conditions within the unit more favorable to oocyst development and survival such as increased humidity and temperature. The number of parasites seen in these young pheasants, although many, was still not sufficient to cause clinical coccidiosis. Environmental conditions within the A and B units where birds of this age were housed were perhaps less stressful or birds of this age may not be as susceptible to the effects of stress. The less severe infections seen in the older birds could have been related to oocyst dose sizes

that were smaller on a relative basis (# of oocysts/# of host tissue cells) and an immunity that should have been established by this age. Movement of the older birds appeared to be the most critical factor in the development of disease as judged by weight-gain reduction, weight loss, elevated oocyst counts and increased mortality during these periods. Stress-related immuno-suppression would account for the dramatic increase in susceptibility among these otherwise resistant hosts.

The Pheasants in the North-D Pen

Eimeria phasiani was observed in all coccidial infections during movement and confinement of the pheasants to the North-D pen. _Eimeria pacifica_ was seen in 50% of the groups within 2-3 days and, by the second week, _E. duodenalis_ was present in all groups except those of the spring hatch. The pathogenicity of _E. pacifica_ is unknown, but its potential as a contributor to the coccidial-related deaths that occurred with increasing frequency from spring to fall is suggested by its appearance in many of the diseased birds. Its action may be an enhancement of biological alterations known to occur within the host as a result of stress-induced coccidiosis or it may help to impair development of immunity, a phenomenon of mixed coccidial infections suggested by Rommel (1970c). There is little doubt, though, that _E. phasiani_ is the species primarily responsible for the high mortality rate during periods of hard rains among pheasants

confined to the North-D pen. Reported to be a pathogen of younger pheasants (Trigg, 1967b), _E. phasiani_ would probably inflict only a moderate degree of damage were the birds not severely stressed.

Hill (1983) writes that physical stress can effect within an animal a chain reaction of biochemical events terminating in suppression of the immune system and an altered glycemic state. The initial stimulus for these complex changes is increased activity of the sympathetic nervous system which affects many functions of the body. Three of these have been related to the General Adaptation Syndrome, which is a group of different stress-related physiological reactions: (1) stimulation of the adrenal medulla with resultant increase in norepinephrine and epinephrine (Zachariasen and Newcomer, 1975), (2) stimulated release of pancreatic glucagon (Freeman and Manning, 1976) and (3) release of adrenocorticotrophic hormone which acts on the adrenal cortex to increase production and secretion of corticosterone. The key role of corticosterone in stress is suppression of the immune system by its depressive effect on the number of circulating leukocytes and on antibody form-ation. As a glucocorticosteroid, corticosterone also acts to a very minor degree with the other two stimulated hormones to ultimately increase blood glucose concentration.

Hyperglycemia has been reported to be a consequence of infection by Pratt (1940) and Herrick (1950), but in severely

diseased birds some investigators have found the reverse. In their study on the physiological basis of mortality among _Eimeria_ _tenella_ infected chickens, Witlock et al. (1981) reported that hypoglycemia was one of the diagnostic features of incipient death. Freeman and Manning (1976) found that birds became hypoglycemic 30 minutes after they were stressed by handling.

A consequence of hypoglycemia is hypothermia, glucose metabolism being essential for maintenance of body temperature (Freeman, 1971a). Herrick (1950) reported that coccidia-infected birds subjected to a cold environment experienced a decreased metabolic rate and a drop of $20^{\circ}C$ in body temperature. Neither Keener (1963) nor Witlock et al. (1981) were able to substantiate these findings; however, they did find that hypothermia was another clinical sign of impending death among infected birds. The condition of hypothermia in birds dying from coccidiosis may be physiologically-based then rather than environmentally induced.

The sequence of events leading to the high death rate among those pheasants confined to the North-D pen may theoretically occur in a somewhat complex manner. As a consequence of the high number of oocysts washed down into the pen, pheasants newly introduced to this area would in a few days ingest high doses of them. The infection level would increase both in numbers and intensity. Coccidia

utilize considerable quantities of carbohydrate during their metabolism (Ryley, 1973) thus a transient reduction in blood glucose concentration may be experienced by these hosts. Hypoglycemia is another pathway for stimulation of the sympathetic nervous system (Guyton, 1963). In this manner, glucose is released from glycogen stores and metabolized from other sources for the energy required during repair of tissue and body temperature regulation. Hyperglycemia would ensue. If at this point the animals were stressed by hard rains, increased corticosterone would suppress the immune system. The coccidia would then be free to reproduce unhampered, overwhelmingly the activity of the medication and inflicting irreparable damage to the intestinal mucosa as was seen in birds that died at the farm following hard rains. Proteins, fluids, nutrients and blood would be lost. Glucose would be utilized rapidly to meet energy requirements of the host while little or none could be absorbed from the intestines leading to hypoglycemia and eventual hypothermia. Both of these conditions, as well as the diminished renal function noted by Witlock et al. in dying birds, are signs of shock resulting from reduced cardiac output (Guyton, 1963). It may be concluded then that the pheasants are dying from irreversible shock enduced by the sequence of events following the stress of exposure to hard rains. Environmental temperatures, although not responsible for the initial hypothermia, may, if low, intensify a state of reduced body temperature.

Guyton (1963) reports that during shock the body temperature tends to decrease even more if the subject is exposed to even the slightest cold. This would support Herrick's findings and may explain, in part, the higher mortality rate among the pheasants in the cooler months.

Summary

1. Four species of coccidia were found to infect the pheasants: _Eimeria_ _duodenalis_, _E_. _phasiani_, _E_. _pacifica_, and _E_. _colchici_.

2. Most infections were mixed but usually did not progress to the disease state.

3. The infection level was more intense in younger birds while mortality was higher in older birds.

4. Infection level and mortality were higher in the non-medicated group.

5. As the seasons progressed from spring through fall, infections were incurred at younger ages and by more of the pheasants.

6. Mortality was highest in both groups following movement into the C unit and the North-D pen.

7. _Eimeria_ _colchici_, a coccidial parasite of pheasant chicks 2-4 weeks old, was rarely seen in birds of this age.

8. _Eimeria_ _phasiani_ was seen most frequently and appeared to be the species with the most serious effect on the pheasants once transferred to the outdoors.

9. *Eimeria* *pacifica* was also observed frequently but pathogenicity studies are required before its potential as a cause of death among the pheasants can be truly ascertained.

10. The coccidiostat amprolium[+] appeared to be quite effective against *E*. *colchici* but only mildly so against the other three species.

11. Hard rains are stressful to the pheasants who tend to be more heavily dosed with oocysts while in the North-D pen thus loss of immunity is of greater consequence.

12. Cooler temperatures could increase the death rate among birds stressed by hard rain.

RECOMMENDATIONS

1. Medicate the birds with a new drug that maintains the efficacy against *E. colchici* while being effective against *E. phasiani* and *E. pacifica*.

2. Reduce the number of oocysts in the initial range pens, e.g. the North-D pen, either by the addition of a drainage system or by treatment of the soil with a chemical that is destructive to coccidial oocysts without adversely affecting the pheasants.

3. Clear the field of all vegetation or other matter that might provide the oocysts shelter from the sun. It is understood that this cannot be entirely effective since the buildings which are close to these pens shade the area rather extensively.

4. Routinely increase the drug dosage for the two weeks that the pheasants are in these pens.

APPENDIX

APPENDIX

1. Additional information on management techniques at the Dansville farm.

   a. Any individual entering an inhabited indoor unit is required to place disposable plastic boots over his/her footwear then step into a liquid disinfectant.

   b. Previous to transfer of the pheasants into the largest indoor unit (C), plastic blinders called "spex" are placed on the bird's beak with a plastic pin inserted through the nasal passage to hold the "spex" in place. These restrict forward vision, thereby reducing the tendency of feather-picking and cannibalism. The "spex" are removed just before the birds are released. (Martin Pollok, "Pheasant Rearing," Michigan Department of Natural Resources.)

   c. Transfer of the pheasants from the largest indoor unit into the first range pen is done at night so that the pheasants can adjust gradually to the change in light.

2. Amprolium[+]: Amprolium attenuated with ethopabate.

3. Modified McMaster's formula for calculating # of oocysts/gm feces.

   a. 2 gm feces + 28 ml saturated zinc sulfate=30ml total volume of sample

   b. 0.15 m./sq.cm in counting chamber

   c. 2 sq.cm counted

   d. formula

$$\frac{\text{\# of oocysts counted}}{(0.15\text{ml/sq.cm})(2\text{sq.cm})} \times \frac{30\text{ml total volume}}{2\text{gm feces}} = \text{\# of oocysts/gm feces}$$

OR

# of oocysts counted X 50 = # of oocysts/gm feces

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VITAE

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