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The Use of a Bacterin Vaccine in Broiler Breeders in the Control of *Ornithobacterium rhinotracheale* in Commercial Broilers

by

Shahn Philip Roosegaarde Bisschop

Submitted in fulfilment of part of the requirements for the degree of Master of Science

Department of Veterinary Tropical Diseases

Faculty of Veterinary Science

University of Pretoria

2003

Supervisor : Professor Moritz van Vuuren

Co-supervisors : Professor Bruce Gummow

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SUMMARY

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Respiratory disease complex is a major cause of mortality and economic losses in the commercial broiler industry. In 1991 a previously unidentified bacterium associated with respiratory disease and cranial cellulitis was isolated from broilers in the then Transvaal Province (van Beek, van Empel, van den Bosch, Storm, Bongers, du Preez, 1994.). In 1994 the organism was

named *Ornithobacterium rhinotracheale* (Vandamme, Segers, Vancanneyt, van Hove, Mutters, Hommez, Dewhirst, Paster, Kersters, Falsen, Devriese, Bisgaard, Hinz, Mannheim, 1994.). Since then *Ornithobacterium rhinotracheale* has been isolated worldwide from chickens and turkeys showing respiratory signs and has become well established as contributing to the respiratory disease complex in both species (van Empel, Hafez, 1999).

In South Africa respiratory disease and *Ornithobacterium rhinotracheale* in particular is routinely controlled by the inclusion of antibiotics such as Oxtetracycline into the feed of broilers during rearing. Concerns about antibiotic residues in poultry meat for human consumption as well as evidence that suggests that *Ornithobacterium rhinotracheale* readily develops resistance to antibiotics (Devriese, Hommez, Vandamme, Kersters, Haesebrouck, 1995), make this strategy unsustainable.

It was with a view to reducing producers' dependence on long term prophylactic antibiotic therapy that this study to determine the safety and efficacy of an OR bacterin vaccine was carried out. Injection of the bacterin into broilers was deemed impractical on a commercial scale, so it was applied to broiler breeder parent stock in order that they could protect their progeny through vertically transmitted immunity developed as a result of vaccination. Breeder flocks were vaccinated intramuscularly at nine and 18 weeks with a monovalent bacterin based on OR serotype A with oil adjuvant.

Vaccine safety was evaluated by palpation of vaccination sites and clinical observation of breeders for two weeks after vaccination. The serological response of breeders to vaccination was monitored using an ELISA test for *Ornithobacterium rhinotracheale* optimised for use under South African conditions. Vaccine efficacy was determined by monitoring of broiler progeny of vaccinated breeders raised under commercial conditions as well as through controlled challenge studies with *Ornithobacterium rhinotracheale* under laboratory conditions. In order to determine the financial consequences of using the test vaccine, a partial farm budget was drawn up from available broiler data and possible outcomes were modelled using a stochastic model.

The vaccine proved to be safe for use in commercial broiler breeders and vaccinated birds developed a good humoral response to vaccination. As a result of cross-contamination of isolators with *Ornithobacterium rhinotracheale* the results of the challenge studies were inconclusive. No evidence of protection of broiler progeny of vaccinated breeder flocks could be detected through the challenge trials. In the absence of in-feed medication, broilers hatched from vaccinated breeders did, however, performed better under commercial conditions than those hatched from unvaccinated breeder flocks.

The partial farm budget showed that broilers raised from OR vaccinated breeder flocks were more profitable than the negative control flocks. The quantitative risk analysis showed that the probability of making a relative profit from broilers as a result of OR vaccination of parent stock was 74%, from the use of in-feed medication in broilers from unvaccinated parents was 70% and

from a combination of the interventions was 99%. It can be concluded that the last of these options was most profitable.

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Funding for the project was received from the National Research Foundation of South Africa as well as from Intervet International. Technical assistance was provided by Dr. Paul van Empel of Intervet with patience and humour over five years.

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Chapter I – Introduction

Avian respiratory disease is a significant cause of mortality as well as morbidity in commercial poultry worldwide, and a major cause of economic losses. The aetiology of respiratory disease is complex and frequently multifactorial, involving infectious agents as well as managemental and environmental factors. Infectious agents include a wide range of fungi, viruses, bacteria and mycoplasmas.

The following micro-organisms are known to play a role in avian respiratory disease; the fungi *Aspergillus fumigatus* and *Aspergillus flavus;* the viruses infectious laryngotracheitis virus (a herpesvirus), infectious bronchitis virus (a coronavirus), Newcastle disease virus (an avian paramyxovirus type 1), avian paramyxovirus types 2, 3 and 6, influenza A viruses, reovirus and turkey rhinotracheitis virus (a pneumovirus) as well as the bacteria *Escherichia coli, Haemophilus paragallinarum, Pasteurella multocida, Riemerella anatipestifer, Bordetella avium,* and the mycoplasmas *Mycoplasma gallisepticum, Mycoplasma synoviae, Mycoplasma meleagridis* and *Mycoplasma iowae* (van Empel, 1998).

In 1991 a previously unidentified bacterium associated with signs of respiratory disease was isolated from the airsacs of broilers in South Africa (Van Beek *et al.,* 1994). It was also isolated in Germany (Hafez, Kruse, Sting, 1993) and in the United States of America in 1993 (Charlton, Channings-Santiago, Bickford, Cardona, Chin, Cooper, Droual, Jeffrey, Meteyer, Shivaprasad, Walker, 1993).

The organism was named *Ornithobacterium rhinotracheale* (OR) in 1994 (Vandamme *et al.,* 1994). Since then the organism has been successfully isolated from chickens and turkeys worldwide, a few isolates have also been made from wild birds in Europe. While OR is often associated with outbreaks of respiratory disease, its exact role in the respiratory disease complex remains unclear (van Empel *et al.,* 1999). The control of OR by means of prophylactic antibiotic treatment, often by means of tetracyclines through the feed, has become widespread in South Africa. In view of growing consumer concern about antibiotic residues in food products for human consumption as well as evidence to suggest that OR rapidly acquires resistance to antibiotics, this approach is clearly not sustainable (Devriese, Hommez, Vandamme, Kersters, Haesebrouck, 1995).

Work in the Netherlands led to the development of an OR bacterin vaccine.
As injection of a bacterin vaccine into broilers is impractical on a large scale, the vaccine was applied to broiler breeder pullets during rearing with the aim of stimulating a strong immune response in the hens that could be transferred to their progeny transovarially. Previous work done under controlled conditions in the Netherlands indicated that breeder hens were able to transfer a significant and protective immunity to their broiler progeny after vaccination with the bacterin (van Empel, van den Bosch, 1998).

The objective of this study was to confirm the safety and efficacy of the OR bacterin under large-scale commercial conditions in South Africa and to determine the potential financial benefits of replacing in-feed tetracycycline medication with OR vaccination.

Chapter II - Literature Review

1. THE HISTORY OF *ORNITHOBACTERIUM RHINOTRACHEALE*

A new respiratory disease in broilers was first observed in 1991 in South Africa by du Preez (Van Beek *et al.*, 1994). Mild respiratory signs starting at about 28 days of age and lasting to the end of the fattening period, together with increased mortality and poor growth were observed. On post mortem examination a foamy "yoghurt-like" exudate was found in the air sacs, predominately in the abdominal sacs; pneumonia was also described. Bacteriological examination revealed a slow growing, capnophilic, Gram-negative, pleomorphic rod, which could not be classified as any known bacterial species (van Empel, 1998).

In 1990 and 1991 an unidentifiable pleomorphic Gram-negative rod, was isolated from 41 cases in turkeys and chickens (as well as four from other avian species) submitted to the laboratories of the California Veterinary Diagnostic Laboratory System. Most isolations were made from the respiratory tract and were commonly associated histologically with a fibrinopurulent inflammation of the respiratory tissue. In 93% of the cases there were other concurrent bacterial infections (Charlton *et al.*, 1993).

In 1992 bacterial isolates, later shown to be *Ornithobacterium rhinotracheale*, were obtained in Germany from 13-week-old turkeys with respiratory problems, pneumonia and increased mortality (Hafez *et al.,* 1993).

Various cases of respiratory problems in meat turkeys and broiler chickens were reported from the Southern Netherlands starting in the middle of 1993. A pleomorphic Gram-negative rod was also isolated from many of these cases. Not all the strains mentioned in these reports reacted with antisera against the South African, German and Hungarian strains, but the appearance, odour and biochemical reactions of all strains were identical (van Beek *et al.,* 1994).

Initially the organism was described as *Pasteurella*- like or *Kingella* –like (Charlton *et al.,* 1993; Cook, Ellis, Huggins, 1991). In 1994 it was initially proposed that the organism be named *Ornithobacterium rhinotracheale* gen.nov.sp. or “Taxon 28”. Later in 1994 the organism was formally described and given the name *Ornithobacterium rhinotracheale* (OR) (Vandamme *et al.,* 1994).

Once the organism had been identified work was done to establish the origin of the organism. A *Pasteurella*-like organism isolated from ducks with respiratory disease in Hungary in 1987 also proved to be OR (van Empel, 1998). Investigation of culture collections in Germany revealed that OR had already been isolated from the respiratory tract of turkeys in 1981 and of rooks in 1983

(Vandamme *et al*., 1994). In Belgium, France and Israel (Bock, Freidlin, Manoim, Inbar, Frommer, Vandamme, Wilding, 1997) OR had also been isolated before 1990. No isolates of OR from before 1981 have been reported (van Empel, 1998).

By 1997 OR had been isolated from all American states with significant poultry populations (Schleifer, 1997b). The disease was reported in broilers in Egypt in 1997 (Elgohary, Awaad, 1998), turkeys in Canada in 1999 (*Ornithobacterium,* 1999), in broilers in Japan in early 1999 (Sakai, Tokuyama, Nonaka, Ohishi, Ishikawa, Tanaka, Taneno, 2000) and in turkeys in Slovenia in June 1999 (Zorman-Rojs, Zdovc, Benčina, Mrzel, 2000).

2. CLINICAL SIGNS

2.1 Turkeys

In turkeys, OR outbreaks have most frequently been observed in male birds over 14 weeks of age and there is reported to be an age susceptibility to the disease with particularly high mortality in turkey toms older than 20 weeks. However, in many cases young poults between the second and eighth week have also been found to be affected, although in these cases the disease may remain subclinical. OR outbreaks are more frequent during winter months – probably a result of poorer ventilation and higher ammonia and dust levels at this time of year (Hafez *et al.,* 1993; Schleifer, 1997b).

Mortality ranges between 1-10% during the acute phase of the disease. Initial signs are coughing, sneezing and nasal discharge followed in some cases by severe respiratory distress, dyspnoea, prostration and sinusitis. The signs are accompanied by a reduction in feed consumption and water intake. In turkey breeder flocks, the disease is associated with drops in egg production and an increase in the numbers of unsettable hatching eggs. In general the disease runs its course in 7-10 days. Clinical signs may only be noticed shortly before death (Hafez, 2000a; Schleifer, 1997b).

2.1.1 Case Studies in Turkeys

A 1992 outbreak of OR in Germany in 23-week-old meat turkeys was described (Hinz, Blome, Ryll, 1994). The birds had been vaccinated against Newcastle disease and *Pasteurella multocida,* and were serologically negative for *Mycoplasma spp.* In this outbreak, a mortality of 5.6% with a morbidity of 16.6 % was reported. Clinical signs were seen only a few hours before death in untreated cases and included weakness, marked dyspnoea, gasping and expectoration of blood-stained mucus. Cyanosis of the bare area of the head occurred immediately before the birds died. The authors report that OR was first isolated in their laboratory in 1981, from five-week-old turkeys with lesions in the respiratory tract.

A series of OR outbreaks occurred on three nearby breeder ranches in the USA in 1996. In these cases increased mortality, especially in stressed or very heavy birds was reported. Birds showed respiratory signs and a drop in egg production. *Escherichia coli* (*E. coli*) was also isolated from most affected birds (De Rosa, Droual, Chin, Shivaprasad, Walker, 1996).

In the American mid-west an OR outbreak in 22-week-old tom turkeys shortly before processing was reported. Birds were depressed and coughed, blood was visible around the beak and nares of some depressed and dead birds. Mortality as a result of the disease reached 5.9% within a few days, before the flock was slaughtered. Other 14 to 22-week-old flocks were also affected by the disease and in these flocks mortality was frequently associated with pneumonia (Roepke, Back, Shaw, Nagaraja, Sprenger, Halvorson, 1997).

In an OR outbreak in turkeys in Quebec, Canada three large white hybrid turkey layer flocks experienced a severe respiratory condition at 52 weeks of age. Birds showed signs of depression, dyspnoea, coughing, nasal discharge and, in some cases, sudden death. During the most severe period, mortality reached 1.4% -7% per week in each of the affected flocks of turkey hens. A significant drop in egg production occurred in each flock for at least four weeks (Joubert, Higgins, Laperle, Mikaelian, Venne, Silim, 1999). In an outbreak of OR in 14-week-old tom turkeys in Ontario, in December 1998, there was a sudden increase in mortality accompanied by a severe snick and reduced activity (*Ornithobacterium,* 1999).

Turkeys in Israel infected with OR typically showed signs of an acute exudative pneumonia (Bock *et al.,* 1997). A 1999 outbreak of OR (concurrently with a *Mycoplasma gallisepticum* infection) in turkeys in Slovenia conformed largely to descriptions elsewhere. In this case the infection started in 10-week-old toms and spread to hens in a nearby house. Morbidity of 50% was reached among the toms and 20% in the hens. Mortality remained below 1% (Zorman-Rojs *et al.,* 2000).

2.2 Broilers

Clinical signs in broilers generally appear between the third and seventh week of age, with a mortality rate of 2-10%. Clinical signs include depression, decreased feed intake, reduced weight gains, nasal discharge, sneezing and facial oedema. Respiratory viruses such as Newcastle disease virus or infectious bronchitis (IB) virus appear to aggravate the severity of the signs (Hafez 2000, Travers, 1996). OR is more frequently linked to problems during winter months (Schleifer, 1997a). It has been associated with increased condemnation of broiler carcasses in the Netherlands (van Veen, Gruys, Frik, van Empel, 2000).

2.2.1 Case Studies in Broilers

OR outbreaks in Egypt have been associated with respiratory signs, depression, anorexia and growth retardation, as well as increased mortality (Elgohary, Awaad, 1998).

Clinical signs described in Europe include mucous discharge from the nostrils, wet eyes and swelling of the infra-orbital sinuses as well as severe growth retardation. In certain cases there was persistent mortality despite medication. In broilers mild to moderate respiratory problems as well as acute deaths were observed (van Empel *et al.*, 1999).

In South Africa, OR has been associated with significant economic losses as a result of condemnations at processing due to chronic airsacculitis and peritonitis. Travers *et al.* (1996) described “three OR associated syndromes” ; the first being primarily an upper respiratory tract syndrome, the second showing few respiratory signs, but a severe peritonitis at post mortem examination, in the third, respiratory signs as well as arthritis and lameness were observed. In all cases there was a poor average daily gain and feed-conversion rate.

OR was isolated from eleven broiler cases showing respiratory signs in the Delmarva Peninsula in the USA. The clinical disease and signs were similar to those described elsewhere. In most cases there was evidence (either serological or from viral isolation) of infectious bronchitis (IB) involvement in conjunction with OR. *E. coli* was also isolated from seven of the eleven cases examined – suggesting that OR is merely a component of the respiratory disease complex in chickens (Odor, Salem, Pope, Sample, Primm, Vance, Murphy, 1997).

In Japan OR has been associated with respiratory disease in seven to eight week old broilers. The disease started with mild sneezing, diarrhoea with green faeces and head tremors and an increased mortality of more than 10% (Sakai *et al.,* 2000).

2.3 Broiler Breeders

The disease primarily affects birds at the peak of egg production, or soon before entering production – mostly between the 24th and the 52nd week of age. Before clinical signs are detected, a slight increase in mortality and decrease in feed intake, may be observed. The first clinical indication of the disease is mild respiratory signs. The mortality is variable and relatively low in uncomplicated cases. The signs are generally accompanied by a drop in egg production, decrease in egg size and poor egg shell quality. Fertility and hatchability are often unaffected (Hafez, 1996). OR was isolated from dead embryos as well as day-old-broiler and turkey chicks in hatcheries in Egypt suffering from a problem of increased embryonic mortality (Elgohary, 1998).

3. PATHOLOGICAL LESIONS ASSOCIATED WITH OR INFECTION

The pathology in cases of OR largely reflects the clinical condition.

3.1 Turkeys

In turkeys OR has been associated with fibrinopurulent pneumonia, sinusitis, tracheitis, airsacculitis, hepatomegaly, splenomegaly and pericarditis (Hafez *et al., 1993;* Back, Rajashekara *et al.,* 1998; De Rosa *et al.,* 1996; Joubert *et al.,* 1999; *Ornithobacterium 1999;* Roepke *et al.,* 1998; Zorman-Rojs *et al.,* 2000).

The most significant lesions associated with the disease are usually seen in the lungs. Lungs are congested, oedematous and frequently consolidated due to pneumonia, either uni-or bilaterally. A fibrinous exudate on the pleura is frequently described. Histologically, there is a fibrino-heterophilic exudate in the lung tissue as well as in the lumen of the parabronchioles (De Rosa *et al.,* 1996). Airways may contain a variable amount of blood. (Hinz *et al.,* 1994; Joubert *et al.,* 1999) Randomly distributed foci of coagulative necrosis seen in the lungs are associated with thrombosis of blood vessels. Bacteria may or may not be observed in the exudate (Joubert *et al.,*1999).

There may be severe congestion of the blood vessels of the nasal sinuses. (De Rosa *et al.,* 1996). In severe cases the birds' heads may be cyanotic (Zorman-Rojs *et al.,* 2000). Airsacculitis is sometimes reported, with the thoracic airsacs usually more severely affected than the abdominal airsacs (*Ornithobacterium*, 1999; Joubert *et al.,* 1999). Airsacs are thickened because of the fibrinous exudate and some birds have caseous exudate in the lumen of the airsacs (De Rosa *et al.,* 1996; Zorman-Rojs *et al.,* 2000).

Hepatomegaly is associated with congestion and, in some cases, acute coagulative necrosis of hepatocytes resulting from thrombosis of blood vessels, especially at the periphery of the liver lobes. Splenomegaly is an occasional finding that results from an accumulation of serofibrinous exudate in the vascular sinuses (De Rosa *et al.,* 1996). Pericarditis may be associated with petechial haemorrhages on the epicardium (De Rosa *et al.,* 1996; Zorman-Rojs *et al.,* 2000). Exudate may be found in the joints and is associated with fibrinoheterophilic inflammation of the synovium (De Rosa *et al.* 1996).

Table 1. Organs affected in natural outbreaks of OR in turkeys

Author	Species	T	Lu	As	Li	Sp	Ov	Ki	J	Pc
De Rosa	Turkey (27-42weeks)		+	+	+	+		+	+	+
Anonymous	Turkey (14 weeks)		+	+	+					+
Hinz	Turkey (23 weeks)	+	+	+						
Joubert	Turkey (52 weeks)	+	+		+	+	+			
Pages-Mante	Turkey (general)		+	+					+	
Roepke	Turkey (22 weeks)		+		+					
Zorman-Rojs	Turkey (10 weeks)		+	+	+	+				+

Key : T-trachea, Lu-lungs, As-airsacs, Li-liver, Sp-spleen, Ov-ovary, Ki-kidney, J-joints, Pc-pericardium.

3.2 Chickens

In chickens infected with OR, lesions include sinusitis, tracheitis, pneumonia, airsacculitis, pericarditis, hepatomegaly and splenomegaly – the most striking lesions are associated with the respiratory tract (Elgohary, Awaad, 1998).

Odor *et al.* (1997) reported in cases of concomitant OR and IB infections in Delmarva in which a profuse yellow to white, foamy airsacculitis containing “islands” of caseous debris was observed. This type of exudate is described by some authors as “yoghurt-like” (Hafez, 1996; Sakai *et al.,* 2000). Odor also described pleuropneumonia, frequently unilateral – these lesions are similar to lesions described from Holland and South Africa (van Beek *et al.,* 1994). Degeneration of heart muscles has been observed (Hafez *et al.,* 1993).

Travers *et al.* (1996) described an outbreak of OR associated with Newcastle disease and severe respiratory signs. It is difficult to determine which lesions in this case were as a result of the viral infection and which were associated with OR.

Table 2 . Organs affected in natural outbreaks of OR in broilers

Author	Species	T	Lu	As	Li	Sp	Ov	Ki	J	Pc
Elgohary	Broilers (not specified)	+	+	+	+	+				+
Hafez	Broilers (general)		+	+	+	+				
Odor	Broilers (various)	+	+	+						
Pages-Mante	Broilers (general)		+	+						
Sakai	Broilers (7-8 weeks)			+						
Travers	Broilers (4 weeks)	+		+						

Key : T-trachea, Lu-lungs, As-airsacs, Li-liver, Sp-spleen, Ov-ovary, Ki-kidney, J-joints, Pc-pericardium.

4. EXPERIMENTAL INFECTION WITH OR

Clarity on the precise role played by OR in the avian respiratory disease complex has not yet been achieved. In experimental OR challenge trials in turkeys and broilers (Van Beek *et al.,* 1994) 10^9 colony forming units (CFUs) were injected directly into the birds' airsacs. Fourteen days after challenge there was a significant growth depression in both groups but re-isolation of the bacterium proved difficult. OR could only be re-isolated from joints showing signs of arthritis. Van Empel (1996a) attempted to reproduce the disease through challenge of turkeys and broilers. When the organism was inoculated into the airsacs of turkeys, there was no mortality, clinical signs or macroscopic lesions at necropsy, but significant growth retardation was recorded. OR could be re-isolated from the brain and the heel joints. When challenged by the aerosol route, 14-day-old

turkeys showed severe airsacculitis seven days after challenge, but no growth retardation. Thirty-one-day-old turkeys under the same challenge conditions showed growth retardation, but airsacculitis was induced only when birds were additionally challenged with turkey rhinotracheitis virus (TRTV) (van Empel *et al.* 1996).

In the case of broilers challenged by the aerosol route at fourteen days, there was a significant decrease in daily weight gain and mild post mortal changes included airsacculitis and exudative tracheitis. The concomitant administration of infectious bronchitis virus (IBV) and to a lesser extent TRTV had an aggravating effect on the development of airsacculitis. The combined administration of OR and Newcastle disease virus (NDV) resulted in more severe pathology than challenge with either single agent. The pneumonia induced in these challenges was usually unilateral with a clear boundary between the affected and unaffected parts of the lung. In turkeys severe fibrinous exudate was found and airsacculitis was characterized by a foamy exudate with large fibrin clots. Bacteria could be reisolated from affected organs (van Empel *et al.,* 1996).

Experimental inoculations into the infra-orbital sinus by Buys (reported in Travers *et al.,* 1996) with South African isolates, resulted in sinusitis with one of the isolates used.

After inoculation of 10^7 CFUs into the caudal airsacs of broilers, Travers (1996) was able to produce clinical disease characterized by respiratory signs, arthritis and peritonitis. He was able to re-isolate the bacterium from the infra-orbital sinuses, the lungs, air sacs and hock joints as well as from the brain in one case. He concluded that there were differences in the pathogenicity of OR strains in so far as the severity of clinical signs was concerned. This could not be confirmed statistically in terms of variation in the growth rates of the birds. He did show, however, that birds infected with OR by inoculation showed growth retardation. After his 1996 efforts at experimental induction of disease with OR van Empel *et al.* (1996) concluded that strains isolated from either turkeys or chickens were equally able to infect both species, and possibly also other avian species.

Travers (1996) reported on the isolation of OR from one of five houses affected by an outbreak of velogenic ND. He indicated that mortality in the house from which the OR was isolated was 7-13% higher than in houses where OR could not be isolated.

Reports from the U.S.A. are largely similar to those from Europe and South Africa – although most work was done on turkeys. In Minnesota, Back and Rajashekara *et al.* (1998) were unable to reproduce respiratory disease by challenging 56-week-old turkeys with OR. The organism could, however, be detected by immunofluorescent antibody assay (IFA) in the trachea, lungs, liver, ovary, oviduct and spleen but not in the intestines or kidneys. OR was also re-isolated

from these organs in some cases. These findings suggest that the organism is able to spread systemically (Back, Rajashekara *et al.,* 1998).

Franz conducted experiments which showed that OR alone was unable to induce disease in broilers. In conjunction with infectious bursal disease, chicken anemia virus and IBV a higher incidence of respiratory lesions occurred. (reported by Schleifer, 1997b).

In Egypt, challenge studies were done in 2 week-old-broilers with OR alone and also a combined infection with *E.coli*. The combined infection resulted in more severe pathology than in the pure OR infection. The most prominent signs were observed between 3 and 4 weeks post challenge (Elgohary, Awaad, 1998).

The findings by different authors regarding the role of OR in the respiratory disease complex vary significantly. Cases have been reported where OR was the only pathogen to be consistently isolated (De Rosa *et al.,* 1996; Roepke *et al.,* 1998; Sakai *et al.,* 2000) and some would argue that the organism is able to induce disease on its own. (van Veen, van Empel, Fabri, 2000). There appears to be more evidence of OR as a principal pathogen in turkeys than in broilers where most authors agree that the concomitant presence of other pathogens results in more severe pathology than where OR alone is involved. (Odor *et al.,* 1997; Travers, 1996; Zorman-Rojs *et al.,* 2000; van Empel *et al.,* 1996).

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5. TRANSMISSION

5.1 Horizontal Transmission

OR is transmitted between chickens horizontally – probably principally by aerosol transmission (van Empel, personal communication, 2003). There is nothing in the literature to confirm this. No evidence of biological vector transmission of the disease has been published. Fomite transmission is assumed.

De Rosa *et al.* (1996) reported on an outbreak of respiratory disease caused by OR in the U.S.A. In this case the first outbreak was observed on a ranch of a particular company, two weeks later the disease broke out on another ranch belonging to the same company, two weeks subsequently the disease spread to an adjacent ranch belonging to a different company 11km away. No human traffic moved between the ranches belonging to the different companies. The authors speculate that airborne transmission by water vapour droplets or an animal vector might account for the transmission.

A case report from Minnesota and Wisconsin (Roepke *et al.,* 1998) indicated that the disease spread from an initial outbreak to 17 other farms in a 160km radius within 5 weeks, despite the application of heightened biosecurity measures.

5.2 Vertical Transmission

Vertical transmission of OR was postulated by Back (1998) when he was able to isolate the organism from the ovaries and oviducts of 56-week-old turkeys post-challenge. He was also able to detect OR by immunofluorescent antibody assay (IFA) post challenge in both the ovary and the oviduct.

As part of his doctoral work on OR, van Empel (van Empel 1998) was able to demonstrate that OR is transmitted from breeder flocks to chicks through the egg. He was able to isolate OR from the egg-shell and from the yolk sac of one day old chicks, but not from the inner parts of the egg (yolk, albumin, membranes). Whether transmission is cloacal or ovarian still remains to be established.

6. BACTERIOLOGICAL FINDINGS

Vandamme and his co-authors (1994) undertook extensive studies on the identification of OR (Vandamme *et al.,* 1994) and found that it is closely related to the *Flavobacterium, Cytophaga, Capnocytophaga* and *Riemerella* genera. Other authors have reported on the use of conventional biochemical characterization of OR, but their results are quite variable as can be seen in table 3. The API system test has also been used for the identification of OR (Charlton *et al.,* 1993; van Beek *et al.,* 1994; De Rosa *et al.,* 1996; Odor *et al.,* 1997; Elgohary, Awaad, 1998; Pages-Mante, 1999).

Table 3. List of biochemical tests used for the identification of OR

	Post *et al.* (1999)	Elgohary Awaad (1998)	Travers *et al.* (1996)	Hinz *et al.* (1994)	Van Beek *et al.* (1994)	Vandamme *et al* (1994)	Charlton *et al.* (1993)
Test Performed		+/n*+	+/n*	+/n*			+/n*
Oxidase	110/110	8/8	2/3	17/18	+	+	+
Catalase			0/3	1/18	-	-	-
MacConkey			0/3		-	-	-
Nitrate red.	0/110		0/3	0/18	-	-	-
Arginine dehydralase		7/8	3/3	16/18	+	+	-
Lysine		0/8	0/3	0/18	-	-	-
Ornithine		0/8	0/3	0/18	-	-	-
Phenylalanine deaminase				0/18		-	-
Gelatinase		0/8		0/18		-	-
Urease	53/110	8/8	3/3	18/18	+	+	-
Indole	0/110	0/8	0/3		-	-	-
H_2S		0/8		0/18		-	-
Esculin			0/3	0/18		-	-
ONPG		8/8	3/3	18/18	+		+
VP		0/8	3/3	17/18	+	+	
O/F						+/-	
Galactose			3/3	17/18	+	+	-
Glucose	0/110	2/8	3/3	15/18		+	
Mannose			3/3	18/18		+	
Lactose			3/3	18/18	+	+	-
Sucrose		3/8	0/3	1/18		+	-
Xylose			0/3			-	-
Mannitol			0/3			-	-
Sorbitol		3/8	0/3			-	-
Malonate						-	
Fructose				17/18	+	+	
Maltose		3/8	3/3	14/18		+	-
Dextrin							
Arabinose		4/8					-
Dulcitol			0/3				-
Trehalose			0/3				-
Inositol		3/8	0/3				-
Salicin			3/3				-

* number of positives/number of isolates

\+ 1/8 isolates identified by Elgohary as OR shows a distinctly different biochemical profile to the other seven isolates – possibly this isolate was mis-identified.

6.1 Antigenic Diversity

Van Empel *et al.* (1996a) used agar gel precipitation (AGP) tests to identify seven different serotypes of OR, typed A-G. In his review article of 1999 van Empel (van Empel, Hafez, 1999) refers to serotypes A-L. As at August 2003, eighteen

serotypes designated A to R have been identified (van Empel, personal communication, 2003). Typing of 514 chicken isolates and 333 turkey isolates from Europe, the USA, South Africa and Israel revealed that serotype A is the most common isolate from both species, although the turkey isolates tended to be more spread throughout the serotypes. Seventy eight of the seventy nine South African isolates (all from chickens) tested were serotype A, the other was serotype C. Odor *et al.* (1997) was able to confirm that serotype A was most prevalent among chickens in the U.S.A. Serotype I was identified in turkeys from the Midwest of the U.S.A. (Schleifer, 1997b). Further work done with the AGP test in Germany using different antigen extractions showed that serotypes could be differentiated successfully using antigens extracted in various ways, although if tests were left for longer than 48 hours cross-reactions among serotypes did occur. (Hafez, Sting, 1999). The Japanese isolate of OR made in 1999 was identified as serotype A (Sakai *et al*., 2000), as were Egyptian isolates made in 1997 (Elgohary *et al.,* 1998). All of twenty-five OR isolates from chickens from Peru were found to be serotype A. (Hung, Alvarado, 2001).

Van Empel developed an ELISA test using boiled extract antigen (B.E.A.) prepared from different OR isolates. Using the ELISA and monovalent antisera, OR could be serotyped in a similar manner to the AGP test. Monovalent antisera were found to contain large amounts of homologous antibodies, resulting in marked background reactions. Cross-reactions between serotypes were observed on the ELISA tests – particularly between serotypes A, B, D and E. All

antisera showed the highest titre against homologous antigen (van Empel *et al.,* 1996a). When testing different OR antigen preparations on ELISA, different cross-reactions were observed between strains, making interpretation difficult. (Hafez, *et al., 1999.)*

Fitzgerald indicated that certain OR isolates have the ability to agglutinate red blood cells and that this could possibly be used as a method to differentiate isolates (Fitzgerald, Greyling, Bragg, 1998). This has not been described elsewhere in the literature.

6.2 Serological Detection of OR

The whole B.E.A. ELISA developed by van Empel is suitable for serological detection of OR, but is limited to detection of antibodies to the OR serotype from which the antigen used in the test is prepared. As shown in table 4, there were high levels of non-specific antibodies to the B.E.A. present in sera.

Table 4. Serotyping of OR by AGP and differentiation of OR from other relevant Gram-negative rods by ELISA (van Empel *et al.*, 1996b - modified)

Strain No.	Species	Monovalent Antiserum Strain	Sero-type	ELISA titre (2 log) against antigens of strain no.: 1	2	3	4	5	6	7	Homo-logous
1	OR	B 3263/91	A	20	15	8	12	12	11	13	20
2	OR	GGD 1261	B	13	19	8	11	13	11	11	19
3	OR	ORV K91-201	C	9	10	17	7	9	11	11	17
4	OR	ORV 94108 no. 2	D	10	11	10	19	11	11	12	19
5	OR	O-95029 no. 12229	E	13	16	11	12	20	11	13	20
6	OR	ORV 94084 K858	F	10	11	11	8	8	20	9	20
7	OR	0-95029 no. 16279	G	11	12	12	9	10	11	20	20
8	P. multocida	X-73	1	<6	<6	<6	-[a]	-	-	-	22
9	P. multocida	P-1059	2	<6	<6	<6	-	-	-	-	21
10	P. multocida	P-1662	3	<6	<6	<6	-	-	-	-	20
11	P. multocida	P-1702	4	<6	<6	<6	-	-	-	-	19
12	R. anatipestifer	PAA CV	1A	7	<6	<6	-	-	-	-	18
13	R. anatipestifer	PAB BRD	6B	<6	7	7	-	-	-	-	20
14	R. anatipestifer	PAD CV	10D	<6	<6	<6	-	-	-	-	21
15	H. paragallinarum	0083	A	8	8	8	-	-	-	-	16
16	H. paragallinarum	Spross	B	8	8	7	-	-	-	-	18
17	H. paragallinarum	H-18	C	9	8	8	-	-	-	-	16
18	H. paragallinarum[b]	281/91	A	10	8	7	-	-	-	-	16
19	H. paragallinarum[b]	4620/91	A	8	8	7	-	-	-	-	12
20	P. gallinarum	Fieldstrain		8	6	7	-	-	-	-	17

a - not determined, b - NADH - independent strain

This means that a high cut-off value for positive samples must be established when using the test. The highest OR titre achieved by a monovalent antiserum that was not an OR strain was 10 log_2 with an antiserum made against an isolate of *Haemophilus paragallinarum.* A value of 10 log_2 was recommended as the negative cut-off value for the test (van Empel *et al.* 1996a).

A serum plate agglutination test (SPAT) was developed for OR in turkeys at the University of Minnesota (Back, Halvorson *et al.,* 1998). The test was serotype specific and later found not to react with all serotypes (van Empel *et al.*, 1999).

Subsequently the team at Minnesota developed an ELISA test for OR using outer membrane proteins (OMP). The ELISA results obtained using OMP from

serotype A of OR were compared with those of the previously developed SPAT. After experimental OR challenge, the SPAT detected antibodies for OR in 65% of birds in the first 2 weeks post infection. The ELISA was able to detect antibodies for up to 8 weeks post infection. In the authors' opinion, this indicated that the SPAT is probably best able to discern high levels of circulating IgM. The ELISA showed good sensitivity to lower levels of antibodies present later in the immune response and good cross-reaction among different OR serotypes (Lopes, Rajashekara, Back, Shaw, Halvorson, Nagaraja, 2000).

6.3 Molecular Characterization

The total protein (TP) profiles and OMP profiles of OR strains show high similarlity levels, with a similarity coefficient (Sd) of more than 84%, despite their differences in origin and serotype (Amonsin, Wellehan, Lin, Vandamme, Lindeman, Edman, Robinson, Kapur, 1997; van Empel *et al.,* 1996a, Hung *et al.,* 2001). This close relationship between isolates is confirmed by PCR studies, sequencing of the 16S rRNA and ribotyping which showed low discriminatory power. The random amplified polymorphic DNA method with OPG11 primer was able to discriminate five RAPD types with an Sd of 50% (Leroy-Setrin, Flaujac, Thénaisy, Chaslus-Dancia, 1998). The amplified fragment length polymorphism (AFLP) method was also found to be discriminative. These results suggest that OR should be divided into at least 5 sub-species and the genus *Ornithobacterium* into 3 species. No other arguments were found that would convincingly support the division of the genus into more species (van Empel *et al.* 1999).

7. TREATMENT AND CONTROL OF OR

7.1 Chemotherapeutics

Reports from various authors indicate that the sensitivity of OR to antibiotics is variable. There is also evidence to suggest that the organism develops resistance to antibiotics rapidly.

Early work to determine the sensitivity of OR to different antibiotics was done by Devriese (Devriese, Hommez, Vandamme, Kersters, Haesebrouck, 1995). Minimum inhibitory concentrations (MICs) were determined using the agar dilution method. The authors argue that disc diffusion tests are not suitable for OR as it grows slowly and fails to grow on recommended antibiotic sensitivity test media (Devriese, De Herdt, Haesebrouck, 2001). The MICs of a range of antibiotics were determined for fourteen OR isolates from domestic gallinaceous birds as well as three strains isolated from rooks. These, as well as the results of other authors, using agar gel diffusion tests, are shown in Table 5.

TABLE 5. Antibiotic sensitivity of OR

Antimicrobial Agent	Hinz (1994) Germany .	Van Beek *et al.* (1994) Holland	Odor *et al.*(1997) U.S.A.	Elgohary (1998) Egypt	Hafez *et al.* (1993) Europe	Devriese *et al.* (1995) Europe	Devriese *et al.* (2001) Belgium[#]
Penicillins		+					
Ampicillin		+		25%		Acquired	Variable
Amoxycillin	+			100%	100%		
Penicillin G			-			Acquired	
Cephalosporins							
Ceftiofur						Acquired	38/45
Macrolides							
Tylosin						Acquired	44/45
Erythromycin			+	50%	90%		
Spiramycin							43/45
Tilmicosin							43/45
Lincosamides							
Lincomycin			+			Acquired	45/45
Tetracyclines		+					
Doxycycline						Acquired	36/45
Tetracycline			+	50%	100%		
Quinolones							
Enrofloxacin		+/-		38%	6%	Acquired	40/45
Danofloxacin				75%			
Sarafloxacin			+				
Nalidixic Acid			+				
Sulphonamides							
Trimethoprim/ Sulpha		+/-	-	0%	0%	-	
Suphisoxazole							
Aminoglycosides							
Streptomycin			-	38%			
Neomycin				0%	0%		
Gentamycin			-	0%	0%		
Spectinomycin						Acquired	
Amphenicols							
Chloramphenicol				100%	100%		
Other							
Novobiocin			+				
Bacitracin			+				
Tiamulin							0/45
Furazolidone					36%		

KEY TO TABLE 5 : (+) indicates sensitivity to the antimicrobial, **(-)** indicates resistance to the antimicrobial, **(Acquired)** indicates where Devriese *et al* suggest that domestic strains have acquired resistance to the antimicrobial which "wild" strains did not have.
n/m n = isolates with resistance or "reduced sensitivity"/m = total isolates tested.
% indicates percentage of isolates tested sensitive to a particular antibiotic.

It was found that the MIC of certain of the antibiotics (notably penicillins and cephalosporins) for the OR strains from domestic birds was significantly higher than in the rook strains. The authors concluded that the MICs of the antibiotics used, on the rook strains represented the natural sensitivity of OR and that all

strains from the domestic birds had acquired resistance. It was also found that more recent isolates were less sensitive to lincosamide and macrolide antibiotics than the type strains (LMG 9086^T from a turkey and LMG 11553 from a rook) that had been isolated earlier.

From these results, the authors concluded that acquired antibiotic resistance is exceptionally frequent in OR. In the case of potentiated sulphonamides MICs were all high, indicating general resistance. This was confirmed by van Empel (pers com., 1998).

More recent work by Devriese *et al.* (2001) on 45 OR isolates from broilers in Belgium confirmed his earlier findings. In terms of his study he defined strains as having acquired resistance if they gave MIC values three or more two-fold dilutions greater than the type strains. In the case of beta lactam antibiotics even the type strains had fairly high resistance to the antibiotics – in these cases he defined OR as "naturally resistant" to the antibiotics. The resistance to beta lactam antibiotics is confirmed by the presence of beta lactamase in all OR isolates studied, with the exception of those originally isolated from rooks.

Other authors have used the disc diffusion technique to determine the sensitivity of OR to various antibiotics. The value of this data may be questionable, but the patterns seem to conform to some degree with that of Devriese (Devriese *et al.,* 2001; Elgohary *et al.,* 1998; Hafez, 2000; Hinz *et al.,* 1994; Odor *et al.,* 1997).

7.2 Treatment

De Rosa *et al.* (1996) indicated that flocks suffering from OR were treated with oxytetracycline in the water, chlortetracycline in the feed and spectinomycin, ceftiofur and penicillin by injection. In response to treatment mortality was reduced in 5-7 days. Hinz *et al.* (1994) reported satisfactory results with amoxicillin. Van Beek *et al.* (1994) reported poor results with enrofloxacin and trimethoprim sulfa but better results with tetracycline and synthetic penicillin. Hafez *et al.,* (1993) reported satisfactory results with chloramphenicol or amoxycillin.

7.3 Vaccination

More recent work has focused on the control of OR by vaccination. Bock *et al.* (1997) indicated briefly that a killed autogenous oil emulsion vaccine has been widely used on turkeys in Israel since 1992 and that a commercial vaccine was developed there in 1997.

Van Empel and van den Bosch (1998) completed preliminary studies on vaccines. He was able to show that a killed bacterin in an oil adjuvant was able to induce high antibody titres and good protection against OR challenge in specific pathogen free (SPF) leghorns which had no antibody titres against OR before vaccination. When the same vaccine was applied to day old commercial broiler

chicks it was found that the antibody titres and the protection against OR challenge was less, possibly as a result of the presence of maternal antibodies.

Although immunity could be induced in broilers using the oil adjuvant vaccine, the method has limited application in the commercial farming environment. Individual injection of oil adjuvant vaccine is not routinely practiced due to the high unit cost of vaccination as well as the traumatic effect of vaccine injection on chicks at an early age.

He then vaccinated broiler breeders and found that the bacterin in mineral oil adjuvant induced high and long lasting immune responses. The vaccine induced a high level of maternal antibodies in the progeny, resulting in good protection against experimental challenge up to 30 days of age. Protection, however, decreased with the ageing of the broiler.

Further work showed that serological and protective response to vaccination increased with age at vaccination and with decrease of maternal antibody levels at time of vaccination. Van Empel concluded that the most practical approach to control of OR infections in broilers would be breeder vaccination with an inactivated vaccine, combined with a live vaccination of broilers at two to three weeks of age.

More recently (Sprenger *et al.,* 2000) trial use of an inactivated bacterin in turkeys demonstrated good protection against challenge at 14 weeks. In the same trial turkeys were exposed to live OR at 7 weeks of age before challenge at 14 weeks. The live bacterial exposure caused no clinical signs and provided good protection against challenge. The authors noted that turkeys in the control groups showed fairly mild reactions to the OR challenge – probably as a result of the low stress conditions in the experimental facilities.

In 1999, eight Belgian broiler breeder flocks were vaccinated using the inactivated bacterin vaccine developed by van Empel under field conditions. This study showed that the bacterin was safe for use in broiler breeders and induced high levels of antibodies to OR. A significant positive correlation was found between antibody titres of breeders and that of their progeny. Progeny of vaccinated breeders demonstrated a lower mortality, a higher production index and a lower percentage of OR infection at slaughter than those derived from similar unvaccinated breeder flocks. As there was no severe OR field challenge during the trial, the authors were unable to determine if the vaccine would protect the progeny of vaccinated breeders (Cauwerts, De Herdt, Haesebrouck, Vervloesem, Ducatelle, 2002).

In 2002 (Lopes, Back, Shin, Halvorson, Nagaraja, 2002) work was done in Minnesota to develop a live vaccine against OR. Field strains of OR were exposed to the mutagenic N-methyl-N'-nitro-N-nitrosoguanidine. Strains were

selected if growth was obtained at 31ºC but not at 41ºC, thus selecting a temperature sensitive mutant able to colonize the upper respiratory tract, but not the deeper tisues of the birds. Preliminary work was also done to evaluate a stable mutant strain developed in this manner, as a vaccine. Colonisation of the upper respiratory tract was studied in day-old turkey poults after administration of vaccine by drinking water and by oculonasal instillation. The temperature sensitive strain could be re-isolated from all groups for 13 days post administration. In 19% of vaccinated birds a humoral immune response could be detected by ELISA. Challenge studies were not carried out.

Chapter III – Materials and Methods

1. VACCINATION OF BROILER BREEDERS AGAINST OR

Broiler breeders were vaccinated twice during rearing with an inactivated OR bacterin vaccine to generate antibodies that could be transmitted transovarially to protect their progeny from OR challenge.

1.1 Broiler Breeders

Seven Ross broiler breeder flocks belonging to EarlyBird Farms were monitored serologically from nine weeks of age to depopulation. These flocks were placed at the rearing farms at approximately monthly intervals, between July and December 1998. Flocks 257, 259 and 261, placed on the farm Vlakfontein, were vaccinated against OR, as described below. Flocks 256, 258, 260 and 262, placed on the farm Kosmos were not vaccinated against OR.

Each breeder flock comprised approximately forty three thousand birds raised on a single site, in climate controlled houses. All birds were supplied to EarlyBird Farms as day old chicks by Ross breeders of Meyerton. At nineteen weeks of age the flocks were transferred from the rearing farms to laying facilities. The houses on the laying farms were open-sided. Eggs from the breeder flocks were hatched at different hatcheries belonging to EarlyBird farms. (Fig.1)

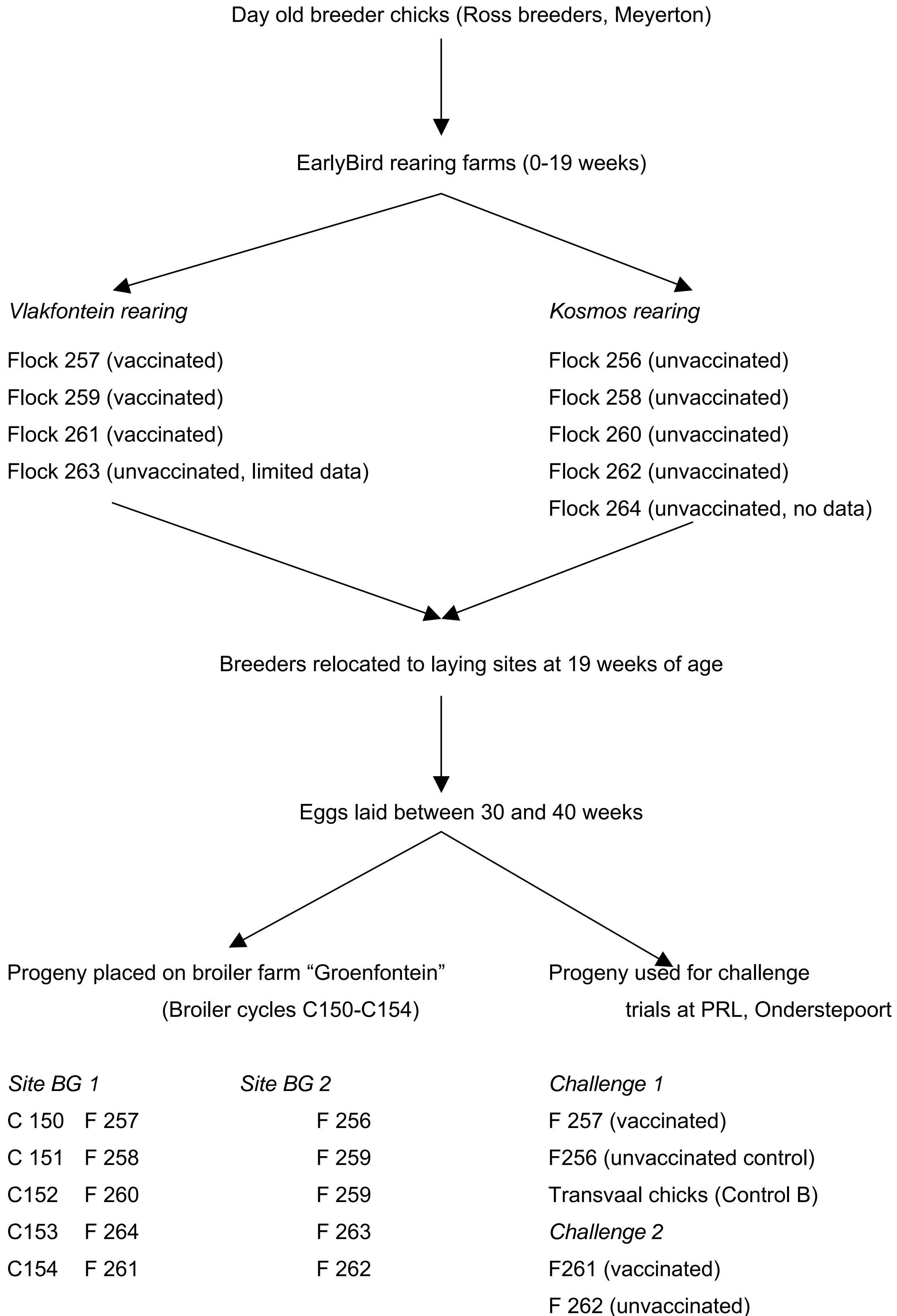
1.2 Vaccination and Treatment of Breeders

An inactivated bacterin vaccine designated Nobilis® ORT Inac was provided by Intervet South Africa. The vaccine contained OR strain B3263/91, a serotype A strain originally isolated from a broiler in South Africa in a mineral oil adjuvant containing approximately 1x 10^9 colony forming units (CFUs) per dose.

Each bird was vaccinated in the right breast muscle with 0.25ml vaccine. Vaccination was carried out by Earlybird vaccination teams. Other vaccinations were given at the same handling of the birds, but always either in the left breast muscle or subcutaneously so that vaccination lesions resulting from different vaccinations could be distinguished. In all cases the vaccine was administered when the birds were between 8-10 weeks and again at approximately 18 weeks of age.

Details of the full vaccination schedule of each flock, as well as clinical findings and treatments are attached as Appendix I. Vaccination was according to the routine schedule of EarlyBird farms, although programmes were slightly modified where necessary in order to fit the additional OR vaccinations into the existing schedule.

Figure 1. Schematic representation of trial procedures



1.3 Vaccination Safety Monitoring Procedures

Birds were monitored daily for clinical signs after vaccination as part of the routine procedures of EarlyBird Farms by the site worker as well as the farm manager. In vaccinated flocks, 15 birds were palpated 7 and 14 days post vaccination by the investigator. It proved to be impracticable to palpate the unvaccinated flocks, given the distances between the investigator and the rearing farms.

Cumulative mortality, percentage egg production per hen housed, mean egg weights and hatchability were recorded for each of the flocks included in the trial. Clinical findings in each flock were recorded by the EarlyBird veterinarian and are included in Appendix I.

1.4 Serological Monitoring of Vaccination

1.4.1 Collection of Specimens for Serological Testing

Blood samples were collected from the brachial vein in 3ml sterile silicon coated test tubes, by the staff of EarlyBird Farms. These samples were then transported on the day of collection to the laboratory of EarlyBird Farms where they were allowed to stand for approximately 6 hours at room temperature to clot. Thereafter the serum was collected into 1ml plastic tubes and frozen at -20ºC. While frozen, samples were transported to the Poultry Reference Laboratory at the Faculty of Veterinary Science of the University of Pretoria where they were

stored at -20ºC before testing. The schedule for collection of blood samples is indicated in Table 6.

Table 6. Blood collection schedule from EarlyBird breeder chickens

Flock	C 256	**V** 257	C 258	**V** 259	C 260	**V** 261	C 262	C 263	Age Group
Actual Age									
9 weeks	30	35	18		24	24	23	16	9 weeks
14 weeks		23	24		25				14 weeks
15 weeks						24			14 weeks
17 weeks			23		23			15	18 weeks
18 weeks	16	16		20		23			18 weeks
20 weeks							23		None
21 weeks	7								None
24 weeks		23					23		25 weeks
25 weeks					12	7			25 weeks
26 weeks				14					25 weeks
29 weeks						6			30 weeks
31 weeks				18				14	30 weeks
41 weeks							24		42 weeks
42 weeks	15	24			22				42 weeks
43 weeks				24					42 weeks
44 weeks			25						42 weeks
47 weeks						24			48 weeks
48 weeks	16				15				48 weeks
54 weeks		24							None
59 weeks	17								60 weeks
60 weeks		24				22			60 weeks

Key to Table 6.
C = unvaccinated parent flock. V = vaccinated parent flock. The number of sera received at each bleed is given in the columns below the flock numbers.

Samples taken within three weeks of each other were deemed similar enough to be grouped for comparisons among flocks. The groups to which samples were assigned are indicated at the right hand-side of the table. In only one case (the 42 week bleed) were samples three weeks apart actually grouped, all other groups varied only by two weeks or less. It was decided to add the 44 week bleed of flock 259 to the "42 week" group as very few samples had been collected from this flock. It was decided not to include the 20 week bleed from

flock 262 to the 18 week group as there were already a large number of samples in this group.

1.5 OR ELISA Test

An indirect ELISA test, developed at Intervet in the Netherlands, by Dr. Paul van Empel, was used to detect antibodies to OR in serum samples. Validation of the test is discussed in the literature review.

1.5.1 Reagents

All reagents used in the test were prepared in accordance with Intervet standard operating procedures (SOPs). Reagents were obtained from Merck chemicals (Fedsure Park, Midrand, Gauteng, South Africa), unless otherwise indicated.

BOILED EXTRACT ANTIGEN (B.E.A.)

A colony of OR (serotype A) bacterium was suspended in 2.5ml sterile saline. 0.1ml of the suspension was seeded on sheep-blood agar and incubated at 37˚C until enough growth was obtained (about 48 hours). The growth was washed off with 2.5ml phosphate buffered saline (PBS) + 0.3% formalin + 8.5% NaCl per agar plate and the optical density (OD) at 660nm measured. The suspension was adjusted with the same diluent so that a dilution of 1 part of the adjusted suspension added to 19 parts of PBS gave an OD reading at 660nm of between 0.15 and 0.3. The adjusted suspension was boiled at 100˚C for one hour then centrifuged (30 min, 15 000g, 4˚C). The supernatant was filtrated and used as

B.E.A. B.E.A. was obtained from Intervet (Boxmeer, Netherlands) and was stored at -70˚C until used.

BUFFERS

A phosphate buffering system was used in the ELISA. Slightly differing buffers include EIA, coating buffer, blocking buffer and wash buffer (includes Polysorbate 20). All buffers were made up shortly before use and stored at 7˚C until used. Indicators were added to buffers so that any pH changes would be immediately obvious. Any buffers showing pH changes in storage were assumed to be contaminated and were discarded.

EIA-TWEEN BUFFER

Prepared by adding 5ml Polysorbate 80 10% to 1l EIA buffer.

TETRA METHYL BENZIDINE (TMB) SOLUTION

Purchased ready to use (Zymed TMB Solution 00-2023, Sterilab, Foreman Str. Kempton Park, Gauteng, South Africa), used according to manufacturer's instructions.

CBB BUFFER (Coating Buffer)

Na_2CO_3 and $NaHCO_3$ buffer.

University of Pretoria etd – Bisschop, S P R (2005)

BLOCKING BUFFER

2M H_2SO_4

CONJUGATE

HRP-Rabbit Anti-Chicken/Turkey IgG 61-3120 (Sterilab, Foreman Str.,Kempton Park, Gauteng, South Africa) conjugate was used. Conjugate was diluted according to ELISA method.

POSITIVE REFERENCE SERUM

Serotype A – serum B3263/91 (titre between $log_2 17$ and $log_2 19$), obtained from Intervet, Boxmeer, Netherlands.

1.5.2 Equipment

Polystyrene flat-bottomed microtitre plates - Greiner, 655001 (Laboratory and Scientific Equipment (Lasec), Kya Sand, Gauteng, South Africa)

Multichannel micropipette – Bibby Sterilin 8 channel pipette (Lasec)

Microelisa reader -Titretek Multiskan MCC 340, Flow laboratories, 450nm filter, connected to an MS Windows compatible computer and printer.

1.5.3 Procedures

PREPARATION OF TEST ARTICLE

- Sera were stored at -20˚C. Shortly before testing, sera were thawed and diluted 1:64 in EIA-Tween buffer.

COATING OF PLATES

- Boiled extract antigen was diluted 1:100 with CBB, just prior to use.
- Plates were filled with 100 µl per well and incubated at 37˚C for approximately 16 hours, with covers.

BLOCKING OF PLATES

- Plates were emptied thoroughly then filled with 200 µl blocking buffer per well.
- Incubated for 20 min. at 37˚C.
- Manually washed 4 times with wash buffer.
- Plates were used immediately after blocking.

ELISA

- All wells except A12-H12 were filled with 100 µl EIA-Tween buffer per well.
- 100µl of pre-diluted positive reference serum was added to well H1, and serial two-fold dilutions were made up to well H12.
- Test-sera were pre-diluted to a final dilution of one part serum to 63 parts EIA-Tween buffer, and 100 µl of the diluted sera was added to wells A1-G1; so the dilution in the first row was 1:128 ($\log_2 7$).
- Serial two-fold dilutions of test sera were made from well A1-G1 to A11-G11, by transferring, after mixing, 100 µl from row 2 to row 3, etc. At the end 100 µl was discarded from wells A11-G11.

- 100 µl of negative control serum, diluted one part serum in 63 parts of EIA-Tween buffer, was added to each of wells A12-H12.
- The plates were incubated at 37˚C for 1 hour.
- The plates were washed with distilled water four times manually.
- 100 µl conjugate, appropriately diluted in EIA-Tween buffer,was added to the wells.
- The plates were incubated at 37˚C for 30 minutes.
- The plates were washed with distilled water four times manually.
- 100 µl of substrate solution was added to all wells.
- The plates were incubated in the dark for 15 minutes at room temperature.

The reaction was stopped by adding 50µl 2 M H_2SO_4 to each well. The same reaction time was ensured for each well.

Figure 2. Schematic representation of ELISA plate layout.

	1	2	3	4	5	6	7	8	9	10	11	12
A	$1:2^7$	$1:2^8$	$1:2^9$	$1:2^{10}$	$1:2^{11}$	$1:2^{12}$	$1:2^{13}$	$1:2^{14}$	$1:2^{15}$	$1:2^{16}$	$1:2^{17}$	–
B	$1:2^7$	$1:2^8$	$1:2^9$	$1:2^{10}$	$1:2^{11}$	$1:2^{12}$	$1:2^{13}$	$1:2^{14}$	$1:2^{15}$	$1:2^{16}$	$1:2^{17}$	–
C	$1:2^7$	$1:2^8$	$1:2^9$	$1:2^{10}$	$1:2^{11}$	$1:2^{12}$	$1:2^{13}$	$1:2^{14}$	$1:2^{15}$	$1:2^{16}$	$1:2^{17}$	–
D	$1:2^7$	$1:2^8$	$1:2^9$	$1:2^{10}$	$1:2^{11}$	$1:2^{12}$	$1:2^{13}$	$1:2^{14}$	$1:2^{15}$	$1:2^{16}$	$1:2^{17}$	–
E	$1:2^7$	$1:2^8$	$1:2^9$	$1:2^{10}$	$1:2^{11}$	$1:2^{12}$	$1:2^{13}$	$1:2^{14}$	$1:2^{15}$	$1:2^{16}$	$1:2^{17}$	–
F	$1:2^7$	$1:2^8$	$1:2^9$	$1:2^{10}$	$1:2^{11}$	$1:2^{12}$	$1:2^{13}$	$1:2^{14}$	$1:2^{15}$	$1:2^{16}$	$1:2^{17}$	–
G	$1:2^7$	$1:2^8$	$1:2^9$	$1:2^{10}$	$1:2^{11}$	$1:2^{12}$	$1:2^{13}$	$1:2^{14}$	$1:2^{15}$	$1:2^{16}$	$1:2^{17}$	–
H	+	+	+	+	+	+	+	+	+	+	+	–

RECORDING OF DATA

- The absorption in the plates was measured in the Multiskan reader at 450nm. (A-450) and the 450nm values for each plate were printed.

EVALUATION OF DATA

The antibody titre of a test serum was defined as the maximum serum dilution with an A-450 of at least 1.5 times the mean background A-450 as determined in the negative control serum (wells A11-H11) and was expressed in $\log_{2.}$. The test was valid if:

- The average of the A-450 values of the negative serum did not exceed 0.30.

- The A-450 values of test and reference sera were regularly decreasing at diluting, and if
- The titre of the positive reference serum was between a titre of 17 and 19.

In broilers, a negative/positive cutoff value of 8 was used.

In breeders a negative/positive cutoff value of 12 was used.

2. FIELD PLACEMENTS OF BROILERS TO COMPARE PERFORMANCE OF PROGENY OF VACCINATED BREEDERS WITH PROGENY OF UNVACCINATED BREEDERS

2.1 Broilers

Ross broiler chicks hatched from breeder flocks in the trial were delivered at day old to commercial broiler rearing sites from hatcheries belonging to EarlyBird Farms. In each broiler cycle, progeny from vaccinated parent flocks were compared with progeny from unvaccinated parent flocks placed immediately before or immediately after the vaccinated breeder flocks.

2.2 Broiler Farm

Broilers were placed on the farm Groenfontein (Fig.1), which is owned by EarlyBird Farms and is located near the Bronkhorstspruit – Delmas road, approximately 80km East of Pretoria in Gauteng Province, South Africa.

The sites designated BG1 and BG2 were used for the broiler trials. These sites lay approximately 300m apart and were identical. Each site contained 3 similar chicken houses with a square area of 892 m^2 per house, giving a total area per site of 2 676 m^2. Approximately 17 840 birds were placed per cycle in each house at a stocking density of 20 birds per m^2. Management procedures on both sites were the same and both were under the control of the same manager.

The houses were closed-sided houses using negative pressure cross ventilation. Fresh air entered the houses through slatted air inlets on the ends of the houses. Air was extracted by banks of extraction fans on either side of the houses. Temperature and ventilation control was automatic.

2.3 Broiler Feed and In-Feed Medication

All feed was supplied by the Randfontein mill of Meadow Feeds Ltd. At the time the trials were run, 600g starter and 1 400g grower were supplied for each broiler chick placed at day-old, for the last five days before slaughter birds went onto an unmedicated post-finisher ration. The balance of feed used was made up of finisher. The exact quantity of finisher and post finisher varied according to the slaughter date of each flock. The nutritional specifications of each ration was the same for both sites used in all the trials, although exact formulations varied slightly depending on the availability of raw materials.

The only practice that was altered from the procedures followed for routine commercial flocks, was the removal of oxytetracycline (OTC) from the feed of certain of the trial groups. During 1998 and 1999, oxytetracycline (OTC) was normally added to the feed at 300 g/tonne from day old until five days before slaughter with the primary objective of controlling OR challenge in the flocks.

2.4 Broiler Placement Schedule

A detailed list of placements is given in Table 7.

Table 7. List of EarlyBird broiler placements at Groenfontein

Cycle	Site	Placement Date	Parent Flock	Parent Vaccination	OTC in Feed
150	BG1	13.04.99	**257**	**vaccinated**	No OTC
	BG2	13.04.99	256	unvaccinated	No OTC
151	BG1	07.06.99	258	unvaccinated	No OTC
	BG2	07.06.99	**259**	**vaccinated**	No OTC
152	BG1	02.08.99	260	unvaccinated	No OTC
	BG2	02.08.99	**259**	**vaccinated**	No OTC
153	BG1	26.09.99	264	unvaccinated	OTC
	BG2	26.09.99	263	unvaccinated	OTC
154	BG1	24.11.99	**261**	**vaccinated**	OTC
	BG2	24.11.99	262	unvaccinated	OTC

2.5 Lighting Programme

All sites followed the same lighting programme throughout the experimental broiler cycles. This was as follows : 24 hours of light on the day of arrival, 23 hours of light and 1 hour of darkness from day 2 to day 20 ; followed by 22 hours light and 2 hours of darkness from day 21 to slaughter.

2.6 Water Supply

The water supply on Groenfontein was from boreholes. The water from the different boreholes mixed as it circulated on the farm. There was no record that any tests were carried out to evaluate the water quality during the period of the trials.

2.7 Vaccination Schedule

The vaccination schedule for broilers at Earlybird Farms at the time of the trials is given in Table 8.

Table 8. EarlyBird broiler vaccination schedule

Day	Vaccination	Application	Product	Supplier
Day 0 (hatchery)	ND inactivated	sc injection	Unknown	
	ND live	coarse spray	Nobilis Clone 30	Intervet
	IB live	coarse spray	+ IB Ma5	
Day 10	Pneumovirus	Fine spray	In-house	EarlyBird
Day 14	IBD live	Drinking water	Bursine Plus	Fort Dodge
Day 20	IBD live	Drinking water	Bursine Plus	Fort Dodge
Day 24	ND live	Fine spray	La Sota	Unknown

Key to table 3.3 : IB = infectious bronchitis, IBD = infectious bursal disease, ND = Newcastle disease, sc = subcutaneous.

2.8 Serological Monitoring

OR serological monitoring of broilers was carried out at slaughter by the Poultry Reference Laboratory, using the OR ELISA test described under 1.5. Values greater than $1:2^8$ were considered positive in broilers.

Serological test results of other infectious agents available from the broilers were supplied by EarlyBird Farms as part of their routine monitoring of flocks. ND titres were calculated using a haemagglutination inhibition (HI) test calibrated at four HA units, in accordance with the Office International des Epizooties (OIE) specifications. Values above $\log_2 3$ were considered to be positive. IB and TRT titres were calculated using commercial Idexx ELISA kits. Values above 4 000 were considered consistent with IB or TRT challenge by the company veterinarian.

2.9 Analysis of Production Parameters

Final production results for each broiler cycle were recorded by EarlyBird Farms on the farm and at the abattoir. These included total live weight, average live weight and age at slaughter, mortality rate, feed consumption and feed conversion ratio. No further data such as individual bird weights or the range of bird weights was made available.

2.10 Partial Farm Budget

Partial farm budgeting was done according to Martin *et al.* (1998) using the following parameters.

Additional returns :

1. Carcass sales/bird = mean live mass at slaughter (kg) x live weight sale price/kg

Foregone returns :

1. Carcass losses during trial = mortality rate x mean live mass at slaughter x live weight sale price/kg.

Additional Costs incurred :

1. Vaccination cost/bird = estimated cost of breeder vaccination/average of 120 chicks per breeder hen
2. Treatment with OTC/bird = cost of Oxytetracycline/tonne of feed x mean feed intake/bird
3. Cost of starter/bird = cost of broiler starter mash/tonne x mean intake of starter/bird slaughtered
4. Cost of grower/bird = cost of broiler grower mash/tonne x mean intake of grower/bird slaughtered
5. Cost of finisher & post finisher/bird = mean of the cost of broiler finisher mash and broiler post finisher mash/tonne x mean intake of finisher and post finisher/bird slaughtered

Inputs for the Partial Farm Budget were as follows :

Mortality rates, average slaughter mass and feed conversion ratios were taken from results supplied by EarlyBird Farms and recorded in Table 13.

Meat sale price = R 7.00. This was the price offered by a commercial abattoir to broiler growers in May 2003.

Estimated cost of breeder vaccination: R 0.40/dose of vaccine x 2 doses/breeder hen = R 0.80/hen + R 0.40 for labour costs associated with vaccination.

Cost of Oxytetracycline/tonne of feed = R43.80. OTC was assumed to be in all rations. In reality OTC would be withdrawn from the post finisher ration. It was not possible to calculate the exact intake of post finisher. This omission could result in a slight overestimation of the cost of OTC for treated birds.

Cost of broiler starter mash = R 2 583/tonne.

Cost of broiler grower mash = R 2 303/onne.

Cost of broiler finisher mash = R 2 228/tonne.

Cost of broiler post finisher mash = R 2 158/tonne.

Prices based on Epol bulk supply prices in May 2003, included cost of transport up to 100km from the feed mill.

Feed intake per bird by ration was extrapolated from the records of EarlyBird farms and is indicated in table 9. EarlyBird farms had a policy, at the time of the trial that 600g of Starter mash and 1.4 kg of Grower mash were ordered for each broiler chick placed. Birds would eat unmedicated Post-Finisher mash for the last five days of the cycle and the balance of the feed would be made up with Finisher mash. It was impossible to calculate the exact use of feed types in the latter part of each cycle, as it had not been recorded. The assumption was therefore made

that the balance of feed required once birds had finished their allocation of Starter and Grower rations was made up equally of Finisher and Post-Finisher rations. Average feed intake of each feed type per bird was calculated by dividing the known amount of feed consumed by the flock by the number of birds slaughtered in the flock.

Table 9. Feed intake by feed type, of broilers on different preventative treatment regimes against OR

Oxytetracycline	**Yes**	**Yes**	**No**	**No**
OR vaccination	**Yes**	**No**	**Yes**	**No**
Starter	0.633 kg	0.662 kg	0.668kg	0.673kg
Grower	1.477 kg	1.545 kg	1.560 kg	1.570 kg
Finisher/Post Finisher	0.862 kg	1.109 kg	1.056 kg	1.033 kg
Total	2.972 kg	3.316 kg	3.284 kg	3.276 kg

2.11 Quantitative Risk Analysis

Quantitative risk analysis was carried out using the software package @ Risk (Palisade Corporation, 31 Dekker Road, Newfield, New York). The programme was set up for 1000 iterations using the Latin Hypercube sampling technique (Vose, 1996). Table 10 shows the inputs used in the model and the distribution function used to model that particular input. The output for the model was the result of the partial farm budget, formulated to describe the economic consequences of the different treatment regimes used on the birds. The triangular distribution function (Triang) uses the parameters, minimum value (a), most-likely value (b) and maximum value (c). The trigen distribution function is a

triangular distribution function with the tails restricted by a bottom percentile and top percentile for the particular input. The percentile values were set to 10% for this model which gives the percentage of the total area under the triangle that falls to the left or right of the minimum or maximum point, respectively. The Beta distribution function uses the parameters α_1 and α_2 where α_1 is set to the value $r + 1$ and α_2 is set to $n - r + 1$. This distribution is used to determine the probability of the occurrence of an event, given a number of trials *n* have been made with a number of recorded successes *r*. The normal distribution uses the parameters mean and standard deviation for the particular input (Vose, 1996). The selection of distribution functions for each input was done according to the guidelines laid out by Vose (1996).

An “impact” (sensitivity) analysis was then performed on the output variable and its associated inputs using a multivariate stepwise regression technique and Spearman’s rank correlation, within @Risk. The “impact” analysis was carried out to identify which inputs had the greatest effect on the output (i.e. the profit margin).

Table 10. Inputs and distribution functions used to simulate a partial farm budget for broiler chickens

Inputs	All groups (per head)
Sale price (R)	Trigen(6.6, 7, 7.4,10, 90)
Price of OTC (R)	Triang(0.03, 0.044, 0.06, risk truncate(0.03, 0.06)
Mean slaughter mass (kg)	Trigen(2000,5,95)
Mortality rate	Beta(8, 1232)

3. CHALLENGE STUDIES IN PROGENY OF BROILER BREEDER FLOCKS VACCINATED AGAINST OR

Two challenge trials were carried out. The first in July 1999 was designated challenge 1 and the second in January 2000 was designated challenge 2. The method for both trials was similar. Where the method for the trials differed, this has been indicated in the text.

3.1 Bacterial Strain.

A South African strain of *Ornithobacterium rhinotracheale* isolated in 1998 from a broiler and designated 955/98 by the Poultry Reference Laboratory was used for the challenge. The strain was serotyped as serotype A by Dr. P. van Empel at Intervet in the Netherlands. The isolate was stored at –20°C. Vials of frozen bacteria were thawed and grown on equine blood tryptose agar plates in a capnophilic environment. Colonies were harvested from the plates and multiplied in serum soup broth (prepared by the Onderstepoort Veterinary Institute) for 36 hours at 37°C. Immediately before challenge, 1ml of the broth was again plated onto equine blood agar to ensure it was free of contaminants. The concentration of bacteria in the broth was determined by measuring the optical density. For all challenges the broth contained in excess of 10^{9} colony-forming units (CFUs) per ml.

3.2 Newcastle Disease Virus Strain

A one-thousand-dose vial of commercial Newcastle disease live La Sota vaccine was reconstituted with distilled water on each treatment day. The vaccine was supplied by Intervet South Africa (Pty) Ltd. According to the registration requirements, the vaccine contained a minimum of 10^6 EID_{50} virus particles per dose. Viral titration of the vaccine was not done, but the integrity of the cold chain was adhered to with caution throughout the handling of the vaccine.

3.3 Experimental Animals

Challenge 1

Broiler progeny of EarlyBird breeder flock 257 were used as the experimental birds and were designated as the "Vaccinated" group. Control broilers were progeny from EarlyBird breeder flock 256 and were designated "Control A". There was a concern that breeders from flock 256 may have been exposed to field challenge by OR during rearing, so an additional control group of broilers was added to the experiment. These broilers came from Transvaal Chicks, an independent breeder company also using Ross birds and situated about 100km East of Pretoria (Fig. 1). This group was designated "Control B". All three parent flocks were between 30 and 40 weeks of age when they laid the eggs used in the trial.

Challenge 2

Broiler progeny of EarlyBird breeder flock 261 were used as the experimental birds and were designated as the "Vaccinated" group. Control broilers were progeny from EarlyBird breeder flock 262 and were designated "Control" (Fig. 1).

3.4 Challenge Placements

The challenge was carried out in the isolators belonging to the Poultry Reference Laboratory at the Faculty of Veterinary Science at the University of Pretoria. Broilers were allocated to the eight available isolators on a random basis. In challenge 1 six birds from each of flocks 256, 257 and Transvaal chicks were placed in each isolator. In challenge 2 ten birds from each of flocks 261 and 262 were placed in each isolator.

3.5 OR Challenge

Challenge 1

On day 21, five days after Newcastle disease vaccination, approximately 200ml of the serum soup broth containing OR was sprayed onto broilers in four of the isolators using either a commercial paint sprayer or an adapted pesticide sprayer commonly used for spray vaccination, known as the "Ulvavac". The other four isolators were sprayed with distilled water in the same manner. The ventilation system of the isolators was switched-off for approximately 25 minutes during challenge.

Challenge 2

On day 14, six days after Newcastle disease vaccination, approximately 200ml of the serum soup broth containing OR was sprayed onto broilers in each isolator using a commercial paint sprayer. Control groups were sprayed with distilled water in the same manner. While birds were challenged, the baffle at the outlet of the individual isolator was closed for 10 minutes, to ensure good exposure to the OR. This change in method from the first trial was allowed by modifications made to the ventilation system of the isolators between the trials.

3.6 Newcastle Disease Vaccination

Each vial of vaccine was diluted with 500ml of distilled water of which 200ml was used for each isolator, thus giving each broiler approximately 20 times the standard dose of vaccine. As the vaccination was used only to elicit inflammation of the respiratory tract, it was felt best to simply use all of the available vaccine. Vaccine was applied in the same way as the bacterial challenge solutions to six of the isolators with the other two isolators acting as negative controls for the vaccination.

In challenge 1 vaccination was done on day 16 and in challenge 2 vaccination was done on day eight.

3.7 Parameters of Infection

Seven and fourteen days after OR challenge in both trials, birds were euthanased and post mortem examinations carried out. In challenge 1, weights were recorded for all birds individually on days one and 16 as well as at euthanasia. Swabs were taken from organs that appeared macroscopically affected (predominantly airsacs). Reisolation of OR was done on equine blood tryptose agar in a capnophilic environment. Identification of reisolated bacteria was done visually. During challenge 2, weights were not recorded.

At post mortem examination a system for scoring lesions after van Empel (1998) was used as follows : for thoracic airsacs, 0=no abnormailties, 1=one airsac seriously affected by fibrinous airsacculitis or limited pin head sized foci of fibrinous exudate in both airsacs, 2=both airsacs seriously affected by fibrinous airsacculitis ; for abdominal airsacs, 0=no abnormalities, 1=pin-head sized foci of fibrinous exudate or slight diffuse fibrinous airsacculitis, 2=severe fibrinous airsacculitis; for lungs, 0=no abnormalities, 1=unilateral; pneumonia, 2=bilateral pneumonia; for trachea, 0=no abnormalities, 1= some exudate in the tracheal lumen, 2=lumen of trachea filled with exudate.

3.8 Serological Tests

Indirect ELISA Test for OR

The same OR ELISA test used for the broiler breeders and described in 1.5 was used to test sera from broilers. A positive/negative cut-off of $1{:}2^8$ was used in the case of broilers.

Haemagglutination Inhibition Test for Newcastle Disease

The test was carried out at the Poultry Reference Laboratory in accordance with OIE guidelines. The antigen was standardized to four Haemagglutination units.

4. STATISTICAL ANALYSIS

In general, continuous parametric data was analyzed using analysis of variance (ANOVA) tests. When ANOVA results indicated statistically significant differences among multiple variables, student t-tests were used to confirm differences between pairs of variables.

In assessment of local reactions after vaccination, no statistical manipulation was attempted as the unvaccinated breeder flocks were not palpated, but any lesion found was assumed to be of importance.

Breeder serological data was complete and repeated measure ANOVA was performed to detect interactions between the age of the breeders at successive sampling times, vaccination and the mean antibody titres to OR. In broilers, the

proportion of positive reactors in different flocks was compared using the Chi-square test. Serological responses to other diseases could not be statistically analyzed as only summary data was made available by EarlyBird Farms.

The data received about broiler production was global and thus prevented any meaningful statistical manipulation, apart from a Chi-square test that was done on mortality rates.

In the challenge trials, group weights, lesion scores and ND HI results were compared using ANOVA. The proportion of serum samples positive for OR, and the percentage of positive bacterial reisolations after challenge were compared using the Chi-square test.

A significance level of $\alpha = 0.05$ was used for all comparisons.

Analyses were carried out with the aid of the NCSS 2000 statistical package and Microsoft Excel spreadsheets.

Chapter IV - Results

1. VACCINE SAFETY

1.1 Clinical Observations

No adverse clinical signs were observed by EarlyBird staff in any of the breeder flocks subsequent to vaccination.

1.2 Palpation for Local Reactions

Table 11. Assessment of local reactions in broiler breeders after vaccination

FLOCK 257	DATE	BIRDS WITH REACTIONS	COMMENT
9 WEEKS	**22.09.98 - 30.09.98**		**VACCINATION**
7 days post vaccination	02.10.98	3/15	2 birds showed mild diffuse swelling of breast muscle. 1 bird showed moderate focal swelling of breast. In 1 other bird vaccine oil could be seen subcutaneously.
14 days post vaccination	09.10.98	0/15	Traces of vaccine oil could be seen subcutaneously on 2 birds. *Salmonella* Enteriditis (SE) oil vaccine had been given in opposite breast on same day. Reactions to SE vaccine were still palpable.
18 WEEKS	**26.11.98 - 04.12.98**		**VACCINATION**
7 days post vaccination	11.12.98	1/15	1 bird showed mild diffuse swelling of breast muscle.
14 days post vaccination	18.12.98	0/15	

Table 11 (continued)

FLOCK 259	DATE	BIRDS WITH REACTIONS	COMMENT
9 WEEKS	**17.11.98 -25.11.98**		**VACCINATION**
7 days post vaccination	26.11.98	3/15	2 birds showed mild diffuse swelling of the breast muscles. 1 bird had a defined lump near caudal part of keel bone.
14 days post vaccination			Not done.
18 WEEKS	**21.01.99 -29.01.99**		**VACCINATION**
7 days post vaccination	02.02.99	2/15	1 bird with diffuse but pronounced swelling of breast muscle. 1 bird with discrete abscess on breast.
14 days post vaccination	08.02.99	9/15	Birds showed circumscribed swellings, often in anterior region of breast. 3 showed tubular elongated swellings in subcutis. Breast muscles were normal size. Able to open 3 dead birds on site and could visualize vaccine oil in subcutis of all three.

FLOCK 261	DATE	BIRDS WITH REACTIONS	COMMENT
9 WEEKS	**12.01.99 -20.01.99**		**VACCINATION**
7 days post vaccination	21.01.99	3/15	1 bird showed a moderate swelling of the breast muscle, while the other 2 showed very mild swelling.
14 days post vaccination	02.02.99	2/15	Both affected birds showed moderate swelling of the breast muscles.
18 WEEKS	**17.03.99 -24.03.99**		**VACCINATION**
* 6 days post vaccination	23.03.99	7/15	In all recorded cases organized circumscribed lumps were palpated. 3 of the affected birds also showed mild diffuse swelling of the breast muscle, this may have been due to OR vaccination.
14 days post vaccination	09.04.99	6/15	Nodular lumps.
* Cockerels prior to vaccination	23.03.99	12/15	Palpation of cockerels prior to vaccination revealed that most had circumscribed lumps in the breast at the position of the OR vaccination site.

* Each flock was housed in eight houses during rearing - seven houses containing hens and one house of cockerels. The vaccination team vaccinated one house per day, thus the eighth house had not yet been vaccinated a week after the first house where the initial seven-day post-vaccination palpations were done.

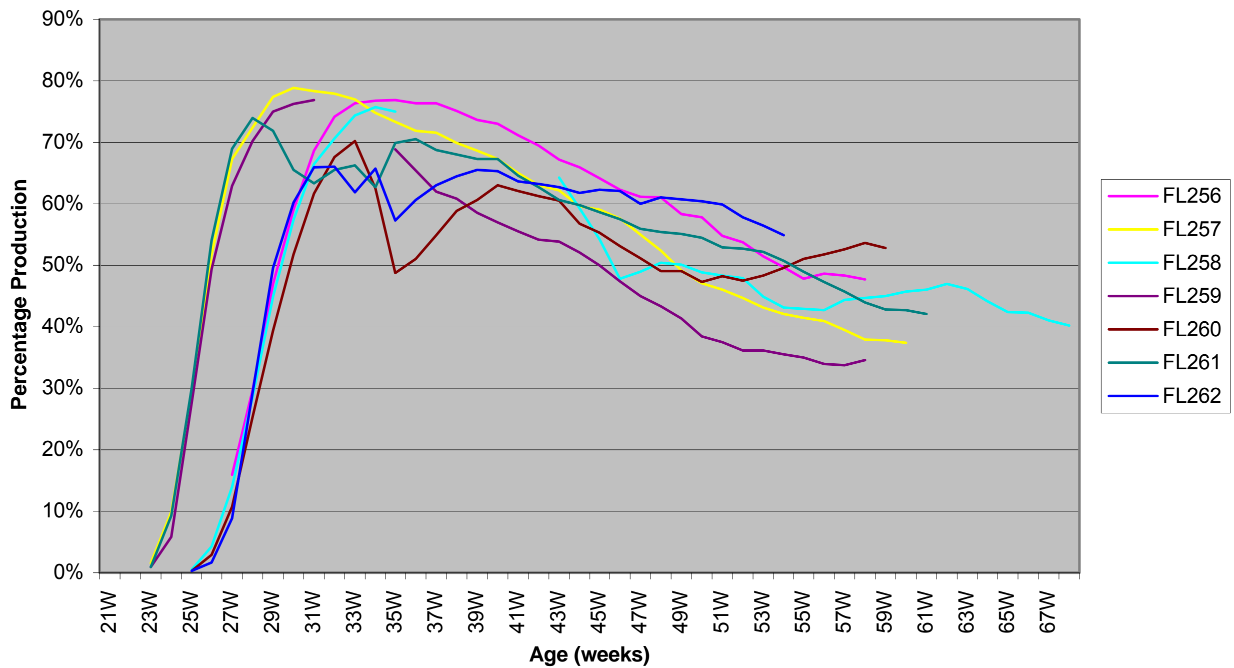
1.3 Broiler Breeder Performance Data

Figure 3 shows percentage hen week egg production for flocks 256-262.

Figures 4 - 7 compare combined percentage hen day egg production, mean egg weights, cumulative mortality and hatchability data of vaccinated flocks with unvaccinated flocks.

Appendix II contains the complete production graphs for flocks 256-262.

Figure 3. Percentage hen week egg production (individual flocks)



Flocks 257, 259 and 261 were OR vaccinated.

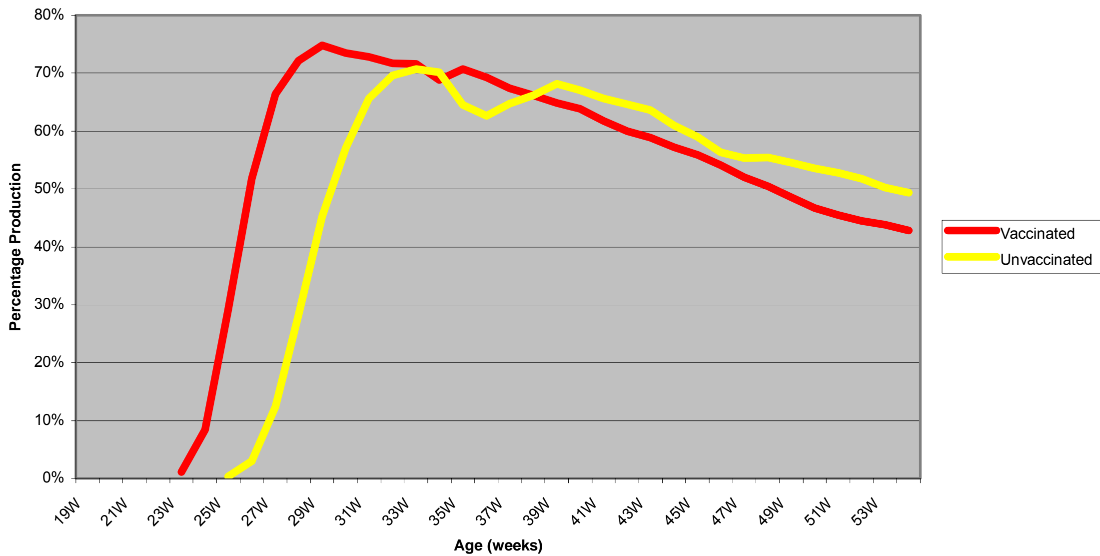


Figure 4. Average percentage hen week egg production - vaccinated vs. unvaccinated Flocks
Percentage Production
80%
70%
60%
50%
40%
30%
20%
10%
0%
19W
21W
23W
25W
27W
29W
31W
33W
35W
37W
39W
41W
43W
45W
47W
49W
51W
53W
Age (weeks)
Vaccinated
Unvaccinated

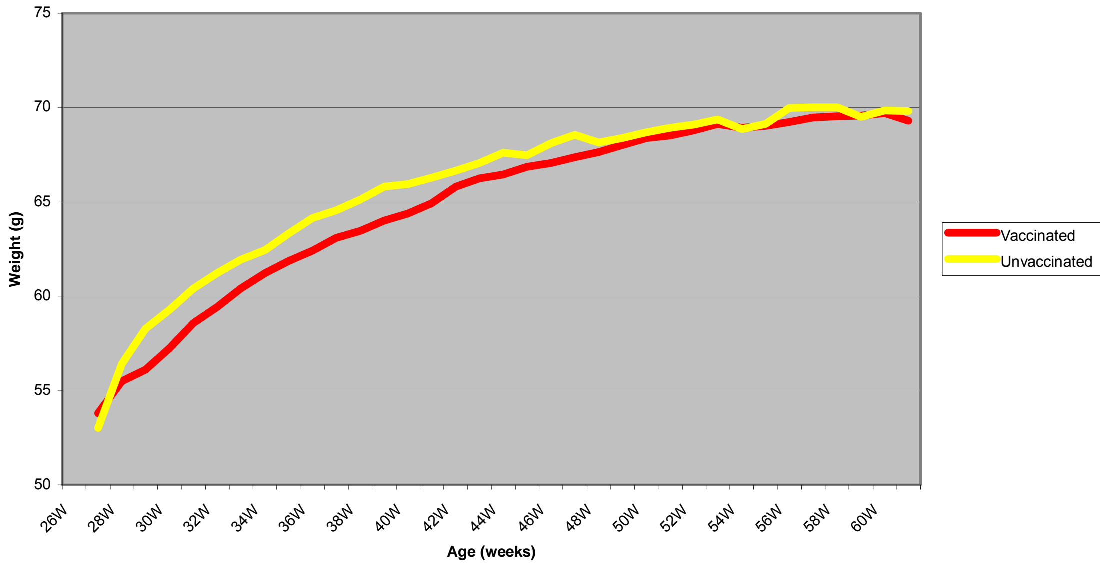


Figure 5. Mean egg weight vaccinated vs. unvaccinated flocks
Weight (g)
75
70
65
60
55
50
26W
28W
30W
32W
34W
36W
38W
40W
42W
44W
46W
48W
50W
52W
54W
56W
58W
60W
Age (weeks)
Vaccinated
Unvaccinated

Figure 6. Cumulative mortality vaccinated vs. unvaccinated Flocks

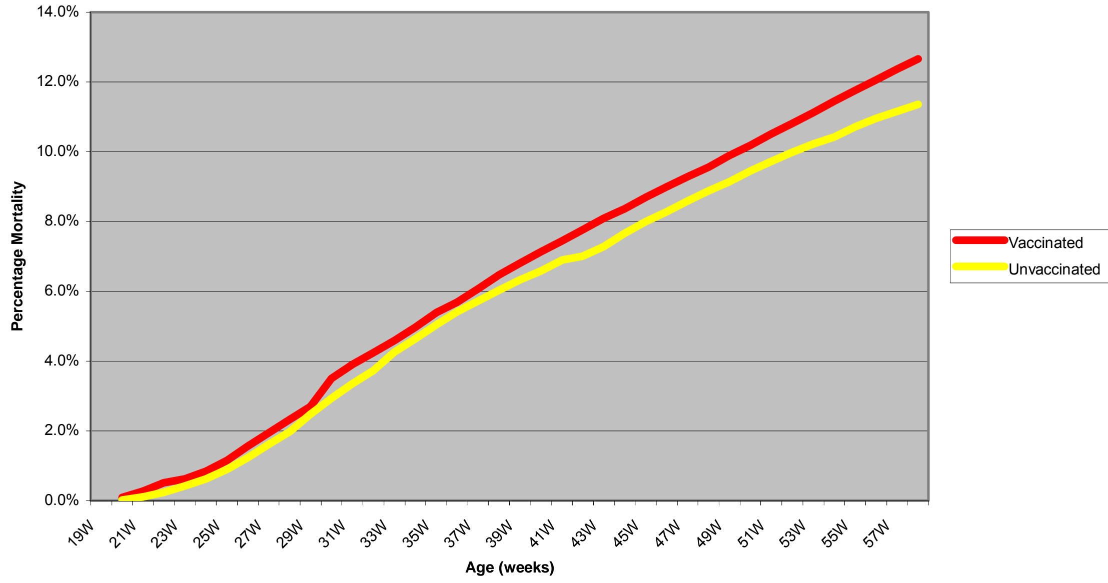
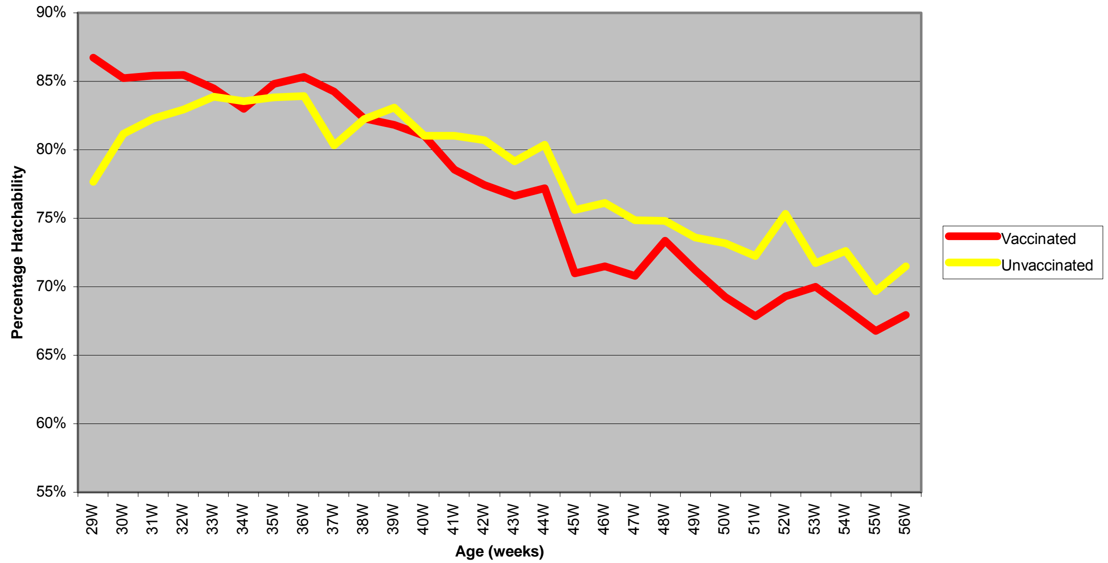


Figure 7. Hatchability vaccinated vs. unvaccinated flocks



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2. VACCINE EFFICACY

2.1 Serological response to vaccination in broiler breeders

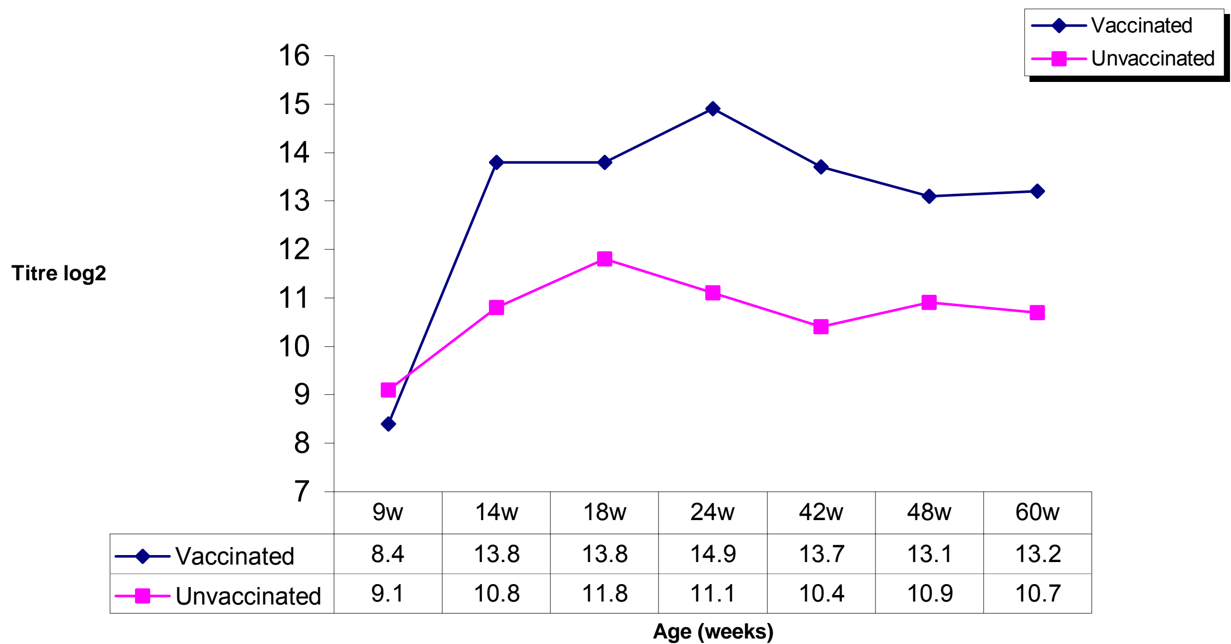
Table 12. Mean OR ELISA titres of EarlyBird breeders by age at bleed

Flock	256	**257**	258	**259**	260	**261**	262	263	**Group**
Age									
9 weeks	9.61	8.97	9.25		8.15	7.64	7.93	9.51	9 weeks
14 weeks		13.00	11.35		10.25				14 weeks
15 weeks						14.51			14 weeks
17 weeks			11.77		11.41			10.63	18 weeks
18 weeks	13.34	14.71		13.77		13.30			18 weeks
20 weeks							11.07		None
21 weeks	14.97								None
24 weeks		14.82					10.85		25 weeks
25 weeks					11.62	14.40			25 weeks
26 weeks				15.75					25 weeks
29 weeks						13.82			30 weeks
31 weeks				15.15				9.19	30 weeks
41 weeks							9.94		42 weeks
42 weeks	12.02	13.99			9.73				42 weeks
43 weeks				13.37					42 weeks
44 weeks			10.36						42 weeks
47 weeks						13.09			48 weeks
48 weeks	11.90				9.92				48 weeks
54 weeks		13.90							None
59 weeks	10.70								60 weeks
60 weeks		13.91				12.52			60 weeks

Key to Table 12.
Vaccinated flocks are indicated by bold type.
The mean log_2 titre for each bleed is given in the columns of the table.

The results of the grouped data are shown in figures 8 and 9.

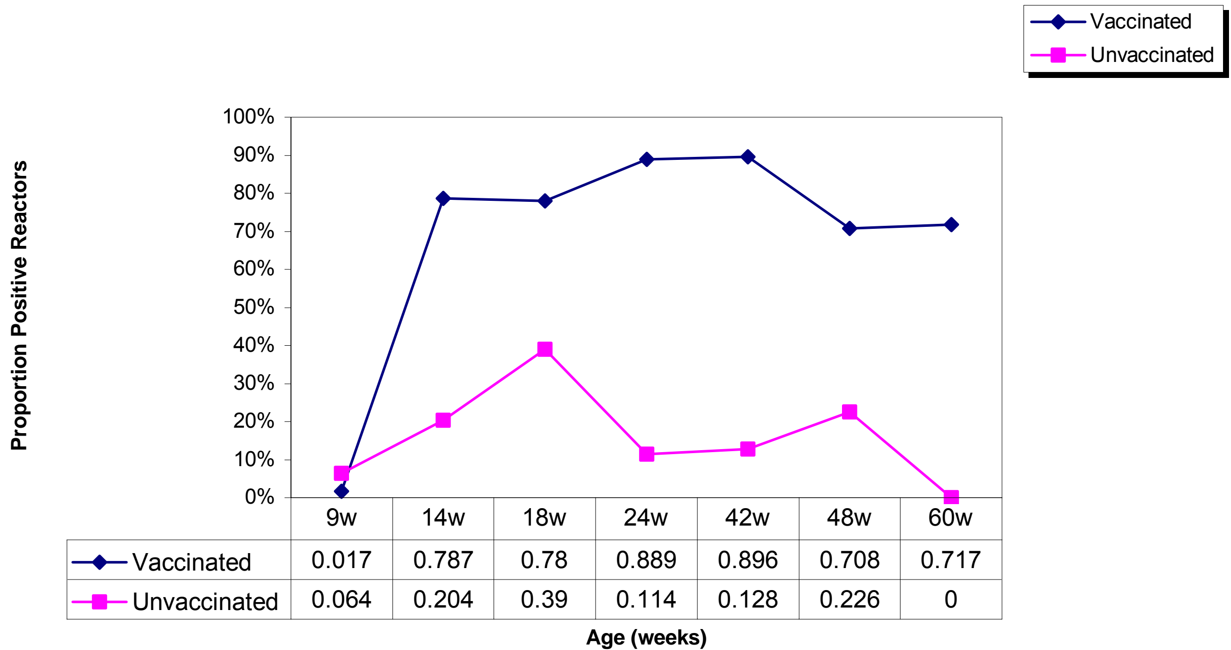
Figure 8. Mean OR ELISA titres in broiler breeders



	9w	14w	18w	24w	42w	48w	60w
Vaccinated	8.4	13.8	13.8	14.9	13.7	13.1	13.2
Unvaccinated	9.1	10.8	11.8	11.1	10.4	10.9	10.7

The difference in titres is highly significant statistically between vaccinated and unvaccinated groups at all ages between 14 and 60 weeks ($p<0.001$) (Figure 8). In the vaccinated group the increase in titres between 9 and 14 weeks is highly significant ($p<0.001$), the increase between 18 and 24 weeks is also significant ($p<0.02$). The subsequent decline in titres is significant only between 24 and 42 weeks ($p<0.01$). In the unvaccinated group the increase in serological response between 9 and 14 weeks, although lower than in the vaccinated group, is also highly significant ($p<0.001$). The further increase between 14 and 18 weeks is also significant ($p<0.05$).

Figure 9. Proportion of broiler breeders with positive (>12) ELISA titres



	9w	14w	18w	24w	42w	48w	60w
Vaccinated	0.017	0.787	0.78	0.889	0.896	0.708	0.717
Unvaccinated	0.064	0.204	0.39	0.114	0.128	0.226	0

The proportion of seropositive birds in the vaccinated group is significantly higher at all ages between 14 and 60 weeks ($p<0.001$) (Figure 9).

2.2 Broiler Production

Table 13. Comparison of production results of different broiler treatment groups

Oxytetracycline in Feed	Yes	Yes	No	No
Vaccination	Yes	No	Yes	No
Number of placements	1	3	3	3
Average number placed	53 520	53 520	52 640	52 640
Ave. Site Area (m^2)	2 676	2 676	2 676	2 676
Average slaughter age (days)	37	39.6	39.7	39.6
Percentage mortality	5.27	9.02	9.48	12.03
Percentage survivors	94.79	90.62	89.79	89.15
Average live weight at slaughter (kg)	1.712	1.730	1.666	1.660
Average daily weight gain (g)	46.27	43.69	41.96	41.92
Feed conversion ratio (F.C.R.)	1.736	1.905	1.955	2.009
Kg/m^2	32.46	31.50	29.69	28.71
Total live weight (kg)	86 856	84 299	79 461	76 826
Total feed used (kg)	150 760	160 833	155 197	153 743

Table 14.

Comparison of production results of broilers placed on sites BG1 and BG2

	BG1 Average	BG2 Average
Number of placements	4	4
Average number placed	52 992	52 992
Total Site Area (m^2)	2 676	2 676
Average slaughter age (days)	39.16	39.59
Percentage mortality	10.09	9.28
Percentage survivors	89.91	90.72
Average live weight at slaughter (kg)	1.681	1.694
Average daily weight gain (g)	42.93	42.79
Feed conversion ratio (F.C.R.)	1.920	1.948
Kg/m^2	29.99	30.45
Total live weight (kg)	80 241	81 482
Total feed used (kg)	153 288	158 728

2.3 Broiler Serological and Clinical Results

Table 15. Serological titres of broilers at slaughter, to various diseases

Cycle	C150		C151		C152		C153		C154	
Site	**BG1**	**BG2**	**BG1**	**BG2**	**BG1**	**BG2**	**BG1**	**BG2**	**BG1**	**BG2**
Parent Flock	257	256	258	259	260	259	264	263	261	262
Vacc.	V	C	C	V	C	V	C	C	V	C
Mean OR Titre	7.07	7.13	7.07	7.31	7.29	7.62			7.64	8.47
% Pos. OR Titre	0%	0%	3%[a]	10%[b]	6%[a]	19%[b]			19%[a]	44%[b]
Mean ND Titre	4.6	5.4	2.1	3.1	6.2	7.9	5.4	4.1	4.1	5.4
Mean IB Titre	2891	1560	1452	1028	2909		8188	4691		
Mean TRT Titre	1127	1404	2424	3060	1160	3536	2590	2659	1019	
Mortality Percent	10.3	7.9	15.4	9.6	9.1	10.8	9.7	8.1	5.2	10.3

In horizontal rows, values with differing superscripts are significantly different ($p<0.05$). In rows where there are no superscripts, no statistically significant differences were found.

Table 16. Broiler serological results for OR by site and by treatment

Site	**% Positive OR Titre**
BG1	6%[a]
BG2	16%[b]
Vaccination	
Vaccinated	11%
Unvaccinated	11%

In vertical columns, values with differing superscripts are significantly different. ($p<0.05$) In columns where there are no superscripts, no statistically significant differences were found.

2.4 Partial Farm Budget

Table 17. Partial farm budget for broilers undergoing different preventative treatments against OR challenge.

	Total (R/head)				Difference from Negative Control (R/head)		
Oxytetracycline	**Yes**	**Yes**	**No**	**No**	**Yes**	**Yes**	**No**
Vaccination	**Yes**	**No**	**Yes**	**No**	**Yes**	**No**	**Yes**
Additional Returns							
Carcass sales (R)	11.98	11.98	11.73	11.82	0.16	0.15	(0.10)*
Foregone Returns							
Mortalities during trial	0.63	1.08	1.11	1.42	(0.79)*	(0.34)*	(0.31)*
Additional Costs Incurred							
Cost of Vaccination	0.01		0.01		0.01		0.01
OTC costs	0.13	0.15			(0.13)*	(0.15)*	
Cost of Starter mash	1.64	1.70	1.71	1.76	(0.13)*	(0.06)*	(0.05)*
Cost of Grower mash	3.40	3.54	3.56	3.67	(0.26)*	(0.12)*	(0.10)*
Cost of Finisher/Post Fin.	1.89	2.42	2.30	2.30	(0.40)*	0.13	0.00
				TOTAL	**1.60**	**0.40**	**0.36**

*Values in parentheses are negative.

The values in columns 2-5 represent the total values for each treatment associated with the partial farm budget. The values in the next 3 columns give comparative values for each treatment group compared to the negative control group of broilers, that is the group hatched from unvaccinated parent flocks and which did not receive OTC in their feed. This second group of figures represents the financial benefit that accrued as a result of the various treatments in the trials that were carried out.

2.5 Quantitative Risk Analysis

Table 18 shows the result of the modeling exercise carried out, based on the available partial farm budget and using the parameters detailed in Chapter 3.

Table 18.

Comparable profitability of broilers undergoing different preventative treatments against OR challenge

OTC	**Yes**	**Yes**	**No**
Vaccination	**Yes**	**No**	**Yes**
Minimum	(R 0.34)*	(R 2.29)*	(R 1.50)*
Maximum	R 3.59	R 2.75	R 2.12
Mean	R 1.60	R 0.40	R 0.35
Standard Deviation	R 0.61	R 0.79	R 0.57
Mode	R 1.53	(R 0.58)*	R 0.39
90%	R 0.80	(R 0.65)*	(R 0.40)*
75%	R 1.18	(R 0.15)*	R 0.04
50%	R 1.62	R 0.42	R 0.37
25%	R 2.05	R 0.97	R 0.76
10%	R 2.39	R1.42	R 1.08
Percentage of cycles where treatment is more profitable than negative control	99%	70%	74%

*Values in parentheses are negative.

Quantitative risk analysis results also show the sensitivity of the possible financial outcomes to the different variables entered into the model. These are listed in Table 19.

Table 19. Regression sensitivity to different variables for profit difference per head

OTC Vaccination	Yes Yes	Yes No	No Yes
Average slaughter mass of test group	0.645	0.798	0.565
Average slaughter mass of negative control group	(0.760)*	(0.590)*	(0.820)*
Carcass price	0.070	0.026	0.015
Price of OTC	(0.03)*	(0.026)*	
Mortality rate of test group	(0.019)*	(0.012)*	(0.015)*
Mortality rate of negative control group	0.016	0.011	0.017

*Values in parentheses are negative.

The ascending cumulative frequency plots in Figures 10-12 show the probability of the profit being less than or equal to the *x* value by means of a cumulative percentile. The cumulative percentile is calculated as $P_x = i/(n+1)$, where *i* is the rank of that data value and *n* is the total number of generated values. The cumulative percentile provides an estimate of the theoretical cumulative distribution function of the output that the data is trying to reproduce.

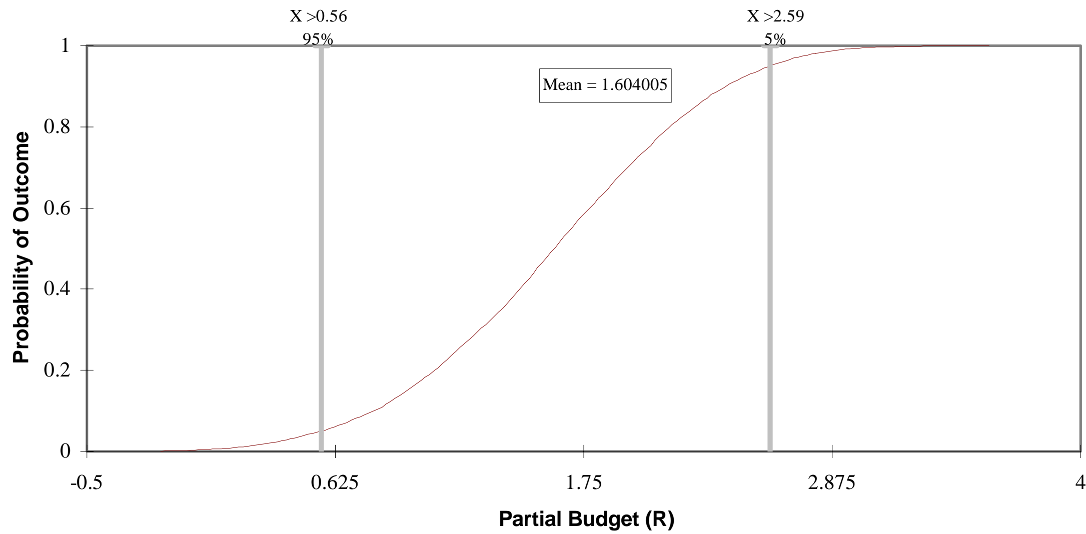


Figure 10. Cumulative distribution of the difference in profit per head between negative control group and OTC medicated and parent OR vaccinated broilers
X >0.56
95%
X >2.59
5%
Mean = 1.604005
Probability of Outcome
1
0.8
0.6
0.4
0.2
0
-0.5
0.625
1.75
2.875
4
Partial Budget (R)

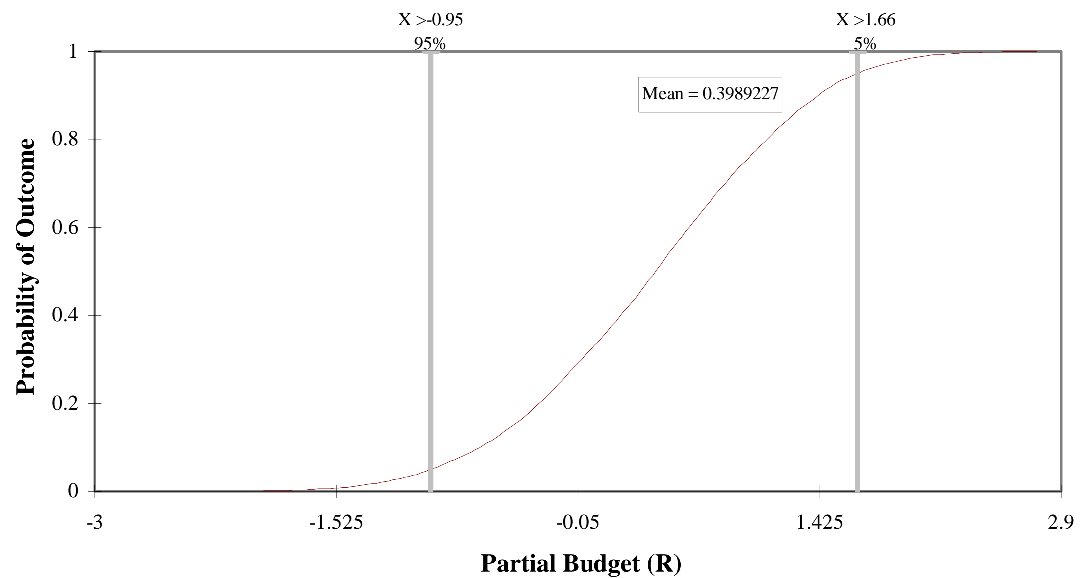
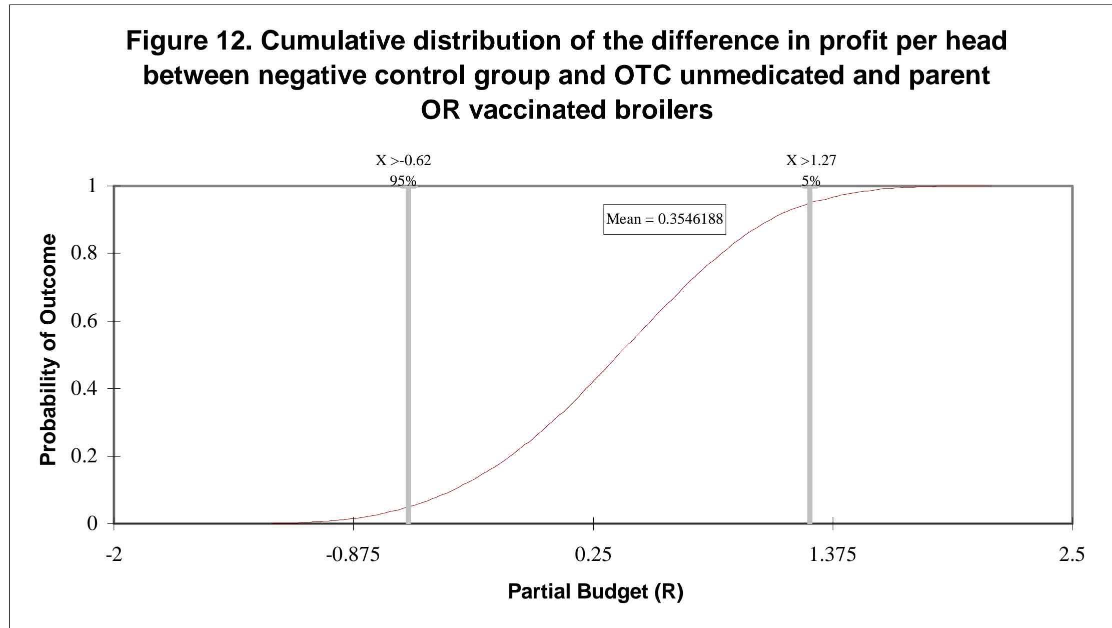


Figure 11. Cumulative distribution of the difference in profit per head between negative control group and OTC medicated and parent OR unvaccinated broilers
X >-0.95
95%
X >1.66
5%
Mean = 0.3989227
Probability of Outcome
1
0.8
0.6
0.4
0.2
0
-3
-1.525
-0.05
1.425
2.9
Partial Budget (R)



2.6 Challenge Studies

Tables 20 and 22 compare the challenge results obtained in the groups of birds that remained unchallenged with results obtained in groups that were exposed to various challenges – either ND vaccination alone or OR challenge in addition to ND vaccination. Tables 21 and 23 compare the challenge results obtained in broilers originating from vaccinated breeder flocks with those obtained in progeny of unvaccinated breeder flocks.

Table 20.

Results of first OR challenge in broilers (Challenge 1), evaluation by challenge treatment group

	No Challenge	Newcastle Vaccination	Newcastle Vaccination and OR Challenge
Mean Weights (g)			
Day 0	42.9	44.4	43.6
Day 16 (pre challenge)	470.1[a]	447.9[b]	464.7[a]
Day 28 (7 days post challenge)	987.1[a]	888.2[b]	913.9[b]
Day 35 (14 days post challenge)	1 289.4	1 255.8	1 280.9
Results from Isolator 1 excluded as weights reduced by water shortage.			
Mean Newcastle Disease HI Titres			
Day 16 (pre-vaccination)	1.5	1.9	2.3
Day 28 (14 days post-vaccination)	7.8	8.8	9.5
Day 35 (21 days post-vaccination)	6.5	6.5	6.7
Percentage of Samples with Positive OR ELISA Titres			
Day 16 (pre-challenge)	25%	17%	25%
Day 28 (7 days post-challenge)	13%	18%	15%
Day 35 (14 days post-challenge)	40%	11%	13%
Mean Total Lesion Score			
Day 28 (7 days post-challenge)	4.9	4.6	4.6
Day 35 (14 days post-challenge)	2.7	2.4	2.7
Percentage of Positive Bacterial Re-Isolation			
Day 28 (7 days post-challenge)	42%	33%	54%
Day 35 (14 days post-challenge)	42%	33%	29%

In horizontal rows, values with differing superscripts are significantly different. ($p<0.05$) In rows where there are no superscripts, no statistically significant differences were found.

Table 21.

Results of first OR challenge in broilers (Challenge 1), evaluation by parent vaccination group

	Vaccinated F 257	Control A F257	Control B Tvl. Chicks
Mean Weight (g)			
Day 0	44.8[a]	45.8[a]	41.3[b]
Day 16(pre-challenge)	444.0	433.6	452.7
Day 28(7 days post-challenge)	901.3	903.2	882.8
Day 35(14 days post-challenge)	1 247.2	1 226.7	1 261.7
Mean Newcastle Disease HI Titres			
Day 16 (pre-vaccination)	2.0	1.6	2.4
Day 28 (14 days post-vaccination)	8.9	9.6	8.3
Day 35 (21 days post-vaccination)	6.4	6.8	6.5
Percentage of Samples with Positive OR ELISA Titres			
Day 0	0%	0%	0%
Day 16 (pre-challenge)	69%[a]	0%[b]	0%[b]
Day 28 (7 days post-challenge)	5%[a]	9%[a]	33%[b]
Day 35 (14 days post-challenge)	22%	12%	32%
Mean Total Lesion Score			
Day 28 (7 days post-challenge)	4.8	4.3	5.0
Day 35 (14 days post-challenge)	2.3	2.9	2.8
Percentage of Positive Bacterial Re-Isolation			
Day 28 (7 days post-challenge)	57%	41%	43%
Day 35 (14 days post-challenge)	24%	43%	35%

In horizontal rows, values with differing superscripts are significantly different. ($p<0.05$) In rows where there are no superscripts, no statistically significant differences were found.

Table 22.

Results of second OR challenge in broilers (Challenge 2), evaluation by challenge treatment group

	No Challenge	Newcastle Vaccination	Newcastle Vaccination and OR Challenge
Mean Newcastle Disease HI Titres			
Day 14 (6 days post vaccination)	2.9	3.1	1.9
Day 21 (13 days post-vaccination)	1.3[a]	4.7[b]	5.1[b]
Day 28 (20 days post-vaccination)	0.5[a]	4.3[b]	4.1[b]
Day 35 (27 days post vaccination)	0.4[a]	2.6[b]	2.7[b]
Day 42 (34 days post vaccination)	0[a]	2.8[b]	2.0[b]
Percentage of Samples with Positive (>8) OR ELISA Titres			
Day 14 (pre-challenge)	0%	17%	0%
Day 21 (7 days post challenge)	0%	8%	14%
Day 28 (14 days post challenge)	19%	17%	36%
Day 35 (21 days post challenge)	42%[a]	29%[a]	79%[b]
Day 42 (28 days post challenge)	44%	38%	73%
Mean Total Lesion Score			
Day 28 (7 days post-challenge)	0[a]	1.3[b]	3.3[c]
Day 35 (14 days post-challenge)	0.3[a]	0[a]	1.8[b]
Percentage of Positive Bacterial Re-Isolation			
Day 21 (7 days post-challenge)	0%	69%	57%
Day 35 (14 days post-challenge)			25%

In horizontal rows, values with differing superscripts are significantly different. ($p<0.05$) In rows where there are no superscripts, no statistically significant differences were found.

Table 23.

Results of second OR challenge(Challenge 2) in broilers, evaluation by parent vaccination group

	Vaccinated	Control
Mean Newcastle Disease HI Titres (all groups combined)		
Day 14 (6 days post vaccination)	2.6	2.3
Day 21 (13 days post-vaccination)	3.4	3.7
Day 28 (20 days post vaccination)	2.9	2.6
Day 35 (27 days post vaccination)	2.2	1.8
Day 42 (34 days post-vaccination)	1.9	1.5
Percentage of Samples with Positive (>8) OR ELISA Titres (challenge group only)		
Day 14(pre-challenge)	0%	0%
Day 21 (7 days post-challenge)	0%	38%
Day 28 (14 days post-challenge)	42%	38%
Day 35 (21 days post challenge)	77%	80%
Day 42 (28 days post-challenge)	83%	67%
Mean Total Lesion Score (challenge group only)		
Day 21(7 days post-challenge)	3.5	3.1
Day 35 (21 days post-challenge)	1.2	2.3
Percentage of Positive Bacterial Re-Isolation (challenged group only)		
Day 21 (7 days post-challenge)	46%	53%

In horizontal rows, values with differing superscripts are significantly different. ($p<0.05$) In rows where there are no superscripts, no statistically significant differences were found.

Chapter V - Discussion

1. VACCINE SAFETY

1.1 Clinical Observations

Clinical evidence indicated that the birds experienced no adverse systemic reactions to the test vaccine or its application. No increase in mortality was reported in any of the vaccinated flocks during rearing.

1.2 Palpation for Local Reactions

It would be expected after application of inactivated vaccines such as the test product, that a proportion of birds would show inflammatory responses to the adjuvant but that this reaction would be transient. As shown in Table 11, this expected pattern was followed in all vaccinated flocks after the nine-week vaccinations. At seven days post vaccination approximately 20% of the birds palpated showed mild diffuse swelling of the breast muscle, while by 14 days post vaccination there was little or no reaction. A similar pattern was observed in flock 257 after the 18-week vaccination.

Fourteen days after the second vaccination in flock 259 circumscribed purulent lesions (abscesses) were found in 60% of birds. Most of these reactions developed between seven and fourteen days after vaccination and are clearly associated with the OR vaccine or vaccine application.

At six and fourteen days after the second vaccination in flock 261, lumps were found in approximately 40% of the birds palpated. When the lesions were noted six days after the second vaccination, cockerels from the same flock, which had not yet had the 18 week vaccination, were palpated. It was found that 80% of the cockerels showed similar lesions prior to vaccination at 18 weeks.

From the finding in the cockerels, it appears likely that the lesions observed in the hens in flock 261 probably did not result from the OR vaccination given at 18 weeks of age, but more probably from an earlier vaccination. The most likely vaccine to have caused the problem was the *Salmonella* Enteritidis/*Pasteurella multocida* combination vaccine given at 15 weeks of age to the birds.

The OR test vaccine was applied a total of six times to three different broiler breeder flocks during this trial. On only one occasion were excessive vaccine reactions associated with the application of this vaccine. It therefore appears more likely that the reactions observed on this single occasion were a result of poor vaccine application and hygiene than a problem with the vaccine itself. It should also be noted that the vigorous reaction observed to another vaccination in flock 261 also only occurred once in the three flocks and may also have been a result of poor application rather than a failure of the vaccine itself.

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1.3 Broiler Breeder Performance Data

Figure 3 shows percentage hen week egg production of individual flocks. Clearly there is variety among flocks, it is also apparent that certain data sets are incomplete. These incomplete data sets for certain flocks contribute to fluctuations when consolidating vaccinated and unvaccinated groups of birds. In terms of production it is apparent that the three vaccinated flocks came into production on average about two weeks earlier than the unvaccinated flocks (Fig. 4). No explanation for this difference can be deduced from the available data. It seems highly unlikely, however, that vaccination could cause birds to come into lay prematurely.

Flocks were exposed to various field challenges during lay, details of challenges may be found in Appendix I. These included *Mycoplasma* infections in various flocks as well as an ongoing problem with myeloid leukosis in flock 258. Most significant, however, were the Newcastle disease challenges that are believed to have affected all of the flocks. Flocks 261 and 262 both failed to achieve good peak production as a result of Newcastle disease challenges at 34 and 28 weeks respectively. In all flocks the Newcastle disease challenge was between 28 and 35 weeks, except for flock 256 which was challenged at 48 weeks. The effect of such challenges would be expected to mask the effects on production of less severe diseases, such as OR.

Figure 4 shows similar egg production in the vaccinated and the unvaccinated flocks, with the vaccinated flocks coming into production earlier and showing initially better results but falling behind later in the cycle. Figure 5 shows that the mean egg mass of unvaccinated flocks was slightly higher than that of vaccinated flocks. No explanation can be given.

Mortality in all EarlyBird breeder flocks monitored for the purpose of the trial was high. This was attributed to the various disease challenges, especially Newcastle disease challenge, the birds had to face at the time. Mortality in vaccinated flocks was slightly higher, culminating at 12.7% at the end of lay compared with 11.4% in unvaccinated flocks. (Figure 6)

Hatchability (Figure 7) fluctuated during production in both vaccinated and unvaccinated flocks. Until 40 weeks of age, average hatchability in vaccinated flocks was better, but thereafter the unvaccinated flocks performed better. When comparing results it should be borne in mind that eggs were hatched at different hatcheries belonging to EarlyBird and that flocks were placed over a six month period. It is likely that hatchery factors influenced these results more than the breeder flocks. It is likely that the various Newcastle disease challenges also affected hatchability.

Overall, there is nothing in the available data to suggest that the OR test vaccine is any less safe for use in broiler breeders than any other oil adjuvant inactivated vaccine, many of which are used routinely in broiler breeder flocks.

2. VACCINE EFFICACY

2.1 Serological Response to Vaccination in Broiler Breeders

(Please refer to Table 12 and Figures 8 and 9.)

At nine weeks of age all flocks tested were serologically negative (mean titre below 12) for OR. In response to the vaccination given at nine weeks of age, the titres of the vaccinated flocks rose from a mean of 8.4 at vaccination to 13.8 at fourteen weeks. There was a further rise to a mean of 14.9 between 18 and 25 weeks of age in response to the repeat vaccination given at 18 weeks. Titres remained high for the duration of lay with a slight decline over time.

In the unvaccinated flocks, mean titres rose from 9.1 to 10.8 from 9 to 18 weeks and then remained constant for the remainder of lay at around 11.

When titres are evaluated in terms of proportion of birds in flocks with positive titres (>12) the trend evidenced by the mean titres is confirmed, with 78.7% of vaccinated birds seroconverting by 14 weeks and 88.9% by 18 weeks. More than 70% of birds remained seropositive to the end of lay. In the case of unvaccinated

flocks only 20.4% had seroconverted by 14 weeks and 39% at 18 weeks. There was a decline with age so that by 60 weeks all birds tested had negative titres.

Clearly the test bacterin caused a highly significant humoral immune response in broiler breeders. The effect of the first vaccination given at 14 weeks was most apparent, while the second vaccination given at 18 weeks although less dramatic was also significant. OR titres in the unvaccinated flocks also rose between nine and 18 weeks. This was possibly due to exposure to OR in these flocks in late rearing or possibly a result of non-specific antibodies developed in response to the numerous other vaccinations given to the birds at this time.

These results were closely similar to those obtained using the same vaccine in Ross broiler breeders in Belgium during 1999 (Cauwerts *et al.,* 2001). In the Belgian study, mean pre-vaccination titres at 12 weeks of age were about 9.5, rising in unvaccinated flocks to 11 during lay. Vaccinated flocks achieved mean titres of 16 at 22 weeks after two vaccinations, dropping away to around 14 later in production. The authors did not specify to what maximum titre was tested. It is possible that it was higher than the 17 used in this study and may account for the slightly higher mean titres obtained in the vaccinated flocks.

2.2 Broiler Production

(Please refer to Tables 13-16 and Appendix III)

This part of the project highlighted many of the difficulties associated with doing scientific research under commercial conditions. After only three flocks of broilers

from vaccinated breeder flocks had been raised (cycles 150-152) without in-feed OTC, the management of EarlyBird Farms determined unilaterally that the broilers from vaccinated parent flocks raised without OTC in the feed were performing worse than flocks raised on other sites at the same time with OTC in the feed. As a result of this they confined the trial work to two small broiler sites and insisted on the inclusion of OTC in the rations of all broilers for the final two cycles (C153 and C154). In cycle 153, EarlyBird placed only birds from unvaccinated flocks, by mistake.

Individual slaughter weights were not supplied and only summary data was made available at the end of each production cycle. Despite these limitations and the limited statistical manipulation that was possible as a result, a number of interesting findings emerged from this work.

Table 13 compares production results of different treatment groups. The unvaccinated group receiving no OTC (negative control group) achieved the poorest results in terms of all production parameters. The progeny of vaccinated breeders receiving no OTC in-feed performed slightly better, gaining 6g more in average bodyweight, 0.054 in F.C.R., and a 2.55% better mortality. The unvaccinated group receiving in-feed OTC performed better than the vaccinated group that did not receive in-feed OTC by gaining an average of 70g more than the negative control group with an improvement in F.C.R. of 0.104 and 3.01%

better mortality. The average slaughter age of each of these three groups was within 0.5 days of 40 days of age.

A single placement of birds received both vaccine and OTC and appeared to perform dramatically better than any of the other groups. Despite being slaughtered three days earlier than the other groups, their average slaughter weight was 52g better, their F.C.R. was 0.273 better and their mortality rate was 6.76% better than the negative control group. The mortality rate of this group was also 3.75% better than that of the group treated with OTC in the feed.

In order to determine the influence of the site on which birds were placed a comparison was done between sites BG1 and BG2 (Table 14). Results on the two sites were similar. On site BG1 mortality was 0.81% higher, F.C.R. 0.028 lower and average weights 13g lower than on site BG2. From these results it was concluded that the site of placement had little effect on the results attained and site was not considered further as a variable.

2.3 Serological and Clinical Findings in Broilers

(results are given in Tables 15 and 16)

2.3.1 Serum Antibody Titres for OR

The results shown in Table 15 show no evidence of OR challenge during cycles 150 or 151. In cycle 154 there appears to have been an OR challenge in site

BG2 where 44% of birds had titres above 8. On site BG1 in cycle 154 and on site BG2 in cycle 152, 19% of birds tested showed seroconversion – the latter suggests some exposure to OR during these cycles as well.

There was no difference in the proportion of seropositive birds between the vaccinated and unvaccinated flocks. The difference between seroconversion between sites was quite significant however, with 16% of the samples from BG2 showing seroconversion while only 6% of samples from BG1 had seroconverted (Table 16). This difference in seroconversion rate did not translate into any difference in production efficacy between the two sites (see 2.2 above).

2.3.2 Serum Antibody titres for Newcastle Disease

Mean ND HI titres ranged from 2.1 to 7.9 with an overall average of 4.8. The company veterinarian considered all of these titres to be a result of ND vaccination.

2.3.3 Serum Antibody Titres for Infectious Bronchitis

Mean IB ELISA titres ranged from 1 028 to 8 188 with a median value of 2 891. Titres above 4 000 were considered by the company veterinarian to indicate challenge. The flocks in BG1 and BG2 during cycle 153 were believed to have undergone IB challenge. These flocks, however, performed well.

2.3.4 Serum Antibody Titres for Turkey Rhinotracheitis Virus

Mean TRT titres ranged from 1 019 to 3 536 with a median value of 2 424. Titres above 4 000 were considered by the company veterinarian consistent with challenge. Based on this criterion none of the broilers were subjected to TRT challenge.

2.3.5 Clincal Findings and Mortality

The highest mortality of 15.4% was in cycle 151 on site BG1. Severe respiratory signs were observed in the flocks together with swollen heads and leg problems. This flock, however remained serologically negative for any of the diseases tested. Its only remarkable result was the low ND titres. Flocks with mortalities between 10 and 15% were C150 in BG1 and C152 in BG 2 where no correlation between mortality and laboratory results could be found. The flock placed in BG2 in cycle 154 also had mortality above 10% and had the highest serological response to OR. The relatively high rate of mortality in this flock may relate to OR challenge.

2.4 Partial Farm Budget

The partial farm budget detailed in Table17 confirms the trends in the broiler production results. The single placement of broiler chicks from a vaccinated parent flock that received OTC in-feed performed dramatically better than the other treatment groups and generated R1.60 per bird more profit than the negative control group. The broilers receiving OTC in-feed also outperformed the

negative control group, by R 0.40 per bird. The broilers hatched from vaccinated breeders that did not get in-feed OTC were R 0.36 more profitable than the negative control group.

2.5 Quantitative Risk Analysis

Table 18 gives a summary of the results of the risk analysis model, based on the data available from the trial. From these results, it can be deduced that if a farmer vaccinated his broiler breeders against OR, using the test vaccine, on average, he would make an additional R 0.35 per bird placed, when compared to the profit he would have made on untreated birds. There is no chance that his relative gain as a result of breeder vaccination would exceed R 2.05 per bird, equally his maximum possible relative loss per bird would be R 1.36. From the various percentiles also given on the table other risk parameters can be calculated. For example there is a 50% chance that he will make a relative profit of R 0.37 or more in any cycle. There is a 74% chance that he will make more money from the treated group in any given cycle than he would have made in the absence of treatment.

Similar extrapolations can be made from the results regarding the other treatment options considered.

With in-feed treatment with OTC (without vaccination) the probability of the farmer making a loss in any individual cycle is 30%. On average he would make

an additional R0.40 per bird placed. These findings are interesting as they suggest that broiler progeny of OR vaccinated breeder flocks would perform quite similarly, from an economic point of view, to broilers originating from unvaccinated parent flocks that receive OTC in their feed. It also shows that while the potential profit that can be made on flocks treated with OTC is slightly larger, the risks are also slightly higher as a result of the greater variability in response to the OTC medication when compared to the OR vaccination.

When OTC is added to the feed of the progeny of OR vaccinated breeder flocks, there appears to be a very good chance of clear benefit for the farmer. On average, he would make an additional profit of R 1.60 per bird placed, and stands a 1% chance of making less money from the treated group than he would make from the control group.

Table 19 shows the sensitivity of the modeled profit outcomes to changes in the input variables. The variables to which the outcomes are most sensitive are the slaughter weight of the treatment groups and the slaughter weight of the untreated group of birds. The average slaughter mass of the test groups is given a positive value as the greater the mass of the treated group, the greater the relative profit made by the farmer. The average slaughter mass of the negative control group is given as a negative value as the relative profit decreases with an increase in this value. Together, these two values account for over 90% of the variability in all groups. It is interesting to note that relative mortality between

groups was not a significant contributor to the variability between treatment and control groups, nor was carcass price or the price of OTC.

Based on the sensitivity of relative profits to the final mass of the birds, the advice that can be given to farmers is that a small change in the final weights achieved by their birds will have a large impact on how profitable a particular cycle will be, financially.

The results shown in table 18 are illustrated in Figures 10-12. The cumulative frequency plot is very useful for reading off quantitative information about the uncertainty of the variable. The range and likelihood of occurrence are directly associated with the level of risk associated with a particular event. By looking at the spread and likelihood of possible results of a given intervention, the decision maker can make an informed decision based on the level of risk he or she is willing to take.

The limited number of broiler trials actually carried out makes validation of the model used, difficult. Particularly problematic was the single cycle of broilers hatched from a vaccinated parent flock that also received in-feed OTC. This flock performed particularly well, but it is impossible to say whether this actually related to either of the treatments given. With only one flock to draw from, minimum and maximum values for different variables were also difficult to determine and had to be estimated based on data available for other treatment groups.

The outcomes of the model are consistent with the apparent benefits seen during the trials in terms of weight gain as well as mortality seen in the treatment groups, when compared to the untreated group. It must also be borne in mind that flocks were slaughtered at different ages and that this would have had a significant impact on feed conversion efficiency as well as final slaughter weights.

2.6 OR Challenge Studies

Challenge 1 (July 1999)

(Please refer to the results in Tables 20 and 21.)

It is apparent that birds in all groups were exposed to both OR and ND. This is evidenced by similar OR ELISA titres, ND HI titres, post mortem lesion scores as well as the rate of bacterial reisolation from necropsy organ specimens in both groups. This problem arose due to airflow from challenged or vaccinated isolators to unchallenged or unvaccinated isolators. The problem was identified and solved before the second challenge was carried out. Differences in weights between groups at 16 and 28 days were a result of "isolator effects".

As a result of the cross-contamination of isolators there was effectively no negative control or vaccinated control group in the challenge and therefore results could not be conclusively attributed to OR challenge. Nonetheless, on the balance of probability certain findings could be made when results were compared between flocks of origin. For this comparison the assumption was

made (as indicated by the results in table 20) that all groups were both vaccinated with ND and challenged with OR.

The response to challenge of progeny of different breeders flocks is given in Table 21. The significantly lower day old mean body weight of chicks hatched from the control B flock reflected the younger age of the breeder flock from which these chicks hatched. From 16 days onwards there was no significant difference among the weights of the different groups.

No OR titres could be detected in any of the broiler chicks from any parent flock in the first day of life. It is likely that chicks were tested before maternal antibodies had been absorbed from the yolksac. By 16 days of age, OR ELISA results suggested that the higher maternal OR titres of the vaccinated breeder hens were transmitted to their progeny. No evidence of maternal antibodies could be detected in the progeny of unvaccinated hens prior to challenge. In control flock B there was a significant serological response to challenge, while in the other flocks there was no evidence of seroconversion seven days after challenge and relatively little at 14 days. It was suspected that a longer period of serological monitoring would be needed after challenge to detect seroconversion. The second challenge trial was modified to allow serological monitoring for 28 days after challenge.

Lesion scores and the rate of bacterial re-isolation in all groups were similar both seven and 14 days after challenge. There was no indication that the higher levels of antibodies to OR found in the vaccinated chicks conferred protection against OR challenge at 21 days. Blood samples were not tested at 21 days of age when the OR challenge was done. It is possible that maternal protection to challenge had declined by that time. To accommodate this concern, the second OR challenge was done in broilers at 16 days of age.

Challenge 2 (January 2000)

Table 22 indicates that a more successful challenge trial was conducted where the unvaccinated negative control group was not exposed to ND vaccination, developed no post mortem lesions and there was no re-isolation of OR from necropsy material. The OR challenged group in contrast showed a good immune response to ND vaccination (as did the vaccinated control group), showed good seroconversion to OR after challenge, developed post mortem lesions and OR could be re-isolated from a large proportion of the specimens collected after challenge.

Both of the control groups showed a steady increase in seroconversion to OR over the duration of the trial and OR could also be re-isolated from the ND vaccinated control group 7 days after challenge. It is probable that a low level of OR contamination was present in all the isolators during the trial and that the ND vaccination was sufficient to trigger pathology in this group of birds while

the unvaccinated controls developed no pathology. This finding is consistent with other investigators who have found that OR is often ubiquitous, even in isolation units.

Table 24 shows that there was no significant difference in the response to OR challenge at 16 days by the progeny of vaccinated breeders and the response of broilers hatched from unvaccinated breeder stock.

Chapter VI – Conclusion

1. SAFETY

No systemic reaction to the vaccine was observed in broiler breeders at any time subsequent to vaccination with the test product. In one instance, circumscribed lesions were found in the breast muscles of the birds that could be attributed to OR vaccination. These lesions also had no noticeable clinical effect on the birds and should most probably be ascribed to poor or unhygienic vaccination technique rather than the test vaccine itself.

While extensive data on the production parameters of the breeder flocks was collected, no conclusions could be reached from it regarding the effect of OR on these parameters. The disease situation was confounded by frequent and severe Newcastle disease challenges during the trial period.

2. EFFICACY

2.1 Serological Response to Vaccination in Broiler Breeders

Serological results show that broiler breeders vaccinated with the test vaccine develop a significantly better humoral immune response to OR than to broiler breeders that remain unvaccinated.

2.2 Broiler Production

In the absence of in-feed medication, broiler progeny hatched from OR vaccinated parent flocks performed better than the progeny of unvaccinated parent flocks. Their performance was similar to that of broilers from unvaccinated parent flocks that received OTC in the feed, but inferior to that of broilers from vaccinated parent flocks which also received in-feed medication.

The partial farm budget shows that broilers raised from OR vaccinated breeder flocks were more profitable than negative control flocks. The quantitative risk analysis shows that the probability of making a relative profit from broilers as a result of OR vaccination of parent stock is 74%, from the use of in-feed medication in broilers from unvaccinated parents is 70% and from a combination of the interventions is 99%. It can be concluded that the last of these options is most profitable.

2.3 Challenge Studies

As a result of cross-contamination of isolators with OR, the results of both challenge studies were inconclusive. Nonetheless, the reaction of the progeny of vaccinated breeder flocks to OR challenge under controlled conditions appeared to be similar to that of the progeny of unvaccinated breeder flocks. There was no indication that any benefit in terms of weight gain, mean lesion scores after challenge or the rate of bacterial reisolation accrued to these chicks in the face of OR challenge as a result of the vaccination, in rearing, of their parents.

3. RECOMMENDATIONS

It would be useful to repeat all aspects of this trial, as the bacterin proved to be safe and to elicit a humoral immune response in broiler breeders that should be passed on to their progeny as maternal antibodies. Additionally, baseline levels of antibodies to OR in broiler breeders needs to be established under South African conditions. Further broiler trials would be useful to determine why broilers hatched from vaccinated breeder flocks appear to perform better than those from unvaccinated breeder flocks. It would also be useful to survey broiler flocks to determine levels of exposure to OR.

Appendix I - Vaccination Schedules and Clinical Findings in EarlyBird Breeders. Flocks (256 - 261)

Records of the vaccination programmes as well as notes made by the Company veterinarian at EarlyBird were recorded for each of the breeder flocks used in the trial.

Key to Abbreviations

Many company and trade names are used in the vaccination schedule. The names on the table correspond with particular proprietary names. Where the names of diseases or companies have been abbreviated, these are explained in the key. Names given in full are not explained.

AE = Avian encephalomyelitis

Coryza = Fowl coryza (*Haemophilus paragallinarum*)

IB = Infectious bronchitis

IBD = Infectious bursal disease

ILT = Infectious laryngiotracheitis

im = by intramuscular injection

MG = *Mycoplasma gallisepticum*

MS = *Mycoplasma synoviae*

ND = Newcastle disease

Past. = *Pasteurella multocida*

RPB = Ross Poultry Breeders South Africa

sc = by subcutaneous injection

Se = *Salmonella* Enteriditis

FLOCK 256

HATCH 24/06/98

AGE	DATE GIVEN	VACCINE	ADMINISTRATION
Day Old	hatchery hatchery	RPB ND Broiler RPB Mareks - Rispens	Injection - neck Injection - thigh
Day old	hatchery	ND Clone 30	Coarse spray
Debeaking	30/6/98	Mareks + TAD Gumboro	Injection sc
5-9 days	1/7/98	Coccidiosis-Paracox	Drinking water
18 days	20/7/98	ND La Sota	Spray
28 days	23/7/98	IBD = TAD Gumboro	Drinking water
5 weeks	3/8/98	ND Oil= Newcavac Fowl Pox IB H120	Injection im Wing web Eye drop
9 weeks	24/8/98	Pasteurella SE - Oil ILT = ASL LT-ivax	Wing web Injection im Eye drop
10 weeks	3/9/98	ND La Sota	Drinking water
12 weeks	18/9/98	Deworm - Askaritox	1g/10kg (4hrs in water)
13 weeks	22/8/98	AE	Drinking water
15 weeks	7/10/98	Pasteurella Avivac Coryza	Injection im Injection sc
17 weeks	23/10/98	IB H120	Drinking water
18 weeks	28/10/98	IBD/NCD - oil SE -oil ILT = ASL LT-ivax Deadline	Injection im Injection im Eye drop 1ml on skin
19 weeks	5/11/98	Deworm - Askaritox	1g/10kg (4hrs in water)

CLINICAL FINDINGS

6 weeks : OR isolated from peritoneum
13 weeks : MS positive : *Staphylococcus* isolated from septic hock arthritis
15 weeks : MG positive
22 weeks : Treat with 600g Oxytetracycline/tonne - to help birds recover after coryza challenge.
48 weeks : Newcastle disease challenge.

FLOCK 257

HATCH DATE : 29.07.98

AGE	DATE	VACCINE	ADMINISTRATION
Day Old	hatchery	RPB ND Broiler RPB Mareks - Rispens	Injection - neck Injection - thigh
Day Old	27-31/7/98	ND Clone 30	Coarse Spray
5-9 days	6/8/98	Coccidiosis-Paracox	Drinking water
18 days	24/8'98	ND La Sota	Spray
28 days	27/8/98	IBD = TAD Gumboro	Drinking water
5 weeks	1-4/9/98	ND Oil= Newcavac Fowl Pox IB H120	Injection im Wing web Eye drop
9 weeks	22-30/9/98 22-30/9/98 22-30/9/98	OR - oil =Nobilis SE-Past. - Oil = Avivac ILT = ASL LT-ivax	Injection im Injection im Eye drop
10 weeks	13/10/98	ND La Sota	Drinking water
12 weeks	?	Deworm - Askaritox	1g/10kg (4hrs in water)
13 weeks	27/10/99	AE	Drinking water
15 weeks	4-12/11/98 4-12/11/98	SE-Past. - Oil = Avivac Coryza =Avivac	Injection im Injection sc
17 weeks	24/11'98	IB H120	Drinking water
18 weeks	26/11-4/12/98	IBD/NCD - oil OR - oil =Nobilis ILT = ASL LT-ivax Deadline	Injection im Injection im Eye drop 1ml on skin
19 weeks	?	Deworm - Askaritox	1g/10kg (4hrs in water)

CLINICAL FINDINGS

34 weeks : MG & MS positive

35-38 weeks : Unusual ND titres - possible challenge.
Broiler farms complained of small chickens, egg size also smaller than expected.

FLOCK 258

HATCH DATE : 19.08.98

AGE	DATE	VACCINE	ADMINISTRATION
Day Old	hatchery hatchery	RPB ND Broiler RPB Mareks - Rispens	Injection - neck Injection - thigh
Day old	19/8/98	ND Clone 30	Coarse spray
Debeaking	25/8/98	Mareks + TAD Gumboro	Injection sc
5-9 days	28/8/98	Coccidiosis-Paracox	Drinking water
18 days	10/9/98	ND La Sota	Spray
28 days	18/9/98	IBD = TAD Gumboro	Drinking water
5 weeks	28/9/98	ND Oil= Newcavac Fowl Pox = FP vax IB H120	Injection im Wing web Eye drop
9 weeks	19/10/98	Pasteurella Se - Oil =Talovac ILT = ASL LT-ivax	Wing web Injection im Eye drop
10 weeks	28/10/98	ND La Sota	Drinking water
12 weeks	13/11/98	Deworm - Askaritox	1g/10kg (4hrs in water)
13 weeks	20/11/98	AE	Drinking water
15 weeks	24/11/98	Pasteurella Coryza =Avivac	Injection im Injection sc
16 weeks	5/12/98	ND Avinew	Drinking water
17 weeks	14/12/98	IB H120	Drinking water
18 weeks	14/12/98	IBD/ND - oil SE -oil ILT = ASL LT-ivax Deadline	Injection im Injection im Eye drop 1ml on skin
19 weeks	2/1/99	Deworm - Askaritox	1g/10kg (4hrs in water)

CLINICAL FINDINGS

9 weeks : MS positive
22 weeks : MG suspect titres
22 weeks : Treated with 600g/tonne OTC to help birds to recover after coryza challenge.
35 weeks : Newcastle disease challenge.

Signs of myeloid leukosis were observed at post mortem examination in this flock throughout its lifetime, this may have accounted for the raised mortality in the flock when compared to the other flocks.

FLOCK 259

HATCH DATE : 16.09.98

AGE	DATE	VACCINE	ADMINISTRATION
Day Old	hatchery	RPB ND Broiler RPB Mareks - Rispens	Injection - neck Injection - thigh
Day old	14-18/9/98	ND Clone 30	Coarse spray
Debeaking	21-25/9/98	Mareks + TAD Gumboro	Injection sc
5-9 days	24/9/98	Coccidiosis-Paracox	Drinking water
18 days	8/10/98	ND La Sota	Spray
28 days	16/10/98	IBD = TAD Gumboro	Drinking water
5 weeks	26-30/10/98	ND Oil= Newcavac Fowl Pox = FP vax IB H120	Injection im Wing web Eye drop
9 weeks	17-25/11/98	OR - oil = Nobilis Se - Past. Oil = Avivac ILT = ASL LT-ivax	Injection im Injection im Eye drop
10 weeks	02/12/98	ND Avinew	Drinking water
12 weeks	?	Deworm - Askaritox	1g/10kg (4hrs in water)
13 weeks	16/12/98	AE	Drinking water
15 weeks	5-8/1/99	Se-Past.-oil =Avimune Coryza =Avimune	Injection im Injection sc
17 weeks	19/1/99	IB H120	Drinking water
18 weeks	21-29/1/99	IBD/ND - oil OR - oil =Nobilis ILT = ASL LT-ivax Deadline	Injection im Injection im Eye drop 1ml on skin
19 weeks	2/1/99	Deworm - Askaritox	1g/10kg (4hrs in water)

CLINICAL DATA

2 weeks : *Staphylococcus* isolated from septic hock arthritis.

29 weeks : Many birds with soiled tail feathers. On post mortem examination peritonitis was observed. Culture appeared to be OR positive but was not identified. Production did not increase as sharply as expected.

30 weeks : Newcastle disease challenge.

FLOCK 260

HATCH DATE : 07.10.98

AGE	DATE	VACCINE	ADMINISTRATION
Day Old	Hatchery	RPB ND Broiler RPB Mareks - Rispens	Injection - neck Injection - thigh
Day old	7/10/98	ND Clone 30	Coarse spray
Debeaking	13/10/98	Mareks + TAD Gumboro	Injection sc
5-9 days	13/10/98	Coccidiosis-Paracox	Drinking water
18 days	29/10/98	ND La Sota	Spray
28 days	4/11/98	IBD = TAD Gumboro	Drinking water
5 weeks	16/11/98	ND Oil= Newcavac Fowl Pox = Intervet IB H120	Injection im Wing web Eye drop
9 weeks	3/12/98	Pasteurella SE Oil ILT = ASL LT-ivax	Wing-web Injection im Eye drop
10 weeks	14/12/98	ND Avinew	Drinking water
12 weeks	3/12/98	Deworm - Askaritox	1g/10kg (4hrs in water)
13 weeks	08/01/99	AE- TAD	Drinking water
15 weeks	21/01/99	Pasteurella Coryza =Avivac Komarov	Wing-web Injection sc Injection im
17 weeks	01/02/99	IB H120	Drinking water
18 weeks	02/02/99	IBD/ND - oil SE oil – Talovac 109 ILT = ASL LT-ivax Deadline	Injection im Injection im Eye drop 1ml on skin
19 weeks	15/02/99	Deworm - Askaritox	1g/10kg (4hrs in water)
NOTE :	House 6	Pasteurella was done at 18 Weeks.	

CLINICAL DATA

4 weeks : *Staphylococcus* isolated from septic hock joints.
9 weeks : IBD challenge observed serologically.
11-15 weeks : Five out of seven houses treated with Avimox, Baytril or Advocin for leg problems – all recovered well.
14 weeks : MS positive.
17 weeks : MG positive.
32 weeks : Newcastle disease challenge.

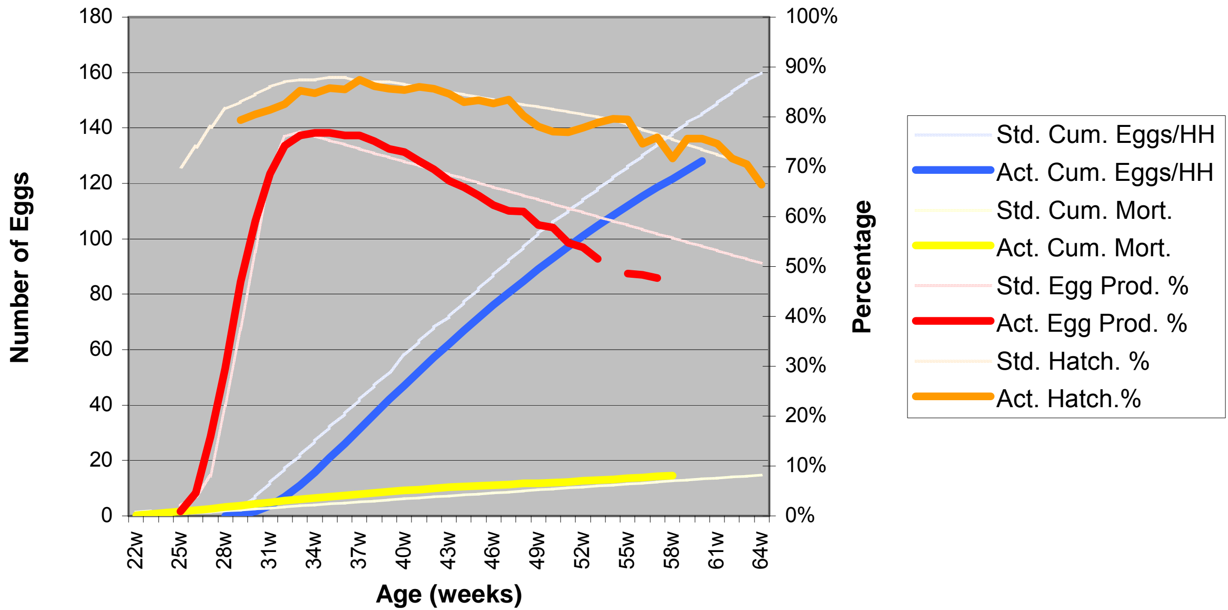
FLOCK 261

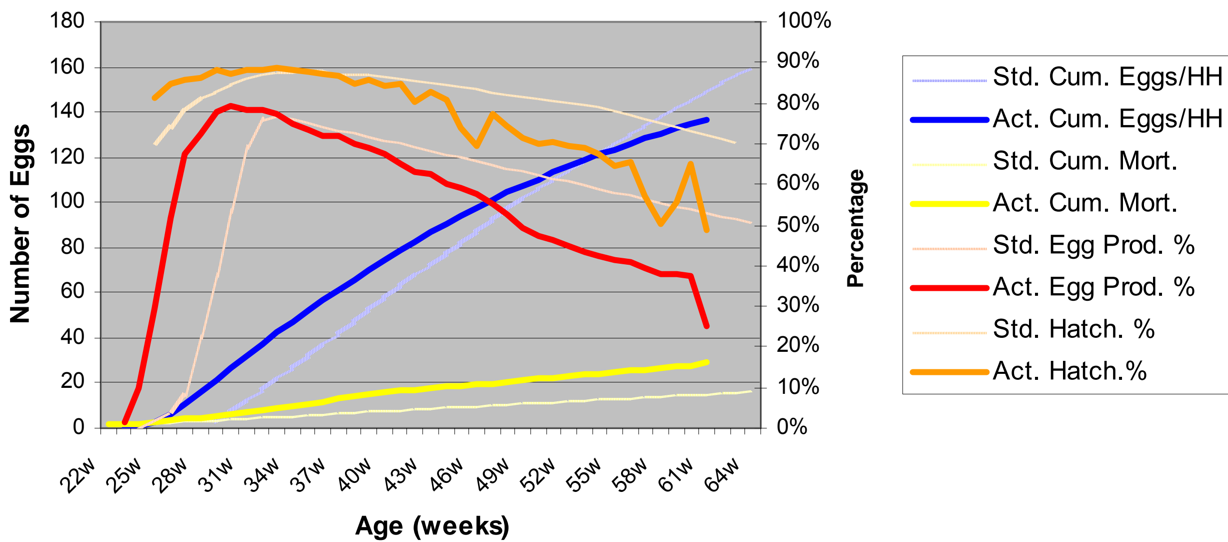
HATCH DATE : 11.11.98

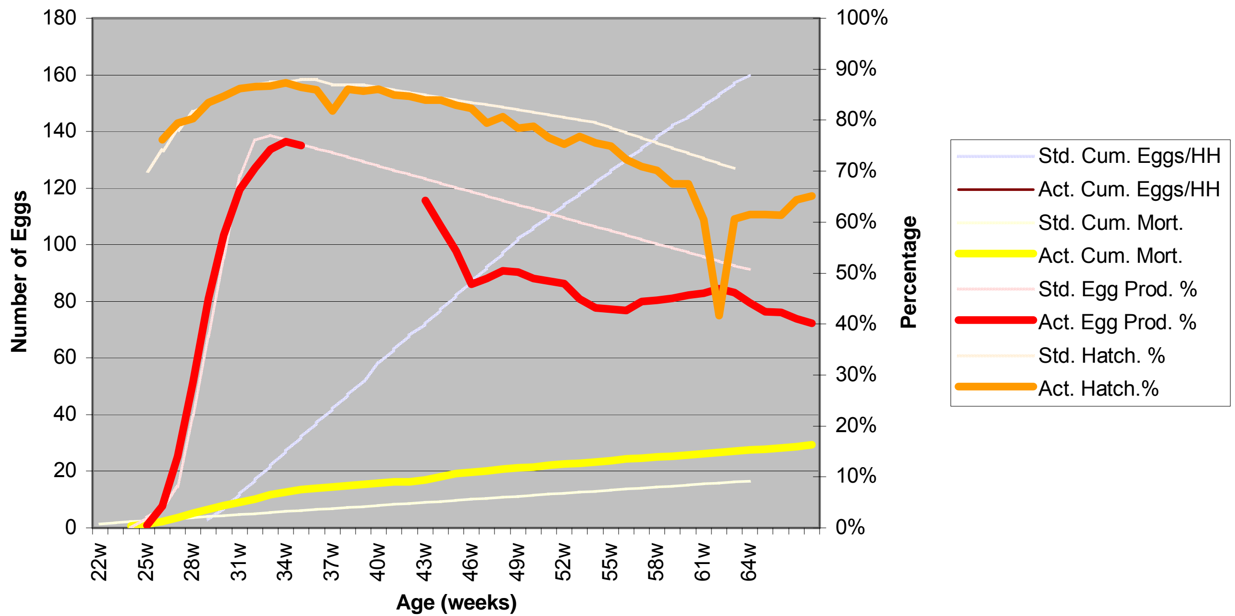
AGE	DATE	VACCINE	ADMINISTRATION
Day Old	Hatchery	RPB ND Broiler RPB Mareks - Rispens	Injection - neck Injection - thigh
Day old	9-13/11/98	ND Clone 30	Coarse spray
Debeaking	17-20/11/98	Mareks + TAD Gumboro	Injection sc
5-9 days	19/11/98	Coccidiosis-Paracox	Drinking water
18 days	04/12/98	ND Avinew	Drinking water
28 days	?	IBD = TAD Gumboro	Drinking water
5 weeks	15-21/12/98	Komarov Fowl Pox IB H120	Injection im Wing web Eye drop
9 weeks	12-20/01/99	OR oil = Nobilis SE - Past. Oil = Avivac ILT = ASL LT-ivax	Injection im Injection im Eye drop
10 weeks	19/01/99	ND Avinew	Drinking water
12 weeks	?	Deworm - Askaritox	1g/10kg (4hrs in water)
13 weeks	09/02/99	AE-TAD	Drinking water
15 weeks	18-26/02/99	SE-Past. oil = Avivac Coryza =Avivac ND Komarov	Wing-web Injection sc Injection im
17 weeks	11/03/99	IB H120	Drinking water
18 weeks	17-24/3/99	IBD/ND - oil OR-oil = Nobilis ILT = ASL LT-ivax Deadline	Injection im Injection im Eye drop 1ml on skin
19 weeks	24/03/99	Deworm - Askaritox	1g/10kg (4hrs in water)

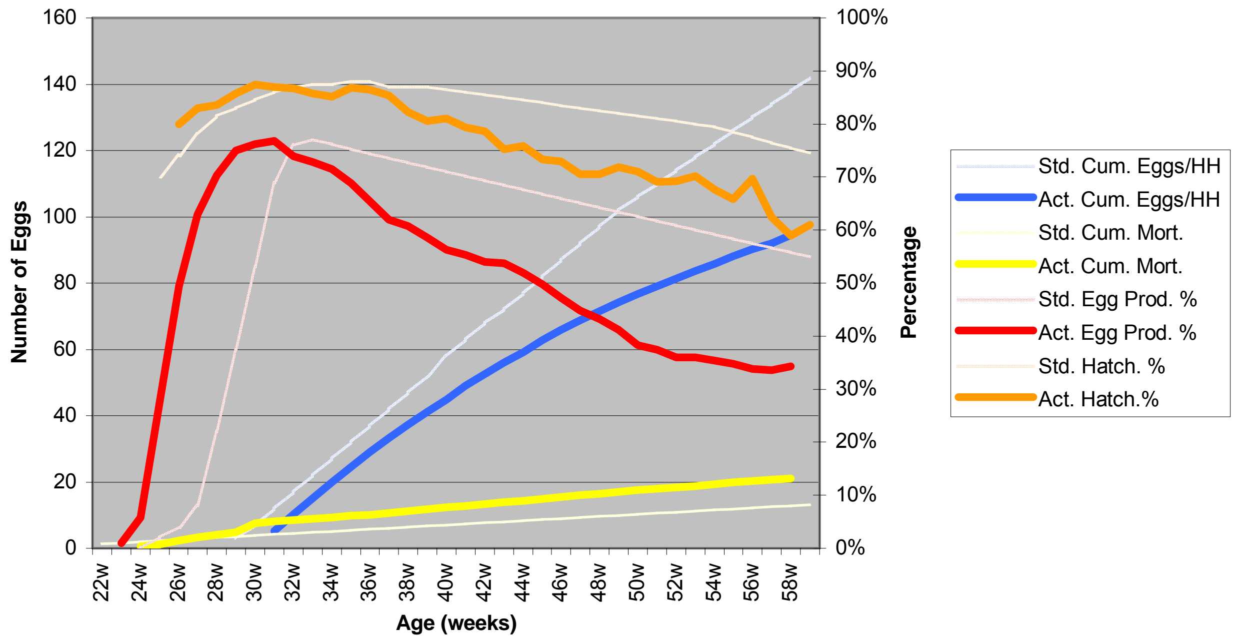
CLINICAL DATA
4 weeks : Leg problems.
10 weeks : Leg problems.
34 weeks : Newcastle disease challenge.

Appendix II - EarlyBird Broiler Breeder Production Graphs (Flocks 256 – 262)

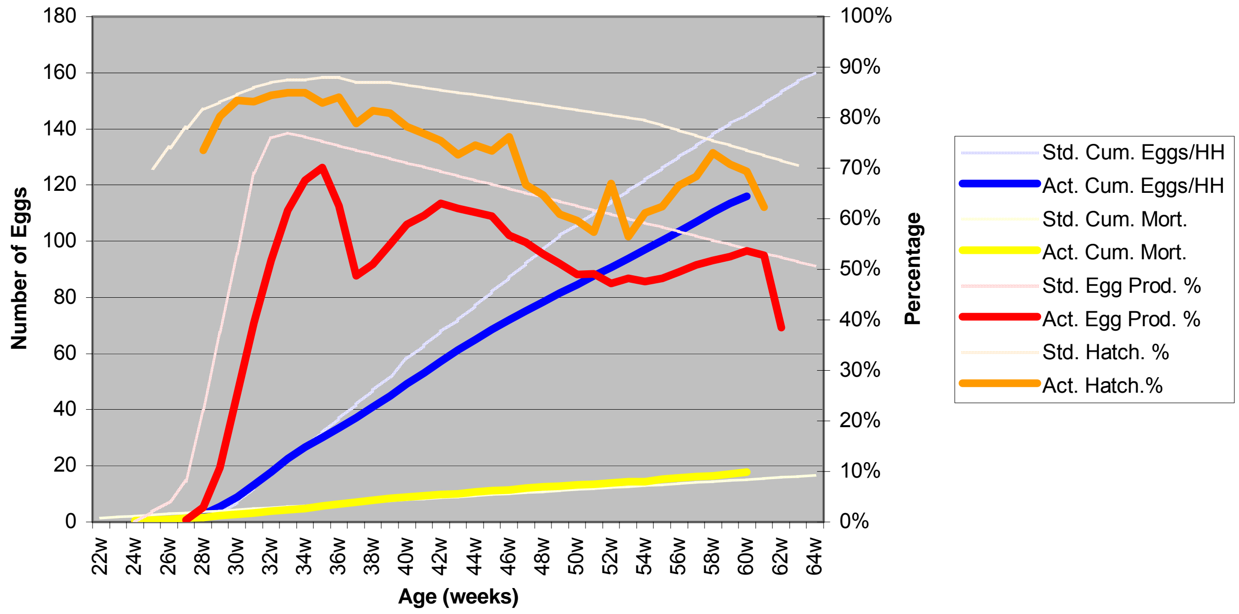




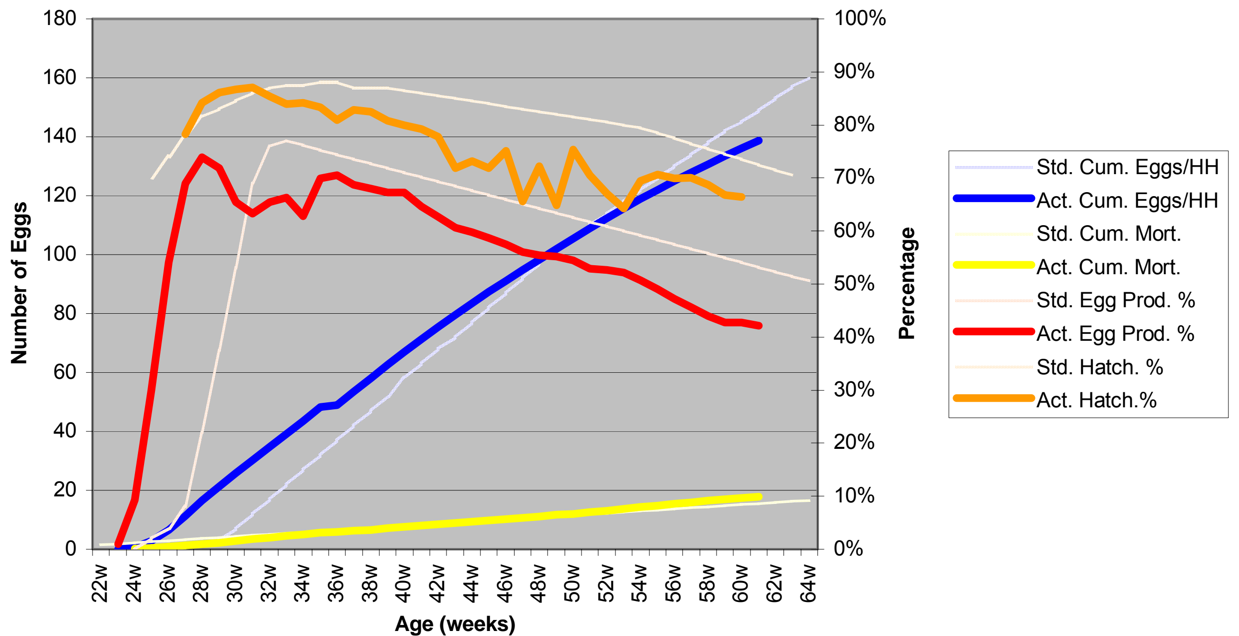

Flock 258
Number of Eggs
Percentage
Age (weeks)
180
160
140
120
100
80
60
40
20
0
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
22w
25w
28w
31w
34w
37w
40w
43w
46w
49w
52w
55w
58w
61w
64w
Std. Cum. Eggs/HH
Act. Cum. Eggs/HH
Std. Cum. Mort.
Act. Cum. Mort.
Std. Egg Prod. %
Act. Egg Prod. %
Std. Hatch. %
Act. Hatch.%

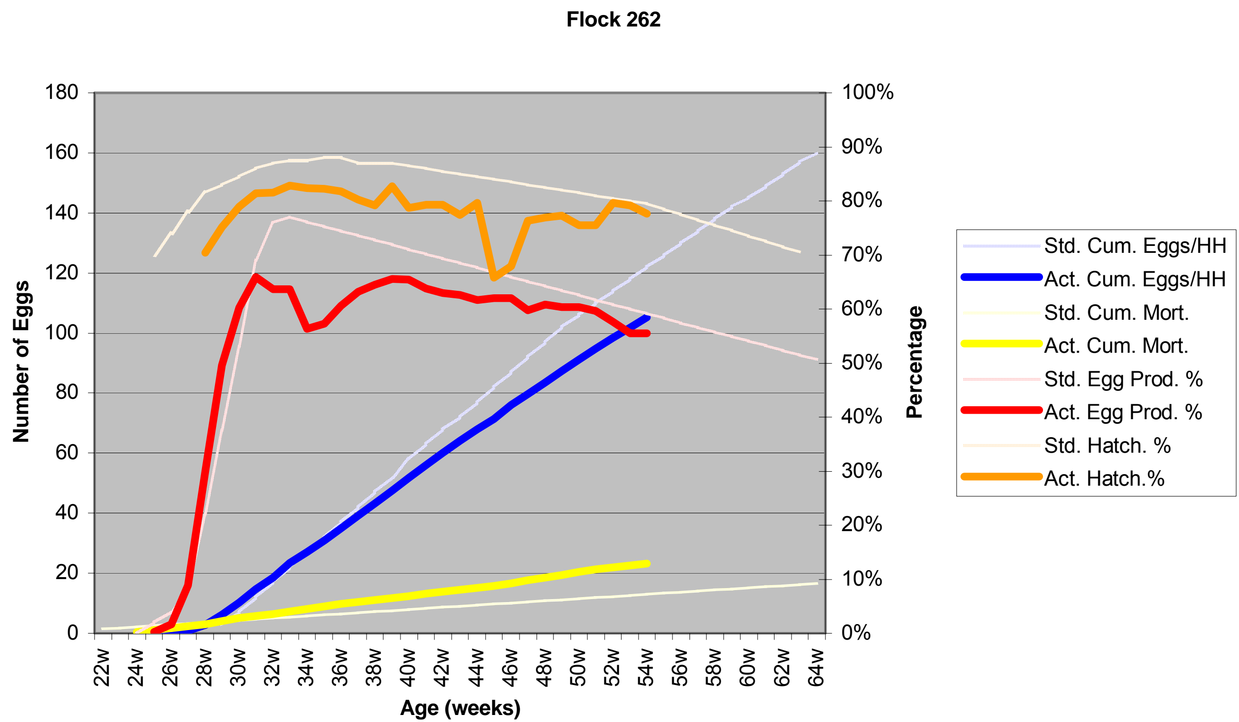

Flock 259
Number of Eggs
Percentage
Age (weeks)
160
140
120
100
80
60
40
20
0
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
22w
24w
26w
28w
30w
32w
34w
36w
38w
40w
42w
44w
46w
48w
50w
52w
54w
56w
58w
Std. Cum. Eggs/HH
Act. Cum. Eggs/HH
Std. Cum. Mort.
Act. Cum. Mort.
Std. Egg Prod. %
Act. Egg Prod. %
Std. Hatch. %
Act. Hatch.%

Flock 260



Flock 261





<u>Flock 262</u> (clinical findings)
4 weeks : leg problems.
9 weeks : MG and MS seropositive.
13 weeks : Three of the houses treated with Baytril.
28 weeks : Newcastle disease challenge.

Appendix III. - Production Results of Individual Broiler Cycles at Groenfontein

Cycle	C150		C151		C152		C153		C154	
Site	BG1	BG2	BG1	BG2	BG1	BG2	BG1	BG2	BG1	BG2
Parent Flock	**FL257***	FL256	FL258	**FL259***	FL 260	**FL 259***	FL 264	FL 263	**FL 261***	FL 262
Medication	No OTC	No OTC	No OTC	No OTC	No OTC	No OTC	OTC	OTC	OTC	OTC
Area (m^2)	2676	2676	2676	2676	2676	2676	2676	2676	2676	2676
Slaughter Age	41	41	38.79	39.12	39	39	40	37.82	37	41
% mortality	9.89	9.66	15.47	7.84	10.96	10.71	8.88	8.37	5.27	9.8
% survivors	90.11	90.34	84.53	92.16	89.04	89.29	91.12	91.63	94.73	90.2
Average live weight	1.762	1.823	1.573	1.643	1.571	1.592	1.785	1.540	1.713	1.872
F.C.R.	1.921	1.933	2.107	1.950	1.981	1.995	1.856	1.877	1.736	1.983
kg/m^2	31.76	32.94	26.59	30.29	26.59	27.03	32.53	28.22	32.46	33.76
Total Liveweight	84 983	88 152	71 160	81 066	71 167	72 333	87 038	75 505	86 856	90 353
Total Feed Used	163 240	170 370	149 900	158 070	140 960	144 280	161 580	141 760	150 760	179 160

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