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# *Mycoplasma gallisepticum* in pheasants and the efficacy of tylvalosin to treat the disease

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Short Title: Tylvalosin for *M. gallisepticum* in pheasants

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## Abstract

Infectious sinusitis, a common condition seen in adult pheasants, is primarily caused by *Mycoplasma gallisepticum.* The aims of this study were to investigate the pathogenicity of *M. gallisepticum* in 14 day old pheasants and evaluate the macrolide antibiotic, tylvalosin (TVN), as a treatment for infectious sinusitis. The minimum inhibitory concentration (MIC) of TVN for five isolates of *M. gallisepticum* taken from pheasants confirmed their susceptibility to TVN (MIC range: 0.002-0.008µg/ml). One of the isolates (G87/02) was inoculated intranasally into 72 pheasants (two groups of 36) at 14 days of age. Eight days later, when 18/72 (25%) of the pheasants showed clinical signs, one group was treated with 25 mg TVN/kg bodyweight daily in drinking water for three consecutive days. An uninfected, unmedicated control group (n=12) was also included. In contrast to the uninfected control group, a range of clinical signs typical of infectious sinusitis with varying severity was observed in challenged birds and *M. gallisepticum* was re-isolated from the infraorbital sinus and the eye/conjunctiva at necropsy, 22 days post-challenge. In comparison to untreated birds, medication with TVN significantly reduced clinical signs and the re-isolation/detection of *M. gallisepticum* ($P \leq 0.0021$). The daily liveweight gain of treated birds was significantly increased in comparison to untreated birds ($P=0.0002$), and similar to daily liveweight gains observed in the uninfected control group. In conclusion, TVN at 25 mg/kg bodyweight daily for three consecutive days in drinking water was efficacious in the treatment of *M. gallisepticum* infection induced by challenging 14 day old pheasants.

## Introduction

Recently, the UK game bird industry has increased dramatically with around 30 to 35 million pheasants released into the wild each year (Canning, 2006). As only 3 million breeding birds are available each spring, this number has to be supplemented by rearing pheasants in captivity in order to produce the required number for release. Therefore it has become common practice to capture wild birds during February and March and transfer them to breeding pens, each holding approximately 100 breeding hens and a smaller number of cocks (Pennycott, 2000). Stress induced by this procedure renders the pheasants more susceptible to a variety of infectious and non-infectious diseases.

Infectious sinusitis, an upper respiratory disease, is one of the most common infectious diseases encountered in adult pheasants in these breeding pens (Pennycott, 2000). It is characterised by swelling of the infra-orbital sinuses below the eye and between the eye and the nostril, causing the conjunctiva and the sinuses to bulge (Welchman, 2008). Other signs include nasal exudate, snicking/sneezing, lacrimation, conjunctivitis and depression. There is usually a loss of condition and in laying birds, a loss of egg production. In affected birds morbidity is high, although mortality is variable (Pennycott, 2000; Benčina *et al*., 2003).

Recent investigations of field outbreaks of upper respiratory disease in UK pheasants and partridges have indicated that *Mycoplasma gallisepticum* is frequently involved (Bradbury *et al.,* 2001; Welchman *et al*., 2002). *Mycoplasma gallisepticum* causes an economically important respiratory disease in chickens and turkeys, resulting in mild to severe disease signs and has been implicated as a primary pathogen for Chukar and red-legged partridges (Jordan 1996; McMartin *et al*., 1996; Ganapathy & Bradbury, 1998). Whilst stress, infection with other bacteria, including other mycoplasma species, and viruses

such as avian metapneumovirus and coronavirus contribute to upper respiratory disease in pheasants, *M. gallisepticum* has been identified as a primary pathogen (Forrester *et al.*, 2004; Forrester *et al*., 2006).

Until recently there have been no licensed products available to treat or control infectious sinusitis caused by *M. gallisepticum* in pheasants. The aims of the current study were to investigate the pathogenicity of a pheasant *M. gallisepticum* strain in 14 day old pheasants, determine the in vitro susceptibility of pheasant field strains of *M. gallisepticum* to the macrolide antibiotic tylvalosin (TVN) and evaluate its efficacy as a treatment for *M. gallisepticum* infection in pheasants.

The present work formed part of the successful submission to the European Medicines Agency for the use of TVN, the active ingredient of the product 'Aivlosin 625mg/g granules for drinking water for pheasants' (ECO Animal Health Ltd; referred to as 'Aivlosin Soluble' throughout) for the treatment of respiratory disease associated with *M. gallisepticum* in pheasants, which subsequently led to approval by the European Commission in December 2009 (European Medicines Agency 2009). The product has an approved dose regimen of 25 mg TVN/kg bodyweight for 3 days, and a meat withdrawal period of 2 days.

## Materials and Methods

***Mycoplasma gallisepticum* strains.** Five *M. gallisepticum* isolates previously recovered from UK pheasants (Table 1) were tested to establish minimum inhibitory concentrations (MIC) for TVN according to the method described by Tanner and Wu (1992). TVN was supplied as the tartrate salt. All these isolates had been filter-cloned once and G87/02 was selected for the challenge infection. It was originally isolated in 2002 from an eye swab, had

undergone eight *in vitro* passages and the inoculum was grown in thallium acetate-free broth (Bradbury, 1977).

**Minimum inhibitory concentration (MIC).** The method of Tanner and Wu (1992) was used with a modification to allow the approximate titres of the challenge inocula to be pre-determined and a set target of $10^4$ CFU/ ml for the assay with a tolerance of $10^3$-$10^5$ CFU/ml. The type strain of *M. gallisepticum* PG31$^T$ was included as a control. The antibiotic product was diluted to produce a range of 0.001 to 1.0 µg/ml TVN. Microtitre plates were inoculated and incubated at 36$^o$ C ± 1$^o$ C. Plates were inspected daily until a pH change was first detected in one or more test wells and then subsequently at the start and finish of the working day until test completion. The antibiotic assay for each strain was read when the challenge control gave a pH change equal to or greater than the endpoint control. Antibiotic dilution wells showing any evidence of a pH shift (i.e. growth) were recorded as positive. The MIC was taken as the lowest antibiotic concentration showing no detectable growth. A volume (0.2 ml) of a 1:2 dilution of challenge inoculum was inoculated onto solid medium and incubated in 95% nitrogen, 5% $CO_2$ for 7 to 14 days, in order to determine the CFU/ml.

**Pheasants.** Eighty-four mixed sex, clinically normal one day old pheasants (Chinese cross, ring necked) were obtained from a UK based closed flock source that had no previous record of respiratory disease or *M. gallisepticum* infection and on which previous screening by ELISA and polymerase chain reaction (PCR) for avian metapneumoviruses and coronaviruses had proved negative (Forrester, 2008). The pheasants were individually identified using uniquely-numbered wing tags and confirmed free of *Mycoplasma gallisepticum* within 24 hours of arrival using choanal cleft swabs tested in a PCR for *M. gallisepticum* using a commercial PCR test (Adiavet Myco AV, Adiagene).

**Housing.** On arrival, pheasants were housed in one room of a temperature controlled, experimental poultry facility. At 13 days of age the pheasants were allocated to one of seven wooden pens (12 birds per pen), balanced according to bodyweight. The pens were located in one of three different rooms each supplied with coarse air filtration (two rooms each with three pens and one room with one pen). Pens, which had solid wooden walls, measured approximately 1m wide x 3m long x 1m high (0.25 $m^3$ per bird) and were fitted with a lid covered in a plastic windbreak material to prevent the birds from escaping once they became flighty. Each pen was provided with an overhead electric heater which also served as a red light source when the main lighting was turned off. Wood shavings were placed on the floor and water and food were provided *ad libitum*. A conventional non-medicated pheasant diet was provided. Care and maintenance was on a daily basis and the challenge study was carried out under Home Office approval. All reasonable measures were taken to avoid pen-to-pen or room-to-room transmission of infection, moreover previous studies (Forrester, 2008) in the same accommodation had demonstrated no transmission of avian pneumovirus or pheasant coronavirus between infected and uninfected groups in pens within a room (Forrester, 2008)

**Experimental design.** The challenge study was a masked (such that individuals performing clinical assessments were unaware of treatment), negatively controlled, parallel design with three treatment groups, conducted according to VICH GCP (VICH 2001). Eighty-four pheasants were ranked in decreasing order of bodyweight and objectively allocated in groups of twelve to the seven pens for balance on mean bodyweight. Two rooms each containing three pens (36 birds) were allocated to either a *M. gallisepticum*-challenged, medicated group (G1) or a *M. gallisepticum*-challenged, unmedicated group (G2). The remaining room containing one pen of 12 birds was allocated to an unchallenged, unmedicated group (G3).

**Challenge infection.** At 14 days of age, each bird in G1 and G2 received 50 µl of the challenge organism containing 1.5 x $10^5$ CFU *M. gallisepticum* strain G87/02. The pheasants in G3 were placebo challenged with 50 µl of sterile thallium acetate-free mycoplasma broth. The inoculation was divided between nostrils with each nostril receiving 25 µl of inoculum. The number of colony-forming units (CFU)/ml was confirmed at the time of challenge by the method described by Bradbury and Jordan (1971).

**Treatment**. Treatment started eight days after challenge, defined as day 0 (D0). Pheasants in G1 were given water medicated with Aivlosin Soluble daily for three consecutive days to provide a dose of 25 mg TVN/kg bodyweight. The medicated water was prepared daily based upon the group bodyweight immediately prior to treatment and water consumption measured over the previous 24 hours, by vigorously stirring cold water whilst adding the product, to form an antibiotic solution, according to label directions. Samples of each fresh antibiotic solution were taken and stored at -20$^{o}$C for later analysis of TVN concentration. A sample of the tap water used to prepare the antibiotic was also stored for analysis. A validated high performance liquid chromatography (HPLC) method was used to analyse the TVN content of the water samples.

**Clinical observations.** Each pheasant was examined daily as part of the routine care and maintenance from the day of challenge infection until necropsy 14 days after the first day of treatment, 22 days after challenge. During examination, birds were scored individually for clinical signs of *M. gallisepticum* infection; the presence or absence of lacrimation, conjunctivitis and depression and the severity (bilateral or unilateral, and mild, moderate or marked) of nasal exudates and sinus swelling. The severity of respiratory signs (sneezing,

snicking and gasping) was assessed on a pen basis and classified as normal (0 = no birds/pen), mild (1 = one to two birds/pen), moderate (2 = three to eight birds per pen) or severe (3 = nine to twelve birds per pen).

The birds were weighed pre-challenge, during treatment and at necropsy, 14 days after treatment started. Feed consumption was measured throughout the study. Before necropsy, blood samples were collected for serology. Birds were then humanely euthanized, aseptically necropsied and examined for gross lesions in the trachea (0 = no exudates, 1 = slight redness and small quantity of exudates, 2 = redness of mucous membrane, exudates), before assessment of the left and right air sacs and the peritoneum. During necropsy, swabs were collected from the eyes and infra-orbital sinuses for mycoplasma isolation and identification.

**Laboratory procedures.** Serum samples were tested for antibodies to *M. gallisepticum* using rapid serum agglutination (RSA) (*M. gallisepticum* stained antigen kindly donated by Intervet International B. V., Boxmeer, Holland).

Swabs collected during necropsy were plated onto mycoplasma agar (Bradbury, 1977), for mycoplasma isolation. Swabs were also inoculated into 1.5 ml of thallium acetate-free broth for detection of *M. gallisepticum* by PCR (Adiavet Myco AV). These broth samples were stored at -20$^{o}$C until required. Culture plates were incubated in 5% $CO_2$ at 37$^{o}$C and examined for mycoplasma growth daily for the first week and weekly thereafter up to 4 weeks. All isolates recovered were identified by indirect immunofluorescence (Rosendal and Black, 1972).

**Statistical analysis**. The MIC results were summarised. For the challenge study, the continuous response variables, bird weight gain (g/day) between the days D0 and D14 and

bird proportion of days with either lacrimation, conjunctivitis, nasal exudates, sinus swelling or depression between D0 to D14 were measured on each bird and analysed with two normal based linear models with the fixed effect of treatment group. Nasal exudates and sinus swelling were defined as a clinical score of mild or more.

A model based one-sided t test was used to compare the variables for groups G1 and G2. A second normal based model was used to compare the non-treated groups G2 and G3. A model based two-sided t test was used to compare groups G2 and G3. The categorical variables tracheal lesions, mortality, presence/absence of *M. gallisepticum* were analyzed using Fischer's Exact test. Two sided Fischer Exact tests were used to compare groups G2 and G3. One-sided Fischer Exact tests were used to compare G1 and G2. The significance level of 0.05 was used throughout.



## Results

**MIC values.** All five field isolates of *M. gallisepticum* and the type strain PG31$^{T}$ were highly sensitive to the effects of TVN (Table 1). The MIC value for three strains was 0.008 µg/ml, and 0.002µg/ml and 0.004µg/ml for the other two strains. Isolate G87/02, selected for the challenge study, was one of the least sensitive with an MIC value of 0.008 µg/ml.

**Challenge infection.** There were no mortalities due to the challenge infection, although one pheasant in untreated-challenged group was humanely euthanized 4 days after medication started because of lameness and consequent difficulty feeding and drinking. No clinical signs were seen at any time in the unchallenged group of pheasants. By contrast, clinical signs were first observed in both challenged groups six days after challenge. On D0, immediately

before treatment, 11/36 pheasants in the G1 group and 7/36 pheasants in the G2 group displayed clinical signs, with mild, unilateral nasal exudate being most commonly observed. By 12 days post-challenge (one day after the end of treatment), all five indicative clinical signs were observed the challenged untreated group (Table 2). At this time-point lacrimation and nasal exudate were seen in the treated group albeit at a greatly reduced incidence in comparison with the untreated control group (Table 2). The most commonly observed sign in the untreated control group was depression (35 birds, 13 days post challenge), with 29 birds still depressed at the time of necropsy 9 days later.

At necropsy, tracheal lesions were identified in eight challenged untreated birds, in six of the treated birds, and in no unchallenged birds. *M. gallisepticum* was detected in all 35 remaining challenged, untreated pheasants, in 20 of the treated birds, and in none of the unchallenged birds (Table 3). Further, all unchallenged birds were negative on serology, whilst all challenged untreated birds were positive for specific *M. gallisepticum* antibodies. In the treated group 29/36 birds were sero-positive. This indicates that the challenge infection was successful in inducing typical clinical signs and infection with *M. gallisepticum*.

**Medicated water analysis and dose determination.** Analysis of each of the medicated water samples indicated that the average dose for all three pens over the three days was 22.86 mg/kg bodyweight (91.4% of the target dose). The un-medicated water sample was confirmed TVN-free.

**Clinical signs after treatment.** Following treatment, there was a significant ($P<0.0001$) improvement in all five clinical signs (nasal exudate, swollen sinus, lacrimation,

conjunctivitis and depression) observed for the treated group compared with the untreated group (Table 4).

The number of pheasants displaying clinical signs in the treated group initially decreased during treatment to a minimum of four birds displaying nasal exudate after only one day of treatment (Table 2). However, after cessation of treatment, the number of birds in this group displaying clinical signs of any type gradually increased to a maximum of 11 with nasal exudate (D13 and D14). At no time during the study did any pheasants in the treated group present with depression, and only one bird was identified with conjunctivitis. In contrast, despite having fewer pheasants with clinical signs on D0, the number of birds in the untreated challenged group with clinical signs continued to increase until all birds presented with depression 13 days post-challenge (D5) in addition to various other clinical signs (Table 2).

Throughout the period of observation, none of the treated pheasants displayed respiratory signs (sneezing, snicking or gasping). However, respiratory signs were first observed 13 days post-challenge (D5) for the untreated group. All three pens of untreated birds were identified with moderate respiratory signs (score 3, three to eight birds per pen) from nine days after the start of treatment (17 days after challenge) until necropsy.

**Mycoplasma detection/isolation.** There was a significant ($P \leq 0.0021$) reduction in the detection and isolation rates for *M. gallisepticum* from swabs taken at necropsy in the challenged treated group compared to the challenged untreated group (Table 3). In all the untreated birds *M. gallisepticum* was isolated from eye swabs and sinuses, but with fewer positive swabs collected from the sinus. The reverse was true for the treated birds with more positive swabs from the sinus than from the eye. The reason for this finding is unclear.

**Serology.** Whilst all 35 pheasants in the untreated groups had seroconverted by 22 days post-challenge infection, only 29/36 (80.6%) of the pheasants in the treated group had specific *M. gallisepticum* antibodies. The seven seronegative pheasants were further tested and five were confirmed seronegative, whilst the remaining two pheasants gave inconclusive results. Testing was with a rapid serum agglutination test.

**Body weight gain and feed efficiency.** Weight gain was significantly (P= 0.0002) improved in the treated pheasants relative to the un-medicated controls, (12.0g/ bird/day and 9.6g/bird/ day respectively (Table 5). An accurate assessment of the feed efficiency could not be made because the pheasants tended to scatter the food without eating it, especially those in the unchallenged untreated control group.

**Gross pathology.** During post mortem examination, lesions were seen only in the trachea. Six of the 36 pheasants in G1 had a slight redness and small quantity of exudates. This compares with seven in G2 with one pheasant showing additional redness of the mucous membrane and exudates.

## Discussion

Infectious sinusitis caused by *M. gallisepticum* is an economically important infectious disease of pheasants. Until recently, there were no products licensed for pheasants to treat this disease and currently there are no commercially available vaccines. The availability of a good challenge disease model is essential for the evaluation of efficacy of new treatments or vaccines. The selection of an appropriate challenge strain is critical. The pathogenicity of

*Mycoplasma gallisepticum* strains is partly dependent upon the host with game birds appearing to experience more severe disease than domestic fowl (Cookson & Shivaprasad, 1994; Benčina *et al*., 2003).

The *M. gallisepticum* isolate (G87/02) selected here was obtained from an eye swab taken from a pheasant during a field outbreak of respiratory disease. It had previously been shown to induce moderate to marked sinusitis and conjunctivitis in young (one-day-old) pheasants (Forrester, 2008). Initial clinical signs, mainly nasal exudate and swollen sinuses were observed 6 to 8 days after infection, peaking at 16 to 19 days post challenge. This compares with previous work in one day-old pheasants where clinical signs were first observed 10 days after infection (Forrester *et al.,* 2004) and in older pheasants (eight to ten weeks old) where clinical signs peaked 14 days post challenge (Forrester *et al*., 2006). There were still marked clinical signs 22 days after challenge, when all of the remaining challenged untreated birds had seroconverted for *M. gallisepticum*. This is the earliest that seroconversion in pheasants has been demonstrated (Forrester *et al.,* 2004; Forrester *et al.,* 2006). At necropsy *M. gallisepticum* was identified in all challenged untreated birds and tracheal lesions were observed. Although *M. gallisepticum* induces marked airsacculitis in chickens, this is not the case in pheasants (Yagihashi *et al.,* 1988). In contrast, unchallenged pheasants of the same origin, maintained in a different airspace remained healthy and sero-negative, thereby validating the challenge model. There may be variability between different strains; however, challenge of 14-day old pheasants with this field strain of *M. gallisepticum* offers a good disease model to investigate the efficacy of antimicrobial therapy or vaccines in the future. It also provides further evidence that *M. gallisepticum* is a primary pathogen for pheasants, and is likely to predispose pheasants to secondary infections as with other poultry species (Forrester *et al*., 2006).

TVN is known to be effective for both the treatment and prevention of respiratory disease associated with *M. gallisepticum* in chickens (Stipkovits and Mockett, 2007). Here we have demonstrated that five tested field isolates of *M. gallisepticum* isolated from UK pheasants were all highly sensitive to TVN (MIC range: 0.002-0.008 µg/ml). The MIC values obtained here are similar to values obtained using *M. gallisepticum* strains isolated from chickens, and lower than the $MIC_{90}$ value reported for chickens (MIC range: 0.002-0.125 µg/ml, $MIC_{90}$ 0.06 µg/ml; Stipkovits and Mockett, 2007).

Whilst still highly sensitive, *M. gallisepticum* G87/02 selected for the challenge infection had one of the highest TVN MIC values (0.008µg/mL), providing robust conditions to evaluate the efficacy of TVN. Three consecutive days of treatment with Aivlosin Soluble at a dose rate of 25 mg TVN/kg bodyweight/day, starting when approximately 25% of the pheasants demonstrated clinical signs, significantly reduced clinical signs ($P<0.0001$) and the re-isolation/detection of *M. gallisepticum* 14 days later ($P\leq0.0021$). In addition, the daily liveweight gain was significantly increased when compared to untreated birds ($P=0.0002$), almost returning to daily body weight gains observed in the unchallenged birds. Similar observations were made in challenged chickens treated with Aivlosin Soluble at the same dose rate used here for pheasants when only 5% of chickens showed clinical signs (Stipkovits and Mockett, 2007). Furthermore, Aivlosin Soluble performed better than tylosin in the challenged chickens.

However, it was still possible to isolate/detect *M. gallisepticum* from treated pheasants 14 days after treatment (one third of eye swabs in treated birds vs. all untreated birds). This also compared to the work in chickens where treatment reduced the isolation rate from 70% in untreated chickens to 26.6% in treated chickens (Stipkovits and Mockett, 2007). It is unrealistic to expect antimicrobial therapy to eliminate mycoplasmas from an infected flock (Levisohn & Kleven, 2000) but it can allow the immune response to control the

infection, helping to prevent associated clinical disease. Interestingly, not all treated birds sero-converted to *M. gallisepticum*. However, rapid serum agglutination test was used to determine sero-conversion and the relative insensitivity to this test may have contributed to this result.

In common with other macrolides, TVN is absorbed from the alimentary tract and enters the lung, especially the epithelial cells lining the respiratory tract. Indeed for TVN, this ability is superior to that of two other macrolides tylosin and tilmicosin when tested in pig cells (Stuart *et al.,* 2007). The poultry mycoplasmas *M. gallisepticum* and *Mycoplasma synoviae* have recently been shown to be capable of invading chicken cells (Winner *et al*., 2000; Vogl *et* al., 2008; Dušanic *et al*., 2009). It is not known if the same phenomenon would occur in pheasants but possible penetration into epithelial cells, combined with the high susceptibility of pheasant *M. gallisepticum* to TVN, would maximise antimicrobial efficacy.

In conclusion, challenge of 14 day old pheasants with a field strain of *M. gallisepticum* was shown to reliably induce clinical signs and pathology typical of infectious sinusitis. This offers a good challenge model for future development of products intended to treat or control infectious sinusitis in pheasants. TVN at a dose rate of 25 mg/kg bodyweight daily for three consecutive days in drinking water was shown to be safe and efficacious for the treatment of *M. gallisepticum* infection, and is the first product to be licensed for this indication in pheasants.



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**Table 1.** *Sources of five UK pheasant* M. gallisepticum *isolates, minimum inhibitory concentrations (µg/ml) for tylvalosin and titres (CFU/ml) of the tested cultures*

| Isolate | Date of isolation | UK county | Site | TVN MIC | Titre tested |
|---|---|---|---|---|---|
| G9/01 | December 2001 | Gloucestershire | Eye | 0.008 | $1.14x10^4$ |
| G42/02 | April 2002 | Lancashire | Eye | 0.008 | $1.11x10^4$ |
| G87/02 | July 2002 | Wiltshire | Eye | 0.008 | $6.1x10^3$ |
| G102/02 | July 2002 | Berkshire | Eye | 0.002 | $1.12x10^4$ |
| G118/02 | July 2002 | Berkshire | Trachea | 0.004 | $1.06x10^4$ |
| PG31[Ta] | | | | 0.002 | $8.4x10^3$ |

[a]Type strain

**Table 2**. *Number of pheasants showing clinical signs by group on days D0 to D14 (8 to 22 days post challenge)*

| Group | Sign[a] | D0 | D1 | D2 | D3 | D4 | D5 | D6 | D7 | D8 | D9 | D10 | D11 | D12 | D13 | D14 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| G1 | L | 2 | 0 | 0 | 0 | 2 | 1 | 1 | 2 | 2 | 0 | 0 | 0 | 1 | 1 | 3 |
| | C | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| | NE | 11 | 4 | 5 | 4 | 5 | 5 | 5 | 7 | 6 | 6 | 5 | 6 | 6 | 11 | 11 |
| | SS | 1 | 0 | 1 | 1 | 0 | 0 | 2 | 2 | 2 | 1 | 0 | 1 | 1 | 4 | 5 |
| | D | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| G2 | L | 0 | 1 | 1 | 0 | 2 | 2 | 3 | 16 | 21 | 23 | 24 | 29 | 31 | 28 | 27 |
| | C | 0 | 0 | 0 | 0 | 5 | 5 | 10 | 15 | 17 | 22 | 24 | 24 | 27 | 25 | 24 |
| | NE | 7 | 14 | 16 | 20 | 23 | 24 | 26 | 26 | 23 | 26 | 25 | 20 | 23 | 23 | 18 |
| | SS | 1 | 1 | 7 | 12 | 13 | 17 | 18 | 21 | 23 | 24 | 26 | 26 | 25 | 27 | 27 |
| | D | 0 | 0 | 0 | 3 | 7 | 35 | 34 | 34 | 32 | 28 | 27 | 32 | 25 | 31 | 29 |

G1 = challenged with *M. gallisepticum,* treated with tylvalosin tartrate

G2 = challenged with *M. gallisepticum*, untreated

[a]Clinical sign noted: L= lacrimation, C= conjunctivitis, NE= nasal exudates, SS= swollen sinus, D=depression (reluctance to move and ruffled feathers).

**Table 3.** *PCR detection and isolation of* M. gallisepticum *from swabs post mortem from treated and untreated, challenge birds.*

| Group | Eye | | Sinus | |
|---|---|---|---|---|
| | PCR | Isolation | PCR | Isolation |
| G1 | 9/36 (25.0%) | 12/36 (33.3%) | 19/36 (52.8%) | 20/36 (55.6%) |
| G2 | 35/35 (100%) | 35/35 (100%) | 31/35 (88.6%) | 33/35 (94.3%) |
| | *P<0.0001* | *P<0.0001* | *P=0.0021* | *P<0.0001* |
| G3 | 0/12 (0%) | 0/12 (0%) | 0/12 (0%) | 0/12 (0%) |

G1 = challenged with *M. gallisepticum,* treated with tylvalosin

G2 = challenged with *M. gallisepticum*, untreated

G3 = not challenged, untreated

P values relate to the comparison of figures for G1 and G2

**Table 4.** *Comparisons of efficacy and productivity variables between* M. gallisepticum-*challenged pheasants untreated or treated with tylvalosin*[a]

| | Treatment Group | |
|---|---|---|
| Measure | Challenged, untreated (G2) | Challenged, treated (G1) |
| Weight gain (g/bird/day)[b] | 9.6 | 12.0 (P = 0.0002) |
| Lacrimation[c] | 0.42 | 0.03 (P < 0.0001) |
| Conjunctivitis[d] | 0.40 | 0.00 (P < 0.0001) |
| Nasal exudate[e] | 0.62 | 0.17 (P < 0.0001) |
| Sinus swelling[f] | 0.54 | 0.04 (P < 0.0001) |
| Depression[g] | 0.64 | 0.00 (P < 0.0001) |

[a]Values in table are model based least squares means for groups, and analysed using a model based one-sided t test.

[b]Measured from D0 to D14 while all other measures are during D1 to D14

[c]Proportion of days in which birds had lacrimation to the days in which they didn't.

[d]Proportion of days in which birds had conjunctivitis to the days in which they didn't.

[e]Proportion of days in which birds had nasal exudates to the days in which they didn't (score of 1 or more; 0 = normal, 1 = mild, 2 = moderate, 3 = marked).

[f]Proportion of days in which birds had abnormal sinus swelling to the days in which they didn't (score of 1 or more; 0 normal, 1 = mild, 2 = moderate, 3 = marked).

[g]Proportion of days in which birds showed depression (reluctance to move or ruffled feathers) to the days in which they didn't.

**Table 5.** *Feed consumed, weight gain, and feed efficiency by pen for D0 to D14*

| Group | Pen | Feed: g | Weight gain: g | Feed efficiency feed/gain: g/g | Group weight gain: g/bird/day (standard deviation) |
|---|---|---|---|---|---|
| G1 (n = 36) | 1 | 9448.0 | 1987.6 | 4.75 | 12.0 (2.4.) |
| | 2 | 7508.3 | 1962.8 | 3.83 | |
| | 3 | 9705.9 | 2102.4 | 4.62 | |
| G2 (n = 35) | 4 | 8082.9 | 1600.1 | 5.05 | 9.6 (2.9) |
| | 5 | 7525.3 | 1607.8 | 4.68 | |
| | 6 | 6210.9 | 1519.2[a] | 4.09 | |
| G3 (n = 12) | 7 | 13220.7 | 2091.3 | 6.32 | 12.4 (2.2) |

[a]Includes weight gained in period D0 to D2 for bird removed on D7