For use in drinking water for chickens and for turkeys

625mg/g Water Soluble Granules

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**Introduction**

Tylvalosin (the active ingredient of Aivlosin® 625 mg/g Soluble Granules) is a second generation macrolide antibiotic developed to address the problems of macrolide resistant strains of bacteria including Mycoplasma species.

The addition of an isovaleryl group to the molecule confers an ability to penetrate the lipid membranes of the host and bacterial cells and bind to the bacterial ribosomes. This strong binding to the ribosomes prevents bacteria from synthesising protein and the compound exerts both a bacteriostatic and bactericidal effect.

Tylvalosin is rapidly absorbed and concentrates in respiratory tract and enteric tissues. The antibiotic also concentrates within circulating heterophil (neutrophil) cells which are drawn to sites of infection within the body. Thus, tylvalosin is concentrated where it is needed.

Tylvalosin has been shown to achieve greater intracellular concentrations than either tylosin or tilmicosin. Whilst tylvalosin is a highly effective intracellular antibiotic achieving concentrations within cells twelve times greater than in the extracellular environment, when extracellular concentrations are low relative to intracellular concentrations, tylvalosin is released into the extracellular environment.

Studies conducted in Japan and the EU showed that strains of *Mycoplasma gallisepticum* resistant to tylosin (a macrolide widely used throughout the poultry industry), remained sensitive to tylvalosin. Additional MIC studies with *M. synoviae* comparing tylvalosin to 6 other antibiotics showed that tylvalosin had the lowest MIC₉₀ value of 0.006µg/ml.

Tylvalosin exerts both a direct and indirect effect on the immune system driving the change from monocytes to macrophages, activating macrophages and concentrating within lysosomes within macrophages. The combination of tylvalosin together with the potent lysosomal enzymes assists the innate immune system to combat pathogens.

This in vitro effect on macrophages was confirmed in vivo. Chickens treated with tylvalosin had significantly increased numbers of macrophages in their lung tissue at necropsy. Macrolides such as tylvalosin can reduce the inflammation caused by pathogens further reducing the severity of lesions.

Clinical efficacy studies were undertaken on commercial poultry units to evaluate tylvalosin for both prevention and treatment of *Mycoplasma gallisepticum*. The data from these studies confirm the unique combination of properties of tylvalosin by showing significantly reduced pathology and a reduction in the amount of *Mycoplasma gallisepticum* isolated at necropsy. In all these studies tylvalosin was significantly better than tylosin, the positive control.

Tylvalosin (Aivlosin 625 mg/g Soluble Granules), has been specifically formulated for both ease of mixing and ease of administration. Simple to use programmes have been designed to suit the commercial poultry industry.

The product is highly palatable thus ensuring that the medication is consumed and correct dose levels achieved. It has a wide margin of safety and a convenient 2 day withdrawal period.

In conclusion tylvalosin (Aivlosin® 625 mg/g Soluble Granules), a novel second generation macrolide in Aivlosin Soluble Granules, offers a better solution to the challenge of preventing and treating mycoplasmosis caused by *Mycoplasma gallisepticum* within the commercial poultry industry, and treatment of respiratory disease associated with tylvalosin sensitive strains of *Ornithobacterium rhinotraceale* in turkeys industry.
What are Aivlosin® 625 mg/g Soluble Granules?

Aivlosin® 625 mg/g Soluble Granules contain a new generation macrolide antibiotic, tylvalosin, and are approved throughout the European Union and in other major poultry-rearing regions of the world for the treatment and prevention of respiratory disease associated with *Mycoplasma gallisepticum* in chickens.

Tylvalosin, developed by Japanese researchers using a patented process, is highly effective against macrolide-resistant strains of bacteria including *Mycoplasma spp.*

![Figure 1: Molecular structure of Aivsolin®](image1)

Significance of the isovaleryl group

The presence of the isovaleryl group on the molecule (Figure 1) increases its lipophilicity, allowing it to rapidly penetrate the lipid membrane of host and bacterial cells and enabling highly effective binding to bacterial ribosomes. Data from this and an earlier study suggested that tylvalosin is more lipophilic than tylosin.

Mode of action

Tylvalosin binds to bacterial ribosomes and prevents protein synthesis (Figure 2). This can lead to inhibition of bacterial growth or death of the bacteria. Tylvalosin's first metabolite, known as 3-AT, also has antimicrobial activity. It attaches to a different site on the ribosome, enhancing the effectiveness and clinical efficacy of tylvalosin. This dual action may be responsible for its favourable resistance profile.

![Figure 2: Tylvalosin binding to ribosome](image2)
Pharmacokinetics and Pharmacology

Introduction

Tylvalosin is rapidly absorbed and metabolised to 3-AT. $T_{\text{max}}$ concentrations for both tylvalosin and 3-AT are achieved by 60 minutes after administration. By 8 hours after administration concentrations of tylvalosin and 3-AT are below the level of quantification.

Once absorbed, tylvalosin concentrates within respiratory and enteric tissues and can also concentrate in phagocytic cells (macrophages and heterophils/neutrophils) which are important cells of the immune system.

Absorption

Ten chickens were given Aivlosin®, 625 mg/g Soluble Granules by gavage at a dose rate of 30 mg tylvalosin/kg bodyweight. Blood samples were collected from each bird pre-dosing, and at regular intervals post-dosing for subsequent analyses of tylvalosin and 3-AT using HPLC.

$T_{\text{max}}$ for both tylvalosin and 3-AT was by 60 minutes post administration. Concentrations of both were below the level of quantification by 8 hours post-dosing (Figure 3).

These data show that tylvalosin is rapidly absorbed following administration of Aivlosin® 625 mg/g Soluble Granules and that metabolism to 3-AT occurs swiftly.

Cellular transportation

Tylvalosin concentrates in phagocytic cells, specifically macrophages and circulating heterophils. It has been shown to concentrate within endosomes/lysosomes in the cell (figure 4). When lysosomes are stained with acridine orange it appears red in acidic conditions but does not change colour in the presence of a high pH. Tylvalosin raises the pH, so no colour change is observed which indicates high concentrations of the antibiotic in the lysosomes.
Heterophils are attracted by chemical signals to the site of infection, taking tyvalosin with them. This may be one reason why tyvalosin concentrates in target tissues. This concentration effect is greater than that seen with other macrolides such as tilmicosin and tylosin⁴.

Avian white blood cells were isolated from heparinised whole chicken blood and continuously mixed on media with three different antibiotics, tyvalosin, tylosin and tilmicosin, at 37°C. After 15, 30 and 60 minutes the material was centrifuged and the resulting cell pellet and supernatant were assayed to determine the levels of antibiotic within them (Results are shown in figure 5).

Tylvalosin achieved significantly higher levels of antibiotic within the chicken white blood cells than both tylosin and tilmicosin.

Concentrations of tyvalosin in tissues

Tyvalosin is taken up rapidly and accumulates in the target tissues, particularly the lungs, airsacs, trachea and gut mucosa (Figure 6). For example, in the tracheal lining, concentrations are 50 fold higher than in plasma. Concentrations of 3-AT in the tissues follow a similar pattern, and 12 hours after medication were greatest in the tracheal lining³.
This is important for the control of infection since therapeutic concentrations of tylvalosin both on the surface of the respiratory tract and intracellularly provide a complimentary effect against target pathogens, such as *M. gallisepticum*.

![Graph](figure6.png)

The equivalent plasma concentration of tylvalosin at these time points (6, 12 and 18 hours) varied from 0.8 – 1.1 ng antibiotic/ml.

**The high concentration of tylvalosin within the mucous lining the epithelial cells of the respiratory system and the gut mucosa increases its effect against target pathogens at the site of entry.**

### Intra- and extracellular activity of tylvalosin

*Mycoplasma gallisepticum* adheres to the surface of respiratory epithelial cells (Figure 7), but recent work has shown that it can enter these cells. Tylvalosin is an effective intracellular antibiotic, achieving intracellular concentrations 12 times greater than extracellular concentrations.

In addition, if the extracellular concentration of tylvalosin is low relative to the intracellular concentration, tylvalosin is released from phagocytic cells into the extracellular microenvironment.

This activity against both intr- and extracellular pathogens such as *M. gallisepticum* makes tylvalosin a highly efficacious antimicrobial.

![Image](figure7.png)
MIC Studies

Tylvalosin at the recommended dose rates can be bactericidal for Mycoplasma. The bactericidal effect of the antibiotic helps the immune system to overcome the infection. This can be important in birds which are immuno-compromised, for example after certain viral infections.

The minimum mycoplasmacidal (bactericidal) concentration (MMC) for tylvalosin is similar to the minimum inhibitory concentration (MIC). The MMC/MIC ratio is often considered highly important in determining cidal effect, and the fact that this is low indicates that tylvalosin has cidal activity in vivo (Table 1)6.

The mycoplasmacidal activity of tylvalosin is both concentration and time dependent.

Cerda et al (2002)7 determined the MIC values for 7 antibiotics (tylvalosin, enrofloxacin, tylosin, tiamulin, kitasamycin, chloracetetracycline, and oxytetracycline) against 8 Argentinean field isolates and two standard strains of Mycoplasma synoviae. Tylvalosin had the lowest MICs with an MIC90 value of 0.006µg/ml.

During early screening, MIC testing was also done to investigate tylvalosin's activity against Clostridium perfringens and Ornithobacterium rhinotracheale (ORT). 34 strains of Clostridium perfringens (21 type A and 13 type C) were tested. The MIC90 value was 1.0 µg/ml for both types8.

12 strains of ORT (turkey and chicken origin) were tested. The MIC values ranged from 0.25 to 1.0 µg/ml with an MIC90 value of 0.5 µg/ml. The MBC90 (Minimum Bactericidal Concentration90) value was 1.0 µg/ml9.

Resistance

*M.gallisepticum* strains resistant to tylosin (MIC≥2.0 µg/ml) have been isolated from Japan and from Europe. These strains were tested and shown to remain sensitive to tylvalosin (Table 2)10,11.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Tylosin MIC* (µg/ml)</th>
<th>Aivlosin MIC* (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Japan</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E-5</td>
<td>10.0</td>
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</tr>
<tr>
<td>E-11</td>
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<td>A-68</td>
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<td>0.31</td>
</tr>
<tr>
<td>A-72</td>
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<td>0.31</td>
</tr>
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<td>UE</td>
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<td></td>
</tr>
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<td>14267-95</td>
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</tr>
<tr>
<td>B139-02</td>
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<td>0.125</td>
</tr>
<tr>
<td>B141-02</td>
<td>4.0</td>
<td>0.25</td>
</tr>
</tbody>
</table>

Table 2: Effect of tylvalosin against tylosin-resistant strains* of *M. gallisepticum*
Aivlosin and the immune system

Introduction
In addition to assisting the immune system by concentrating within phagocytic cells tylvalosin directly affects the immune system by:

- Driving the differentiation of monocytes to macrophages
- Activating macrophages from the resting state
- Concentrating in lysosomes within the cells

Tissue macrophages are derived from blood monocytes and can exist in either a resting or an activated state. Once in an activated state, the macrophage is more metabolically active, has more lysosomes and has greater phagocytic activity and thus has a greater ability to destroy invading pathogens.

The in vitro studies reported below were conducted in collaboration with the University of Cambridge and demonstrate in vitro the action of tylvalosin on macrophages and the lysosomes within them.

The results from the in vitro studies were confirmed in vivo. Chickens treated with tylvalosin showed a 67% increase in macrophages in lung tissue at necropsy.

Studies have also shown that macrolides can reduce inflammation in tissues by their action on parts of the inflammatory cascade, namely Interleukin-8, and by reducing the release of inflammatory products during cell death.

The beneficial effects of tylvalosin on the immune system are confirmed by the results of clinical studies for both prevention and treatment of chickens naturally infected with *Mycoplasma gallisepticum*.

In vitro studies

1. Tylvalosin drives the differentiation of monocytes to macrophages

A human monocyte cell line (THP-1) was grown in the presence of tylvalosin. The metabolic activity increased as did the expression of certain surface activation markers, especially DAF which is a protein that helps protect cells against the effects of the complement system (Figure 8).

The metabolic activity induced by tylvalosin was greater than that of other macrolides tested (tylosin and tilmicosin). Various cell lines were used and only the macrophage cell lines showed increased metabolic activity in the presence of tylvalosin.

The increased expression of these surface activation markers in the presence of tylvalosin indicates that tylvalosin drives the differentiation of monocytes to macrophages.
2. Tylvalosin activates macrophages from the resting state

A porcine macrophage cell line (3D4/31) was grown in the presence of either tylvalosin or tilmicosin for 48 hours. Cells were stained for a marker protein of porcine tissue macrophages, CD68, a lysosome-associated protein.

The results showed that tylvalosin increased the expression of CD68 more effectively than tilmicosin (Figure 9). Up-regulation of CD68 is an important event that leads to more of this lysosomal protein, and hence more lysosomes being made in the cell.

*Increased lysosome activity is associated with activation of the macrophage*.

3. Concentrations of tylvalosin in lysosomes

Lysosomes are acidic vesicles containing potent enzymes, which help to destroy invading pathogens. They are contained in large numbers within macrophages and heterophils. Both these cell types are phagocytic, and both are important in the non-specific or innate immune system.

*Tylvalosin concentrates within endosomes and lysosomes in the cell*. The combination of tylvalosin with the potent enzymes helps the innate immune system in its battle against invading pathogens.
In vivo studies

1. Tylvalosin has been shown to increase the numbers of macrophages in chicken lungs

Chickens were either medicated with Aivlosin® 625 mg/g Soluble Granules (20 mg tylvalosin/kg bwt) or left unmedicated. Five days later the chicks were euthanased and their lungs fixed then selectively stained. The macrophages (and granulocytes) were stained using a monoclonal antibody (MAC-1) (Figure 10).

Chickens treated with tylvalosin had a 67% increase in macrophage numbers in lung tissue at necropsy16.

2. Reduction of localised inflammation

Macrolides can reduce the secretion of interleukin-8 and hence reduce inflammation17. In addition, macrolides can induce the programmed cell death (apoptosis) of neutrophils which prevents the sudden release of chemicals into the microenvironment and hence reduces inflammation18.

Tylvalosin drives the differentiation of monocytes to macrophages, specifically increases the metabolic activity of macrophages, and increases the production of lysosomal proteins.

Aivlosin® 625 mg/g Solubes Granules and the immune system

In addition to being highly effective against Mycoplasma, tylvalosin has a beneficial effect on the innate immune system thus helping to ensure a rapid recovery.
Clinical Efficacy in broilers

Introduction
The following section on clinical trials confirms the effectiveness of tylvalosin in both prevention and treatment of disease caused by *Mycoplasma gallisepticum* in comparison to both tylosin (positive control) and untreated birds.

The trials were conducted in commercial units in Europe with naturally infected birds. The results are particularly significant as the standard of management on these units was high.

The prevention studies evaluated clinical scores as the primary variable with necropsy lesion scores and isolation of *M. gallisepticum* at day 34 as the secondary variables.

The treatment studies evaluated post mortem lesion scores of 20% of randomly selected birds from each group 14 days post treatment as the primary variable and respiratory lesion scores and isolation of *M. gallisepticum* as the secondary variables at the end of the study.

Aivlosin® 625 mg/g was significantly better than tylosin in both prevention and treatment trials.

Clinical trials
Following dose determination studies in experimentally infected birds, more extensive confirmatory controlled field studies, two treatment and two prevention, were conducted in naturally infected birds.

General design of studies
- Conducted in commercial production units in Slovakia and Hungary
- Randomised, masked, parallel group design
- 3,000 day-old chicks per study
- Same protocol followed on both sites for each claim
- Positive (tylosin) and negative control groups
- The maximum MIC<sub>90</sub> of strains from all the field trials was 0.125 µg/ml for tylvalosin and 0.25 µg/ml for tylosin, demonstrating that all strains were sensitive to both macrolides
- Scoring system for lesions at necropsy:
  - 0 = no lesions
  - 1 = foci of lesions
  - 2 = diffuse lesions
  - 3 = pronounced diffuse lesions
  - Scores from 4 to >6 indicate pronounced lesions in several or all tissues

Prevention trials
- Treatments evaluated:
  - Aivlosin® 625 mg/g Soluble Granules at: 25 mg tylvalosin/kg bodyweight (bw) daily for 3 days on arrival at unit followed by 15 mg/kg bw daily for 4 days at 16 days of age.
  - Tylosin: 500 ppm in water for 3 days on arrival at unit and again once at 22 days of age (authorised dose).
- Primary variable: clinical scores on day 34.
- Secondary variables: total lesion scores on day 34 and *M. gallisepticum* isolation.
**Results:** Aivlosin® was significantly better than tylosin (P=0.002) on both sites (Table 3) for the primary variable.

Lesion scores of the Aivlosin® and tylosin treated birds on day 34 were significantly reduced (p<0.001) relative to the untreated birds (Figures 11 & 12).

The Aivlosin® group was also significantly (p<0.001) better than the tylosin group.

<table>
<thead>
<tr>
<th>Grupo/site</th>
<th>Slovakia</th>
<th>Hungary</th>
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<tr>
<td>Aivlosin®</td>
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<td>tylosin</td>
<td>34.2</td>
<td>37.5</td>
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<td>Unmedicated control</td>
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<td>50</td>
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<tr>
<td>P Value: Aivlosin vs. tylosin</td>
<td>P=0.002</td>
<td>P=0.002</td>
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Lesion scores of the Aivlosin® and tylosin treated birds on day 34 were significantly reduced (p<0.001) relative to the untreated birds (Figures 11 & 12).

The Aivlosin® group was also significantly (p<0.001) better than the tylosin group.
Isolation of *Mycoplasma gallisepticum* was significantly less in all respiratory tissues examined at day 34 in medicated chickens (*p*=0.001) (Figure 13). The Aivlosin® group had significantly fewer isolations than the tylosin group in the lung (*p*=0.013) and in the trachea (*p*=0.020).

Both prevention field trials showed that the Aivlosin regimen was more effective in preventing the adverse effects of congenitally transmitted *M. gallisepticum* infection than the tylosin regimen (*P*=0.002).

**Treatment trials vs *M. gallisepticum***

- **Treatments evaluated:**
  - Aivlosin® 625 mg/g Soluble Granules at 25 mg tylvalosin/kg (bw) daily for 3 days when 2-5% of birds showed clinical signs (21/22 days of age)
  - Tylosin: 500 ppm in water for 3 days (authorised dose)
- **Primary variables:** lesion scores of respiratory tissues at necropsy of 20% of the birds in each treatment group 14 days after treatment.
- **Secondary variables:** *M. gallisepticum* isolation.
- **Results:** Aivlosin® treatment significantly reduced the pathology (lesion scores) at post-mortem compared to unmedicated chickens and was also significantly better (*P*=0.001) than tylosin (Figures 14 & 15).

There were significantly (*p*<0.05) fewer lesions in the Aivlosin® treated chickens compared with the tylosin treated chickens.

Figure 13: Percentage of *M. gallisepticum* positive samples isolated from the respiratory tract on day 34

Figure 14: Lesion scores (trachea, airsacs, peritoneum) on day 14 in Slovakia
There was a significant reduction in the isolation of mycoplasma from both the respiratory (Figure 16) and internal organs in both medicated groups. The untreated group had significantly lower bodyweight than the medicated groups.

Both treatment field trials showed that Aivlosin® 625 mg/g was more effective than tylosin at reducing the adverse effects of *M. gallisepticum* infection on respiratory tissues (*p*<0.001).

**Prevention**

The Aivlosin® prevention programme was significantly better than the tylosin programme at preventing the clinical signs of mycoplasmosis.

**Treatment**

When given at 25 mg/kg bwt for 3 days, Aivlosin® was significantly better than tylosin at reducing the lung pathology (lesions and histology) associated with mycoplasmosis.
Aivlosin: the only one approved for ORT

ORT: a serious disease

- Respiratory disease in turkeys is a multi-factorial disease with ORT as one of the major causes. *Ornithobacterium rhinoatraceale* is transmitted vertically and horizontally.

- ORT occurs when turkeys move to the grower phase, at about 4-6 weeks of age, and later on in the finishing stage. Many other significant factors contribute to turkey respiratory disease:
  - Individual susceptibility, with declining maternal immunity
  - Stressful move from starter to grower phase at about 4-6 weeks of age
  - Increasing bird density as production progresses
  - Poor ventilation
  - Other complicating bacteria like *Mycoplasma spp.* and viruses like Avian Pneumovirus, Paramyxovirus and Influenza virus

Incidence of ORT in turkeys

Results have shown a high incidence of ORT found at post-mortems from 4 weeks of age onwards

Review of Post-Mortem findings 11 333 Turkey Carcasses submitted May 2001 - April 2011

P.F. Mc Mullin, Personal Communication, 2013

More cases as turkeys move into the grower phase at about 4-6 weeks of age

Passive immunity for TRT decreases at around 4 weeks of age

TRT: Turkey (or Avian) Rhinotracheitis
Respiratory disease increases significantly from 4-5 weeks of age

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<tr>
<td>sinusitis</td>
<td>22 days</td>
<td>0.39 kg</td>
<td>17%</td>
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<td>airsacculitis</td>
<td>38 days</td>
<td>1.37 kg</td>
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Significantly decreased sensitivity of various anti-microbials against ORT

Avian Pathology (2001) 30, 197– 200

Antibiotic sensitivity and resistance in Ornithobacterium rhinotracheale strains from Belgian broiler chickens

Authors - L. A. Devriese*, P. De Herdt & F. Haesebrouck Faculty of Veterinary Medicine, Department of Pathology, Bacteriology and Poultry Diseases, Ghent University, Salisburylaan 133, B-9820 Merelbeke, Belgium

Outline - The minimal inhibitory concentrations of 10 antibiotics were determined for 45 strains of O. rhinotracheale from Belgian broiler chickens collected from 45 farms between 1995 and 1998. They were compared with the type strain, which was isolated from a turkey, and a strain isolated from a rook.

Results - All the strains were resistant to lincomycin and to the β-lactams, ampicillin and ceftiofur. Less than 10% of the strains were sensitive to the macrolides tylosin and spiramycin, tilmicosin and flumequine. A few strains were sensitive to enrofloxacin and doxycycline.


Outline - As part of the basic characterization of Ornithobacterium rhinotracheale, the minimal inhibitory concentrations of 10 antimicrobial drugs were determined for reference strains and isolates by a broth microdilution method. For optimal growth of the organisms, a supplemented brain–heart infusion broth was used.

Results - The susceptibility of O. rhinotracheale to amoxicillin, enrofloxacin, and oxytetracycline was variable. Result obtained from among the isolates indicate a marked antimicrobial drug resistance trend.

Serious signs appear with ORT
- Nasal exudate, tracheitis in the upper respiratory tract
- Pneumonia, pleurisy in the lower respiratory tract
- Often evolving to tenosynovitis

Serious financial losses due to ORT
- Increased mortality and reduced growth rates
- Worse feed conversion rates
- Lame birds at older ages
- More rejects at processing
- Lowered carcass quality and grading
- Lost medication costs from ineffective anti-microbial treatments
Outstanding Performance and Financial Results

Commercial trial results versus amoxicillin water soluble powder, performance and financials

Trial outline

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<td>6 weeks of age</td>
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<td></td>
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<td>Group</td>
<td>Amoxicillin</td>
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<td>10 mg/kg BW for 5 days</td>
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Great performance results in high challenge operations

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<th>Group Aivlosin®</th>
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<td>No. Poults at start of trial</td>
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<td>1600</td>
<td></td>
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<td>Mortality %</td>
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<td>Ave weight gain kg</td>
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<td>**FCR (whole production time)</td>
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<td>63.9</td>
<td>67.9</td>
<td></td>
</tr>
<tr>
<td>Rejects at slaughter %</td>
<td>1.5%</td>
<td>3.8%</td>
<td>2.30%</td>
</tr>
<tr>
<td>Total kill weight kg</td>
<td>29,016</td>
<td>26,630</td>
<td></td>
</tr>
<tr>
<td>Processing A Grade kg</td>
<td>28,729</td>
<td>25,911</td>
<td>10%</td>
</tr>
<tr>
<td>Treatment costs</td>
<td>£ 2,267</td>
<td>44</td>
<td></td>
</tr>
<tr>
<td>Kgs feed kg</td>
<td>92,480</td>
<td>94,960</td>
<td>3%</td>
</tr>
<tr>
<td>Feed Cost £/tonne</td>
<td>£ 276.45</td>
<td>£ 25,566.10</td>
<td>£ 26,251.69</td>
</tr>
</tbody>
</table>

* ADG: Average Daily Gain, ** FCR: Feed Conversion Rate

MIC results for ORT

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>*MIC (ug/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>tylvalosin</td>
<td>0.062</td>
</tr>
<tr>
<td>amoxicillin</td>
<td>1</td>
</tr>
<tr>
<td>chlortetracycline</td>
<td>1</td>
</tr>
<tr>
<td>tilmicosin</td>
<td>0.5</td>
</tr>
</tbody>
</table>

* MIC: Minimum Inhibitory Concentration

Great financial benefits

<table>
<thead>
<tr>
<th>Aivlosin® benefits</th>
<th>Group Aivlosin®</th>
<th>Group Amoxicillin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Feed Saving</td>
<td>£ 685.60</td>
<td></td>
</tr>
<tr>
<td>p/Bird</td>
<td>£ 3.38</td>
<td></td>
</tr>
<tr>
<td>ADG, Additional Weight kg</td>
<td>£ 1,888</td>
<td></td>
</tr>
<tr>
<td>Revenue Benefit</td>
<td>£ 3,397.68</td>
<td></td>
</tr>
<tr>
<td>Less Rejects, Additional Weight kg</td>
<td>£ 574</td>
<td></td>
</tr>
<tr>
<td>Revenue Benefit</td>
<td>£ 1033.56</td>
<td></td>
</tr>
<tr>
<td>Less Mortality, Additional Weight kg</td>
<td>£ 337</td>
<td></td>
</tr>
<tr>
<td>Revenue Benefit</td>
<td>£ 660.741</td>
<td></td>
</tr>
<tr>
<td>Total Liveweight Payment @ p/kg</td>
<td>£ 51712.2</td>
<td>£ 46,639.26</td>
</tr>
<tr>
<td>Additional kg Live</td>
<td>£ 5,072.94</td>
<td></td>
</tr>
<tr>
<td>Flock Benefit on Feed and Additional weight</td>
<td>£ 5,758.54</td>
<td></td>
</tr>
<tr>
<td>Flock NET benefit after cost of treatment</td>
<td>£ 3,535.54</td>
<td></td>
</tr>
<tr>
<td>Benefit Per bird slaughtered</td>
<td>£ 2.41</td>
<td></td>
</tr>
<tr>
<td>Benefit Per kg grade A</td>
<td>£ 0.12</td>
<td></td>
</tr>
</tbody>
</table>
Controlled trial at Veterinary Faculty, University of Gent, Belgium

20th WVPA Congress. Nantes, France, 19-23 August 2013

Outline

<table>
<thead>
<tr>
<th>GCP Floor-pen study</th>
<th>4 pens of 17 Turkeys</th>
</tr>
</thead>
<tbody>
<tr>
<td>Challenges</td>
<td>34 days of age</td>
</tr>
<tr>
<td></td>
<td>37 days of age</td>
</tr>
<tr>
<td>Groups</td>
<td>TVN</td>
</tr>
<tr>
<td></td>
<td>38-42 days of age</td>
</tr>
<tr>
<td></td>
<td>UC</td>
</tr>
<tr>
<td></td>
<td>42 days of age</td>
</tr>
<tr>
<td></td>
<td>Aivlosin® at 25 mg activity/kg BW; 5 days in drinking water</td>
</tr>
<tr>
<td></td>
<td>untreated control</td>
</tr>
</tbody>
</table>

Results

<table>
<thead>
<tr>
<th>Groups and Dosage</th>
<th>Tylvalosin 25.3 mg/kg BW</th>
<th>Control</th>
<th>Improvement grams/day</th>
<th>Improvement %</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parameter</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ADG (g/day) day 0-14 after ORT challenge</td>
<td>93.56</td>
<td>84.08</td>
<td>9.48</td>
<td>11.3%</td>
<td>0.0015</td>
</tr>
<tr>
<td>Mortality (%)</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ORT counts [log10 (cfu/g)] from tracheal tissue (Day 6 after ORT challenge)</td>
<td>3.54</td>
<td>7.29</td>
<td>0.0073</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Incidence of ORT in air sac swabs (Day 6 after ORT challenge)</td>
<td>0/10</td>
<td>4/10</td>
<td>0.0433</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Really active

Sustained powerful activity for ORT, other key respiratory pathogens and Clostridium perfringens

MIC’s for ORT Strains in Europe

<table>
<thead>
<tr>
<th>Organism</th>
<th>MIC Geometric mean</th>
<th>MIC range</th>
<th>MIC50*</th>
<th>MIC90*</th>
<th>Mode</th>
</tr>
</thead>
<tbody>
<tr>
<td>tylvalosin</td>
<td>0.44</td>
<td>0.016-32</td>
<td>0.5</td>
<td>4</td>
<td>0.5</td>
</tr>
<tr>
<td>tilmicosin</td>
<td>3.19</td>
<td>0.06-128</td>
<td>1</td>
<td>128</td>
<td>1</td>
</tr>
<tr>
<td>tylosin</td>
<td>0.88</td>
<td>0.06-64</td>
<td>0.5</td>
<td>32</td>
<td>0.125</td>
</tr>
</tbody>
</table>

MIC data for Mycoplasma spp.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Country</th>
<th>Year</th>
<th>n</th>
<th>Antimicrobial</th>
<th>MIC [µg/mL]: % of isolates at each MIC*</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. gallisepticum</td>
<td>Germany</td>
<td>2003-2006</td>
<td>10</td>
<td>TVN</td>
<td>0.06</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.12</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.25</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.5</td>
</tr>
<tr>
<td>M. meleagridis</td>
<td>UK</td>
<td>2005</td>
<td>2</td>
<td>TVN</td>
<td>0.06</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.12</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.25</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.5</td>
</tr>
</tbody>
</table>

1 minimum inhibitory concentration; 2 TVN = tylvalosin
Ref: In vitro susceptibility to tylvalosin of various turkey respiratory pathogens, JB Tasker, ECO Animal Health, PO Box 47542, London N14 6WS, UK
Ref: ECO Internal Report
### Distribution (%) of MIC values for tylvalosin against turkey and chicken isolates

<table>
<thead>
<tr>
<th>Organism</th>
<th>Country</th>
<th>Year</th>
<th>n</th>
<th>Antimicrobial</th>
<th>MIC (µg/mL): % of isolates at each MIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. synoviae</td>
<td>Italy, Catania</td>
<td>2012</td>
<td>11</td>
<td>TVN</td>
<td>0.00375: 8  0.0075: 1  0.015: 1  0.03: 1  0.12: 1  0.25: 1  0.5: 1</td>
</tr>
</tbody>
</table>

1 minimum inhibitory concentration; 2 TVN = tylvalosin
Ref: In vitro susceptibility to tylvalosin of various turkey respiratory pathogens, JB Tasker, ECO Animal Health, PO Box 47542, London N14 6WS, UK
Ref: ECO Internal Report

### MIC data for Clostridium perfringens strains

### Distribution (%) of MIC values for tylvalosin and tylosin against turkey isolates

<table>
<thead>
<tr>
<th>Antimicrobial</th>
<th>Origin MIC 0.125</th>
<th>0.25</th>
<th>0.5</th>
<th>1</th>
<th>2</th>
<th>4</th>
<th>8</th>
<th>16</th>
<th>32</th>
<th>&gt;64</th>
<th>total</th>
<th>Geometrical mean</th>
<th>range</th>
<th>MIC 50</th>
<th>MIC 90</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tylvalosin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>30</td>
<td>0.338</td>
<td>0.125-2</td>
<td>0.250</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>2003-2009 %</td>
<td>7%</td>
<td>67%</td>
<td>10%</td>
<td>10%</td>
<td>7%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>30</td>
<td>0.871</td>
<td>0.25-64</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>cum%</td>
<td>7%</td>
<td>73%</td>
<td>83%</td>
<td>10%</td>
<td>10%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>30</td>
<td>0.871</td>
<td>0.25-64</td>
<td>0.5</td>
</tr>
</tbody>
</table>

Tylosin

<table>
<thead>
<tr>
<th>%</th>
<th>0%</th>
<th>30%</th>
<th>47%</th>
<th>0%</th>
<th>0%</th>
<th>7%</th>
<th>7%</th>
<th>3%</th>
<th>0%</th>
<th>7%</th>
</tr>
</thead>
<tbody>
<tr>
<td>cum%</td>
<td>0%</td>
<td>30%</td>
<td>77%</td>
<td>77%</td>
<td>77%</td>
<td>83%</td>
<td>90%</td>
<td>93%</td>
<td>93%</td>
<td>100%</td>
</tr>
</tbody>
</table>

1 minimum inhibitory concentration
Ref: In vitro susceptibility to tylvalosin of various turkey respiratory pathogens, JB Tasker, ECO Animal Health, PO Box 47542, London N14 6WS, UK
Ref: ECO Internal Report
Additional information

Dosing Recommendations

For treatment of respiratory disease associated with *Mycoplasma gallisepticum*:
The dosage is 25 mg tylvalosin per kg bodyweight per day in drinking water for 3 consecutive days.

When used as an aid in the prevention strategy (where infection in ovum with *Mycoplasma gallisepticum* is likely):
The dosage is 25 mg tylvalosin per kg bodyweight per day in drinking water for 3 consecutive days at 1 day old.

This is followed by a second treatment with 25 mg tylvalosin per kg bodyweight per day in drinking water for 3 consecutive days at the period of risk, i.e. at times of management stress such as administration of respiratory vaccines, typically when birds are 2-3 weeks old.

Broilers and Pullets: typical prevention programme

Dose rate is 25 mg tylvalosin/kg bodyweight daily for three consecutive days from one day old, followed by a second three-day treatment in the 3rd week of life.

The timing of the second medication is dependent upon known disease patterns and stress periods including environmental factors on the premises.

<table>
<thead>
<tr>
<th>Weeks</th>
<th>Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>5</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td></td>
</tr>
</tbody>
</table>

Get ahead in tackling ORT

- Make sure you’re ahead: Ahead of complications, before it’s too late
- Target your treatment: Carefully diagnose and select the correct time to medicate according to the farm’s disease history and avoid vertical transmission

<table>
<thead>
<tr>
<th>Turkey (stage of life)</th>
<th>Daily Dosage of Aivlosin®625 mg/g Water Soluble Granules (mg activity and mg product/kg bodyweight)</th>
<th>Days of administration in drinking water</th>
</tr>
</thead>
<tbody>
<tr>
<td>GROWING turkeys</td>
<td>25 mg Tylvalosin activity/kg body weight, corresponding to 40 mg Aivlosin® 625 mg/g Water soluble granules /kg bodyweight</td>
<td>5 days, repeat if necessary with a further pulse</td>
</tr>
<tr>
<td>DAY-OLD turkey poults</td>
<td>25 mg Tylvalosin activity/kg bodyweight, corresponding to 40 mg Aivlosin® 625 mg/g Water soluble granules /kg bodyweight</td>
<td>5 first days of life</td>
</tr>
</tbody>
</table>
Administration

One sachet of 40 g is sufficient to treat a total of 1000 kg weight of chickens for one day e.g. 20,000 birds with an average bodyweight of 50 g. A 400 gram pack will treat 4,000 kg of turkeys live bodyweight per day.

The product should be added to a volume of water that the chickens will consume in one day. No other source of drinking water should be available during the medication period.

Mixing instructions

The product may be mixed directly into the drinking water system or first mixed as a stock solution into a smaller amount of water, which is then added into the drinking water system.

When mixing the product directly into the drinking water system, the contents of the sachet should be sprinkled onto the surface of the water and mixed thoroughly until a clear solution is produced (usually within 3 minutes).

When preparing a stock solution the maximum concentration should be 1 sachet per 1500 ml and it is necessary to mix the solution for 10 minutes. After this time, any remaining cloudiness will not affect efficacy of the product.

Incompatibilities

- Aivlosin®, at the approved dose rate, is compatible with the approved in-feed ionophore anti-coccidials for turkeys
- No interference with salmonella monitoring programmes as Aivlosin® has no activity Salmonella spp

Palatability

Even when administered at a dose rate of 150mg tylvalosin per kg bodyweight daily for 5 days in safety studies chicks voluntarily consumed the medicated water emphasising that tylvalosin at high doses is palatable.

Safety

No signs of intolerance were observed in chickens at dose rates up to 150 mg tylvalosin per kg bodyweight given daily for 5 days.

Withdrawal Periods

A 2-day chicken / turkeys meat withdrawal period.

Tylvalosin is not authorised for use in birds laying eggs for human consumption. The time period during which treatment is not allowed before onset of egg laying for human consumption is 14 days. Tukeys: Do not use within 21 days of the onset of laying.

Development of Resistance: risk to man

The main bacterial species of concern as zoonotic organisms are E. coli, Salmonella spp, Campylobacter spp and Enterococci spp. Tylvalosin has no activity against Enterobacteriaceae including E. coli and Salmonella spp and therefore it will not influence the development of resistance in man.

The regulatory authorities have concluded that the impact of the risk of the use of tylvalosin in relation to development of resistance in human medicine was considered acceptable.

Aivlosin® 625mg/g granules for use in drinking water for chickens are registered for veterinary use only.
Summary of Product Characteristics

NAME:
AIVLOSIN 625 mg/g Water Soluble Granules.

ACTIVE SUBSTANCE:
Tylvalosin as tartrate 625 mg/g. White granules.

INDICATIONS:

Chickens: Treatment and prevention of respiratory disease associated with Mycoplasma gallisepticum in chickens. As an aid in the prevention strategy to reduce the clinical signs and mortality from respiratory disease in flocks, where infection in ovum with Mycoplasma gallisepticum is likely because the disease is known to exist in the parent generation. The prevention strategy should include efforts to eliminate the infection from the parent generation.

Turkeys: Treatment of respiratory disease associated with tylvalosin sensitive strains of Ornithobacterium rhinotracheale in turkeys.

CONTRAINDICATIONS:
None.

ADVERSE REACTIONS:
None known. If you notice any serious effects or other effects not mentioned in this package leaflet, please inform your veterinary surgeon.

TARGET SPECIES:
Chickens / turkeys.

DOSAGE FOR EACH SPECIES, ROUTE AND METHOD OF ADMINISTRATION:

For use in drinking water.

Chickens: For treatment of respiratory disease associated with Mycoplasma gallisepticum:

The dosage is 25 mg tylvalosin per kg bodyweight per day in drinking water for 3 consecutive days. When used as an aid in the prevention strategy (where infection in ovum with Mycoplasma gallisepticum is likely):

The dosage is 25 mg tylvalosin per kg bodyweight per day in drinking water for 3 consecutive days at 1 day old. This is followed by a second treatment with 25 mg tylvalosin per kg bodyweight per day in drinking water for 3 consecutive days at the period of risk, i.e. at times of management stress such as administration of vaccines (typically when birds are 2–3 weeks old). Determine the combined bodyweight (in kg) of all the chickens to be treated. Select the correct number of sachets according to the amount of product required.

One sachet of 40 g is sufficient for a total of 1,000 kg of chickens (e.g. 20,000 birds with an average bodyweight of 50 g). One sachet of 400 g is sufficient to treat a total of 10,000 kg of chickens (e.g. 20,000 birds with an average bodyweight of 500 g). In order to achieve a correct dose, the preparation of a concentrated (stock) solution might be required (e.g. to treat a total of 500 kg total bird weight, only 50 % of the prepared stock solution prepared from the 40 g sachet should be used). The product should be added to a volume of water that the chickens will consume in one day. No other source of drinking water should be available during the medication period.

Turkeys: For treatment of respiratory disease associated with tylvalosin-sensitive strains of Ornithobacterium rhinotracheale:
The dosage is 25 mg tylvalosin per kg bodyweight per day in drinking water for 5 consecutive days. Determine the combined bodyweight (in kg) of all the turkeys to be treated. Select the correct number of sachets according to the amount of product required. One sachet of 40 g is sufficient for a total of 1,000 kg of turkeys (e.g. 10,000 birds with an average bodyweight of 100 g). One sachet of 400 g is sufficient to treat a total of 10,000 kg of turkeys (e.g. 10,000 birds with an average bodyweight of 1 kg).

In order to achieve a correct dose, the preparation of a concentrated (stock) solution might be required (e.g. to treat a total of 500 kg total bird weight, only 50 % of the prepared stock solution prepared from the 40 g sachet should be used). The product should be added to a volume of water that the turkeys will consume in one day. No other source of drinking water should be available during the medication period.

**ADVICE ON CORRECT ADMINISTRATION:**

The veterinary medicinal product may be mixed directly into the drinking water system or first mixed as a stock solution into a smaller amount of water, which is then added into the drinking water system.

When mixing the product directly into the drinking water system, the contents of the sachet should be sprinkled onto the surface of the water and mixed thoroughly until a clear solution is produced (usually within 3 minutes).

When preparing a stock solution the maximum concentration should be 40 g per 1,500 ml or 400 g of product per 15 litres and it is necessary to mix the solution for 10 minutes. After this time, any remaining cloudiness will not affect the efficacy of the veterinary medicinal product. Only a sufficient amount of medicated drinking water should be prepared to cover the daily requirements. Medicated drinking water should be replaced every 24 hours.

**WITHDRAWAL PERIOD:**

Meat and offal: 2 days. Not authorised for use in birds producing eggs for human consumption.

**Chickens:** Do not use within 14 days of onset of laying. **Turkeys:** Do not use within 21 days of the onset of laying.

**SPECIAL STORAGE PRECAUTIONS:**

Keep out of the sight and reach of children. 40 g sachet: do not store above 30°C. 400 g sachet: do not store above 25°C.

Shelf-life after opening the immediate packaging: Open sachets should not be stored. Do not use this veterinary medicinal product after the expiry date, which is stated on the label as “EXP”. Shelf-life of the medicated drinking water: 24 hours.

**SPECIAL WARNINGS:**

**Special precautions for use in animals:**

Good management and hygiene practices should be introduced in order to reduce the risk of re-infection. It is sound clinical practice to base treatment on susceptibility testing of the bacteria isolated from the animal. If this is not possible, therapy should be based on local (regional, farm level) epidemiological information about susceptibility of target bacteria. Use of the product deviating from the instructions may increase the risk of development and selection of resistant bacteria and decrease the effectiveness of treatment with other macrolides due to the potential for cross-resistance.

Special precautions to be taken by the person administering the veterinary medicinal product to animals:

Tylvalosin has been shown to cause hypersensitivity (allergic) reactions in laboratory animals; therefore, people with known hypersensitivity to tylvalosin should avoid contact with this product. When mixing the veterinary medicinal product and handling the medicated water, direct contact with eyes, skin and mucous membranes should be avoided. Personal protective equipment consisting of impervious gloves and a half-mask respirator conforming to European Standard EN 149 or a non-disposable respirator conforming to European Standard EN 140, with a filter conforming to European Standard EN 143 should be worn when mixing the product. Wash contaminated skin.
In case of accidental ingestion, seek medical advice immediately and show the package leaflet or the label to the physician. Lay: Use only in accordance with risk/benefit assessment by the responsible veterinarian.

**SPECIAL PRECAUTIONS FOR THE DISPOSAL OF UNUSED PRODUCT OR WASTE MATERIALS, IF ANY:**

Any unused veterinary medicinal product or waste materials derived from such veterinary medicinal product should be disposed of in accordance with local requirements.

**DATE ON WHICH THE PACKAGE LEAFLET WAS LAST APPROVED:** 12/2013

Detailed information on this product is available on the website of the European Medicines Agency http://www.ema.europa.eu

**OTHER INFORMATION:**

Aivlosin® 625 mg/g granules for use in drinking water for chickens / turkeys is available in sachets containing 40 g or 400 g. Not all pack sizes may be marketed. For any information about this veterinary medicinal product, please contact the local representative of the marketing authorisation holder:

United Kingdom: ECO Animal Health Limited, The Grange, 100 The High Street, London, N14 6BN, United Kingdom. Tel: +44(0)20 8447 8899. Email: enquiries@ecoanimalhealth.com

**Marketing Authorisation Holder:** ECO Animal Health Limited, 78 Coombe Road, New Malden, Surrey, KT3 4QS, United Kingdom.

Aivlosin® 625mg/g Water soluble powder is a prescription only medicine: POM-V

**Distributed by:**

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email: esteveveterinaria@esteve.es

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Telef. 21 424 60 27 Fax: 21 417 59 74

**Esteve Spa:** Italy

Via Ippolito Rosellini, 12, 1º piano
20124 - Milano (Italia)
Tel.: 02 699.64.224
Fax: 02 699.64.250
email: estevespa@esteve.es

**Esteve GmbH:** Germany - Austria

Max-Planck Straße 11 – D-85716 Unterschleißheim
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Fax: +49 (0)89/ 32 14 19 65
email: estevegmbh@esteve.com

USE MEDICINES RESPONSIBLY
Tylvalosin (Aivlosin) sensitivity discs - Guidelines in ORT

Use of 25 \( \mu g \) TVN discs to evaluate the sensitivity of *O. rhinotracheale* to tylvalosin

**Preparation of bacterial cultures for disc susceptibility test**

1. Culture each ORT isolate on Nutrient Agar or Columbia Blood Agar and incubate in air + 5% \( \text{CO}_2 \) at 37°C ± 1°C for 24-48 h, until bacterial colonies are readily visible.

2. Inspect each plate to confirm the presence of a pure culture. Prepare further subcultures if necessary using the incubation conditions described above.

3. From each pure culture, sample three to five well-isolated colonies of a single morphological type by touching with a sterile bacteriological loop. Suspend these bacterial cells in 5-10 mL of MRD\(^1\), with periodic vortex mixing, until homogenous turbidity equivalent to that of a 0.5 McFarland Standard is attained. Comparison with the McFarland turbidity standard can be made against a white card bearing contrasting black lines. Each bacterial suspension adjusted in this way should contain at least \( 1 \times 10^8 \) cfu per mL.

4. Use each standardized inoculum within 15 minutes of preparation.

**Procedure: preparation of disc susceptibility plates**

1. For each ORT isolate, susceptibility to a 25 mg TVN disc will be determined on CBA.

2. Remove discs from the refrigerator and allow them to equilibrate at room temperature for 1-2 h.

3. Within 15 min of adjusting the turbidity of the inoculum suspension (see above), dip a sterile cotton swab into the adjusted suspension. Rotate the swab several times and press firmly on the inside wall of the tube, above the fluid level, to remove excess inoculum from the swab.

4. For each ORT isolate, inoculate the CBA plate by streaking the swab over the entire sterile agar surface. Repeat this by streaking two more times, rotating the plate approximately 60 degrees each time, to ensure an even distribution of inoculum. As a final step swab the rim of the agar.

5. Leave the lid of each agar plate ajar for 3 to 5 minutes, (no more than 15 mins) to allow the excess surface moisture to be absorbed before applying the TVN discs.

6. Dispense discs onto each inoculated agar plate, ensuring that they are of equal distance apart. Gently press each disc with a sterile loop to ensure they are in close contact with the agar surface. After a disc has been placed on the agar surface it must not be moved.

**Procedure: incubation of disc susceptibility plates**

Once the discs are in place invert the plates and incubate at 35±1°C in an atmosphere enriched with 5 - 7% \( \text{CO}_2 \) for 16-18 h. For each plate, incubation must start within 15 minutes of applying the discs.

**Procedure: reading and interpretation of disc susceptibility plates**

1. Incubate the plates for 16 - 18 hours before examining the inhibition zones.

2. If plates have been satisfactorily streaked and the inoculum is correct, incubated plates should yield a confluent lawn of growth with uniformly circular zones of inhibition. In any case where the appropriate plate appearance is not obtained, the test will be considered invalid and should be repeated.

3. Measure the diameter of the zones of growth inhibition to the nearest mm preferably using digital callipers. Hold plates between 10 and 20 cm above a black, non-reflective background, illuminated with reflected light and read using the naked eye.

\(^1\)MRD = Maximum Recovery Diluent
Interpretation of results

<table>
<thead>
<tr>
<th></th>
<th>MIC</th>
<th>Inhibition zone (mm)</th>
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<tbody>
<tr>
<td>Sensitive</td>
<td>2</td>
<td>&gt; 26</td>
</tr>
<tr>
<td>Intermediate</td>
<td>&lt; 2 - 16</td>
<td>18 – 26</td>
</tr>
<tr>
<td>Resistant</td>
<td>&gt; 16</td>
<td>&lt; 18</td>
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</tbody>
</table>

**Note:** this interpretation is for guidance only and is based on validation of relatively few isolates of *O. rhinotracheale*. The tentative interpretation will be refined, if necessary, in the light of further clinical experience.
Tylvalosin (Aivlosin®) sensitivity discs - Feedback Form

Please return this form to ESTEVE by fax or email

Date of test:       Your reference:
Veterinarian Name:
Address:
Country:
E-mail address:
Phone number:

FLOCK DETAILS
Turkeys or chickens  Breed of bird
Type of flock
No. birds in flock
ORT status of farm
Clinical signs (what and when?)
Medication strategy (what, when, how much?)
Is vaccine used and in what segment?
No. birds to be treated and when

RESULT OF DISC TEST

<table>
<thead>
<tr>
<th>Disc no.</th>
<th>Diameter (mm)</th>
<th>Disc no.</th>
<th>Diameter (mm)</th>
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</thead>
<tbody>
<tr>
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<td>6</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>2</td>
<td>7</td>
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<td>5</td>
<td>10</td>
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<td></td>
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</tbody>
</table>

RESPONSE TO TREATMENT
Aivlosin® treatment programme
Clinical response
Zootechnical / productivity

Signed:       Date:
References

Reference 1:

Reference 2:

Reference 3:
ECO Internal Report PKD.UK.050077
Aivlosin Soluble: A Target Animal Pharmacokinetic Study in Broiler Chickens.

Reference 4:
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A report on the intracellular accumulation of Aivlosin, 3-AT, tylosin and tilmicosin in pig cells (pig kidney and white blood cells) and chicken cells (white blood cells).

Reference 5:
In Vitro cell invasion of Mycoplasma gallisepticum. Infection and Immunity, 68: 4238-4244.

Reference 6:
ECO Internal report Windsor (2007)
Determination of the minimum mycoplasmacidal concentration (MMC) of aivlosin against nine strains of Mycoplasma gallisepticum.

Reference 7:

Reference 8:
ECO internal report PKD.UK.050032
Aivlosin water soluble: to determine the minimum inhibitory concentration of aivlosin (La tilvalosina) and comparator compounds against 36 Clostridium perfringens strains.

Reference 9:
ECO internal report PKD.UK.050033
Aivlosin water soluble: to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of Aivlosin (AIVT) against Ornithobacterium rhinotraceae.

Reference 10:

Reference 11:
ECO internal report Windsor (2005)
Determination of the minimum inhibitory concentration of Aivlosin, tylosin and 3-AT for eight recent field isolates of Mycoplasma galliseptium from chickens and five recent field isolates from turkeys.
Reference 12:
ECO internal report PKD.UK.080163
Stuart A.D and Brown T.D.K. (2008a). Aivlosin increases the metabolic activity of macrophage cell lines and peripheral blood mononuclear cells

Reference 13:
ECO internal report PKD.UK.080162

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Reference 15:

Reference 16:
Cerda RO, Barbeito CG, Portiansky EL (2000)
Effect of a treatment with Bromesol and a macrolide on present lung’s macrophage number of healthy broilers. Vetanco Technical Bulletin n.15

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Reference 18:

Reference 19:
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Reference 21:
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Aivlosin Granules for oral solution for chickens. Prevention of Mycoplasmosis (Mycoplasma gallisepticum) in chickens. Field trial: Slovakia

Reference 22:
ECO internal report EFF.HU.050055
Testing the efficacy of Aivlosin for the treatment of Mycoplasmosis (Mycoplasma gallisepticum) in chickens in field conditions (Site 1: Hungary)

Reference 23:
ECO internal report EFF.SK.060103
Aivlosin Granules for oral solution for chickens. Treatment of Mycoplasmosis (Mycoplasma gallisepticum) in chickens. Field trial: Slovakia