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Antibiotic treatment of *Streptococcus bovis* infections in pigeons

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SUMMARY

The antibiotic susceptibility pattern of *S. bovis* strains isolated from pigeons was studied. *In vitro*, *S. bovis* strains were sensitive to penicillins, macrolides, lincomycin, tetracyclines, chloramphenicol and nitrofurans. However, the prevalence of acquired resistance against tetracyclines was approximately 40%. Sulphonamides and trimethoprim had little *in vitro* activity against *S. bovis* while activity of the quinolone enrofloxacin and the aminoglycoside antibiotics, neomycin and gentamicin, were in or near to the intermediate range.

The comparative efficacy of 5 antimicrobials administered via the drinking water for the treatment of experimental *S. bovis* infection in pigeons was also tested. Morbidity after intravenous inoculation of *S. bovis* in groups of pigeons treated with ampicillin, erythromycin, doxycycline, enrofloxacin and trimethoprim was 20%, 30%, 20%, 70% and 90%, respectively. Morbidity in an untreated control group was 90%.

INTRODUCTION

In pigeons, *Streptococcus bovis* is an important facultative pathogen (De Herdt et al., 1992a, 1993). The bacterium was isolated from the intestinal tract of approximately 40% of healthy pigeons of all ages (De Herdt et al., 1993). Pigeons that carry *S. bovis* in the intestinal tract usually do not develop clinical disease. Some unknown factor(s), however, may predispose pigeons to septicaemia and disease. Clinical signs of *S. bovis* septicaemia include sudden death in pigeons of all ages, inability to fly, lameness, emaciation, polyuria and green, slimy droppings (Devriese et al., 1990; De Herdt et al., 1991, 1992b). From January 1990 to November 1992, *S. bovis* septicaemia was diagnosed in 10% of the pigeons that were necropsied at the Faculty of Veterinary Medicine of the University of Gent (De Herdt et al., 1993). Epizootiological and pathological aspects of *S. bovis* infections in pigeons have already been studied (De Herdt et al., 1992a, b, 1993). Data on the treatment of *S. bovis* septicaemia, however, are not available. Some guidance about the antibiotic to use can be gained from the *in vitro* sensitivity...
pattern of isolates to a range of different antimicrobial drugs. Therefore, in this study *S. bovis* strains from pigeons were assessed for their sensitivity to a range of antibiotics belonging to different groups. However, although a pathogenic organism is sensitive *in vitro* to an antibiotic, therapy may not necessarily be successful (Mackie et al., 1988). Therefore, we studied the efficacy of 5 antimicrobials that are frequently used orally in pigeon practice on experimental *S. bovis* infections in pigeons. These antimicrobials were administered via the drinking water in appropriate dosages for pigeons.

**MATERIALS AND METHODS**

**In vitro antibiotic sensitivity testing**

Eighty strains of *S. bovis* isolated from pigeons were tested for their *in vitro* antimicrobial sensitivity. Forty were obtained from organ lesions of pigeons that died from *S. bovis* septicaemia. The others were isolated from crop or cloaca samples of clinically healthy pigeons, all from different lofts.

For antibiotic sensitivity testing, lyophilized samples of each bacterial isolate were rehydrated with phosphate-buffered saline (PBS), streaked onto Columbia agar (Gibco, Paisley, Scotland) with 5% bovine blood and incubated overnight at 37°C in a 5% CO$_2$ enriched environment to check for purity. Isolated colonies were suspended in PBS to achieve a turbidity equal to McFarland 0.5 standard. These suspensions were applied to the entire surface of Iso-sensitest agar plates (Oxoid, Basingstoke, England) using sterile swabs. Neo-sensitabs® (Rosco, Taastrup, Denmark) containing a diffusable amount of 5 µg penicillin G, 78 µg erythromycin, 19 µg lincomycin, 80 µg oxytetracycline, 60 µg chloramphenicol, 240 µg sulphonamides, 5.2 µg trimethoprim, 120 µg neomycin, 40 µg gentamicin, 260 µg nitrofurantoin or 10 µg enrofloxacin were placed on the inoculated plates. After 24 h incubation at 37°C in a 5% CO$_2$ atmosphere, the zones of inhibition were measured and interpreted according to the criteria of the manufacturer. *In vitro* sensitivity of streptococci to penicillin G is identical to sensitivity to ampicillin and amoxycillin (Washington, 1980). Sensitivity of *S. bovis* strains to macrolides, tetracyclines and nitrofurans was deduced from their sensitivity to erythromycin, oxytetracycline and nitrofurantoin, respectively (Washington, 1980). All tests were performed at least twice.

**Antibiotic treatment of pigeons experimentally infected with *S. bovis***

**Pigeons**

Sixty squabs derived from healthy racing pigeons that did not carry *S. bovis* in the intestinal tract, were raised in a disease containment building for 3–4 months. Every week, crop and cloaca samples were taken and examined bacteriologically for the presence of *S. bovis*. The samples were consistently negative. One week before experimental infection, pigeons were transferred into individual wire-floored cages and received water and food *ad libitum*.
Antimicrobials

Antimicrobials used in these studies were ampicillin anhydrous (Certa, Braine l'Alleud, Belgium), erythromycin thiocyanate (VMD, Arendonk, Belgium), doxycycline HCl (VMD), enrofloxacin (Baytril®, 10% oral solution, Bayer, Leverkusen, Germany) and trimethoprim base (VMD).

S. bovis strain

Experimental infections were carried out with the reference S. bovis strain STR 357 (De Herdt et al., 1992b). This strain was isolated from the liver of a pigeon that died from S. bovis septicaemia. In vitro, S. bovis strain STR 357 was sensitive to penicillins, macrolides, lincomycin, tetracyclines, chloramphenicol and nitrofurans, and resistant to trimethoprim and sulphonamides. Sensitivity to enrofloxacin was intermediate.

The isolate was grown for 24 h at 37°C on Columbia agar (Gibco, Paisley, Scotland) with 5% bovine blood in a 5% CO₂ enriched environment. Bacteria were harvested in PBS, centrifuged, and resuspended in RPMI 1640 (Gibco) with 10% foetal calf serum. The suspension was checked for purity by plating 10-fold dilutions on Columbia agar (Gibco) with 5% bovine blood and counting the number of colony-forming units (CFU). The suspensions were stored overnight at 4°C. The next day, the bacteria were washed once in PBS and used for experimental infections.

Experimental design

The pigeons were distributed randomly over six test groups of 10 pigeons each. All pigeons were inoculated intravenously in the V. axillaris with 1 × 10⁹ CFU S. bovis in 0.25 ml inoculum as described earlier (De Herdt et al., 1992b). In five groups of pigeons, ampicillin 2 g/l, erythromycin 1 g/l, doxycycline 500 mg/l, enrofloxacin 150 mg/l or trimethoprim 200 mg/l, respectively, was added to the drinking water from 48 h before experimental infection to 72 h post-inoculation. The mean daily intake of ampicillin, erythromycin, doxycycline, enrofloxacin and trimethoprim was 174 mg/kg, 71 mg/kg, 40 mg/kg, 12 mg/kg and 16 mg/kg, respectively. One group of pigeons received no antimicrobials and served as an inoculated untreated control group.

The pigeons were followed clinically for 3 weeks. They were released daily in a room under confinement and observed for lameness and inability to fly. The aspect of the droppings and the existence of polyuria was noted. The pectoral muscle was palpated to detect areas of necrosis. Every 2 or 3 days, the body weight was measured and a 10% decrease in body weight was regarded as weight loss. Every day, crop and cloaca swab samples were taken and examined bacteriologically for the presence of S. bovis as described below.

Pigeons were necropsied when they showed clinical disease or when a necrotic area in the pectoral muscle was detected by palpation. Euthanasia was by
intravenous administration of T61® (Hoechst, Brussels, Belgium). At post-mortem examination, macroscopic lesions were noted, and samples were taken for bacteriological examination from brain, pectoral muscles, M. iliotibialis cranialis, lungs, heart, liver, spleen, kidneys, duodenum, stifle and shoulder joints, and left and right hock and canalis triosoese with sterile disposable inoculating loops (Nunc Intermed, Kamstrup, Denmark).

Bacteriological examination

Bacteriological examination of all samples was performed on Slanetz and Bartley agar (Oxoid, Basingstoke, England) that had been cooked until it turned pink (approximately 3 min). Samples collected at necropsy were also inoculated on Columbia agar (Gibco) with 5% bovine blood. All plates were incubated for 24 h at 37°C in a 5% CO₂ atmosphere. S. bovis colonies were identified by the method of Devriese et al. (1990).

Statistical analysis

Student’s t-test was used to determine significant differences in morbidity after experimental S. bovis infection between untreated pigeons and groups treated with ampicillin, erythromycin, doxycycline, enrofloxacin or trimethoprim. A significance level of 0.05 was used.

RESULTS

In vitro antibiotic sensitivity testing

In vitro, all strains of S. bovis were sensitive to penicillin G, ampicillin and amoxyccillin. Acquired resistance to macrolides, lincomycin, chloramphenicol, nitrofurans and tetracyclines was observed in 5, 7, 2, 1 and 33 of the 80 strains tested, respectively. All other strains were sensitive to these antibiotics. For all strains, the size of inhibition zones for neomycin, gentamicin and enrofloxacin was in or near to the area of intermediate sensitivity without clear cut separation between sensitive, intermediately sensitive and resistant strains. None of the strains tested was sensitive to trimethoprim or sulphonamides.

Influence of antibiotic treatment on experimental S. bovis infections

Clinical signs

Clinical signs observed after experimental S. bovis infection in the 5 groups of pigeons treated with different antimicrobials and in the untreated control group, are shown in Table 1. The symptoms included sudden death, lameness, inability to fly, emaciation, polyuria and production of slimy, green droppings. The clinical signs observed were similar to those described earlier after experimental S. bovis infections in pigeons (De Herdt et al., 1992b). Morbidity in groups of pigeons
treated with ampicillin, erythromycin, doxycycline, enrofloxacin and trimethoprim was 20%, 30%, 20%, 70% and 90%, respectively and 90% in the untreated group. Morbidity in pigeons treated with ampicillin, erythromycin and doxycycline was significantly lower than in untreated pigeons (P-values 0.0001, 0.0123 and 0.0001, respectively). Differences in morbidity after experimental infection between pigeons treated with erythromycin or ampicillin and pigeons treated with doxycycline, were not significant (P = 0.2327).

Pathological findings
Lesions observed in necropsied pigeons were similar to those described earlier after experimental *S. bovis* infections in pigeons (De Herdt et al., 1992b).

Bacteriological examination
*S. bovis* was isolated from the crop or cloaca in eight of 10 pigeons belonging to the infected untreated control group. In these pigeons, excretion lasted for 3 to 13 days. In two of the 10 pigeons treated with erythromycin, *S. bovis* was demonstrated in the crop or cloaca on the day post-inoculation. Crop or cloaca samples were also positive for *S. bovis* in 7 of 10 pigeons treated with enrofloxacin and in eight of 10 pigeons treated with trimethoprim but *S. bovis* was recovered for only 1 or 2 days. *S. bovis* was not isolated from the crop or cloaca of pigeons treated with ampicillin or doxycycline.

The results of bacteriological examination are presented in Table 2. *S. bovis* was isolated from organs and joints of seven untreated pigeons and one, four and six pigeons treated with doxycycline, enrofloxacin and trimethoprim, respectively. *S. bovis* was not demonstrated in samples of necropsied pigeons treated with ampicillin or erythromycin.

DISCUSSION

Difficulties appeared in the interpretation of results of *in vitro* sensitivity tests with enrofloxacin, neomycin and gentamicin. For most strains, inhibition zones were in the category of intermediate sensitivity. For the other strains, zone sizes were in the area of normal sensitivity or resistance but near to the zone of intermediate sensitivity. Upon repeated testing, differences between strains in sensitivity to enrofloxacin, neomycin and gentamicin proved to be more apparent than real. Strains which were found sensitive in one test appeared intermediate or resistant upon repetition and vice versa. The intermediate category of sensitivity has been created for strains with a borderline sensitivity. Such strains, in systemic infections, would probably be sensitive when a high dose (up to the limits of toxicity) of antibiotic is used, or in the treatment of localized infections at sites where the agent is concentrated by physiological processes or local application (Ericsson & Sherris, 1971). The results of our study demonstrate the difficulties which may arise in the interpretation of *in vitro* antibiotic sensitivity tests of streptococci with
Table 1. Clinical observations after intravenous inoculation of $1 \times 10^6$ CFUs. bovis per bird in groups of pigeons treated with antimicrobials and in an untreated control group

<table>
<thead>
<tr>
<th>Clinical observation</th>
<th>Ampicillin</th>
<th>Erythromycin</th>
<th>Doxycycline</th>
<th>Enrofloxacin</th>
<th>Trimethoprim</th>
<th>Untreated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mortality</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Weight loss</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Lameness</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>2</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Inability to fly</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>4</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Polyuria</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Morbidity</td>
<td>20%</td>
<td>30%</td>
<td>20%</td>
<td>70%</td>
<td>90%</td>
<td>90%</td>
</tr>
</tbody>
</table>

*For each treatment and the control group there were 10 pigeons.
Table 2. Bacteriological examination of organs and joints after experimental S. bovis infection in groups of pigeons treated with different antibiotics and in an untreated control group

<table>
<thead>
<tr>
<th>Treatment</th>
<th>None</th>
<th>Ampicillin</th>
<th>Doxycycline</th>
<th>Erythromycin</th>
<th>Enrofloxacin</th>
<th>Trimethoprim</th>
</tr>
</thead>
<tbody>
<tr>
<td>Necropsied pigeons</td>
<td>8</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>7</td>
<td>9</td>
</tr>
<tr>
<td>None</td>
<td>3 3 1 2 1 3 4 2 1 2 1 2 3 0 0 0 0 0 0 0 0 0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ampicillin</td>
<td>0 0 0 0 0 0 0 0 0 0 0 0 0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Doxycycline</td>
<td>0 1 0 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Erythromycin</td>
<td>0 0 0 0 0 0 0 0 0 0 0 0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enrofloxacin</td>
<td>2 1 1 1 1 1 2 2 1 1 1 1 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>9 1 2 2 3 2 3 3 2 3 2 3 3 3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>


*Number of pigeons that were necropsied because they showed clinical disease.

*Number of necropsied pigeons from which S. bovis was isolated from organs.
aminoglycosides and quinolones. Results of in vivo tests may provide a solution to this problem. No difficulties however occurred in the interpretation of results of the in vitro sensitivity tests with erythromycin, lincomycin, chloramphenicol, nitrofurantoin and oxytetracycline. Our studies showed a clear bimodal distribution of strain sensitivities to these antimicrobials; inhibition zones were always in the area of normal sensitivity or in the zone of resistance and not in the category of intermediate sensitivity. Therefore, all strains in the less sensitive group can be considered to have acquired resistance (Devriese & Dutta, 1981).

In the present study, an intravenous infection model was used for testing the efficacy of antibiotic treatment of S. bovis infections in pigeons as this had previously been shown to be acceptable (De Herdt et al., 1992b). The infective dose was high in order to determine the most protective antibiotic, but test groups were treated 48 h in advance of infection, allowing them to obtain (if possible) a blood antibiotic level which should inhibit septicaemia. Antibiotics were administered via the drinking water and although dosages were high, no problems in acceptance of the drugs were observed.

For the treatment of bacterial infections in birds, pharmacokinetic aspects of antimicrobials must be taken into account. Ampicillin, doxycycline, erythromycin, enrofloxacain and trimethoprim are often used in pigeon practice. Since the pharmacokinetics of these drugs in pigeons indicated a possible good in vivo response for the treatment of S. bovis septicaemia (Dorrestein et al., 1986a, b, 1987, 1988; Vanhaecke et al., 1990), their efficacy in the treatment of an experimental S. bovis infection was examined. Other antibiotics were not tested because of their poor in vitro activity against S. bovis or unfavourable pharmacokinetic aspects in pigeons. Trimethoprim was not active in our in vitro tests. It was included in the treatment series because in vitro tests with trimethoprim may produce misleading results as was amply demonstrated in the case of enterococci (Hamilton-Miller & Purves, 1986).

Morbidity after experimental infection with S. bovis was 20%, 20%, 30%, 70% and 90% in groups of pigeons treated with ampicillin, doxycycline, erythromycin, enrofloxacain and trimethoprim, respectively. Thus, variations in in vivo activity appeared to correlate closely with in vitro sensitivity for these antibiotics. Differences in outcome after experimental infection in groups of pigeons treated with ampicillin, doxycycline and erythromycin were not significant. In field conditions, however, ampicillin is probably the antibiotic of first choice for the treatment of S. bovis septicaemia since no acquired resistance against penicillins occurs among strains of S. bovis. Acquired resistance of S. bovis against tetracyclines was very high (42%). The majority (94%) of the strains tested in our studies were sensitive to erythromycin. The excellent in vitro activity of penicillins and macrolides has also been documented with human S. bovis strains (Thornsberry et al., 1974). In vitro it has been shown, however, that many pigeon crop lactobacilli with acquired macrolide and lincosamide resistance, are able to inactivate the macrolides erythromycin and spiramycin (Vanhaecke et al., 1986). Possibly, this inactivation may have an adverse effect on the oral bioavailability of macrolide antibiotics in pigeons carrying macrolides-inactivating lactobacilli among their crop flora.
TREATMENT OF S. BOVIS INFECTIONS IN PIGEONS

S. bovis is a facultative pathogenic agent which can belong to the normal intestinal flora of clinically healthy pigeons (De Herdt et al., 1993). Factors predisposing to septicaemia and disease are not known. Some indications were found for a possible relationship between the occurrence of S. bovis septicaemia and poor hygiene on pigeon-lofts. For the treatment of S. bovis septicaemia in field conditions, administration of antibiotics should be accompanied by hygienic measures such as keeping the pigeons on wire floors, since the lowest prevalence of S. bovis was found in pigeon-lofts with wire floors (De Herdt et al., 1993).

The results obtained in our study indicate that certain antibiotics offer good possibilities for the treatment of S. bovis septicaemia in pigeons. In field conditions, however, treatment may be more difficult than our experimental results suggest, since S. bovis infections are usually initiated before treatment starts and poor hygienic conditions may interfere with the treatment. In field outbreaks, it was repeatedly seen that S. bovis excretion quickly resumed after the cessation of successful antibiotic treatment.

ACKNOWLEDGEMENTS

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REFERENCES


RESUME

Traitement antibiotique des infections à Streptococcus bovis chez les pigeons

Une étude a été menée sur le modèle de sensibilité aux antibiotiques des souches de S. bovis isolées à partir de pigeons. In vitro, les souches de S. bovis étaient sensibles aux pénicillines, macrolides, lincomycine, tetracyclines, chloramphenicol et nitrofuranes. La prévalence de résistance acquise vis à vis des tetracyclines s'élevait à 40%. In vitro, les sulfamides et la trimethroprime avaient peu d'effet contre S. bovis alors que l'activité de la quinolone enrofloxacine, des antibiotiques aminoglycosidiques, de la néomycine et de la gentamicine se situait à un niveau intermédiaire.

L'efficacité comparative de 5 agents antimicrobiens administrés dans l'eau de boisson en traitement de l'infection expérimentale à S. bovis chez les pigeons a également été testée. Après inoculation intraveineuse de S. bovis à des groupes de pigeons traités avec l'ampicilline, l'érythromycine, la doxycycline, l'enrofloxacine et la trimethroprim, le taux de morbidité s'élevait respectivement à 20, 30, 20, 70 et 90%, alors que dans le groupe témoin non traité, il était de 90%.

ZUSAMMENFASSUNG

Antibiotische Behandlung von Streptococcus bovis-Infektionen bei Tauben


Außerdem wurde die Wirksamkeit von 5 Bakteriostatika, die zur Behandlung von experimentellen S. bovis-Infektionen bei Tauben über das Trinkwasser verabreicht wurden, vergleichend untersucht. Nach intravenöser Inokulation von S. bovis war die Morbidität in den Taubengruppen, die mit Ampicillin, Erythromycin, Doxycyclin, Enrofloxacin oder Trimethoprim behandelt wurden, 20%, 30%, 20%, 70% bzw. 90%. Die Morbidität in einer unbehandelten Kontrollgruppe war 90%.
RESUMEN

Tratamiento de la infección por *S. bovis* en palomas

Se estudió la susceptibilidad a antibióticos de cepas de *S. bovis* aisladas de palomas. Las cepas de *S. bovis in vitro* fueron sensibles a las penicilinas, macrólidos, lincomicina, tetraciclinas, cloranfenicol y nitrofuranos. No obstante, la prevalencia de la resistencia adquirida frente a las tetraciclinas fue aproximadamente del 40%. Las sulfonamidas y el trimetoprim tuvieron poca actividad in vitro frente a *S. bovis* mientras que la actividad de la quinolona enrofloxacina y los antibióticos aminoglucósidos, neomicina y gentamicina, estuvieron en o cerca del rango intermedio.

Se comprobó también la eficacia de las cinco sustancias administradas a través del agua de bebida en el tratamiento de la infección experimental con *S. bovis* en las palomas. La morbilidad tras la inoculación endovenosa de *S. bovis* en grupos de palomas tratados con ampicilina, eritromicina, doxiciclina, enrofloxacina y trimetoprim fue del 20%, 30%, 20%, 70% y 90%, respectivamente. La morbilidad del grupo control no tratado fue del 90%.