Examinations on the efficacy of chemical disinfectants on Ornithobacterium rhinotracheale in vitro

Untersuchungen über die Wirksamkeit chemischer Desinfektionsmittel gegen Ornithobacterium rhinotracheale in vitro

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Dedicated to Prof. Dr. Hellmut Woernle, on behalf of his 80th birthday

Introduction

Ornithobacterium rhinotracheale (ORT) is an acute highly contagious disease of chickens and turkeys. The infection has been recognised in many countries worldwide and incriminated as additional causative agent in respiratory disease complex (CHARLTON et al., 1993; HAFEZ et al., 1993; VAN BEEK et al., 1994, BOCK et al., 1995, TRAVERS et al., 1996; ARNS et al., 1998; HAFEZ and FRIEDRICH, 1998; ZORMAN ROIS et al., 2000; HUNG and ALVARADO, 2001; EL-SUKHON et al., 2002; SORIANO et al.; 2002, TURAN and AK, 2002). The disease is mostly accompanied with heavy economic losses by increased mortality rates, increased medication costs, increased condemnation rate and drops in egg production (VAN EMPEL and HAFEZ, 1999). Initially, the bacterium was designated as Pasteurella-like, Kingella-like, Taxon 28 or pleomorphic gram-negative rod (PGNR) before the name Ornithobacterium rhinotracheale gen. nov. sp. nov. in the rRNA-Superfamily was suggested (VANDAMME et al., 1994). The early history of Ornithobacterium rhinotracheale (ORT) was described by Hinz and Hafez (1997).

The disease spreads horizontally by direct and indirect contact. Vertical transmission is suspected since ORT has been isolated at very low incidence from reproductive organs, hatching eggs, infertile eggs and dead embryos (TANYI et al., 1995, VAN EMPEL, 1998; EL-GOHARY, 1998). Currently, 18 serotypes designated A to R exist. Neither the origin nor the serotype of the Ornithobacterium rhinotracheale strains affects its pathogenicity (RYLL et al., 1996; TRAVERS, 1996; VAN EMPEL et al., 1996).

The treatment of ORT infections is very difficult because different strains have variable susceptibility to antibiotics. ORT acquires resistance easily against antibiotics (VAN EMPEL and HAFEZ, 1999). Currently, ORT infection appears to have become endemic and can affect every new restocking even in previously cleaned and disinfected houses especially in areas with intensive poultry production and on multiple age farms (HAFEZ, 2000). As a general principle, good biosecurity is essential in preventing the introduction and spread of ORT onto poultry farms. Disinfection of poultry houses and equipments should be routine practice. Currently, no reports on the efficacy of chemical disinfectants on ORT exist.

The present investigation was undertaken to study the efficacy of different chemical disinfectants on ORT in vitro.

Material and Methods

Disinfectants

Disinfectant A (VENNO-VET 1 super, Menno-Chemie GmbH, Nordersted) based on different organic acids (formic and glyoxyl acids) and disinfectant B (VENNO-FF super, Menno-Chemie GmbH, Nordersted) an aldehydes-based product (20% glutaraldehyde) were tested.

ORT Strain

ORT strain GG1261/91 isolated from meat turkey flocks in 1991 (HAFEZ et al., 1993) was used. This isolate belongs to serotype B (HAFEZ and STING, 1999).

Examination on the efficacy in vitro

The efficacy was estimated according to the method described in the guideline for testing chemical disinfectant by The German Veterinary Medical Society (ANON, 1984).

Bacterial suspension

The ORT isolate was grown on blood agar containing 10% sheep blood and incubated under microaerophil condition at 37 °C for 48 hours. Thereafter the plate was flooded with phosphate-buffered saline (PBS). The bacterial suspension was used to inoculate 95 ml Pasturella broth. After incubation at 37 °C for 48 hours under microaerophil condition the suspension was used to contaminate the bacterial carriers. The total viable counts were determined on blood agar and were ranged between 7.5 × 10⁸ and 1.4 × 10⁹ colony forming units/ml.

Contamination of bacterial carriers

Sterile lime thin wood blocks (size: 1 cm²) were used as bacterial carriers. The lime wood carriers were immersed in the bacterial suspension for 20 seconds, and then allowed to dry at room temperature for 30 minutes.

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Determination of bactericidal effect

Contaminated, dried bacterial carriers were immersed for 2 minutes in the corresponding disinfectant concentrations (Contact time). Thereafter they were removed and kept in sterile empty Petri dishes for 15, 30, 60 and 120 minutes (Effect time) at room temperature. At the end of each effect time bacterial carrier was transported to 10 ml Pasteurella broth and incubated at 37°C for 48 hours under microaerophilic condition. 100 µl of each Pasteurella broth tube was streaked on blood agar, incubated as mentioned above and then controlled for bacterial growth. As negative control contaminated dried bacterial carriers were immersed in Aqua-desf. instead of disinfectant. As positive control 3% formalin solution was used. All trials were repeated twice at two different days.

Results

Both products were able to completely inactivate ORT at lowest concentrations (0.5%) used and minimum effect time tested (15 minutes). Also 3% formalin was able to completely inactivate the growth of ORT after 15 minutes (Tables 1 and 2).

Discussion

Currently, ORT infection appears to have become endemic and can affected every new restocking even in previously cleaned and disinfected houses especially areas with intensive poultry (chickens and turkeys) and on multiple-age farms. The poultry industry has long realised the value of all-in-all-out rearing system. Increasing the production costs has enhanced the intensification of production. It has been a common practice to develop multiple-age farms. On these multiple-age farms, birds are placed in brooding houses, while older birds are in adjacent houses. This practice results in birds of different ages and possibly from different microbiological sources being on the same farm and attended by the same caretakers. This method of rearing will not allow one to apply effective cleaning and disinfection, and as a consequence, disease continually cycles from house to house.

The results obtained in the present investigation revealed that both tested products were able to completely inactivate ORT in vitro at concentrations of 0.5% after 15 minutes effect time. The results indicate that ORT can be easily inactivated under laboratory condition. The extreme concentration of turkey within integrated enterprises and/or in defined geographic area promoted the spread of infection. Also multiple-age production does not allow applying of an effective cleaning and disinfection. Similar situation appears to be taking place with turkey rhinotracheitis (TRT). The virus has been proved to be highly sensitive to different chemical disinfectants. Disinfectant based on aldehyde, phenol and alcohol combinations or disinfectant based on different organic acids as well as H₂O₂ were able to inactivate the TRT virus at concentrations of 0.5% within 15 minutes (Hafez and ArnS, 1991), however, the disease appears to have become endemic in several areas (Hafez, 2000).

Table 1. Bactericidal effect of disinfectant A based on different organic acids (Formic and Glyoxyl acids) on ORT in vitro

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Table 2. Bactericidal effect of disinfectant B based on different aldehyde on ORT in vitro

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Disease control is a challenge in areas with intensive poultry production as well as in multiple age farms, and prevention must continuously practise to minimise the losses from infections. Whenever possible poultry producers should practice all-in and all-out production and more attention must be paid on biosecurity (Bermudez and Stewart-Brown, 2003). Biosecurity procedure implies a cost. We should ready to weighting the benefits of implementing biosecurity versus avoiding it and saving money. Further investigations on the distribution of the ORT in the environmental samples in poultry houses are required.

**Summary**

The present paper describes the efficacy of chemical disinfectants on Ornithobacterium rhinotracheale (ORT) in vitro. Turkey ORT isolate (GGD1261/91) which belongs to serotype B and wood pieces as bacterial carriers were used. Two disinfectants, one based on different organic acids (formic and glyoxyl acids) and one containing different aldehydes (20% glutaraldehyde) were tested. The efficacy was estimated according to the method described in the guideline for testing chemical disinfectant by The German Veterinary Medical Society.

Both products were able to completely inactivate ORT at concentration of 0.5% after 15 minutes effect time.

**Key words**

Ornithobacterium rhinotracheale (ORT), chemical disinfectants, Poultry

**Zusammenfassung**

In der vorliegenden Arbeit wurde die Wirksamkeit von Desinfektionsmitteln zur Inaktivierung von Ornithobacterium rhinotracheale in vitro untersucht.


Die zwei untersuchten Mittel wiesen gute Wirksamkeit auf. Eine Konzentration von 0.5% und eine Einwirkungszeit von 15 Minuten führten zur vollständigen Inaktivierung von ORT.

**Stichworte**

Ornithobacterium rhinotracheale (ORT), chemisches Desinfektionsmittel, Geflügel

**References**


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