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Isolation of *Mycoplasma bovis* from broiler chickens in Turkey

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Abstract

Mycoplasma bovis normally affects cattle, in which it causes pneumonia in calves, mastitis, arthritis and other diseases. Here we report the isolation of this bovine pathogen from the tracheas of broiler chickens with no clinical signs. The most likely source of infection was the cattle herd sharing the farm with the chickens.

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Introduction

Mycoplasmas are ubiquitous throughout the animal kingdom and have been found in many mammals, and birds, as well as reptiles, amphibians and fish. For a long time it was believed that mycoplasmas were highly host specific because of their small genome, limited biosynthetic capability and parasitic habitat. There were, however, some well known exceptions of mycoplasmas crossing the species barrier, including *M. gatae* which is found in both cats and dogs, *M. canis* in dogs and cattle, and *M. felis* in horses and cats (Pitcher & Nicholas, 2006).

Mycoplasma gallisepticum probably has the largest host range of all mycoplasmas, infecting chickens, ducks, geese, game birds, pigeons and more recently, songbirds (Bradbury, 2005). This wider host range may well be a function of its genetic heterogeneity (Santha *et al.*, 1988). In the majority of cases, the crossing of species barriers almost certainly represents an opportunistic infection brought about by close proximity of two animal species. It is more difficult to explain the appearance of a mycoplasma in a highly unusual host species, and the temptation in these cases is to suggest that the greater likelihood, particularly with single isolates, is that cross contamination took place in the laboratory or during handling of the sample.

Perhaps the isolation of *M. iowae*, normally found in turkeys, from an apple seed falls into this doubtful category (Razin, 1992). A recent and rare example of a mycoplasma crossing the species barrier was reported by Bencina *et al.* (2005), where a mycoplasma with a 99% similarity to *M. capricolum* subspecies capricolum, usually found in goats, was isolated from several tissues of chickens. There is some uncertainty about the origin of a mycoplasma isolated in chicken embryos that had been inoculated with bovine placental tissue taken from a cow that had delivered a weak calf (Page *et al.*, 1972). Laboratory investigations subsequently identified the isolate, strain 3222, as *M. gallisepticum*, suggesting that it was derived from the egg, rather than from the bovine placenta, but serological studies with strain 3222 detected seroconversion in cattle in several herds. No further studies have been reported on this strain to further clarify its identity.

Here we report the isolation from chickens in Turkey of numerous isolates of mycoplasmas that were identical to *M. bovis*, a common cause of pneumonia, mastitis and arthritis in cattle.

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Materials and methods

The necks were collected from 500 broiler chickens at a commercial poultry abattoir in Eastern Turkey between February and June 2007. The broilers, selected randomly, originated from 14 different flocks in the region. The samples were stored in a cool box and transported to the laboratory within 2 hours. The necks were aseptically opened to expose the trachea. Swabs taken from the trachea were streaked onto modified Hayflick's medium, consisting of PPLO broth without crystal violet (21 g/l), 20% heat treated horse serum, 10% fresh yeast extract, 0.2% glucose, 0.4% sodium pyruvate, 0.04% ampicillin and 1% agar noble. Additionally, the medium was supplemented with gentamicin at 10 µg/ml to inhibit growth of other bacteria. It should be noted that gentamicin may inhibit the growth of some mycoplasmas. The presence of *Mycoplasma synoviae* was specifically investigated by culturing samples in the above medium supplemented with 1% nicotinamide adenine dinucleotide (NAD). The samples were incubated at 37 °C in 5% CO₂ for 7-10 days. The plates were checked daily by stereomicroscopy for the appearance of colonies and isolates were inoculated into a stock suspension containing 50% broth and 50% horse serum for further analysis.

A few suspected mycoplasma colonies from modified Hayflick's medium were transferred into a microcentrifuge tube containing 300 µl distilled water. The bacterial suspension was then treated with 300 µl TNES buffer (20 mM Tris-HCl pH 8.0, 150 mM NaCl, 10 mM EDTA, 0.2 % SDS) and proteinase K (200 µg / ml), and was incubated at 56 °C for 1 hour. The suspension was then heated at 95 °C for 10 min to inactivate the proteinase K.

Two different polymerase chain reaction (PCR) procedures were used. In the first, DNA samples were tested using a group-specific PCR for *Mycoplasma species* (Botes *et al.*, 2005), and in the second they were tested using specific PCR assays for *M. gallisepticum* and *M. synoviae* (Garcia *et al.*, 2005; Ramirez *et al.*, 2006). The PCRs were performed in a TC 512 Temperature Cycling System (Techne, Staffordshire, UK) in a reaction volume of 50 µl, containing 5 µl 10_x PCR buffer (750 mM Tris-HCl, pH 8.8, 200 mM (NH₄)₂SO₄, 0.1% Tween 20), 5 µl of 25 mM MgCl₂, 250 µM of each deoxynucleotide triphosphate, 1.25 U Taq DNA Polymerase

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(MBI Fermentas, St. Leon-Rot, Germany), 20 pmol of each primer (IDT) and 25 ng of template DNA. The group specific PCR was incubated through one cycle of 94 °C for 2 minutes (min), then 35 cycles of 94 °C for 15 seconds (sec), 53 °C for 15 sec and 72°C for 15 sec, and finally one cycle of 72°C for 5 min. The amplified products were detected by staining with ethidium bromide (0.5 µg/ml) after electrophoresis at 80 V for 2 hours (7 V/cm) in 1.5% agarose gels. Reference strains of *M. gallisepticum* and *M. synoviae* were included as positive controls and distilled water was used as a negative control in all assays.

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DNA sequence analysis was carried out on the amplicons from samples yielding a product in the group specific PCRs. For this, DNA samples were amplified with 16S rRNA gene primers and PCR products were sequenced one both strands using the ABI 310 Genetic Analysis System (Iontec, Istanbul, Turkey).

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In addition, DNA was also sent to the Veterinary Laboratories Agency (Weybridge) for confirmation by PCR/denaturing gradient gel electrophoresis (DGGE) using the method of McAuliffe *et al.* (2005).

Results and Discussion

Isolates were obtained from 4% (20/500) of the tracheal samples of the broilers. These isolates were all confirmed to belong to the *Mycoplasma* genus by the group-specific PCR. Three of the isolates from a single flock were identified as *M. gallisepticum* by species-specific PCR; *M. synoviae* was not detected in any sample. The 16S rRNA genes of the remaining 17 isolates, which were biochemically similar and originated from three flocks from a single farm, were sequenced and compared to those available in the GenBank DNA database (www.ncbi.nih.gov) using the nucleotide-nucleotide BLAST algorithm. There was 99% sequence identity between these isolates and *M. bovis* (sequence accession number AF332754.1). In addition, a product was amplified from these isolates using a specific PCR for *M. bovis* (Foddai *et al.*, 2005). Results of DGGE testing confirmed the identity of the isolates as *M. bovis* (Figure 1).

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This report of the isolation of *M. bovis* from chickens is another example of mycoplasmas crossing the species barrier. The farm from which the infected birds originated was a mixed farm on which poultry and cattle had close contact, but there were no indications that the poultry were affected by the bovine mycoplasma as no clinical or pathological signs were reported. Further investigations will examine the similarity between these chicken isolates and those isolated from the cattle on the farm.

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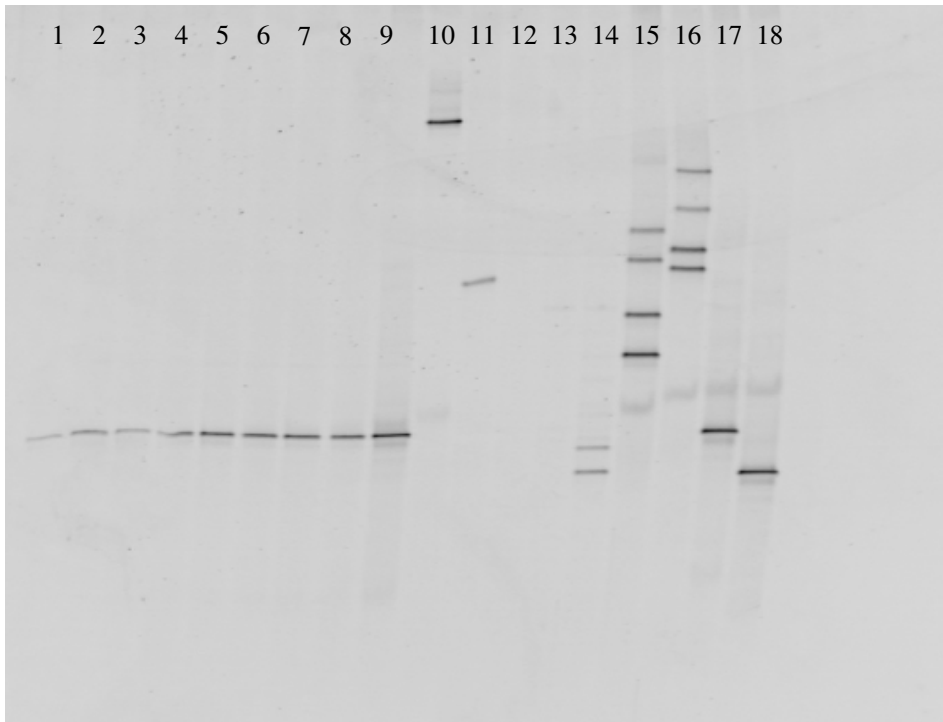


Figure 1. PCR/DGGE of 16S rDNA PCR products. Lanes 1 to 9, chicken *M. bovis* isolates; lanes 10 to 18, positive control PCR products, (*M. gallisepticum*, lane 10, *M. iners*, lane 11, *M. arginini*, lane 14, *M. bovirhinis*, lane 15, *M. capricolum* subspecies *capricolum*, lane 16, *M. bovis*, lane 17, and *M. glycyphilum*, lane 18).

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