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COMMUNICATION I

Survival and Isolation of Avian Mycoplasmas from Drinking Water of Infected Chickens.

ABSTRAK

Mikoplasma telah disuntik ke dalam air minum ayam dan diambil contoh pada waktu yang berlainan. Pada kepekatan yang tinggi, organisma-organisma boleh didapati kembali daripada air 24 jam pos inokulasi. Mikoplasma tidak boleh didapati kembali daripada air minum ayam yang berinfeksi.

ABSTRACT

Mycoplasma was inoculated into the drinking water of chicken and sampled at different time intervals. At a higher concentration, the organisms could be recovered from the water 24 hours post inoculation. Mycoplasma could not be recovered from the drinking water of infected chickens.

INTRODUCTION

The survivial of avian mycoplasmas within and outside the body of the host is important in epidemiology (Jordan, 1985). In the study of the epidemiology of avian mycoplasmas, little has been reported on the survival of these organisms in the environment. Perlstein (1969) cited by Polak–Vogelzang (1977) reported that *Mycoplasma gallisepticum* could be recovered from drinking water of infected chickens and several strains of *M. gallisepticum* remained viable for at least 48 hours after inoculating broth cultures into water. Polak–Vogelzang (1977) stated that survival of *M. gallisepticum* in mains water at room temperature depended on the initial number of organisms and the incorporation of mycoplasma broth in the suspending medium.

In a survey on the prevalence of mycoplasmas in the indigenous fowls, Shah-Majid and Nihayah (1987) isolated mycoplasmas from 64 out of 205 fowls sampled. The mycoplasmas were isolated from the choanal cleft of these fowls. Perhaps, at the time of drinking, the mycoplasmas that are present in the choanal cleft may be introduced into the water and other fowls that share the same drinker may be infected. This study was conducted to determine if mycoplasma could survive in the drinking water of chicken and to isolate mycoplasmas from the drinking water consumed by infected chickens.

MATERIALS AND METHODS

Mycoplasma spp. isolated from the choanal cleft of chicken during the preliminary survey was cultured in mycoplasma broth (Mycoplasma Supplement–G, Oxoid Limited, England). After 2–3 days of incubation, the viable count was determined using the plate count method and was diluted to contain approximately 10^2 and 10^8 colony–forming units (CFU) per ml. The mycoplasma was dispensed in 1 ml aliquot, frozen at –20 C. On the day of inoculation, the vial was thawed before use. After inoculation, the exact numbers of mycoplasmas used was determined by the plate count method.

In the first study, a rectangular and round drinkers were filled with tap water (ph 7.6) and kept at room temperature. The rectangular (62 x 5 x 7.5 cm^3) drinker has a capacity of 2.16 litres of water when filled up to the brim while the round drinker has a capacity of 3.28 litres. One millilitre of 4 x 10^2 cfu/ml of mycoplasmas were inoculated into the rectangular drinker and another 1 ml of the same concentration was inoculated into the round drinker. The mycoplasmas were inoculated into the drinking water with a 1 ml pipette and attempt were made to cover the entire length of the water surface with mycoplasma. A sterile dry

cotton swab was used to sample the water surface for mycoplasmas. At the time of sampling, about 1 inch of the cotton swab was dipped into the water and was dragged slowly to the other end and back to its starting point. A different swab was used for different drinkers and for sampling at different time intervals. The swabs were then inoculated onto Mycoplasma Supplement –G agar medium. The water was sampled after 10, 20, 30 mins and 1, 4, 8, 12 and 48 hours after initial inoculation. The same procedure was repeated with 5.5×10^8 cfu/ml inoculum. All plates were incubated at 37 C in the presence of 5 per cent carbon dioxide and were examined for growth at days 2, 4, 7, 10 and 14 of incubation.

In the second study, two-month-old indigenous fowls belonging to the university farm were initially swabbed for the presence of mycoplasmas in the choanal cleft region. Ten mycoplasma positive birds were then selected and kept in individual cages. These birds were allowed to drink and the remaining water was immediately sampled for mycoplasma using a dry cotton swab. Samples were then inoculated onto Mycoplasma Supplement–G agar medium. Five water samples (after drinking) were taken from each bird.

RESULTS AND DISCUSSION

Table 1 shows the recovery of mycoplasmas from the drinking water at different time intervals following inoculation. Mycoplasmas remain viable in the drinking water when the inoculum used is high. Mycoplasmas could be recovered from the tap water 48 hours post inoculation and this is similar to the observation made by Perlstein (1969) and Polak–Vogelzang (1977). Polak–

TABLE 1

Recovery of mycoplasmas from the drinking water at different time interval following inoculation

Inoculum	Time interval after initial inoculation	Isolation of mycoplasmas from: rectangular water drinker	round water drinker
4×10^2 organisms/ml	10 mins	–	–
	20 mins	–	–
	30 mins	–	–
	1 hr	–	–
	4 hrs	–	–
	8 hrs	–	–
	12 hrs	–	–
	24 hrs	–	–
5.5×10^8 organisms/ml	10 mins	+++	+++
	20 mins	++	+++
	30 mins	++	+++
	1 hr	++	+++
	4 hrs	+	++
	8 hrs	+	++
	12 hrs	+	++
	24 hrs	+	+
	48 hrs	–	+

\+ 0 to 10 colonies
++ 10 to 100 colonies
+++ >100 colonies
– No Isolation

Vogelzang (1977) stated that the survival of mycoplasma strains in mains water at room temperature depend on the number of initial organisms used and the suspending medium of the mycoplasma broth. Polak–Vogelzang (1977) was able to isolate mycoplasmas at 6 to 8 $\log_{10}$ cfu/ml for 4 to 5 days when suspended in 1% broth in sterile water at ph 7.7. However, at concentrations less than 6 $\log_{10}$ cfu/ml, the mycoplasmas survive for less than one day. In the present study, mycoplasma could not be reisolated from both drinkers as early as 10 mins post inoculation when the inoculum used was 10^2 cfu/ml.

Mycoplasmas could not be recovered from all the water samples taken from all the infected birds. The infected birds used in this study were clinically normal with no nasal discharges, sneezing or coughing but only the presence of mycoplasmas in the choanal cleft region. Polak–Vogelzang (1977) suggests that under certain environmental conditions or when well protected by exudates or tissue debris, the survival of mycoplasma in the environment may be longer. However, this study shows that mycoplasma that were present in the choanal cleft region cannot be isolated from the water sample at the time of drinking using the swabbing technique. A small number of mycoplasmas could have been transmitted into the water but the technique of swabbing the water surface may not be efficient in detecting small numbers of mycoplasmas as shown in the drinker studies (Table 1). Perlstein (1969) cited by Polak-Vogelzang (1977) observed than when infected nasal discharges were placed in water, the *Mycoplasma gallisepticum* could be recovered from the water for two days and drinking water of infected chicks yielded *Mycoplasma gallisepticum*. It is probable that the transmission of *Mycoplasma gallisepticum* into the water at the time of drinking is from the nasal discharges. In the present study the infected birds were clinically normal with no nasal discharges. Under farm conditions and at room temperature, it is probable that the important factors which will influence the survival of mycoplasma in drinking water are the presence of agents which would protect against osmotic lysis (Polak-Vogelzang, 1977) and high concentration of mycoplasmas as shown in this study.

REFERENCES

JORDAN, F.T.W. (1985): Gordon Memorial Lecture: People, poultry and pathogenic mycoplasmas. *Br. Poult. Sci.* **26**: 1–15.

POLAK-VOGELZANG, A.A. (1977): Survival of *Mycoplasma gallisepticum* in mains water. *Avian Pathol.* **6**: 93-95.

SHAH-MAJID. M and M. NIHAYAH. (1987): Choanal cleft as an alternative site for the isolation of avian mycoplasmas. *Vet. Rec.* **121**: 153.

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