

Research Note

**Effects of Antibiotics on the Development of  
*Metastrongylus apri* in Vitro**

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We reported that the exsheathed 3rd stage larvae of *M. apri* were cultured to the preadult worms *in vitro* (Hata, 1978; Hata *et al.*, 1980, 1981). In the course of these studies, we found a great deal of difficulty in removing the 3rd stage larvae of *M. apri* without contaminant from experimentally infected earthworms. Antibiotics have routinely been incorporated in the media for the culture of parasitic helminths to protect them from bacterial and fungal contamination. However, high concentration of antibiotics in culture media may inhibit the growth and development of parasitic helminths. This investigation was designed to examine the effect of antibiotics on the development of *M. apri* from the 3rd to 5th stage larvae *in vitro*. The 3rd stage larvae collected from experimentally infected earthworms were exsheathed in 0.1% sodium hypochloride solution. The larvae were washed three times in Hank's solution containing penicillin (200 units/ml) and streptomycin (100

µg/ml) and then introduced aseptically into tubes (16×150 mm) containing NCTC 109 (Difco) supplemented with 20% calf serum (Gibco). The medium was renewed twice a week. Antibiotics tested were (1) penicillin G potassium (Takeda), (2) streptomycin sulfate (Kaken), (3) chloromycetin (Yamanouchi), (4) kanamycin sulfate (Meiji), (5) amphotericin B (Fangizone, Gibco) and (6) nystatin (Mycostatin, Gibco). All the antibiotics were dissolved in the culture medium, pH of which were adjusted to 7.4 with 7.5% NaHCO<sub>3</sub> or 1/10N HCl. Cultures were transferred to Leighton tube after 2 weeks to examine their survival under an inverted microscope. They were then fixed in 10% neutral formaldehyde solution to count the number of 5th stage worms. All media and cultures at termination were tested in thioglycollate broth for microbial contaminant.

As shown in Table 1, worms cultivated in media containing 100 to 800 units/ml penicillin were identical in growth and survival to the control parasites. With streptomycin at a concentration of 100 µg/ml of medium, the result was similar to that in the control. However, in medium at levels higher than 200 µg/ml fewer parasites attained to the 5th stage. No worms could develop to the 5th stage at a

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Table 1 Effects of penicillin G and streptomycin sulphate on the development and the survival of *M. apri* in NCTC 109 supplemented with 20% calf serum

Antibiotic	Concentration	Percent 5 th stage worms	Percent mortality	Total No. of worms tested
Penicillin	100 (units/ml)	76.9	9.1	432
	200	78.0	10.7	475
	800	73.1	10.5	583
	3200	67.6	17.0	401
Streptomycin	100 ( $\mu\text{g/ml}$ )	76.6	10.4	389
	200	68.6	21.3	432
	800	26.8	47.1	470
	3200	0	82.3	455
Control	—	77.7	8.8	298

Observations were made after 2 weeks in culture. Results represent mean values from 3 tubes at each concentration.

Table 2 Effects of kanamycin and chloromycetin on the development and the survival of *M. apri* in NCTC 109 supplemented with 20% calf serum

Antibiotic	Concentration	Percent 5 th stage worms	Percent mortality	Total No. of worms tested
Kanamycin	100 ( $\mu\text{g/ml}$ )	28.1	55.8	472
	200	9.2	81.2	561
	800	3.8	86.4	520
	3200	0	97.8	379
Chloromycetin	100 ( $\mu\text{g/ml}$ )	54.1	8.4	387
	200	49.4	41.0	439
	800	1.2	79.8	578
	3200	0	59.5	460
Control	—	82.3	8.8	567

Observations were made after 2 weeks in culture. Results represent mean values from 3 tubes at each concentration.

Table 3 Effects of amphotericin B and nystatin on the development and the survival of *M. apri* in NCTC 109 supplemented with 20% calf serum

Antibiotic	Concentration	Percent 5 th stage worms	Percent mortality	Total No. of worms tested
Amphotericin B	20 ( $\mu\text{g/ml}$ )	81.3	10.0	466
	100	66.0	13.3	241
Nystatin	20 (units/ml)	84.0	9.3	431
	100	71.5	11.1	379
Control	—	84.7	10.0	423

Observations were made after 2 weeks in culture. Results represent mean values from 3 tubes at each concentration.

level of 3200  $\mu\text{g/ml}$ . The incorporation of kanamycin or chloromycetin to the medium gave retardatory or inhibitory effect on the growth of *M. apri* even at a level of 100  $\mu\text{g/ml}$  (Table 2). All worms were dead at a level of 3200  $\mu\text{g/ml}$ . Kanamycin at the concentration of 200  $\mu\text{g/ml}$  which is recom-

mended for the control of mycoplasma in culture medium (Perlman *et al.*, 1967) had an inhibitory effect on the development of *M. apri*. Also it was reported that the use of 30  $\mu\text{g/ml}$  chloromycetin can be recommended for the control of mycoplasma in culture medium (Perlman *et al.*, 1967).

Though inhibitory effect for *M. apri* was observed at 100  $\mu\text{g/ml}$  of chloromycetin, the effect to *M. apri* in lower concentration than 100  $\mu\text{g/ml}$  was not examined in this investigation. Further studies into the lower concentration levels are needed. Amphotericin B or nystatin at the concentration of 20  $\mu\text{g/ml}$  or 20 units/ml respectively had no significant effect on the development and the survival of *M. apri* (Table 3). Although the tolerance of parasitic nematodes for antibiotics has not been well investigated, it has been reported that the difference in tolerance may exist among the species of parasites (Hansen and Hansen, 1978). Consequently, in the case limited to *M. apri* the use of 200 units/ml penicillin and 100  $\mu\text{g/ml}$  streptomycin can be recommended for the control of bacterial contaminants in the culture. Nystatin and amphotericin B may be added to the medium for eliminating fungus at a

level of 20 units and 20  $\mu\text{g/ml}$ , respectively.

It is preferable to avoid the use of kanamycin for the cultivation of *M. apri*, because of unfavorable effects on it.

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#### 短 報

### 豚肺虫 *Metastrongylus apri* の *in vitro* 下での発育に対する抗生物質の影響

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豚肺虫の第3期仔虫からの培養に際し、medium中へ添加される抗生物質の虫体の発育に対する影響について検討した。

その結果、用いた6種の抗生物質のうち、penicillinおよびstreptomycinが比較的虫体に対する影響が少なく、200 units/mlおよび100  $\mu\text{g/ml}$ 以下の濃度で、

また抗カビ剤であるnystatinやamphotericin Bでも20 units/mlおよび20  $\mu\text{g/ml}$ 以下の濃度で虫体への影響はほとんど認められなかった。よって豚肺虫の培養に際しこれら抗生物質を上記濃度以下でmedium中へ添加できることが明らかとなった。