Drug Susceptibility of *Brucella canis* Isolated from Dogs

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Abstract. *Brucella canis* strains isolated from house dogs, stray dogs and beagles in breeding or experimental colonies were examined for their susceptibility to 38 antibacterial drugs by an agar dilution method. All of 90 strains tested were found to be highly susceptible to tetracyclines and aminoglycoside antibiotics. These were also susceptible to chloramphenicol, spectinomycin, rifampicin and sulfonamide. However, in these isolates, decreased susceptibility was found to cephalosporin antibiotics and nalidixic acid, and almost insensitivity to peptide antibiotics and cycloserine. On the other hand, 22 (24.4%) of 90 strains tested were found to be multiply resistant to macrolide antibiotics, some of penicillins, novobiocin and lincomycin. These multiple drug-resistant strains were isolated from stray dogs and beagles in various districts, indicating that the prevalence of such resistant strains is relatively wide-spread in dogs in Japan.

Brucella canis is a causative agent of canine abortion which is recognized as an important economic problem in dog breeding kennels [3, 4, 7, 8, 11]. Natural *Br. canis* infection was also reported in human beings [9, 10, 13], suggesting that this organism will create serious problems not only for dog breedings but also for public health. In Japan, the first occurrence of canine brucellosis was observed in 1971 in a beagle colony [1, 2] and the isolation of *Br. canis* was succeeded by some workers [6, 15, 16]. Little information is available, however, on the drug susceptibility of this organism. This paper deals with the drug susceptibility of naturally occurring *Br. canis* strains.

Materials and Methods

***Br. canis* strains:** Ninety strains of *Br. canis* were supplied for the assay of drug susceptibility. These strains were isolated during survey for 5 years from 1972 to 1976 from house dogs, stray dogs and beagles in various districts; Asahigawa, Sapporo, Sendai, Tokyo and Kyoto. The reference strain (RM6/66) was used as a control. This strain was kindly supplied by Dr. L. E. Carmichael.

Media: Trypticase soy broth (BBL), trypticase soy agar (BBL) and Mueller-Hinton medium (Difco) were employed for liquid or solid cultures.

Drugs and drug susceptibility test: Drugs used in this study are listed in Tables 1-3. Drug susceptibility test was done by an agar dilution method [14]. A loopful of one hundredfold dilution (about 10^6 bacteria/ml) of broth culture incubated for 2 days was spotted onto trypticase soy agar plates containing serial twofold dilutions of each drug. For the assay of sulfadimethoxine (SA) susceptibility, Mueller-Hinton medium was used. The minimum inhibitory concentration (MIC) of each

Table 1. Susceptibility of 90 strains of *Brucella canis* to various antibacterial drugs-I

Drug tested	Minimum inhibitory concentration (MIC, $\mu\text{g}/\text{ml}$)						
	0.04	0.09	0.19	0.39	0.78	1.56	3.13
CTC	•	21*	53	26	•	•	•
OTC	14	59	27	•	•	•	•
TC	1	51	48	•	•	•	•
MTC	•	1	66	33	•	•	•
DXC	•	9	76	15	•	•	•
SM	•	6	56	38	•	•	•
KM	•	4	61	35	•	•	•
ADKM	•	•	54	46	•	•	•
VSM	•	•	27	69	4	•	•
GM	6	89	5	•	•	•	•
CP	•	•	•	•	1	43	56
SPC	•	•	1	16	81	2	•
RFP	•	•	4	57	22	17	•
SA	•	14	16	20	39	11	•

Remarks.

CTC: chlortetracycline. OTC: oxytetracycline. TC: tetracycline. MTC: methacycline. DXC: doxycycline. SM: streptomycin. KM: kanamycin. ADKM: amino deoxykanamycin. VSM: vistamycin. GM: gentamicin. CP: chloramphenicol. SPC: spectinomycin. RFP: rifampicin. SA: sulfadimethoxine.

*: The percentage of strains to various MICs.

Table 2. Susceptibility of 90 strains of *Brucella canis* to various antibacterial drugs-II

Drug tested	Minimum inhibitory concentration (MIC, $\mu\text{g}/\text{ml}$)					
	3.13	6.25	12.5	25.0	50.0	100
CER	3*	29	68	•	•	•
CET	4	89	6	1	•	•
CEX	•	13	80	7	•	•
CEG	•	1	12	85	2	•
NA	•	27	69	4	•	•
CL	•	•	18	82	•	•
PM-B**	•	•	•	3	31	66
BC**	•	•	•	59	41	•
CS	•	•	•	•	•	100

Remarks.

CER: cephaloridine. CET: cephalothin. CEX: cephalexin.

CEG: cephaloglycin. NA: nalidixic acid. CL: colistin.

PM-B: polymyxin-B. BC: bacitracin. CS: cycloserine.

*: The percentage of strains to various MICs.

** : Potency of the drug is expressed by unit/ml.

drug was read after incubation at 37°C for 48 hours, aerobically.

Results

The MICs of 5 tetracyclines (chlortetracycline, CTC; oxytetracycline, OTC; tetra-

Table 3. Susceptibility of 90 strains of *Brucella canis* to various antibacterial drugs-III

Drug tested	Minimum inhibitory concentration (MIC, $\mu\text{g/ml}$)											
	0.04	0.09	0.19	0.39	0.78	1.56	3.13	6.25	12.5	25	50	<100
EM	17*	48	11	.	7	17	.	.	.	.	.	.
OM	.	.	21	55	.	.	.	8	16	.	.	.
KT	.	.	9	46	21	.	4	19	1	.	.	.
SP	.	.	.	.	.	6	70	.	.	8	15	1
TS	.	.	.	.	3	59	14	.	11	12	1	.
PC-G	.	.	.	25	52	22	1	.	.	.	.	.
PE-PC	.	.	.	.	.	12	64	.	3	20	.	1
MPI-PC	.	.	.	.	.	.	.	9	67	.	2	22
MCI-PC	.	.	.	.	.	.	.	.	29	47	.	24
AB-PC	.	.	20	59	21	.	.	.	.	.	.	.
AC-PC	.	.	.	.	.	.	18	64	10	8	.	.
CB-PC	.	.	.	.	.	10	71	18	1	.	.	.
AMX-PC	.	.	43	57	.	.	.	.	.	.	.	.
NB	.	22	49	5	.	19	4	1	.	.	.	.
LCM	.	.	.	.	.	.	.	2	7	67	5	19

Remarks.

EM: erythromycin. OM: oleandomycin. KT: kitasamycin. SP: spiramycin. TS: tylosin. PC-G: penicillin-G. PE-PC: phenethicillin. MPI-PC: oxacillin. MCI-PC: cloxacillin. AB-PC: ampicillin. AC-PC: cyclacillin. CB-PC: carbenicillin. AMX-PC: amoxicillin. NB: novobiocin. LCM: lincomycin.

*: The percentage of strains to various MICs.

cycline, TC; methacycline, MTC; doxycycline, DXC), 5 aminoglycoside antibiotics (streptomycin; SM, kanamycin; KM, amino deoxy-kanamycin; ADKM, vistamycin; VSM, gentamicin; GM), chloramphenicol (CP), spectinomycin (SPC), rifampicin (RFP) and SA are shown in Table 1. The tetracyclines and aminoglycoside antibiotics exhibited marked activities against all of *Br. canis* strains tested; MICs of these drugs ranged from 0.04 to 0.78 $\mu\text{g/ml}$. There was also susceptibility to the other 4 drugs (CP, SPC, RFP, SA); all strains were inhibited at a concentration of less than 3.13 $\mu\text{g/ml}$. However, 4 cephalosporin antibiotics (cephaloridine, CER; cephalothin, CET; cephalixin, CEX; cephaloglycin, CEG) and nalidixic acid (NA) were not so effective; MICs of these drugs ranged from 3.13 to 50 $\mu\text{g/ml}$ (Table 2). As is also clear from Table 2, all strains were found to be almost insensitive to 3 peptide antibiotics (colistin, CL; polymyxin-B, PM-B; bacitra-

cin, BC)) and cycloserine (CS). The distribution of MICs of 5 macrolide antibiotics (erythromycin, EM; oleandomycin, OM; kitasamycin, KT; spiramycin, SP; tylosin, TS), 8 penicillins (penicillin-G, PC-G; phenethicillin, PE-PC; oxacillin, MPI-PC; cloxacillin, MCI-PC; ampicillin, AB-PC; cyclacillin, AC-PC; carbenicillin, CB-PC; amoxicillin, AMX-PC), novobiocin (NB) and lincomycin (LCM) is shown in Table 3. With 5 macrolide antibiotics, 3 penicillins (PE-PC, MPI-PC, MCI-PC), NB and LCM, there were two peaks in the distribution of their MICs, indicating that 22 (24.4%) of 90 strains tested are resistant to these drugs. The resistance levels of 5 representative strains to these drugs are shown in Table 4. It is apparent from this table that the resistance levels to macrolide antibiotics (EM, OM, KT, SP, TS) were ten or more times higher than that of the control strain RM6/66. Similar results were obtained with the other 5 drugs (PE-PC, MPI-PC,

Table 4. Resistance levels of 5 representative strains of *Brucella canis*

Name of strain	Minimum inhibitory concentration (MIC, $\mu\text{g/ml}$)									
	EM	OM	KT	SP	TS	PE-PC	MPI-PC	MCI-PC	NB	LCM
S610	1.56	6.25	12.5	100	50	12.5	100	>100	6.25	50
S805	1.56	6.25	6.25	50	25	100	100	>100	3.13	100
EK1	1.56	12.5	6.25	50	25	25	100	>100	1.56	100
EK5	1.56	12.5	6.25	50	25	25	100	>100	1.56	100
QE13	0.78	12.5	6.25	25	12.5	25	100	>100	1.56	50
RM6/66 (control)	0.19	0.39	0.39	3.13	1.56	1.56	12.5	12.5	0.39	12.5

Remarks.

EM: erythromycin. OM: oleandomycin. KT: kitasamycin. SP: spiramycin. TS: tylosin. PE-PC: phenethicillin. MPI-PC: oxacillin. MCI-PC: cloxacillin. NB: novobiocin. LCM: lincomycin.

MCI-PC, NB, LCM). It was also found that there was a complete cross-resistance between 5 macrolide antibiotics and the other 5 drugs. Most of these multiple drug-resistant strains were isolated from beagles reared on certain colonies in Tokyo, but some strains were from stray dogs in Sapporo.

Discussion

Various antibacterial drugs have been used in the treatment of *Br. canis* infection, although some workers [4, 7, 8] reported that complete elimination of causative agent was not achieved successfully. An explanation usually given for such unsuccessful therapy was characteristic feature of this organism which was an intra-cellular parasite.

The present studies showed that all of *Br. canis* isolates tested exhibit high susceptibility to tetracyclines. This finding is in agreement with that obtained by Hall and Manion [5], indicating that tetracyclines remain the drug of choice for the treatment of *Br. canis* infection. It was also found that aminoglycoside antibiotics exhibit marked activity against *Br. canis* isolates. However, a comparison of MICs among this group indicates that gentamicin is the most effective one. Recently, Robert-

son et al. [12] also mentioned that gentamicin may be more effective than streptomycin when used in combination with tetracyclines for the treatment of *Br. abortus* infection. Chloramphenicol, spectinomycin and rifampicin are another antibiotics that showed in-vitro activities against *Br. canis* isolates. However, little information is available on in-vivo efficacy of these drugs for canine brucellosis. Further clinical evaluation may be required in this regard.

It should be noted that 24% of *Br. canis* isolates tested exhibited a cross-resistance to macrolide antibiotics, some of penicillins, novobiocin and lincomycin. These multiple drug-resistant strains were isolated from stray dogs and beagles in various districts, indicating that the prevalence of such resistant strains is relatively wide-spread in dogs. Although the detailed history of drug-using in these dogs was not clear, there is no doubt that the use of some drugs has brought about the appearance and selection of these multiple drug-resistant bacteria. This is the first report on the occurrence of such resistant strains in *Br. canis*, suggesting that the choice of drug for the treatment of *Br. canis* infection must be handled carefully. Genetic and biochemical studies on these drug-resistant

strains are now in progress and will be described elsewhere.

References

- [1] Azuma, R., and Isayama, Y. (1973). Canine brucellosis. *J. Jap. vet. med. Ass.*, **26**, 111-119 (in Japanese).
- [2] Azuma, R., Isayama, Y., Tanaka, S., Suto, T., and Morgan, W. J. B. (1977). Identification of microorganisms isolated from an enzootic case of canine abortion in Japan. *Ann. Sclavo.*, **19**, 83-88.
- [3] Carmichael, L. E. (1966). Abortion in 200 beagles. *J. Amer. vet. med. Ass.*, **149**, 1126.
- [4] Carmichael, L. E., and Kenney, R. M. (1968). Canine abortion caused by *Brucella canis*. *J. Amer. vet. med. Ass.*, **152**, 605-616.
- [5] Hall, W. H., and Manion, R. E. (1970). In vitro susceptibility of *Brucella* to various antibiotics. *Appl. Microbiol.*, **20**, 600-604.
- [6] Hayashi, T. T. A., and Isayama, Y. (1977). Detection of *Brucella canis* infection in dogs in Hokkaido. *Microbiol. Immunol.*, **21**, 295-298.
- [7] Moore, J. A. (1969). *Brucella canis* infection in dogs. *J. Amer. vet. med. Ass.*, **155**, 2034-2037.
- [8] Moore, J. A., and Gupta, B. N. (1970). Epizootiology, diagnosis, and control of *Brucella canis*. *J. Amer. vet. med. Ass.*, **156**, 1737-1740.
- [9] Morisset, R., and Spink, W. W. (1969). Epidemic canine brucellosis due to a new species, *Brucella canis*. *Lancet.*, **2**, 1000-1002.
- [10] Munford, R. S., Weaver, R. E., Patton, C., Feeley, J. C., and Feldman, R. A. (1975). Human disease caused by *Brucella canis*: A clinical and epidemiologic study of two cases. *J. Amer. med. Ass.*, **231**, 1967-1969.
- [11] Pickerill, P. A., and Carmichael, L. E. (1972). Canine brucellosis: Control programs in commercial kennels and effect on reproduction. *J. Amer. vet. med. Ass.*, **160**, 1607-1615.
- [12] Robertson, L., Farrell, I. D., and Hinchliffe, P. M. (1973). The sensitivity of *Brucella abortus* to chemotherapeutic agents. *J. med. Microbiol.*, **6**, 549-557.
- [13] Swenson, R. M., Carmichael, L. E., and Cundy, K. R. (1972). Human infection with *Brucella canis*. *Ann. intern. Med.*, **76**, 435-438.
- [14] Terakado, N., Azechi, H., Ninomiya, K., and Shimizu, T. (1973). Demonstration of R factors in *Bordetella bronchiseptica* isolated from pigs. *Antimicrob. Agents Chemother.*, **3**, 555-558.
- [15] Ueda, K., Saegusa, J., Fujiwara, K., Muto, S., Okada, K., Hasegawa, A., Saegusa, S., and Usui, K. (1974). Detection of *Brucella canis* infection in dogs from the Tokyo area. *Jap. J. vet. Sci.*, **36**, 539-542.
- [16] Yamaguchi, C., Suzuki, T., Nomura, T., Kukita, Y., Iwaki, T., Kazuno, Y., and Ghoda, A. (1974). Canine brucellosis in a beagle breeding colony. *Jap. J. vet. Sci.*, **36**, 175-182 (in Japanese with English summary).