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Comparison by PFGE of *Escherichia coli* from the Uterus and Feces of Bitches with Pyometra

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Abstract: To compare *Escherichia coli* (*E. coli*)-DNA profile isolated from the uterus and feces of bitches suffering from pyometra, Plsed-Feld Gel Eectrophoresis (PFGE) was used. *E. coli* from seven bitches suffering from pyometra was isolated as 8 strains from the uterus and 15 from feces, respectively. In three out of seven bitches same DNA-profiles of *E. coli* colony types from the uteres and feces were shown by PFGE. It was first demonstrated by PFGE that *E. coli* strains isolated from the uterus and feces of bitches suffering from pyometra had the same DNA-profile. Therefore, the pathogen of pyomotra has reconfirmed as *E. coli* from feces by PFGE with higher homogeneity.

Key words: Bitch, *E. coli*, fece, PFGE, pyometra, Japan

INTRODUCTION

Pyometra is regarded as one of the most common illnesses in bitches (Hagman and Kuhn, 2002; Pretzer, 2008; Smith, 2006). Its etiology and pathogenesis are complex and only partly understood (Choi and Kawata, 1975; Grindlay *et al*., 1973; Hardy and Osborne, 1974; Nomura and Funahashi, 1999; Pretzer, 2008; Schoon *et al*., 1992; Smith, 2006). The most common bacterium isolated in cases of pyometra is *Escherichia coli* (*E. coli*) (Bjurstrom, 1993; Frasson *et al*., 1997; Grindlay *et al*., 1973; Hardy and Osborne, 1974; Pretzer, 2008) which is also usually found in the feces of affected bitches (Watts *et al*., 1996). In their studies, all *E. coli* were typed with the aid of an automated typing system for biochemical fingerprinting. The biochemical type of uterine and fecal *E. coli* isolated from bitches with pyometra showed higher homogeneity. It was concluded that *E. coli* associated with canine pyometra derived from the fecal flora. Hagman and Kuhn (2002) compared *E. coli* isolated from the uterus and urinary bladder of bitches suffering from pyometra by Pulsed Field Gel Electrophoresis (PFGE). However, there is no report that compares *Escherichia coli* (*E. coli*)-DNA profile isolated from the uterus and feces of bitches suffering from pyometra by Pulsed-Field Gel Electrophoresis (PFGE).

In this study using PFGE, researchers compared *E. coli* isolated from the uterus and feces of bitches suffering from pyometra.

MATERIALS AND METHODS

Sample collection: Each sample from the uterus and feces of seven bitches (7-12 years old, mean 8.5 years old, 3 Chihuaha, 2 dachshund and 2 Pekingese) suffering from pyometra was plated onto Desoxycholate-Hydrogen sulfide-Lactose (DHL) agar. After inocubation at 37°C for 18 h, from 2-4 red colonies were identified using miniaturized Biochemical Systems (API System^{R}; bioMerieux, Lion, France). As a result, eight *E.coli* isolates from the uterus, sixteen from feces and one from cholecystis were obtained.

PFGE: DNA profiles of all *E. coli* were analyzed by PFGE. Techno Suruga Laboratory Co., Ltd. (Shizuoka, Japan) performed this analysis, as described by Rice *et al*. (1999). Briefly, a single colony on LB agar (Becton Dickinson, MD, USA) was inoculated with 5 mL Tripticase Soy Broth (Becton). Growth was marked in all broth cultures after incubation at 30°C for 18 h in an incubator. A 1.5 mL sample of the bacterial culture was centrifuged at 14000 g min^{-1}, the supernatant was discarded and the bacterial pellet was suspended in 150 μL EET buffer (100 mM EDTA, 10 m MEGTA, 10 mM Tris; pH 8.0). The bacterial suspension was then mixed with the same volume of 1% Seakem Gold Agarose (Takara, Shiga, Japan) in EET, placed into disposable agarose plug molds (BioRad, Hercules, CA, USA) and cooled at 4°C for 30 min. Agarose plugs for each isolate were then placed

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into sterile 50 mL centrifuge tubes containing 1 mL of EET with 200 μg mL^{-1} lysozyme and 0.05% N-lauroylsarcosine sodium salt (EET-SP) and incubated at 37°C 3 h. The EET-SP was removed and 1 mL EET with 1 mg mL^{-1} proteinase K and 1.0% (w/v) lauryl sulfate sodium salt (EET-SP) was added to each tube and incubated in a water bath at 50°C overnight. The EET-SP was then removed and the plugs were rinsed by a 30 min soak in 40 mL of 10 mM Tris 1 mM EDTA disodium dehydrated pH 8.0 (TE) repeated 2 times. Agarose embedded chromosomal DNA was cleaved with XbaI (Toyobo, Osaka Japan) following the manufacturer's directions. PFGE was performed on a CHEF-DR III PFGE apparatus (BioRad) using the following parameters; 1% agarose-0.5×TBE gel (PFGE agarose; BioRad) at 14°C for 19 h at 6.0 V cm^{-1} and a linear ramp of 2.2-54.2 sec Lambda concatameres (BioRad) were used as molecular weight markers in the first and last lane of each gel. Agarose gels were stained with SYBR Green solution for 30 min and photographed on a CheimiDocXRS (BioRad).

RESULTS AND DISCUSSION

DNA-profiles of *E. coli* colony types from the uteri and feces of 3 bitches with pyometra are shown in Fig. 1. The DNA profiles of all *E. coli* from the uterus and feces in No. 1 and seven bitches were the same. In No. 4 bitch, one *E. coli* sample from the uterus and feces, respectively had the same profile. In three out of seven bitches, the same DNA-profiles of *E. coli* colony types from the uteri and feces are shown. Furthermore, *E. coli* from cholecystis had the same profiles as *E. coli* from the uteri and feces (bitch No. 7). In four of seven bitches, different DNA-profiles of *E. coli* from the uteri and feces were shown.

The common finding of *E. coli* in pyometra in bitches may simply be due to the fact that *E. coli* belongs to the normal intestinal flora and thus is likely to invade the vagina and uterus. *E. coli* is frequently isolated from the normal vaginal and uterine bacterial flora of bitches (Baba *et al*., 1982; Bjurstrom, 1993; Watts *et al*., 1996). In a previous study of *E. coli*, the phenotypic diversity of *E. coli* isolated from pyometra infection was shown to be homologous to the *E. coli* strains isolated from feces which could indicate that related bacteria caused the infections (Wadas *et al*., 1996).

In this study, the same DNA-profile of *E. coli* was isolated from three out of seven dogs. This indicated that *E. coli* from the uterus and feces of bitches with pyometra had the same origin from the DNA-profile, however researchers examined only two or three colonies from feces. If researchers examined many bacteria from feces, it seems that probably all *E. coli* of pyometra would be of feces origin. Therefore, the vagina of bitches could be polluted by feces and it is thought that *E. coli* from feces might invade the uterus. *E. coli* infect the uterus and are thought to lead to pyometra.

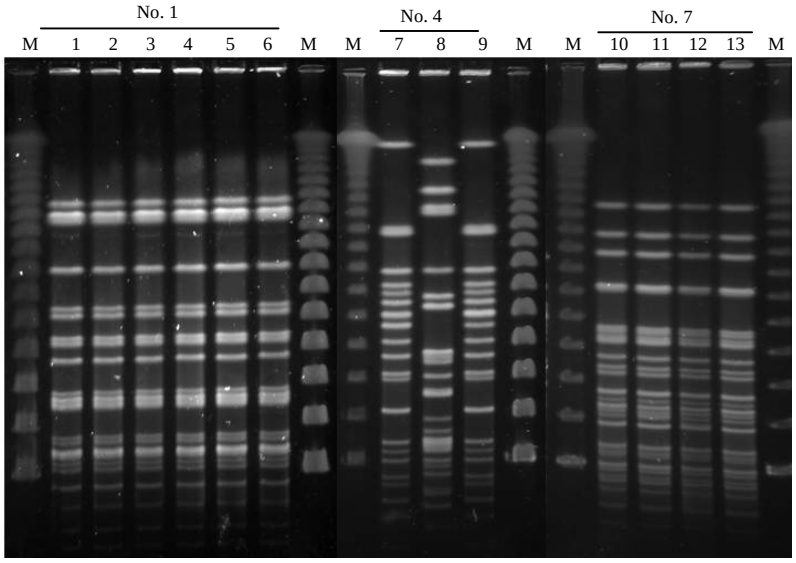

Fig. 1: DNA-profiles of *E. coli* from the uteri and feces of 3 bitches with pyometra; the animals are numbered 1, 4 and 7; lanes 1 and 2 were isolated from the uteri of dog No. 1 and lane 3-6 from feces of the same dog; lane 7 from the uteri of No. 4 and lane 8 and 9 from feces of the same dog; lane 10 from the uteri of No. 7 and lane 11 and 12 from feces and lane 13 from cholecystis of the same dog. M: CHEF DNA size standard, lambda ladder

In addition, *E. coli* as those from cholecystis showed the same profiles of *E. coli* from the uteri and feces but this is unknown whether it is a shift from the blood circulation or intestinal tract.

CONCLUSION

The study suggests that it is important that the reproductive organs of bitches should be kept to clean to prevent pyometra.

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