ブルセラ血清反応陽性ラクダにおけるYersinia enterocolitica 09の検出

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NOTE

Detection of *Yersinia enterocolitica* 09 Infection in Camels Serodiagnosed as Brucellosis

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The strong serological cross-reaction between *Yersinia enterocolitica* 09 and *Brucella abortus* was first described by Ahvonen, *et al.* [1]. *Y. enterocolitica* was isolated from various kinds of animals including livestock [12]. Under these circumstances, it is necessary to pay attention to *Y. enterocolitica* 09 infection when the serological examinations for brucellosis are performed.

Thirteen camels were introduced from China in March, 1981. One male and four females of them were diagnosed as brucellosis by agglutination and complement fixation (CF) tests. The samples of blood and feces were collected from these five animals at frequent intervals thereafter. But no clinical symptoms such as diarrhea and fever were detected among them. These five camels were slaughtered as brucellosis on the 77th day after their introduction. The following samples of viscera and lymph nodes, along with the fecal samples, were obtained for the isolation of Brucella organism and *Y. enterocolitica*. They were lung, liver, spleen, kidney, testis, ovary and uterus, and parotid, mandibular, retropharyngeal, superficial cervical, mediastinal, tracheobronchial, iliac, inguinal, subiliac, ischiadic, popliteal, hepatic, gastric, mesenteric and ileocolic lymph nodes.

For the isolation of Brucella organism, both serum-dextrose-brucella agar (DIFCO) and the selective media described by Farrell [4] were employed. One or two pieces of viscera and lymph nodes were smeared directly on them. These samples were divided into three groups: one group of viscera and two groups of lymph nodes. The emulsion of each group was inoculated subcutaneously into one guinea pig.

On the other hand, for the isolation of *Y. enterocolitica*, both DHL agar and SS agar containing sucrose (10 g/l) were employed. These agar plates, on which the samples of viscera and lymph nodes were smeared directly, were incubated at 22°C for 48 hours. Moreover, phosphate buffer solution (M/15, pH 7.6) was used as enrichment medium. Each sample of viscera, lymph nodes and feces, as well as each group of viscera and lymph nodes above-mentioned, were transferred into this solution and cultured at 4°C for 3 weeks.

No Brucella organism could be recovered from either the samples of viscera or lymph nodes of five camels. Moreover, no rise of agglutinin titer to *B. abortus* was found in the sera of any guinea pigs on the 39th day after the inoculation of these samples, and no Brucella organism could be isolated from

the viscera of any guinea pigs.

On the other hand, *Y. enterocolitica* was recovered from four of five camels, and their serovars were 09 by the slide agglutination tests using diagnostic sera (Toshiba Chemical Co.). With one of four camels, *Y. enterocolitica* 09 was isolated from the iliac and inguinal lymph nodes and the feces. With the others, these organisms were isolated from the feces, from the group of lymph nodes—mandibular, superficial cervical and tracheobronchial ones, and from the subcutaneous abscess of a guinea pig inoculated the group of lymph nodes—parotid, mandibular, retropharyngeal and superficial cervical ones, respectively. This organism, however, could not be recovered from the individual lymph nodes included in these two groups. *Y. enterocolitica* 09 was detected in the state of pure culture on the agar plates where the samples of iliac lymph nodes and abscess were smeared directly. The biochemical characteristics of six isolates at 22°C were indole −, Voges-Proskauer + (37°C −), Simmons citrate −, urease +, ornithine decarboxylase +, motility + (37°C −), raffinose −, rhamnose −, sucrose +, melibiose −, salicin −, esculin −.

For the demonstration of pathogenicity of six isolates of *Y. enterocolitica* 09, the enterotoxin production and the invasive ability were examined according to the method of Feeley, *et al.* [5]. As the result, heat-stable enterotoxin could be demonstrated in four isolates from the feces, the iliac and inguinal lymph nodes and the group of lymph nodes. But neither heat-labile enterotoxin nor mucosal invasiveness could be detected in any isolates.

Serological examinations were conducted on the sera from five camels to compare the antibody titers to *Y. enterocolitica* 09 with those to *B. abortus*. *Y. enterocolitica* 09 isolates from the iliac lymph nodes were offered to the preparation of antigens for agglutination and CF tests. *Y. enterocolitica* OH antigen (YeOH), O antigen (YeO) and OH antigen for H agglutination tests (YeH) were prepared, and YeOH and YeO agglutination tests were performed by the method of Mittal & Tizard [9]. YeH agglutination tests, however, were conducted at 56°C for 2 hours to stabilize the reactions. YeOH suspensions used for the agglutination tests gave 50% agglutination with a 1/400 dilution of the National Standard *B. abortus* antiserum. CF soluble antigen of *Y. enterocolitica* (YeCF) was prepared by the method of Corbel & Cullen [3] and CF tests were performed by the LBCF micro technique [11]. Brucella agglutination antigen (BrO) and CF antigen (BrCF) employed in this study were supplied by National Institute of Animal Health. The agglutination tests were carried out by the regular method, while the LBCF micro tests were employed to compare BrCF titers with YeCF.

The results of agglutination and CF tests for one camel are given in Fig. 1. The agglutinin titers to YeOH, YeO and BrO decreased steadily with the passage of time, respectively. YeOH agglutinin titers always exceeded BrO titers, and YeO agglutinin titers paralleled with BrO titers. YeH agglutinin titers, which were detected in the sera from four of five camels, were 1:40 or 1:20 and held almost the same levels. On the other hand, YeCF antibody titers were almost equivalent to BrCF titers and they remained at the levels of 1:40 or 1:20 on the 77th day.

The serum collected on the 14th day in Fig. 1 and that obtained from another camel, which had almost equivalent antibody titers each other, were offered to the absorption with YeOH and BrO by

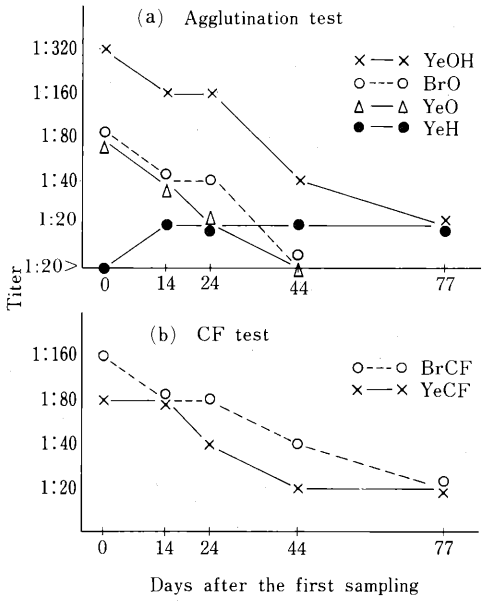

Fig. 1. Comparison of anti-yersinia titers with anti-brucella titers.

the method of Mittal & Tizard [9]. YeOH was able to absorb out the agglutinins to YeO, YeOH, YeH and BrO. YeOH also removed YeCF and BrCF antibodies from both sera completely. On the other hand, BrO failed to remove YeOH agglutinins completely, but BrO could absorb out the agglutinins to YeH, YeO and BrO as well as YeCF and BrCF antibodies.

Serovar 09 of *Y. enterocolitica* has been relatively rare in Japan and isolated from only human enteritis [6, 13], dogs [7] and small wild animals [8]. This study revealed that the foreign animals might introduce *Y. enterocolitica* 09 into our country, and suggested the existence of this organism in China.

Some useful information was given recently on the difference of antibody to *Yersinia* from that to *Brucella*. Mittal & Tizard [9] indicated that bovine exposed to *Y. enterocolitica* 09 possessed YeH agglutinins, along with higher titers of YeOH agglutinins than BrO agglutinins, and the absorption of their sera with BrO failed to remove YeOH or YeH agglutinins completely. Corbel [2] pointed out that the antibody titers to both organisms steadily declined after their maximum in the cattle exposed to *Y. enterocolitica* 09, compared with the persistence of these reactions in the animals inoculated *B. abortus*. These facts prove that the camels in this study had been infected with *Y. enterocolitica* 09, although they were serodiagnosed as Brucella infections at the initial stage of their quarantine. The pathological studies on these camels will give us much valuable knowledge about Yersinia infection.

The distribution of antibodies to *Y. enterocolitica* 09 demonstrated that the animal populations such as goat and pig were naturally infected with this organism to a considerable extent and cattle was suspected of the exposure to this organism [10]. This evidence leads to the conclusion that Yersinia infection must be taken into consideration when the animals that possess the antibodies to *Brucella* are detected. It is urgently necessary to device the satisfactory technique of distinguishing Yersinia infection from Brucella infection.

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要　約

ブルセラ血清反応陽性ラクダにおける *Yersinia enterocolitica* 09 の検出(短報)：須永　裕・谷藤子・向井清孝[1)](動物検疫所，[1)]動物検疫所成田支所)——中国産のラクダ5頭が，ブルセラ病の血清学的検査で陽性と診断されたが，ブルセラ菌は分離されなかった．しかし，4頭のリンパ節，糞便から *Y. enterocolitica* 09 (Ye) が分離された．　また，分離された Ye を抗原として凝集試験を行った結果，YeH に対する抗体が存在し，YeOH に対する抗体価は，ブルセラに対する抗体価よりも明らかに高値を示した．