TYPING OF *CORYNEBACTERIUM RENALE* ISOLATED FROM CATTLE IN A HERD WITH PERSISTENT PYELONEPHRITIS

Takashi HIRAMUNE, Tsuneo KUME and Nobuo MURASE
*Hokkaido Branch Laboratory, National Institute of Animal Health,
Hitsujigaoka, Sapporo*

Ryo YANAGAWA
*Department of Veterinary Hygiene and Microbiology, Faculty of Veterinary Medicine,
Hokkaido University, Sapporo*

(Received for Publication July 14, 1969)

Pyelonephritis in cows due to *Corynebacterium renale* has been reported in many countries¹⁾, including Japan^{5,8,9)}. It seems that one may not highly estimate the importance of this disease because the occurrence of the disease is usually sporadic, and that the etiological organism is susceptible to antibiotics. Nevertheless, once the disease occurs in a herd, it is difficult to eliminate the disease completely from the herd.

On a stock farm (N herd) in Hokkaido, pyelonephritis caused by *C. renale* was first noticed in 1951. Since then it has been found intermittently in the past 17 years. Cases of the disease amounted to 10 in 1962. Many strains of *C. renale* were isolated from apparently healthy cattle²⁾ and diseased cows in this herd.

Recently, *C. renale* has been classified into three types¹⁰⁾. Furthermore, the type I strains were found to be lysogenic¹¹⁾ by YANAGAWA et al. Therefore, phage typing of the organism has become possible. HIRAMUNE and YANAGAWA⁴⁾ made a report on the presence of 3 phage-types in *C. renale* of type I.

A total of 50 strains of *C. renale* isolated from the N herd were examined serologically and by applying a temperate phage, RP 6, which had been obtained from a strain derived from this herd, and which was the only phage to lyse the isolates from this herd. The results of the classification of *C. renale* and the resultant epizootiological findings are described in this paper.

MATERIALS AND METHODS

Isolation of *C. renale*: *C. renale* was isolated from the urine, urethra, urinary bladder, ureter, and kidney of cows manifesting pyelonephritic symptoms, as well as from the urine and vagina of apparently healthy cows, in the N herd. Urine samples sometimes were collected by the aid of a catheter. Materials were collected from the vaginal mucosa by a sterile cotton wool swab introduced into the vagina. Cultures were made by plating on blood agar and incubated at 37°C for 2 days. It was on the basis of BERGEY's Manual (1957) that the strains isolated were identified as *C. renale*.

Strains: A total of 50 strains isolated from cattle of the N herd were used. Ten strains of them were isolated from diseased cows. The remaining 40 were isolated from some of about 200 apparently healthy cattle of the same herd.

Serological typing of *C. renale*: Typing of *C. renale* was done on the basis of the precipitin reaction in gels conducted by the methods reported by YANAGAWA et al.¹⁰⁾.

Response to phage RP 6: Phage RP 6 was used. It had been obtained from *C. renale* strain No. 6 isolated from the N herd¹¹⁾. This was the only type of phage to lyse *C. renale* type I strains isolated from the N herd. All the strains isolated were examined to elucidate whether or not they produced any phage similar to phage RP 6 in host range, and whether or not they were susceptible for or resistant to the same phage. Production of phages was done by ultraviolet irradiation. The produced phages were examined for host range in the same manner as reported by YANAGAWA et al.¹¹⁾

RESULTS

Isolation of *C. renale*

About 200 cattle had been raised in the N herd. Since the first occurrence of pyelonephritis in 1951, 16 of them had been found to manifest the same symptoms until 1968.

C. renale was isolated from the urine of all the 16 animals. In seven animals on which post mortem examination was conducted by the authors, *C. renale* was isolated from the urethra, urinary bladder, ureter, and kidney. In these organs, urethritis, cystitis, ureteritis, and pyelonephritis were observed, respectively. The results of isolation of *C. renale* coincided with the presence of lesions in those organs. The remaining 9 cows could not be examined by the authors, because seven of them had been sacrificed in the abattoir and two recovered from the disease.

Serological typing of *C. renale*

The results of serological typing of the isolates are shown in Table 1. All the strains isolated from cows manifesting pyelonephritic symptoms belonged to type I. The strains isolated from apparently healthy cattle belonged either to type I or to type II at nearly the same frequency. Neither type III nor serologically non-typable strains were isolated from this herd.

Response of *C. renale* to phage RP 6

As shown in Table 2, about two-thirds of the strains isolated from pyelonephritic and apparently healthy animals produced phages similar to phage RP 6 in host range. The remaining strains were either resistant to or susceptible for phage RP6. There was no difference in response pattern to phage RP 6 between the strains isolated from the diseased and those from the apparently healthy cattle.

Typing of *C. renale* isolated from each barn of the N herd

The results of the serological classification of the organisms isolated from the animals housed in each barn of the N herd are shown in Table 3. The population of cattle in each barn consisted of animals of the same sex and similar age. The cows with clinical pyelonephritis were found in barns A, B, and C where only females (above 24 months of age) were housed. All the cows suffering from pyelonephritis were proved to have been infected with *C. renale* type I. Furthermore, type I organisms were isolated from apparently healthy cows housed in the same barns and from females (under 12 months of age) in barn F. None of them, however, were isolated from barn D or G, both of which housed only males.

Type II organisms were isolated from the animals of all the barns, except B, regardless of sex and age. It is of interest to note that all the strains isolated from males belonged to type II.

Table 1. Serological Types of *Corynebacterium renale* Strains Isolated from Cattle in the N Herd

Cattle	Number of animals	Type of <i>C. renale</i> isolated		
		I	II	III
Cows with pyelonephritis symptoms	10	10*	0	0
Apparently healthy cattle	40	22	18	0

* Number of strains.

Table 2. Response to Phage RP 6 of Type I Strains Isolated from Cattle in the N Herd

Cattle	Response to phage RP 6			Total No. of strains
	Productive	Susceptible	Resistant	
Cows with pyelonephritis symptoms	6*	1	3	10
Apparently healthy cattle	15	4	3	22

* Number of strains.

Table 3. Types of *C. renale* Strains Isolated from Cattle in Each Barn in the N Herd

Barn	Average number of animals housed	Sex	Age (months)	Type I		Type II
				Cows with pyelonephritis symptoms	Apparently healthy cattle	Apparently healthy cattle
A	40	Female	Above 24	2*	7	2
B	30	"	"	6	1	0
C	30	"	"	2	9	6
F	20	"	Under 12	0	5	3
E	30	"	12~24	0	0	1
D	40	Male	"	0	0	4
G	10	"	Above 24	0	0	2
Total				10	22	18

* Number of strains.

No distinct relation was found between their hosts (as grouped according to the barn) and their response to phage RP 6 in *C. renale* type I.

Additional epizootiological findings

Any relationship was investigated between the occurrence of this disease and pregnancy or parturition. Of ten cows which showed symptoms of pyelonephritis, seven were found to have been affected with this ailment during 1~2 months before and after parturition.

DISCUSSION

Pyelonephritis in cows due to *C. renale* occurred intermittently in the N herd in Hokkaido in the last 17 years. Serological typing was carried out on *C. renale* organisms isolated from diseased cows and apparently healthy cattle in this herd. These organisms were also examined for response to phage RP 6 derived from one of them. All the strains

isolated from the diseased cows were found to belong to type I. On the other hand, type I organisms were isolated from apparently healthy cows at almost the same frequency as type II organisms. In addition, there was no difference in response pattern to phage RP 6 of type I strains between the strains isolated from the diseased and those from the apparently healthy cattle. Thus, it is obvious in this herd that type I caused pyelonephritis in some cows, while it induced infection of the urinary tract in others, without causing that disease. Type II did not seem to cause any disease, but was parasitic to healthy cattle.

It is of interest, from an epizootiological point of view, that type I was isolated from females, while type II was isolated not only from females but also from males. Since each barn of the herd housed animals of the same sex and similar age, the relations of the bacterial type to the sex of animals were clearly demonstrated.

The authors³⁾ previously reported that there was no difference in the pathogenicity of *C. renale* organisms for cows between type I organisms isolated from a cow with symptoms of pyelonephritis and those isolated from a healthy cow. From the viewpoint of pathogenesis, it may be said that the establishment of the disease due to type I organisms depends generally on such factors on the host side as pregnancy and parturition, but not on any factor on the part of bacterial strains. This was confirmed by the authors on the basis of more detailed data on *C. renale*, including serological types and response to phage RP 6. JONES and LITTLE^{6,7)} described that many cases might really be originated from infection during early life, and that pregnancy or parturition might serve as a favorable factor for the occurrence of the disease.

Application of the serological typing and phage typing of the strains of *C. renale* made it possible to clarify the nature of the disease to a considerable extent. It should be emphasized that the serological types and phage types of *C. renale* are important to understand the epizootiology of the disease and the ecology of the causative organism.

The distribution of *C. renale* among cattle in Japan with special reference to the serological types will be reported in another paper to come.

SUMMARY

Corynebacterium renale strains were isolated from cows with pyelonephritis and apparently healthy cattle in the N herd, where pyelonephritis was noticed continually in the last 17 years. They were identified as those of types I and II by the precipitin reaction in gels. Classification of the strains of type I was done by using a phage, RP 6, which had been obtained from a strain isolated in the same herd. The strains isolated from the diseased cows were found to belong to type I, without exception, and those from the apparently healthy cattle to belong to either type I or type II. There was no difference in the response pattern of the type I strains to phage RP 6 between the strains isolated from the diseased cows and those from the apparently healthy cattle. There were some relations between the barn where animals were housed and the type of *C. renale* isolated from these animals. Type I strains were isolated from all the cows suffering from pyelonephritis and also from the apparently healthy cows housed in the same barns with the diseased cows. Type II strains were isolated from apparently healthy cattle in all the 7 barns, except one, regardless of sex and age. Furthermore, all the strains isolated from males were proved to belong to type II.

From the findings mentioned above, discussion was made on the importance of the serological types and phage types of *C. renale* in understanding the epizootiology of the disease and the ecology of the etiological microorganism.

REFERENCES

- 1) GIBBONS, W. J. (1963): Disease of cattle 2nd Ed., 254 Amer. vet. Publ. Illinois.
- 2) HIRAMUNE, T., KUME, T. and MURASE, N. (1967): *Bull. nat. Inst. anim. Hlth*, No. 54, 13~18. (in Japanese. English summary: *Nat. Inst. anim. Hlth Quart.*, 7, 124 (1967)).
- 3) HIRAMUNE, T., KUME, T., ISAYAMA, Y. and MURASE, N. (1968): *Nat. Inst. anim. Hlth Quart.*, 8, 187~194.
- 4) HIRAMUNE and YANAGAWA, R. (1969): *Jap. J. vet. Res.*, 17, 25~31.
- 5) HIRATO, K. (1933): *J. Jap. Soc. vet. Sci.*, 12, 264~289 (in Japanese with English summary).
- 6) JONES, F. S. and LITTLE, R. B. (1926): *J. exp. Med.*, 44, 11~20.
- 7) JONES, S. F. and LITTLE, R. B. (1930): *J. exp. Med.*, 51, 909~920.
- 8) KUBOTA, K., KITAHARA, T. and AOKI, M. (1958): *J. Japan vet. med. Ass.*, 11, 375~376 (in Japanese).
- 9) KUME, T., SASAKI, N. and MURASE, N. (1959): *J. Japan vet. med. Ass.*, 12, 119~125 (in Japanese with English summary).
- 10) YANAGAWA, R., BASRI, H. and OTSUKI, K. (1967): *Jap. J. vet. Res.*, 15, 111~119.
- 11) YANAGAWA, R., SHINAGAWA, M. and NEROME, K., (1968): *Jap. J. vet. Res.*, 16, 121~127.

腎盂腎炎が長期間認められた 1 牧場の牛から分離された *Corynebacterium renale* の型別

平棟孝志・久米常夫・村瀬信雄
家畜衛生試験場北海道支場

梁 川 良
北海道大学獣医学部家畜衛生学教室

(昭和 44 年 7 月 14 日受付)

過去17年間、続いて腎盂腎炎の発生があった北海道の1牧場において、病牛および健康牛から分離された *Corynebacterium renale* を、血清学的に型別し、さらにファージ RP 6 に対する態度も調べた。

病牛から分離された菌株はすべて type I であったが、健康牛由来のそれは、type I か、または type II であった。ファージ RP 6 に対する態度は、病牛と健康牛由来の菌株では、差がみられなかった。この牧場では、性別、年令別に7牛舎に飼育しているが、発生はいずれも24カ月以上の雌牛のいる牛舎で認められた。見かけ上健康な牛から分離された株のうち、type I はすべて雌牛(病牛のいた牛舎および他の1牛舎の)に由来した。

これに反して、type II は性・年令に関係なく、いずれの牛舎の牛からも分離された。しかし、雄牛に由来した菌株のすべてが、type II であったことは注目される。このように、*C. renale* 菌株の牛舎別由来と血清型の間には、明らかな関連がみられた。しかしファージ RP 6 に対する態度と、菌株の牛舎別由来には、別に関連はなかった。

以上、この牧場に長い間発生が見られた牛の腎盂腎炎は、*C. renale* type I によること、発症には生体側の条件の関与の必要なこと、およびこの菌の血清学的型別、ファージ型別は、本病の疫学や、この菌の ecology を知る上に、重要であることについて考察した。