Corynebacterium renale 各タイプのわが国の牛に分ける分布

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DISTRIBUTION OF THE TYPES OF *CORYNEBACTERIUM RENALE* IN COWS OF JAPAN

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Corynebacterium renale has been isolated not only from cows with cystitis and pyelonephritis but also from apparently healthy cows[1,4,8,13,14]. In Japan, isolation of *C. renale* from diseased cows has been reported in Hokkaido (20 cases) by HIRATO[7] and ICHIMURA (personal communication), in Yamagata (6 cases) by KUME et al.[12], in Saitama (2 cases) by AZUMA and INOUE (personal communication), in Kanagawa (1 case),[9] in Nagano (1 case),[10] and in Tottori (1 case) by TSUBOKURA (personal communication). The actual number of cases in Japan may probably be more than that mentioned above. However, many cases have been reported particularly in Hokkaido. Since the report of HIRATO (1933),[7] a disease caused by this organism has appeared in many parts of Hokkaido, such as Sapporo, Hayakita, Hidaka, Iwamizawa, Asahikawa, and Kitami.

Recently *C. renale* was divided into 3 types by YANAGAWA et al.[15] serologically and biochemically. There are differences among the 3 types of *C. renale* in nutritional requirements,[3] piliation,[16] DNA base composition,[11] and lysogeny[17].

The typing of *C. renale* isolated from cattle in a herd with persistent pyelonephritis was reported by the authors[6]. Type I strains were isolated from both diseased and apparently healthy females, and type II strains only from apparently healthy females and males. There was a distinct relationship between the types and the distribution of the organism in the same herd.

An attempt was made by the authors to survey the distribution of *C. renale* in apparently healhty cows in different districts of Japan and to determine the types of *C. renale* isolated. Stock cultures of *C. renale* which had been isolated from cows with pyelonephritis symptoms were also classified. This paper deals with the results of the survey and bears discussion on the epizootiology of *C. renale* infection.

MATERIALS AND METHODS

Cows examined: Surveys were mostly conducted on apparently healthy Holstein-Friesian cows over a period from January to May in 1968. Most of the cows surveyed were more than 24 months of age. The prefectures, herds, cows, and specimens examined are indicated in Table 1. In short, a total of 740 cows were examined in 23 herds in 4 prefectures, Hokkaido, Aomori, Tochigi, and Hyogo. Of the 740 cows, 421 were subjected to both urine and vaginal smear examinations, and 140 and 179 to only

Table 1. Prefectures, Herds, Cows, and Specimens Examined

Prefecture	Number of herds	Number of cows	Number of specimens	
			Urine	Vaginal smear
Hokkaido	14	598	428	460
Aomori	5	58	58	58
Tochigi	1	29	27	27
Hyogo	3	55	48	55
Total	23	740	561	600

Table 2. Isolation of C. *renale* from Urine and Vaginal Smears of Apparently Healthy Cows

Prefecture	Urine	Vaginal smear
Hokkaido	57/428 (13.3)	37/460 (8.0)
Aomori	4/ 58 (6.9)	1/ 58 (1.7)
Tochigi	0/ 27	0/ 27
Hyogo	1/ 48 (2.1)	0/ 55

Numerator: Number of specimens from which C. *renale* was isolated.
Denominator: Number of specimens examined.
In parentheses is shown percentage.

Table 3. Number of Herds where C. *renale* was Detected from Apparently Healthy Cows

Prefecture	Number of herds examined	Number of herds where C. *renale* was detected
Hokkaido	14	14
Aomori	5	3
Tochigi	1	0
Hyogo	3	1

urine and vaginal smaer examination, respectively. Thus, a total of 561 urine and 600 vaginal specimens were tested. They had been collected from 23 herds located in Yakumo, Noboribetsu, Hayakita, Hidaka, Sapporo, Shintoku, Kamishihoro, and Wakkanai in Hokkaido, Shichinohe and Hachinohe in Aomori, Nasu in Tochigi, and Wadayama in Hyogo. Of the 23 herds, four in Hokkaido (2 in Hayakita, 1 in Sapporo, and 1 in Hidaka) had had cows with pyelonephritis symptoms, and the remaining herds had been free from such clinical cases.

Collection of specimens: Urine specimens were collected mostly at the time of natural urination. A few of them were obtained by the aid of a sterilized catheter. Vaginal smears were collected by a sterile cotton-wool swab introduced into the vagina.

Detection of *C. renale*: The cotton swab was plated directly on blood agar. One-tenth ml of urine was inoculated onto the same medium. The plates were incubated at 37°C for 2 days. Usually, one typical colony of *C. renale* was picked up from among colonies of the same appearance and used for identification and typing. When there were colonies of *C. renale* different in such features as color and form, another representative colony was also picked up and examined. Ten or 20 colonies were obtained from the urine of 1 apparently healthy and 2 diseased cows to determine the rate of each type of *C. renale* in one specimen. Identification of the strains isolated as *C. renale* was done according to BERGEY'S Manual (1957).

Typing of *C. renale*: Typying of *C. renale* was done by the precipitin reaction in gel according to the methods reported by YANAGAWA et al.[15]

RESULTS

Isolation of *C. renale* from the urine and vaginal smears of apparently healthy cows is presented in Table 2. The rate of isolation of *C. renale* from urine and vaginal smears was high in Hokkaido, but not so high in any other prefecture. There is a statistically highly significant difference ($P<0.01$) in the rate of isolation of *C. renale* from the urine and vaginal smears of apparently healthy cows between Hokkaido and any other prefecture.

The rate of herds positive for *C. renale* was 14/14 (100%) in Hokkaido, 3/5 in Aomori, 1/3 in Hyogo, and 0/1 in Tochigi (Table 3). Thus, in Hokkaido, the distribution of *C. renale* in apparently healthy cows was observed in all the herds surveyed.

The results of typing of *C. renale* are shown in Table 4. Type I was isolated from the urine (17.4%) and vaginal smears (21.7%) of apparently healthy cows in herds where pyelonephritis occurred (4 herds in Hokkaido). It was not isolated, however, from cows in any herd where no pyelonephritis had occurred. Type II was isolated from urine at such rate as ranging from 3.8 to 7.2%, and from vaginal smears at such rate as ranging from 0.7 to 4.9%. Type II was isolated even from apparently healthy cows in many herds, regardless of the occurrence of pyelonephritis. Type III was never isolated from apparently healthy cows. Two strains obtained in Hokkaido were non-typable.

Such colonies of type I and II organisms as different in color and form were isolated from 2 urine samples. One colony (type I) was yellow and the other colony (type II) white in the case of sample T-11. One colony (type I) was smooth and the other colony (type II) rough in case of sample N-211. These colonies were derived from apparently healthy cows in herds where pyelonephritis had occurred before (Table 5). The presence of both types I and II in the same sample prompted the authors to examine more colonies derived from 3 urine samples containing many *C. renale* organisms, even though such colonies looked very similar to one another. It was found, as shown in Table 5, that when obtained from the urine samples of 2 diseased cows, each 10 colonies were all of type I, and that when isolated from the urine samples of a healthy cow, 20 colonies were of type II, without exception.

The numbers of colonies of *C. renale* detected from the urine and vaginal smears of apparently healthy cows are shown in Tables 6 and 7. The number of colonies grown from 0.1 ml of urine was less than 100 in most of the urine specimens. Statistically, no difference was found between types I and II in the number of colonies detected from urine or vaginal smears.

In Table 8, the strains of *C. renale* isolated in Japan are summarized and grouped into types in relation to pyelonephritis symptoms. They include those obtained during this survey and the stock cultures kept in the authors' laboratories which had been isolated in Japan. Types I, II, and III have been isolated from cows with pyelonephritis symptoms. Type I was predominant in number. Type I strains were isolated from some apparently healthy cows in herds exclusively where pyelonephritis had occurred. Type II strains were isolated from apparently healthy cows in any herd, regardless of whether pyelonephritis had occurred previously or not. Type III strains were never isolated from any apparently healthy cow.

Table 4. Incidence of Types of C. *renale* Isolated from Urine and Vaginal Smears of Apparently Healthy Cows

Type of C. *renale*	Herds where pyelonephritis occurred		Herds where no pyelonephritis occurred			
	Hokkaido (4 herds)		Hokkaido (10 herds)		Aomori, Tochigi, and Hyogo (9 herds)	
	Urine 178*	Vaginal smear 69	Urine 250	Vaginal smear 391	Urine 133	Vaginal smear 140
I	31** (17.4)	15 (21.7)	0	0	0	0
II	9 (5.1)	3 (4.3)	18 (7.2)	19 (4.9)	5 (3.8)	1 (0.7)
III	0	0	0	0	0	0

* Number of specimens examined.

** Number of specimens from which C. *renale* was isolated.

Percentage is shown in parentheses.

Table 5. Results of Typing of More than 2 Colonies of C. *renale* in the Same Specimens

Cow No.	Number of C. *renale* cells in 0.1ml of urine	Number of colonies examined	Number of colonies of corresponding type	
			I	II
T-11 (Healthy)	130	2*	1	1
N-211 (Healthy)	3×10^3	2*	1	1
D-752 (Diseased)	8×10^5	10	10	0
D-756 (Diseased)	8×10^5	10	10	0
S-17 (Healthy)	1×10^5	20	0	20

* The two colonies were different from each other in character.

Table 6. Incidence of Number of Colonies of C. *renale* Detected from Urine of Apparently Healthy Cows

Type of C. *renale*	Number of colonies detected from urine*			Total
	100 and less	101-1,000	1,001 and more	
I	23** (74.2)	4 (12.9)	4 (12.9)	31
II	21 (65.6)	9 (28.1)	2 (6.3)	32

* One-tenth ml of urine was examined.

** Number of specimens from which C. *renale* was detected.

Percentage is shown in parentheses.

Table 7. Incidence of Number of C. *renale* Colonies Detected from Vaginal Smears of Apparently Healthy Cows

Type of C. *renale*	Number of colonies detected from vaginal smears*			Total
	+	++	+++	
I	10** (66.7)	5 (33.3)	0	15
II	15 (65.2)	5 (21.7)	3 (13.1)	23

* The number of colonies obtained by direct plating of smear on blood agar is:
+, 100 and less
++, 101-1,000
+++, 1,000 and more

** Number of specimens from which C. *renale* was detected.

Table 8. Types of C. *renale* Isolated in Japan in Relation to Pyelonephritis Symptoms

Type of C. *renale*	Isolated from cows with pyelonephritis symptoms	Isolated from apparently healthy cows	
		in herds where pyelonephritis occurred	in herd where no pyelonephritis occurred
I	27*	63	0
II	13**	30	43
III	18	0	0

* Number of strains.

** Of the 13 strains of type II isolated from cows with pyelonephritis symptoms, 2 were isolated in Saitama, 1 was in Tottori, and the remainder were in Hokkaido.

DISCUSSION

Although a considerable number of papers have been published on the distribution of *C. renale* among apparently healthy cattle, little is known about the types of *C. renale* distributed among them. In the present survey, the authors examined the distribution of *C. renale* among cows in different parts of Japan with spinal reference to the types of this organism.

There was a clear relationship between the distribution of the types of *C. renale* in apparently healthy cows of a herd and the presence or absence of pyelonephritis in this herd. Type I strains were isolated from apparently healthy cows exclusively in herds where pyelonephritis had occurred, and type II strains from those in herds, regardless of the occurrence of pyelonephritis. Type III was never isolated from any apparently healthy cow. These results suggest that type II may normally be distributed widely among cows, but that, on the contrary, type I may be distributed among cows of a limited area. This suggestion is supported more strongly by the authors' previous report on phage-types of *C. renale*[5], which revealed that the lytic pattern of 3 phage-types of *C. renale* type I was distinctly related to the source of strains which was in a limited area, such as herd and barn.

MORSE[13] reported that the rate of isolation of *C. renale* from apparently healthy cattle was higher in herds where clinical cases had been observed than in herds where no clinical cases had occurred. The results of the present investigation are similar on this point, but suggest that the high rate of isolation of *C. renale* in herds where clinical cases had occurred may have been induced by the prevalence of type I organisms in these herds. The following observation lends support to the above conclusions: Type I strains were isolated from the urine or vaginal smears of apparently healthy cows at the rate of about 20% in herds where pyelonephritis had occurred, and type II strains at the rate of several percent in these and other herds where no pyelonephritis had occurred. The rate of isolation of *C. renale* from apparently healthy cows was higher in Hokkaido than in any other prefecture surveyed. This may also have resulted from the fact that clinical cases of *C. renale* infection have been found only in Hokkaido.

According to GIBBONS[2], this infection appears to be prevalent in the colder regions of the United States and is seen most frequently in winter. In Japan, too, many cases of the disease have occurred in the colder districts, such as Hokkaido. It may also be due to cold weather that the isolation of *C. renale* type I from apparently healthy cows was frequent in Hokkaido.

Typing of *C. renale* must be done carefully, because types I and II were present in the same samples obtained from herds where pyelonephritis had ever occurred. In

such samples, however, the rate of isolation may not exceed several percent, which was the average rate of recovery of type II in this investigation.

Of the types of *C. renale* isolated from diseased cows, type I was predominant, type II the lowest in frequency of appearance, and type III isolated only from among the diseased cows. These results suggest that there may be a differenc in pathogenicity for cows bewteen the types of *C. renale*. Such difference in pathogenicity for cows is now under investigation and will be reported in a paper to come.

SUMMARY

The distribution of the types of *Corynebacterium renale* in apparently healthy cows in different parts of Japan was examined by the precipitin reaction in gel according to the method of YANAGAWA et al.

Twenty-three herds were surveyed in 4 prefectures: Hokkaido, Aomori, Tochigi, and Hyogo. Of them, 4 herds in Hokkaido had had cows with pyelonephritis symptoms and the remaining 19 herds had been free from such clinical cases. A total of 561 urine samples and 600 vaginal smears were collected from 740 cows for examination. The results obtained are summarized as follows.

(1) The rate of isolation of *C. renale* was high in Hokkaido (13.3% for urine samples; 8.0% for vaginal smears), but not so high in Aomori (6.9%; 1.7%), and low in Hyogo (2.1%; 0%) and Tochigi (0%;0%). (2) There was a clear relationship between the distribution of the types of *C. renale* in the apparently healthy cows of a herd and the history of occurrence of pyelonephritis in the herd. Type I strains were isolated from apparently healthy cows exclusively of herds where pyelonephritis had occurred, and type II strains from cows of many herds, regarless of the occurrence of pyelonephritis. Type III strains were never isolated from apparently healthy cows. (3) Colonies of both types I and II were isolated from two of the urine samples collected from cows of herds where pyelonephritis had occurred. Usually, however, only one type was recovered from one sample. (4) The number of organisms of *C. renale* (types I and II) recovered from 0.1 ml of urine of apparently healthy cows was less than 100 in most of the specimens. (5) Additional data on the isolates from diseased cows showed that type I was predominant, type II the lowest in frequency of appearance, and type III isolated only from diseased cows.

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健康牛や病牛から分離される *Corynebacterium renale* の型や分布を明らかにすることは，本菌の ecology や腎盂腎炎の疫学を知る上に必要である．著者らは，北海道を中心に，青森，栃木，兵庫各県のホルスタイン牛（雌）の尿561例，膣垢600例を集めて，本菌の検出を行ない，得られた菌株と，わが国で病牛から分離され，保存されていた菌株とについて，型別を行なった．

(1)健康牛からの本菌検出率を地域別にみると，北海道では，他の3県のいずれよりも，はるかに高かった．(2)健康牛からの本菌のタイプ別の検出率をみると，I型は病牛のあった牧場の牛からのみ検出され，尿から17.4%，膣から21.7%となっている．これに反し，II型は，本病の発生があった牧場の牛からも，発生がなかった牧場の牛からも分離され，その率は尿から3.8～7.2%，膣からは0.7～4.9%であった．III型は，健康牛からは全く分離されなかった．(3) I型とII型が混在している尿材料が，たまたま認められたので，病牛2例と健康牛1例の尿から得た多くのコロニーのうち，それぞれ10または20個について型別した．病牛2例の各10個のコロニーは，いずれもI型で，健康牛由来の20個のコロニーは，すべてII型であった．(4)健康牛の尿や膣から分離される *C. renale* の菌数は，一般に多くはなく，尿 0.1ml 中，ほとんど100個以下であった．(5)病牛からは，I，IIおよびIII型ともに分離されている．このうちI型に属する株が最も多く，III型がこれにつぎ，II型は最も少なかった．またIII型は，病牛のみから分離された．