Capsular Serotypes of *Corynebacterium equi*

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ABSTRACT

Antisera were prepared against 26 isolates of *Corynebacterium equi.* A capsular antigen preparation was made by washing a heavy suspension of individual *C. equi* isolates in saline overnight. Ninety-seven isolates from a variety of sources were tested by a double diffusion immunoprecipitin test and seven capsular serotypes identified. The majority of isolates belonged to capsular serotype 1 (59.8%) and 2 (25.8%). No clear relationship was established between capsular serotype and the source of origin of the isolates (disease processes in horses, pigs, man, cattle, dogs or cats).

RÉSUMÉ

Cette expérience consistait à préparer des antisérums à l'endroit de 26 souches de *Corynebacterium equi.* L'auteur prépara un antigène capsulaire en lavant une suspension concentrée de souches individuelles de *C. equi,* dans de la saline, durant toute une nuit. Il soumit 97 souches, isolées de diverses sources, à l'épreuve d'immunodiffusion en gélose et identifia ainsi sept sérotypes capsulaires. La majorité des souches appartenaient aux sérotypes capsulaires # 1 (59.8%) et # 2 (25.8%). Il ne réussit toutefois pas à clarifier la relation entre le sérotype capsulaire et la provenance des souches, i.e. une maladie d'origine équine, porcine, humaine, bovine, canine ou féline.

INTRODUCTION

Corynebacterium equi is an important cause of chronic pneumonia in foals. Recent work has contributed some understanding of the epidemiology (9, 14) and pathogenesis (11) of the disease. Studies on the epidemiological aspects of the disease have been hampered in part by lack of a method for further characterizing isolates, such as by bacteriophages or serologically.

Various attempts have been made to classify *Corynebacterium equi* serologically. Magnusson (8) found that all his isolates were agglutinated by the same antiserum. Later workers (3) found that their 34 isolates from horses, pigs and cattle possessed a species specific antigen, extracted by hot acid, on complement fixation and also group and type specific antigens demonstrable by either agglutination or by tube precipitation tests following hot acid extraction of soluble antigen (100°C for 40 minutes). Twenty-nine of 34 isolates fell into four distinct groups and five other individual types. Within the four groups agglutinin-absorption revealed at least 14 serological types. The group specific antigens were thought to be superficial and probably of capsular origin. *Corynebacterium equi* is a mucoid organism with a distinct lamellar polysaccharide capsule (13). The serological findings of Bruner and Edwards were generally confirmed by others (7). Woodroffe (12) found 18 of 21 isolates were divisible by the precipitation test (hot acid extraction) into two major groups and three individual strains.

Other workers (2) identified precipitinogens by tube precipitation in broth culture supernatants or in saline suspensions of two strains of *C. equi* isolated from horses. The capsular antigens of these isolates were shown to diffuse into broth or saline, and the capsular antigen of each strain was shown to be distinct (2). Heating the antigen at 120°C for two and one-half hours did not affect its ability to induce precipitins in rabbits. Others (4) recovered what was also considered to be capsular antigen after moderate heating (56°C, 30 minutes) of a saline suspension of *C. equi.* This polysaccharide antigen was absorbed onto red blood cells for the purpose of a passive haemagglutination test. All the isolates examined possessed the same capsular antigen when tested by either passive haemagglutination or precipitation (4).

The purpose of the work described here was to develop a capsular serotyping scheme for future epidemiological studies of *C. equi* infections. The results of this scheme were also compared to those of biochemical identication of the organism. This study may also have relevance for the production of suitable vaccines (10).

MATERIALS AND METHODS

C. EQUI ISOLATES

The species and source of origin of *C. equi* isolates is shown in Table I. Isolates were from unrelated animals or individuals. Isolates

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were obtained from the Clinical Microbiology Laboratory, Ontario Veterinary College and from the Veterinary Services Branch, Ontario Ministry of Agriculture and Food, Guelph, Ontario. Numbered isolates were also obtained from the following donors: 21-26, — G.R. Carter, Michigan State University; 60, — T.F. Kramer, Auburn University, Alabama; 66, 67, — C.O. Thoen, National Animal Disease Center, Iowa; 73-80, — R.E. Weaver, Center for Disease Control, Atlanta; 92-101, — T.W. Swerczek, University of Kentucky; 83, 102, 105, 106, — R. Higgins, University of Montreal. There were 97 isolates.

BIOCHEMICAL TESTS

Tests for nitrate reduction, urease production, H_2S production (lead acetate strip), glucose fermentation and indole production were done by standard methods (5).

CAPSULAR SEROTYPING

1. Antisera production. The following isolates: 2, 5, 8, 11, 12, 17, 19, 34, 35, 36, 37, 39, 40, 43, 45, 64, 71, 76, 80, 81, 83, 95, 97, 99, 103 and 108, were inoculated onto Mueller

TABLE I. Identification, Species of Origin, Source and Capsular Serotype of *Corynebacterium equi* Isolates

Number	Source	Serotype	Number	Source	Serotype
1	Horse: Lung	1	52	Horse: Lung	1
2	Horse: Lung	1	53	Horse: LN	1
3	Horse: Lung	1	54	Horse: Lung	1
4	Horse: Lung	1	55	Horse: Lung	1
5	Dog: Skin	1	56	Horse: Joint	1
6	Horse: Lung	1	57	Cow: LN	1
7	Horse: Lung	1	60	Horse: Auburn	1
8	Dog: Skin	2	63	Horse: Lung	1
9	Horse: Abscess	1	64	Horse: Lung	1
10	Horse: Lung	1	65	Pig: LN 2141-TB	5
11	Horse: Lung ATCC 6939	1	66	Pig: LN 2160-D-TB	4
12	Pig: LN[a]	1	67	Pig: LN 45-32	4
13	Pig: LN	1	70	Pig: LN 21364-B_2	4
14	Pig: LN	1	71	Horse: Lung	1
15	Pig: LN	2	73	Man: Lung B9967	1
16	Pig: LN	2	74	Man: Lung B6408-5-22	1
17	Pig: LN	3	75	Man: Lung A6916	1
19	Pig: LN	1	76	Man: Blood A6784	2
20	Horse: Lung EC 273-72	1	77	Man: Lung A9855	1
21	Horse: Lung EC 151-73	1	79	Man: Spinal fluid A4826	2
22	Pig: ATCC 7699	1	80	Man: Abscess B1480	7
23	Cat: FD-118	1	81	Horse: Feces	6
24	Horse: Lung 1910	1	83	Horse: Lung 4104-79	1
25	Horse: Lung EC-360	1	84	Horse: Cellulitis	1
26	Horse: Lung 72E-691	1	85	Horse: Cellulitis	2
27	Pig: LN	1	86	Horse: Lung	2
28	Pig: LN	2	87	Horse: Lung	1
29	Pig: LN	2	88	Horse: Lung	1
30	Pig: LN	1	89	Horse: Lung	2
31	Pig: LN	1	90	Horse: Cellulitis	2
32	Pig: LN	1	91	Horse: Lung	1
33	Pig: LN	2	92	Horse: Lung 79E 78E	2
34	Pig: LN	2	93	Horse: Lung 79E 820	2
35	Pig: LN	1	94	Horse: Lung 79E 828	1
36	Pig: LN	2	95	Horse: Lung 79E 931	2
37	Pig: LN	2	96	Horse: Lung 79E 869	1
38	Pig: LN	1	97	Horse: Lung 79E 902	6
39	Pig: LN	5	98	Horse: Lung 79E 775	2
40	Pig: LN	5	99	Horse: Lung 79E 705	2
41	Pig: LN	1	100	Horse: Lung 79E 283	2
42	Pig: LN	2	101	Horse: Lung 79E 858	1
43	Pig: LN	4	102	Cat: Abscess	6
44	Pig: LN	1	103	Horse: Lung	6
45	Pig: LN	6	104	Horse: Lung	2
46	Pig: LN	1	105	Horse: Lung	1
47	Pig: LN	1	106	Pig: LN	2
48	Pig: LN	1	107	Horse: Lung	1
49	Horse: Lung	1	108	Dog: Abscess	2
50	Horse: Lung	1			

[a]LN = lymph node

Hinton agar in Petri dishes[1] so as to yield nearly confluent growth after incubation at 37°C for 48 hours. Growth was washed from plates with 5 mL of 0.15 M NaCl solution. Rabbits were given saline suspensions intravenously of live *C. equi* prepared as described, incrementing every three to four days from 0.5 mL by 0.5 mL to a maximum of 3 mL. After a total of seven to eight inoculations, an equal volume of 1.5 mL of *C. equi* suspension and 1.5 mL Freund's complete adjuvant was injected subcutaneously and intramuscularly in equal parts into the rabbits. Sera were checked for precipitins ten to 14 days after this procedure, when they were generally present. Occasionally, precipitins were absent but were induced by two to three further intravenous inoculations of 0.5 mL of a live suspension of *C. equi*. Sera were then harvested and stored in small aliquots at -70°C until used.

There was an occasional faint line of precipitation when antisera prepared against isolates 43 and 103 were tested against capsular serotype 2 supernatants. Antisera prepared against isolates 43 and 103 were therefore absorbed with an equal volume of 10^{10} *C. equi* per mL of 28 or 29. The suspensions were incubated for one hour at 37°C, centrifuged and supernatants retained.

2. Antigen preparation. Every isolate was cultured as described above, and one Petri plate of organisms growing on Mueller Hinton agar was then washed off with 5 mL of 0.15 M NaCl solution and incubated at 37°C overnight. The suspension was centrifuged, the supernatant removed, preserved with 0.01% merthiolate and stored at 4°C.

3. Double diffusion precipitin test. A micro agar gel diffusion test was carried out using 1% Noble agar[2] in barbital buffer 0.075 M, pH 8.8 (6). Samples were applied by filling wells in a 2.5 cm square plastic template 0.5 cm thick placed on the surface of the agar. The template contained seven wells, one central and six peripheral. The well diameter was 3 mm and the distance between the central and peripheral wells was 4 mm. Slides were incubated in a moist atmosphere for 48 hours. Antigen preparations from all isolates were tested against all the antisera.

RESULTS

BIOCHEMICAL REACTIONS

All isolates failed to metabolize dextrose, were indole negative, and produced hydrogen sulphide. All isolates except number 103 produced urease. All isolates except 24, 37, 41, 44, 49, 42, 57-61, 66, 76, 87 and 103 completely or partially reduced nitrate to nitrite.

CAPSULAR ANTIGENS

One line of precipitation was seen with the majority of isolates when tested against homologous antisera or that prepared against organisms considered to belong to the same capsular serotype. On occasion a further faint line was also present (Fig. 1).

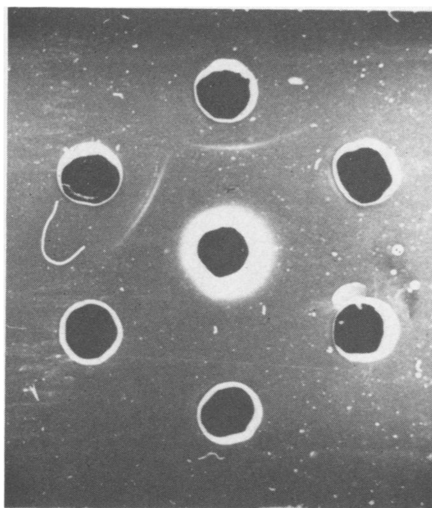

Fig. 1. Double immunodiffusion precipitation test of antisera prepared against isolate 1 (serotype 1, central well) against antigen prepared from isolates 8, 17, 65, 67, 1 and 2 (peripheral wells). 1 and 2 show typical line of precipitation of capsular antigen: in addition, a faint additional precipitation line is present with antigen preparation of isolate 1.

Fifty-seven isolates were classified at serotype 1 since antigen prepared from these isolates consistently showed one line of precipitation only with antisera prepared against isolates 2, 5, 11, 12, 19, 34, 64, 71 and 83. Twenty-five isolates were classified as serotype 2 since antigen preparations showed a single line of precipitation only with antisera prepared against 8, 34, 36, 37, 76, 95, 99 and 108 but not with antisera prepared against serotype 1 isolates.

Antisera prepared against isolate 17 gave only a single precipitin line with antigen from isolate 17 and with no other antigens; this isolate was designated serotype 3.

Antisera prepared against isolate 43 gave a faint line of precipitation with supernatant prepared from isolates of serotype 2 but a further and more marked precipitin line with antigen prepared from isolates 43, 66, 67 and 70. These isolates were designated serotype 4. Absorption of antisera prepared against 43 with organisms belonging to serotype 2 did not remove the marked line of precipitation seen with 43, 66, 67 and 70 but did remove the faint line of precipitation seen with serotype 2 strains.

Capsular serotype 5 contained three isolates — 39, 40 and 65, all of which showed single lines of precipitation when antigen preparations were tested with antisera prepared against 39 and 40.

The sixth distinct capsular antigenic group contained strains 45, 81, 97, 102 and 103. Antisera prepared against strains 81, 97 and 103 produced a marked single line of precipitation when tested against antigen prepared from these five isolates. However, antisera prepared against isolate 45 precipitated antigen prepared from 45 only, and antisera prepared against 45, 81 and 103 did not precipitate antigen preparations of 97. Antisera prepared against 103 showed in addition a faint precipitin line with supernatant prepared from the majority

[1]Difco Laboratories, Detroit, Michigan.
[2]Difco Laboratories, Detroit, Michigan.

TABLE II. Capsular Serotypes of *C. equi* Recovered from Different Species

Serotype	Source of Serotype (Number of Strains)					
	Horse	Pig	Man	Dog	Cat	Cow
1	35	16	4	1	1	1
2	11	10	2	2		
3		1				
4		4				
5		3				
6	3	1			1	
7			1			

of isolates belonging to capsular serotype 2. This line could be removed by absorption with organisms of capsular serotype 2 without affecting the marked precipitin line with capsular group 6 strains.

Antisera prepared against isolate 80 produced only a single precipitin line with antigen from 80; this isolate was designated serotype 7.

Antigen preparations were stable at 4°C for at least one year, provided they did not become contaminated.

The relationship between the source of origin and the capsular serotype of isolates is given in Table II.

DISCUSSION

All isolates listed in Table I and tested for capsular serotype had colonial characteristics typical of *C. equi*, notably a mucoid tear-drop appearance and a pink tinge after prolonged incubation. The biochemical behaviour of isolates was also consistent with those described for *C. equi* (1), the one exception being strain 103 which was apparently urease-negative. A small number of strains failed to reduce nitrate to nitrite, but this biochemical property is variable for *C. equi* strains (1). All isolates examined were thus thought to be *C. equi*.

The antigen present in the supernatant of saline suspensions of *C. equi* isolates was assumed to be of capsular origin since it was removed so readily and it was almost certainly that described by others (2, 4) and shown to be heat-stable (2). A single capsular serotype may have been detected previously because only low numbers of isolates were tested (4). Other workers described two serotypes (2). In the current series each strain tested had only one capsular antigen, but a total of seven different antigens were detected among the 97 isolates. The secondary faint lines occasionally observed in some supernatants was not thought to be capsular antigen.

The superficial or capsular antigens described were probably the same as the group specific antigens previously identified by either agglutination or by precipitation tests using hot acid extracts of the organisms (3, 7, 12). The antigen was stable for at least one year at 4°C, but stability to hot acid was not tested. The double immunodiffusion precipitation test was used since the tests were easily read and the relationship between the isolates so clearly shown, particularly when compared to (unpublished) results of slide or tube agglutination reactions. A micromethod of the double immunodiffusion precipitation test was used since some strains produced less capsular antigen than others, and low quantities of antigen were not readily detected by macromethods. The only exception to the clearness of this qualitative test for the presence of capsular antigen was the occasional presence of a faint line of precipitation when antisera prepared against isolates of capsular serotype 4 (#43) and 6 (#103) were tested against isolates of serotype 2. The lines were, however, less marked than positive control precipitation lines and could be absorbed out by capsular serotype 2 organisms without affecting the major precipitation line seen with the homologous serotype. The group contained within capsular serotype 6 were all classified as such since antisera prepared against 81, 97 and 103 gave marked precipitin lines when tested against preparations from all five isolates. It is, however, clear that the relationship between isolates in this group requires further investigation.

No clear relationship was apparent between the capsular serotype and the source of origin of the isolates (Table II), given the comparatively small numbers of isolates tested and the possibility of geographical variation in the predominance of different serotypes. It was noted that the majority of isolates from different farms in Kentucky (#92-101) belonged to serotype 2. A tendency was also apparent for horse isolates to belong to serotype 1, whereas pig isolates were more evenly distributed among the serotypes. Serotype 1 was the most common isolate from all sources (59.8%). It has been established that *C. equi* is commonly present in the feces of normal horses, cattle and pigs (9, 14), but the relationship between isolates of intestinal origin and those involved in disease remains to be determined. The only fecal isolate studied (#81) was isolated from a six month old foal with suspected *C. equi* enteritis; it was classified as serotype 6.

The relationship between the presence of a capsule and the pathogenicity of the organism is not known. If the capsule is important in the pathogenesis of infection in foals, then a more specific vaccine than the currently used whole cell vaccine might be developed (10). If such vaccines were shown to be effective, then it is apparent that they should include the several different serotypes.

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BOOK REVIEW/ ANALYSE DE VOLUME

DRUG CARRIERS IN BIOLOGY AND MEDICINE. *Edited by G. Gregoriadis. Published by Academic Press, London, England. 1979. 363 pages. Price $46.00.*

The book, Drug Carriers in Biology and Medicine, is essentially a collection of papers by 27 authors reviewing up-to-date information on the general subject of drug carriers as adjuncts to organ and target directed therapy. A fairly large number of carrier systems were described in the document, naturally occurring molecules and complex molecules, albumen, antibodies, lectins, glycoproteins, DNA and dextrans; cellular drug carriers, erythrocytes, neutrophils and lymphocytes as well as some synthetic drug carriers such as lactic-glycolic acid polymers and artificial cells.

The major push behind research on drug carriers is the need for physicians and veterinarians to produce very high levels of drug at specific sites of need. Most frequently this site is a tumor cell. The hope is to require very low dosages of extremely toxic drugs that will accumulate specifically in the tumor in order to kill the tumor without harming the host. Without appropriate carrier systems high enough levels of drug cannot be given to be effective against the tumor. The use of tumor specific antibody as the carrier of cytotoxic drugs for the treatment of certain tumors appears to have great promise. The use of cells such as neutrophiles as carriers of antibacterial drugs, red blood cells as carriers of antineoplastic drugs, ie, in the treatment of red blood cell consuming tumors, and lymphocytes as carriers of drugs to treat lymphoid tumors, appears after additional development, also to be very promising.

This book is not one for the practitioner who is only interested in reading information which he can apply in his veterinary practice. This technology is not in a sufficiently advanced stage to be of practical use. It is interesting reading however, for the individual interested in learning about new vistas in science. The book is well written, in the style of scientific reports, and gives us many clues about one of the next steps, in the advancement of chemotherapy.

Wm. D. Black.