Proposal for Standardization of the Designations Used for Serotypes of *Erysipelothrix rhusiopathiae* (Migula) Buchanan

GYÖRGY KUCSERA

State Institute for the Control of Veterinary Serobacterial Products, Budapest, X, Szállás U, 8, Hungary

A serological investigation of 45 swine erysipelas strains showed that among *Erysipelothrix rhusiopathiae* strains of different origin there are some with the same serotype designation that differ serologically, and there are others which have different serotype designations but which belong to the same serotype. To put an end to the resultant confusion, it is necessary to use both a uniform serological method (the double agar-gel diffusion precipitation test which employs autoclaved antigen and type-specific sera seems to be the most suitable) and a uniform system for designating the serotypes. It is proposed that Arabic numerals instead of letters be applied to serotypes of *E. rhusiopathiae* according to the chronological order in which the serotypes are described. Strains belonging to one serotype but differing in their antigenic structure can be differentiated by small letters placed after the numerals. The letter N is used to designate swine erysipelas strains which do not have a type-specific antigen.

On the basis of the agglutination test Watts (22), Atkinson (1), and Gledhill (9) established serotypes among strains of *Erysipelothrix rhusiopathiae* (Migula) Buchanan and used various designations to refer to them such as "smaller group," "bigger group," and Roman numerals I, II, III, and IV. Dedié (3) established two swine erysipelas serotypes (A and B) on the basis of heat-resistant, acid-soluble antigens. He also described a third group (N) for those strains which showed no reaction with either A or B type-specific serum.

Dedié's classification of *E. rhusiopathiae* serotypes became generally accepted. Later when Heuner (8: A₂, B₂, C, D), Murase and his co-workers (16: E, F; 17; 18; 19: C, D, Fr), Kucsera (12: E; 13: G; 14: I, J, K, L, M, O; 15: undesignated), Truszczyński (21: P), Ewald (6: H; 7: J) and Castro (2: I, J, K; A. F. Castro, Ph.D. thesis, Univ. de São Paulo, 1969: I, II, III) described new, or supposedly new, serotypes different from those of Dedié, they designated them by using the letters of the alphabet in consecutive order. Unfortunately, many of the authors who described new serotypes did not compare their strains with those belonging to previously described serotypes. Furthermore, the serological methods

used in the investigations were also varied (4, 5, 8, 11, 12, 14, 17). Thus it is necessary to carry out comparative studies by using the same method with *E. rhusiopathiae* strains from different parts of the world to determine the uniqueness of the previously described serotypes. It is also desirable to establish a uniform system for designating these serotypes.

MATERIALS AND METHODS

Bacterial strains. The strains used in this study are listed in Table 1. Cultures of the strains were grown in horse-meat broth containing 20% horse serum for 24 h at 37 C and were maintained in the freeze-dried state.

Preparation of antigens. Homogeneous cultures of each strain were prepared by inoculating horse-meat broth containing 10% normal horse serum with growth from a smooth colony from a 48-h agar plate culture and incubating for 48 h at 37 C. The use of horse-meat broth is recommended for the production of good antigen. Each suspension was centrifuged (5,000 rpm for 30 min) and then washed three times with physiological saline.

Autoclaved antigens are needed for the precipitation tests: the washed suspensions were resuspended in distilled water to 1/30 of the original volume and autoclaved at 120 C for 1 h (5, 14) and preserved by adding sufficient Merthiolate to give a 1:10,000 final

TABLE 1. Origin of strains of *Erysipelothrix rhusiopathiae* examined

Designation of strain	Origin of strain			Described by	Year	Type
	Isolated by	Country	Species			
422/1	Unknown	U.S.A.	Unknown	Heuner	1958	A ₂
NF 4	Unknown	U.S.A.	Unknown	Heuner	1958	B ₂
EW 2	Gladhill	U.K.	Unknown	Heuner	1958	A ₂
EW 24	Gladhill	U.K.	Unknown	Heuner	1958	A ₂
MEW 22	Gladhill	U.K.	Unknown	Heuner	1958	N
Seelachs	Wellmann	G.F.R.	Fish	Heuner	1958	B ₂
Wittling	Wellmann	G.F.R.	Fish	Heuner	1958	C
Doggerschabe	Wellmann	G.F.R.	Fish	Heuner	1958	D
Heilbutt	Wellmann	G.F.R.	Fish	Heuner	1958	D
Kuniyasu	Murase	Japan	Pig	Murase	1959	A
A-1	Murase	Japan	Pig	Murase	1959	B
P-190	Murase	Japan	Fish	Murase	1959	C
P-32	Murase	Japan	Fish	Murase	1959	D
P-43	Murase	Japan	Fish	Murase	1960	E
P-92	Murase	Japan	Fish	Murase	1960	F
P	Truszczyński	Poland	Ground squirrel	Truszczyński	1963	P
G-4 (S-52)	Castro	Brazil	Pig	Castro	1969-70	I (i)
JAC-10 (S-66)	Castro	Brazil	Pig	Castro	1969-70	II (J)
4-PR (S-4)	Castro	Brazil	Pig	Castro	1969-70	III (K)
T 131	Ewald	G.F.R.	Pig	Ewald	1967	H
A 360	Unknown	Unknown	Unknown	Dedié	1949	A
B 4	Unknown	Unknown	Unknown	Dedié	1949	B
Szentpál-2	Kemenes	Hungary	Goose	Kucsera	1971	A ₂
Kiskunlacháza	Kemenes	Hungary	Goose	Kucsera	1971	A ₂
Tótkomlós	Kemenes	Hungary	Goose	Kucsera	1971	A ₂
Kaparek	Csontos	Hungary	Fish	Kucsera	1962	E
IV.12/8	Kucsera	Hungary	Pig	Kucsera	1964	G
Pécs 52	Forray	Hungary	Pig	Kucsera	1970	G
Pécs 100	Forray	Hungary	Pig	Kucsera	1970	G
Pécs 9	Forray	Hungary	Pig	Kucsera	1970	I
M 2	Forray	Hungary	Cow	Kucsera	1970	i
Pécs 18	Forray	Hungary	Pig	Kucsera	1970	J
Pécs 56	Forray	Hungary	Pig	Kucsera	1970	J
Izszap-4	Kemenes	Hungary	From mud of zoo pond	Kucsera	1970	K
Pécs 67	Forray	Hungary	Pig	Kucsera	1970	L
Pécs 4586	Forray	Hungary	Pig	Kucsera	1971	L
Pécs 4587	Forray	Hungary	Pig	Kucsera	1971	L
Tuzok	Kemenes	Hungary	Zoo great bustard (<i>Otis tarda</i>)	Kucsera	1970	M
Vadkacsa	Kemenes	Hungary	Zoo mellard (<i>Anas platyrhynchos</i>)	Kucsera	1970	M
M 1	Forray	Hungary	Cow	Kucsera	1970	M
Pécs 110	Forray	Hungary	Pig	Kucsera	1970	N
Pécs 112	Forray	Hungary	Pig	Kucsera	1970	O
Goda	Kemenes	Hungary	Zoo black-tailed godwit (<i>Limosa limosa</i>)	Kucsera	1970	O
Pécs 3596	Forray	Hungary	Pig	Kucsera	1971	—
Pécs 3597	Forray	Hungary	Pig	Kucsera	1971	—

concentration of Merthiolate. Suspensions thus prepared can be stored in a refrigerator for a long time.

Two types of antigen are needed for preparing precipitating sera: live and Formalin-killed bacterial suspensions. Live antigens were prepared by resus-

pending the washed suspensions in physiological saline to give a density of Brown degree 4. Formalin-killed suspensions were prepared by resuspending the washed suspensions in physiological saline containing 0.2% Formalin to give the same density. After treatment

with Formalin for 24 h at 37 C, a sterility test was made and the suspension was stored in a refrigerator.

Preparation of precipitating sera. Rabbits (2.5-3.0 kg) were inoculated intravenously with Formalin-treated antigen at 3- to 4-day intervals with increasing doses (1, 2, 3, and 4 ml). Subsequently the rabbits were treated with the live antigen on the same schedule. On the eighth day after the last inoculation and after having starved the animals for at least 24 h, the rabbits were killed by bleeding. The sera collected after clotting were preserved with sufficient 1% Merthiolate in saline to give a final concentration of 1:10,000 Merthiolate and were stored in a refrigerator. Sera were judged suitable only when they reacted with the homologous antigen at least at dilutions of 1:32 to 1:64 and did not react with the heterologous antigens even when undiluted.

Precipitation tests. These tests were performed in duplicate and carried out by the double agar-gel diffusion method of Ouchterlony (20). Melted agar (15 ml) was poured into petri dishes (10 cm in diameter). After solidification, wells 7 mm in diameter spaced equidistantly (6 mm) from each other were cut into the agar plate for holding serum and antigen. The plates were read twice, once after incubation for 48 h at 37 C and again when stored at room temperature for 72 additional hours.

RESULTS AND DISCUSSION

The results of the precipitation tests are summarized in Table 2. The strains of each serotype described by an author gave the same

TABLE 3. *Proposed scheme and serotype-designation of the Erysipelothrix rhusiopathiae strains*

Type	Designation of strains	Described by		
		Name	Year	Type
1	A-360	Dedié	1949	A
	EW-2	Heuner	1958	A ₂
2	B ₄	Dedié	1949	B
	NF-4	Heuner	1958	B ₂
3	Wittling	Heuner	1958	C
4	Doggerschabe	Heuner	1958	D
5	P-190	Murase	1959	C
	Jac-10	Castro	1969	II
	(S-66)		(1970)	(J)
	Pécs-67	Kucsera	1970	L
6	P-32	Murase	1959	D
	G-4	Castro	1969	I
	(S-52)		(1970)	(I)
	Tuzok	Kucsera	1970	M
7	P-43	Murase	1960	E
	T-131	Ewald	1967	H
8	P-92	Murase	1960	F
	Goda	Kucsera	1970	O
9	Kaparák	Kucsera	1962	E
10	P	Truszczyński	1963	P
11	IV 12/8	Kucsera	1964	G
12	4-PR	Castro	1969	III
	(S-4)		(1970)	(K)
	Pécs-9	Kucsera	1970	I
13	Pécs-56	Kucsera	1970	J
14	Iszap-4	Kucsera	1970	K
15	Pécs-3597	Kucsera	1971	—

results, hence only the data from a single strain of each serotype are given in Table 2. From these data it is apparent that among the *E. rhusiopathiae* serotypes described throughout the world there are some which have the same designation but which belong to different serotypes, whereas there are others which have different designations but which belong to the same serotype. For example, serotypes C and D described by Murase do not correspond with Heuner's C and D, but they are the same as Castro's serotypes II (J) and I (I) and Kucsera's L and M, respectively. Murase's serotype E is not identical with Kucsera's E, but Murase's strain cross-reacts with the H strain described by Ewald. Murase's F serotype and Kucsera's O belong to the same group. There is serological correspondence between Castro's serotype III (K) and Kucsera's serotype I.

To put an end to this confusion, it is necessary to standardize the designations of the serotypes of *E. rhusiopathiae*. Because the number of serotypes may ultimately exceed the number of letters in the alphabet, it is proposed that the serotypes be designated by Arabic numerals instead of letters. Variations of the antigenic pattern within a given serotype are designated by lower-case letters following the numerals. Those strains which do not have a type-specific antigen and thus do not induce antibody production in rabbits even against themselves are designated by the letter N.

Correlation of previous serotype designations with those proposed here and assignment of the reference strains used in this study to the newly designated serotypes are given in Table 3.

REPRINT REQUESTS

Address requests for reprints to: Dr. György Kucsera, State Institute for the Control of Veterinary Serobacterial Products, Budapest, X, Szallas U, 8, Hungary.

LITERATURE CITED

- Atkinson, N. 1941. A study of some Australian strains of *Erysipelothrix*. Aust. J. Exp. Biol. 19:45.
- Castro, A. F., L. B. Trabulsi, O. F. Campedelli, and C. Troise. 1970. Isolation of three new serotypes of *Erysipelothrix*. Rev. Microbiol. (Brasil) 1:95.
- Dedie, K. 1949. Die säurelöslichen Antigene von *Erysipelothrix rhusiopathiae*. Mh. Vet. Med. 4:7.
- Dinter, Z. 1949. Der Haemagglutinationstest als eine Hilfsmethode bei der Bestimmung immunogener Rotlaufstämme. Berlin München. Tierärztl. Wochenschr. 62:177.
- Ewald, F. W. 1962. Typing *E. rhusiopathiae* in agar gel. Berlin. München. Tierärztl. Wochenschr. 75:71.
- Ewald, F. W. 1967. Untersuchungen über die Gruppe "N" der Rotlaufbakterien (*Erys. insid.*) unter Verwendung autoklavierter Bakterien in Agargel. Berlin. München. Tierärztl. Wochenschr. 80:335.
- Ewald, F. W. 1972. Über neue Serotypen von *Erysipelothrix insidiosa* und ihre Stellung zur Gruppe "N." Arb. aus dem Paul-Ehrlich-Institut zu Frankfurt a. M. 67:40.
- Heuner, F. 1958. Über serologische Untersuchungen an Rotlaufstämmen. Arch. Exp. Veterinaarmed. 12:40.
- Gledhill, A. W. 1945. The antigenic structure of *Erysipelothrix*. J. Pathol. Bacteriol. 57: 179.
- Kast, A., and L. Still. 1970. Serotypen von *Erysipelothrix rhusiopathiae* bei der Endocarditis des Schweines. Berlin. München. Tierärztl. Wochenschr. 83:485.
- Kucsera, G. 1962. Die Untersuchung von Schweinerotlaufbakteriumstämmen mit der Präzipitationsprobe unter Anwendung verschiedener Antigenextraktionsmethoden. Acta Vet. Hung. 12:43.
- Kucsera, G. 1963. Über einen aus Fischery isolierten Schweinerotlauf-Bakterienstamm von neuem Serotypus. Acta Vet. Hung. 13:61.
- Kucsera, G. 1964. *Erysipelothrix rhusiopathiae* Stämme eines neuen Serotyps und ihre Bedeutung in der Serodiagnostik der Rotlaufbakterien. Acta Vet. Hung. 14:293.
- Kucsera, G. 1971. Detection of new serotypes among *Erysipelothrix rhusiopathiae* strains of different origin. Acta Vet. Hung. 21:211.
- Kucsera, G. 1972. Comparison of *Erysipelothrix rhusiopathiae* strains of uncommon serotype from Hungary and abroad. Magy. Allatorv. Lapja 27:95.
- Murase, N., and Y. Ebi. 1960. Studies on the typing of *Erysipelothrix rhusiopathiae*. IV. Epizootiological significance of *Erysipelothrix rhusiopathiae* harboured in the tonsils of apparently healthy pigs. Jap. J. Vet. Sci. 1:22.
- Murase, N., K. Suzuki, Y. Isayama, and M. Murata. 1959. Studies on the typing of *Erysipelothrix rhusiopathiae*. III. Serological behaviours of the strains isolated from the body surface of marine fishes and their epizootiological significance in swine erysipelas. Jap. J. Vet. Sci. 21:215.
- Murase, N., K. Suzuki, and T. Nakahara. 1959. Studies on the typing of *Erysipelothrix rhusiopathiae*. II. Serological behaviours of the strains isolated from fowls including those from cattle and humans. Jap. J. Vet. Sci. 21:177.
- Murase, N., K. Suzuki, T. Nakahara, W. Araumi, and K. Hashimoto. 1959. Studies on the typing of *Erysipelothrix rhusiopathiae*. I. Serological behaviours of *Erysipelothrix rhusiopathiae* isolated from pigs. Jap. J. Vet. Sci. 21:113.
- Ouchterlony, O. 1949. Antigen-antibody reaction in gels. Acta Path. Microbiol. Scand. 26:507-515.
- Truszczyński, M. 1963. Investigations on the antigenic structure of the new *Erysipelothrix insidiosa* serotype. Bull. Vet. Inst. Pulawy 7:85.
- Watts, P. S. 1940. Studies on *Erysipelothrix rhusiopathiae*. J. Pathol. Bacteriol. 50:355.