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THE TAXONOMIC STATUS OF THE CAUSATIVE AGENT OF HEARTWATER

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ABSTRACT

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A fresh pragmatic classification of the rickettsias has been derived by applying the simplest techniques of numerical taxonomy. One order, the Rickettsiales, containing 3 families, the Rickettsiaceae, Phagosomaphilaceae, and the Bartonellaceae (?) is proposed. *Cowdria* is classified as a genus along with *Chlamydia, Coxiella* and *Anaplasma* in the tribe Chlamydieae in the family Phagosomaphilaceae.

INTRODUCTION

Although typhus has had a centuries-old influence on the history of mankind (Zinsser, 1935), the search for the taxonomic pigeonhole for the causative organism has been turbulent. The word "rickettsia" was coined in 1916 and honours Dr H. T. Ricketts, the pioneer investigator who, in collaboration with Dr R. M. Wilder, first characterised the pathogenic agents causing spotted fever and epidemic typhus in man, and who died in Mexico from typhus acquired accidently in the laboratory. The causative agent of Rocky Mountain spotted fever had earlier been classified as a protozoal piroplasm (Wilson & Chowning, 1902, cited by Cox, 1959) and for many years thereafter some agents, now accepted as being rickettsias, were considered to be protozoa and were studied primarily by protozoologists, e.g. *Anaplasma marginale*. Other rickettsias were clearly obligate intracellular parasites and, as such, were largely studied by virologists. The chlamydia, for example, were classified as Chlamydozoaceae (mantle viruses) in the 3rd edition of the classic virology text by Rivers & Horsfall (1959).

The rickettsias were long considered as bridging the taxonomic gap between bacteria and viruses and purist bacteriologists ignored them. William Bulloch in his well-known and respected *History of bacteriology*, published in 1938, neither lists the word "rickettsia" in the index nor refers to Dr Ricketts in his biographical notices of early workers in bacteriology. Even as late as 1977 Buxton & Fraser in their textbook, *Animal microbiology*, only grudgingly concede that rickettsias are probably very small bacteria which have developed an ultra-parasitic mode of life.

Most of us who have worked with these intriguing organisms are now agreed that rickettsias are bacteria. Moreover, most of us will echo the sentiments expressed by Emilio Wiess & Gregory Dasch (1981) that many of these organisms have been improperly classified but a sound basis for re-classification is not yet available. The identification of phylogenetic relationships by DNA/DNA and DNA/RNA hybridization and oligonucleotide cataloguing lies largely in the future although a start has been made.

A simple phenotypic classification (Table 1) lays stress on the arthropod links of the rickettsias and reminds us that the vertebrate hosts are man, animals (including birds) and plants. The better known classification is that given in Volume 1 of Bergey's manual of systemic bacteriology edited by J. G. Holt and published in 1984 under the general editorship of N. R. Krieg (Fig. 1). It is manifestly unsatisfactory in many respects lumping together, as it does, obligate intracellular parasites, epicellular parasites and organisms that grow axenically. *Coxiella* does not belong to the tribe Rickettsieae if only because it multiplies inside intracytoplasmic vacuoles whereas *Rickettsia* multiplies freely in the cytoplasm and/or nucleus of host cells. By the same token, members of the tribe Ehrlichieae differ from the tribe Rickettsieae and consequently probably do not belong to the family Rickettsiaceae. *Anaplasma* and *Aegyptianella* differ fundamentally from *Haemobartonella* and *Eperythrozoon* by being intracellular instead of epicellular. The *Chlamydia* are not arthropod-associated but, nevertheless, they possess many of the characteristics of the other rickettsias that multiply in intracytoplasmic vacuoles; Mohan (1968) in his review of diseases and parasites of buffaloes firmly placed *Cowdria* with the *Chlamydia*. Gerrit Uilenberg (1983) went further by suggesting that *Cowdria* and *Ehrlichia* shared characters with *Chlamydia* and *Rickettsia* and he proposed that the order Chlamydiales be abolished by reintegrating the *Chlamydia* into the Rickettsiales. The aim of this paper is to flesh Dr Uilenberg's skeletal proposals.

TABLE 1 Arthropod and associated hosts of rickettsias

Arthropod hosts		Associated hosts
Insecta:	Anoplura	Man and rodents
	Coleoptera	
	Diptera	
	Dictyoptera	
	Homoptera	Plants
	Lepidoptera	
	Orthoptera	
	Scutoria	Man and rodents
Arachnida:	Araneae	
	Scorpionida	
	Acarina	
Crustacea:	Isopoda	
	Amphipoda	
	Decapoda	

MATERIALS AND METHODS

Operational taxonomic units

Eight genera of the rickettsias were selected as operational taxonomic units (OTUs) for comparison, viz., *Rickettsia* (Ri), *Coxiella* (Cb), *Ehrlichia* (Eh), *Cowdria* (Cr), *Anaplasma* (An), *Eperythrozoon* (Ep), *Haemobartonella* (Ha) and *Chlamydia* (Ch). A 9th genus, *Cytoecetes (incertae sedis)* was added because we Scots have long begged to differ with the pundits contributing to Bergey's manual of systematic bacteriology (Foggie, 1962).

Taxonomic characters

The dearth of convincing or meaningful quantitative characters in all the OTUs was such that the characters selected had to be two-state qualitative ones. They were coded "1" for presence and "0" for absence. No attempt was made to weight the characters.

A total of 63 characters were used; 16 were organism-related, 6 were arthropod-related, 34 were animal host-related, and 7 were associated with the behaviour of the organism in experimental hosts and cultures (Table 2). Brandt & Snedecor's formula for calculating chi-square

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TABLE 2 Selected two-state qualitative characters

Organism-related

1. Intracellular parasite
2. Surface-associated parasite
3. Arthropod association
4. Environmental stability
5. Pleomorphism
6. Elementary bodies
7. Chains
8. Coccal forms ≥0,5 μm
9. Non-motile
10. Trilaminar cell wall
11. Haemagglutination
12. Binary fission
13. Gram negative
14. Acid fast
15. Acridine orange
16. Tetracyclic susceptibility

Arthropod-related

1. Multiply in gut epithelial cells
2. Parasitize salivary glands
3. Transovarian transmission
4. Faecal transmission
5. Bite transmission
6. Toxicity of infected arthropods

Animal host-related

1. Human pathogen
2. Animal pathogen
3. Carriers
4. Age-related innate resistance
5. Aerosol transmission
6. Oral transmission
7. Lactogenic transmission
8. Vertical transmission
9. Intradermal transmission
10. Subcutaneous transmission
11. Intravenous transmission
12. Fever
13. Thrombocytopaenia
14. Anaemia
15. Reticulocytosis
16. Rash
17. Nervous signs
18. Abortion
19. Organisms free in cytoplasm
20. Organisms in cytoplasmic vacuoles
21. Organisms in nucleus
22. Organisms in/on non-nucleated cells
23. Organisms in monocytes
24. Organisms in granulocytes
25. Organisms in/on erythrocytes
26. Organisms in endothelial cells
27. Organisms in epithelial cells
28. Dense clusters
29. Morulae
30. Resist homologous challenge
31. Resist heterologous challenge
32. Weil-Felix antibodies
33. Vaccines available
34. Immunosuppression

Experimental host systems

1. Guinea pig
2. Strauss reaction
3. Mouse
4. Embryonated hen eggs
5. Monocyte cultures
6. Monolayer cultures
7. Plaque formation

TABLE 3 Similarity between rickettsias: characters present/absent

Rickettsia	Characters present	Characters absent	Proportion present
Rickettsia	34	29	0,54
Coxiella	36	27	0,57
Ehrlichia	34	29	0,54
Cytoecetes	35	28	0,56
Cowdria	32	31	0,51
Anaplasma	31	32	0,49
Eperythrozoon	25	38	0,40
Haemobartonella	24	39	0,38
Chlamydia	32	31	0,51

$\chi^2_{[8]} = 9{,}157; P>0{,}05$

TABLE 4 Similarity between rickettsias: simple matching coefficients (× 100)

	Ri	Cb	Eh	Cy	Cr	An	Ep	Ha	Ch
Ri	1								
Cb	68	1							
Eh	49	59	1						
Cy	54	63	89	1					
Cr	59	71	71	70	1				
An	51	60	76	75	73	1			
Ep	46	51	63	62	51	75	1		
Ha	52	62	59	60	62	76	89	1	
Ch	61	71	59	67	68	63	51	59	1

Ri = *Rickettsia*
Cb = *Coxiella*
Eh = *Ehrlichia*
Cy = *Cytoecetes*
Cr = *Cowdria*
An = *Anaplasma*
Ep = *Eperythrozoon*
Ha = *Haemobartonella*
Ch = *Chlamydia*

TABLE 5 Similarity between rickettsias: Jaccard's coefficients (× 100)

	Ri	Cb	Eh	Cy	Cr	An	Ep	Ha	Ch
Ri	1								
Cb	56	1							
Eh	36	46	1						
Cy	41	51	82	1					
Cr	43	58	57	56	1				
An	35	46	62	61	58	1			
Ep	26	33	43	43	30	56	1		
Ha	32	43	38	40	40	57	75	1	
Ch	47	58	43	52	52	46	30	36	1

Ri = *Rickettsia*
Cb = *Coxiella*
Eh = *Ehrlichia*
Cy = *Cytoecetes*
Cr = *Cowdria*
An = *Anaplasma*
Ep = *Eperythrozoon*
Ha = *Haemobartonella*
Ch = *Chlamydia*

was used to test homogenicity between the proportions of the character states in the OTUs.

Simple matching coefficients

Simple matching coefficients were computed for every pair of OTUs after the character counts for any 2 OTUs were summarized in a 2 × 2 table such that "a" was the number of characters where both OTUs were coded "1" (present), "d" was the number of characters where both OTUs were coded "0" (absent), "b" was the number of characters where OTU_i was coded "0" and OTU_j was coded "1", and "c" was the number of characters where OTU_i was coded "1" and OTU_j "0", the total number of binary characters being "p". The Sokal & Michener's formula (1958) used to compute the simple matching coefficients was as follows:

$$S_{ij} = \frac{a + d}{p} \qquad (1)$$

Jaccard's coefficients (1908)

Jaccard's coefficients were computed from the same summary 2 × 2 tables using the formula:

$$S_{ij} = \frac{a}{a + b + c} \qquad (2)$$

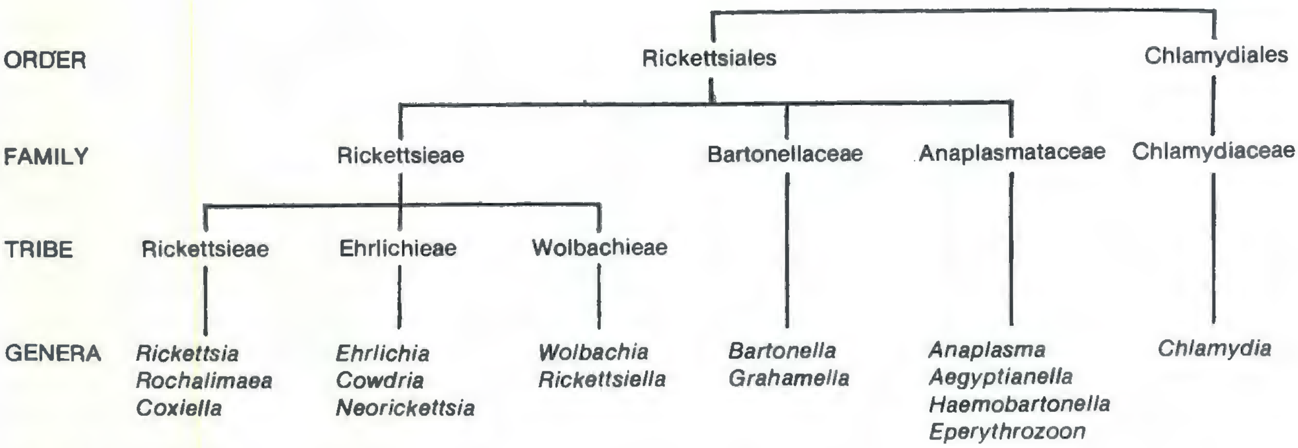

FIG. 1 Current classification of the rickettsias

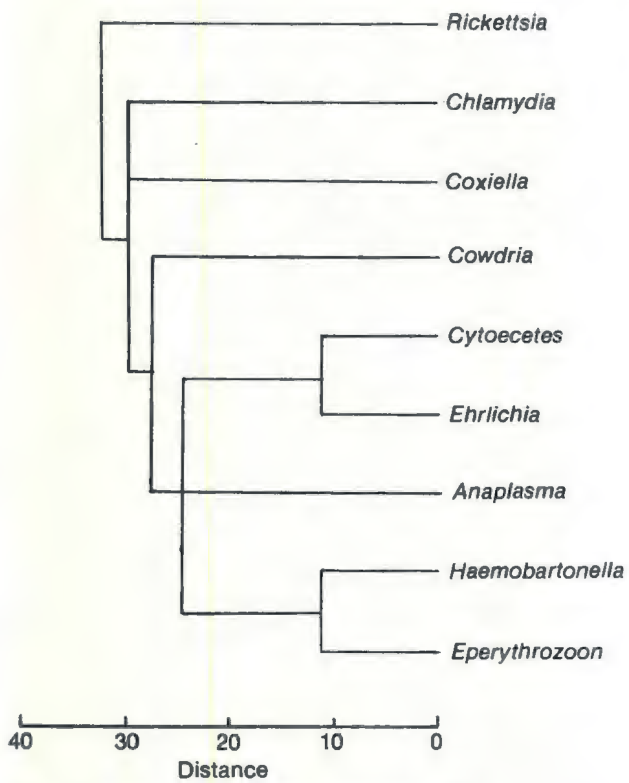

FIG. 2 Dendrogram from simple matching coefficients

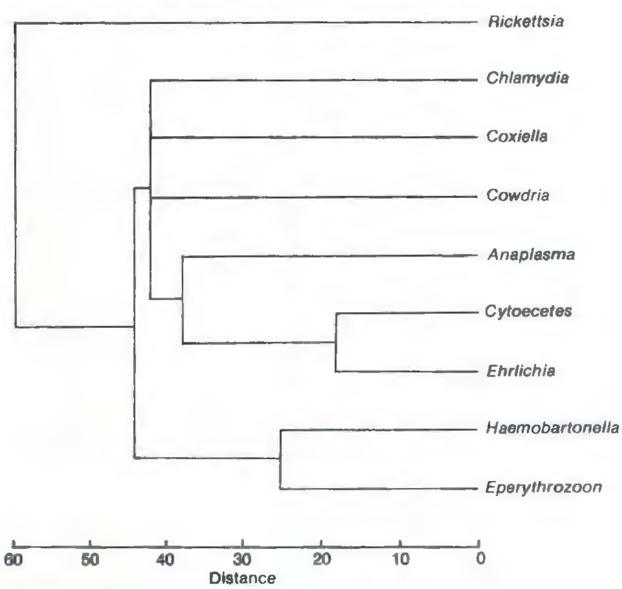

FIG. 3 Dendrogram from Jaccard's coefficients

TABLE 6 Similarity between rickettsial families: characters present and absent

Family	Characters present	Characters absent	Proportion present
Rickettsiaceae	34	29	0,54
Phagosomaphilaceae	200	178	0,53
Bartonellaceae	49	77	0,39

$\chi^2_{(2)} = 7{,}898^*; P>0{,}05$

Dendrograms

The similarity coefficient matrices were transformed into dissimilarity matrices to ease the construction of single-linkage dendrograms (or "family trees") with the hope of revealing apparent hierarchical relationships between the OTU.

RESULTS

Homogeneity

The propositions of the 63 taxonomic characters present and absent in the 9 genera of rickettsias ranged from 25 to 36 and 27 to 39 respectively (Table 3). Differences between the proportions were not significant (chi^2 = 9,157; P > 0,05).

Simple matching coefficients

The simple matching coefficients ranged from 0,46 for the match between *Rickettsia* and *Eperythrozoon* to 0,89 for the matches between *Ehrlichia* and *Cytoecetes* on the one hand and between *Eperythrozoon* and *Haemobartonella* on the other (Table 4). *Cowdria's* best match was with *Anaplasma* and its worst matches were with *Rickettsia* and *Eperythrozoon*.

Jaccard's coefficients

The spread of Jaccard's coefficients of 0,26 to 0,82 was greater than that of the simple matching coefficients but the best and worst matches were virtually the same, the only shift being the *Eperythrozoon-Haemobartonella* match which fell into the 2nd best position of 0,75 (Table 5). *Cowdria's* best match with *Anaplasma* was now equalled by its match with *Coxiella*. *Cowdria's*

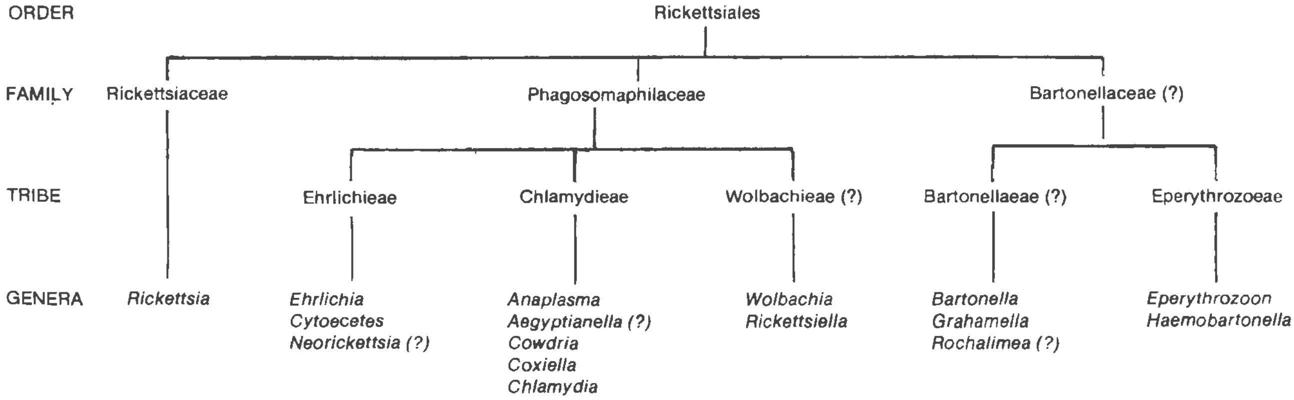

FIG. 4 Proposed reclassification of the rickettsias

worst match remained unaltered being 0,30 with *Eperythrozoon*.

Dendrograms

The dendrograms (Fig. 2 and 3) derived from the simple matching coefficients and Jaccard's coefficients differ in 3 areas. First, differences between genera are maximized by using Jaccard's coefficients. Secondly, *Eperythrozoon* and *Haemobartonella* are linked in the simple matching dendrogram to *Anaplasma, Ehrlichia* and *Cytoecetes* whereas in the Jaccard dendrogram the *Eperythrozoon-Haemobartonella* cluster falls in between *Rickettsia* and the rest. Thirdly, *Cowdria* in the simple matching dendrogram is linked to the *Eperythrozoon-Cytoecetes* cluster and thereafter to the *Chlamydia-Coxiella* match. In the Jaccard dendrogram *Cowdria* is linked, on the one hand, to *Chlamydia* and *Coxiella* and equally to the *Ehrlichia-Cytoecetes-Anaplasma* cluster on the other hand.

Rickettsia, in both dendrograms, is the most distantly related genus.

DISCUSSION

Jaccard coefficients and the dendrogram based on them yield more logical relationships between the rickettsias than simple matching coefficients if only because they separate the epicellular parasites in the *Eperythrozoon* and *Haemobartonella* genera from the intracellular parasites. *Ehrlichia* and *Cytoecetes* are closely linked and both form a cluster with *Anaplasma*. This cluster, in turn, is linked to the other rickettsias that multiply by binary fission inside membrane-lined intracytoplasmic vacuoles, viz., *Chlamydia, Coxiella* and *Cowdria*.

The homogeneity found between the proportions of the 63 selected taxonomic characters supports Uilenberg's proposals (1983) to scrap the order Chlamydiales and to restore the *Chlamydia* to the order Rickettsiales.

The Jaccard dendrogram delineates 3 major clusters or families which I have labelled the Rickettsiaceae, the Phagosomaphilaceae and the Bartonellaceae(?) (Fig. 4). The family Rickettsiaceae consists only of one genus. The family Phagosomaphilaceae has at least 2 tribes and perhaps 3, viz. Ehrlichieae, Chlamydieae and Wolbachieae(?). The Wolbachieae may be misplaced because at least one species *W. melophagi* is an epicellular parasite in the lumen of the alimentary tract of the sheep ked. The family Bartonellaceae(?) comprises rickettsias that grow axenically in the tribe Bartonellaeae(?) and the epicellular haemoparasites in the tribe Eperythrozoeae.

The classification into 3 families is supported by an analysis of the proportions of the 63 taxonomic characters present and absent in the families (Table 6). The differences are significant ($chi^2 = 7{,}898$, $P < 0{,}05$) such that the Bartonellaceae have a very significantly smaller proportion of characters present than the Rickettsiaceae and Phagosomaphilaceae ($chi^2 = 7{,}874$, $P < 0{,}01$). The proportions in the Rickettsiaceae and Phagosomaphilaceae are similar ($chi^2 = 0{,}024$, $P > 0{,}80$).

The proposed reclassification of the rickettsias is crude but it has more taxonomic merit than the existing classification. It will undoubtedly be modified, and even discarded, as quantitative taxonomic characters become available.

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