

Epidemiology of *Salmonella typhimurium*: ribosomal DNA analysis of strains from human and animal sources

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SUMMARY

Salmonella typhimurium is the most frequently identified serovar of *Salmonella* in Italy. This serovar is characterized by the widespread dissemination among human and non-human sources of phenotypically and genetically well-differentiated clones.

In this study 457 strains of *S. typhimurium* isolated in Italy in the years 1982–91 from human and animal sources were submitted to characterization by the rDNA fingerprinting technique. Application of this typing method, after digestion of chromosomal DNA with *HincII* endonuclease, confirmed the greatest genetic differentiation of clones of *S. typhimurium*, allowing reliable identification of 45 rDNA patterns linked into 9 major clusters. rDNA pattern clusters or ribotypes specific to man were not recognized, whereas some rDNA patterns were characteristically related to ducks, pigeons and pet birds. The ribotyping results for isolates from animal hosts suggest that pig and cattle are the main source of human infection.

INTRODUCTION

Salmonella typhimurium is the most frequently identified serovar of *Salmonella* at the Centres of Enterobacteriaceae in Italy. This serovar, which is characterized by a widespread dissemination and by its differentiation in phenotypically and genetically distinct clones, has been studied by various typing techniques to obtain reliable strain discrimination. In particular, combined use of biotyping and phage typing [1] and plasmid profile analysis [2, 3] have proved to be useful tools in tracing of *S. typhimurium* infections.

Because of the involvement of man and of a wide range of animal hosts in the spread of this serovar, possible relationships between bio-phage types of strains of *S. typhimurium* and sources have been investigated [4–6].

In our study, the rDNA fingerprinting technique, which has been applied in recent years to many bacterial species [7–10], was used on strains of *S. typhimurium* isolated over the last 10 years in Italy from human and non-human sources, in order to investigate the epidemiological features of interchange of clones between man and animal hosts.

MATERIALS AND METHODS

Bacterial strains

Four hundred and fifty-seven isolates identified from human and non-human sources at the Northern and Southern Italy Centres of Enterobacteriaceae in the years 1982–91 were examined. Epidemiological correlations were not apparent among the isolates selected for the study. They included 127 isolates from man, 80 from poultry, 62 from cattle, sheep and goats, 55 from pigeons, 36 from pet birds, 23 from pigs, 21 from hedgehogs, 14 from wild birds, 13 from cats and dogs, 9 from rabbits and 17 from other animal sources.

rDNA gene pattern analysis

rDNA fingerprinting of the strains of *S. typhimurium* was carried out by digesting DNA with *HincII* restriction endonuclease, electrophoresing the fragments onto agarose gels, transferring them by vacuum to nitrocellulose filters and hybridizing them with photobiotin labelled rRNA of *Escherichia coli* [7, 11, 12]. The hybridized bands were visualized by the 'Blugene' non-radioactive nucleic acid detection system (Gibco-BRL, Ltd.).

HincII rDNA patterns were visually screened for bands between 6.5 and 2.0 Kb and the presence or absence was coded as 1 and 0, respectively. The similarity coefficient between each pair of ribotypes was expressed as the fraction of zero and non-zero matches to the total number of the hybridized bands used in the comparison. Clustering was carried out from a matrix of coefficients of pairwise genetic distances by using the commercial program STATPRO (Penton Software, Inc.).

RESULTS

rRNA hybridization of the *HincII* digested chromosomal DNA of the 457 isolates under study yielded 45 different ribotypes, of which the most frequent are represented in Fig. 1. A remarkable degree of genetic heterogeneity was detected, similarity coefficients being distributed between 0.04 and 0.29. Nine major clusters and seven additional ribotypes closely related to their next cluster were identified at the 0.11 similarity coefficient level (Fig. 2).

Fig. 3 shows the subdivision of the isolates from man and from the main animal sources into the nine clusters identified. Within the human isolates, ribotypes belonging to clusters 1 and 8 predominate (73 and 27 of 127 isolates, respectively), but at a relatively lower incidence all the remaining clusters are represented. Isolates from pigs exhibit a very similar distribution to that of strains from human sources, whereas ribotypes belonging to cluster 1 are particularly common within isolates from cattle, sheep and goats. Clusters 3 and 4 predominate within *S. typhimurium* strains from pigeons, and cluster 5 within those from pet birds, whereas the major clusters are more regularly found within isolates from poultry, but with a slightly higher proportion of cluster 1.

The features of the most representative rDNA pattern clusters and of the most common ribotypes are shown in Fig. 4*a–g*. Cluster 1 contains 216 strains, that are grouped into 9 ribotypes (1, 14, 3, 5, 6, 23, 28, 39 and 21). It is the largest cluster and the most heterogeneous with respect to the source of isolates. Of the 5 most

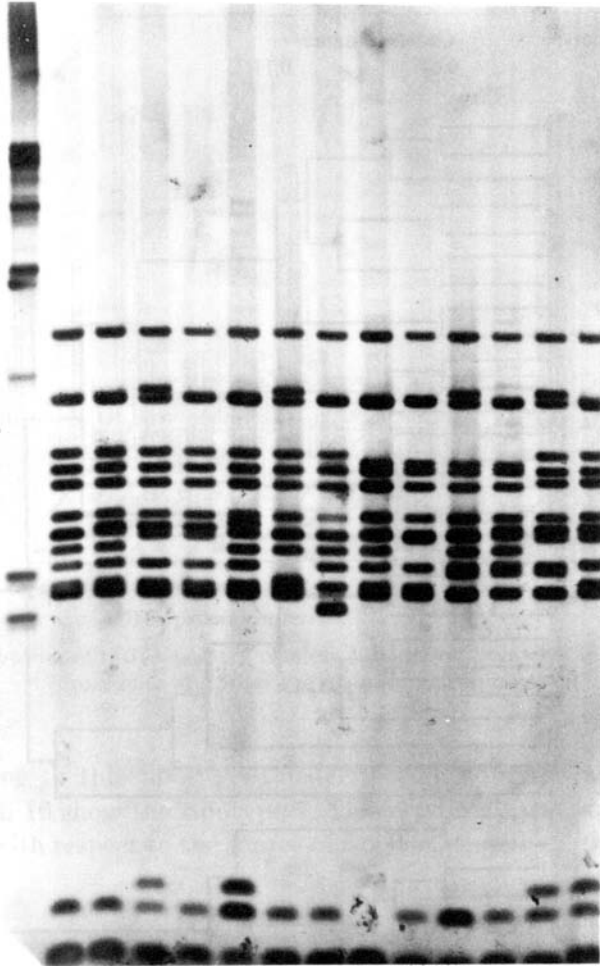


Fig. 1. rDNA gene restriction patterns of the *HincII* digests of strains of *S. typhimurium* belonging to the most frequently identified ribotypes. Lane 1, lambda DNA *HindIII* fragments (molecular weights); lanes: 2, ribotype 1; 3, ribotype 14; 4, ribotype 6; 5, ribotype 5; 6, ribotype 21; 7, ribotype 31; 8, ribotype 16; 9, ribotype 15; 10, ribotype 32; 11, ribotype 12; 12, ribotype 8; 13, ribotype 2; 14, ribotype 7.

frequent ribotypes, rDNA pattern 1 includes 96 strains from man and from a large variety of animal sources, whereas ribotypes 5, 6 and 14 appear to encompass a high proportion of human isolates (53.3–70.0%). Finally, isolates from hedgehogs account for 70.0% of strains grouped into ribotype 21. Cluster 2 includes 3 ribotypes (41, 31 and 36) and 23 isolates. Sixteen of 21 strains belonging to ribotype 31 are from poultry; cluster 2 and, in particular, ribotype 31 are characteristically associated with isolates from ducks. Cluster 3 includes 39 isolates that are distributed into 5 ribotypes (33, 10, 16, 34 and 43). Ribotype 16, which contains 26 isolates, is made up of non-human isolates only, of which 69.2% are from pigeons. Cluster 4 contains 66 isolates grouped into 7 ribotypes (4, 20, 29, 32, 15, 35 and 22). Ribotypes 15 and 32, which include 46 and 11 isolates, respectively, comprise a large number of strains from pigeons (50.0–54.5%).

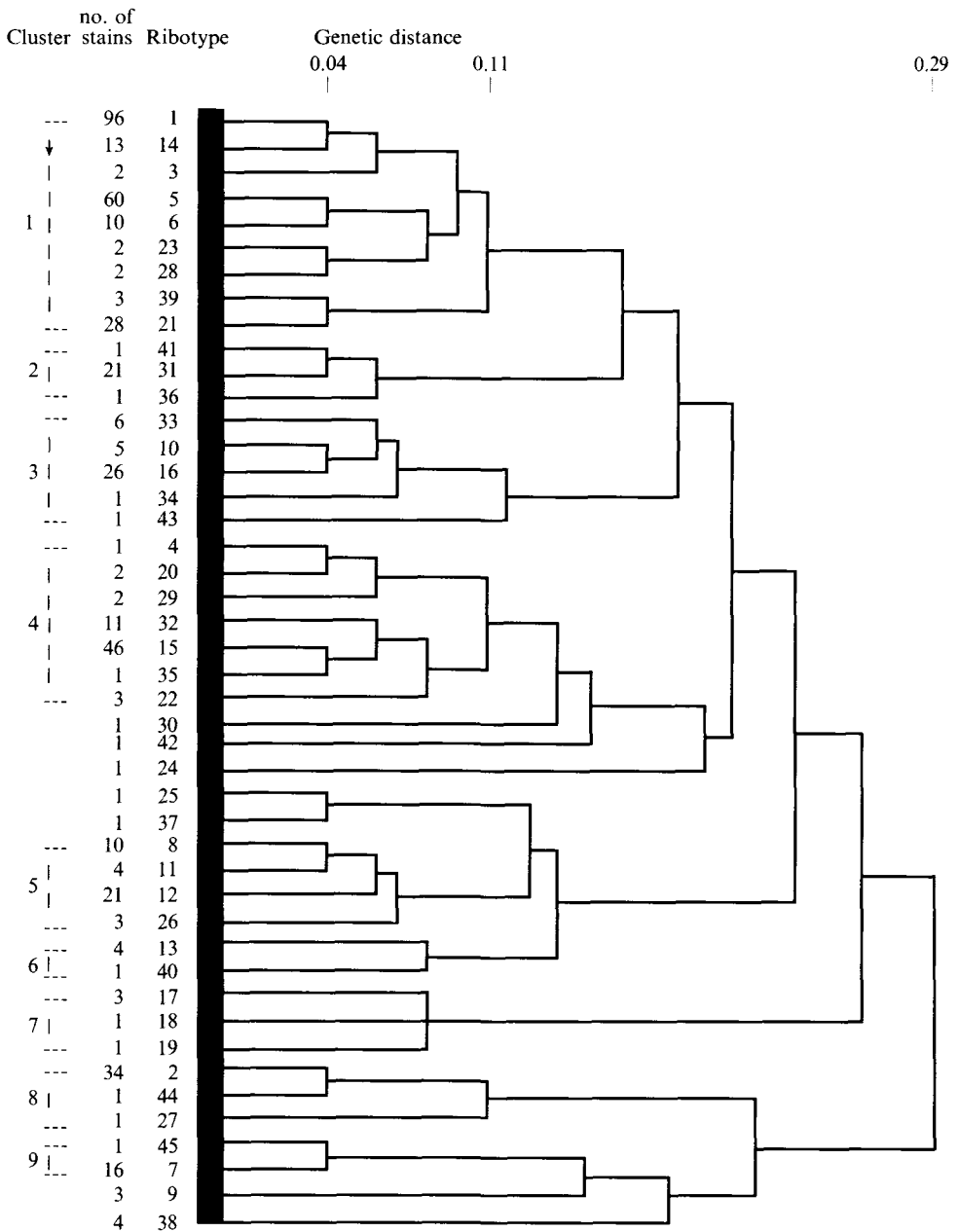


Fig. 2. Dendrogram of genetic relatedness among the *HincII* rDNA patterns of strains of *S. typhimurium*.

Cluster 5 contains 4 ribotypes (8, 11, 12 and 26) and 38 isolates, 21 of which are grouped into ribotype 12. 57.2% of these latter strains are from pet birds. Clusters 6 and 7 comprise a small number of isolates from different sources. Cluster 8 (ribotypes 2, 44 and 27) contains 36 isolates, of which 34 fall into ribotype 2. Strains from man and from pigs account for 76.5% and 11.9%, respectively, of

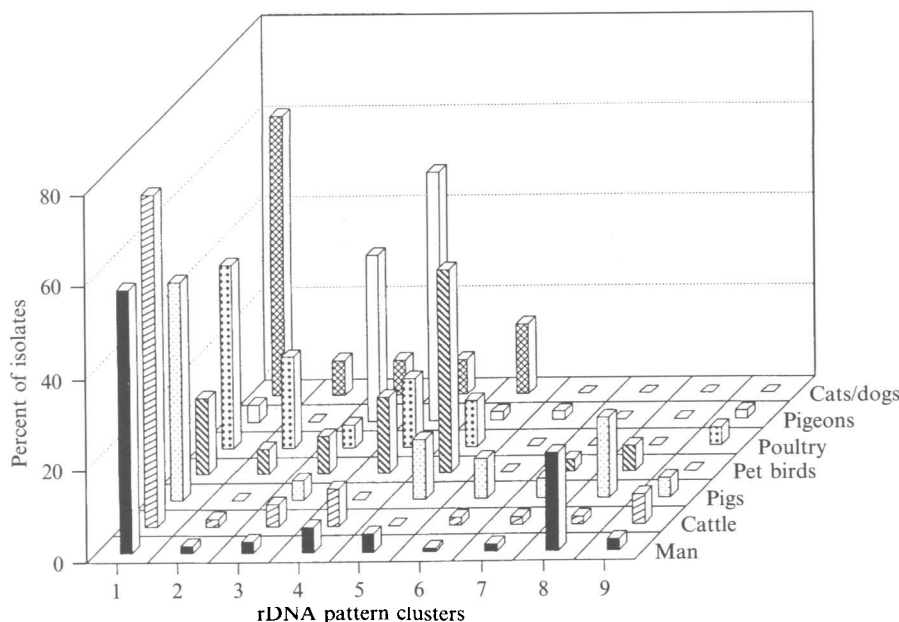


Fig. 3. Distribution of rDNA pattern clusters 1–9 among isolates of *S. typhimurium* from man and from the main animal sources.

isolates belonging to this ribotype. Cluster 9 (ribotypes 45 and 7) includes 17 isolates of which 16 show the ribotype 7. This cluster appears to be particularly heterogeneous with respect to the source of isolates, despite of its small numerical size.

DISCUSSION

The results obtained by the analysis of the restriction polymorphisms of the chromosomal DNA fragments containing rRNA coding sequences confirm the widespread diffusion within human and non-human sources of highly differentiated strains of *S. typhimurium*.

The distribution of the human isolates throughout all clusters and the absence, at least within the most frequently encountered ribotypes, of rDNA patterns specific to man suggest an active role of the human host in the spread of this serovar of *Salmonella*.

While the rDNA pattern on the animal strains might invite some interesting speculations, analysis of the data is complicated by the wide variation in the number of strains per animal source. Only strains from pigs showed relatively similar patterns of ribotypes and clusters to those of man, though the greatest proportion of cattle strains were in cluster 1. Thus the data is in accordance with epidemiological studies, in which other typing methods were used, which suggest that cattle and pigs are a major source of *S. typhimurium* infection in man [5, 13].

Finally, some ribotypes were found to be particularly common in avian hosts, most notably amongst pigeons, pet birds and ducks. This special behaviour of

(a)

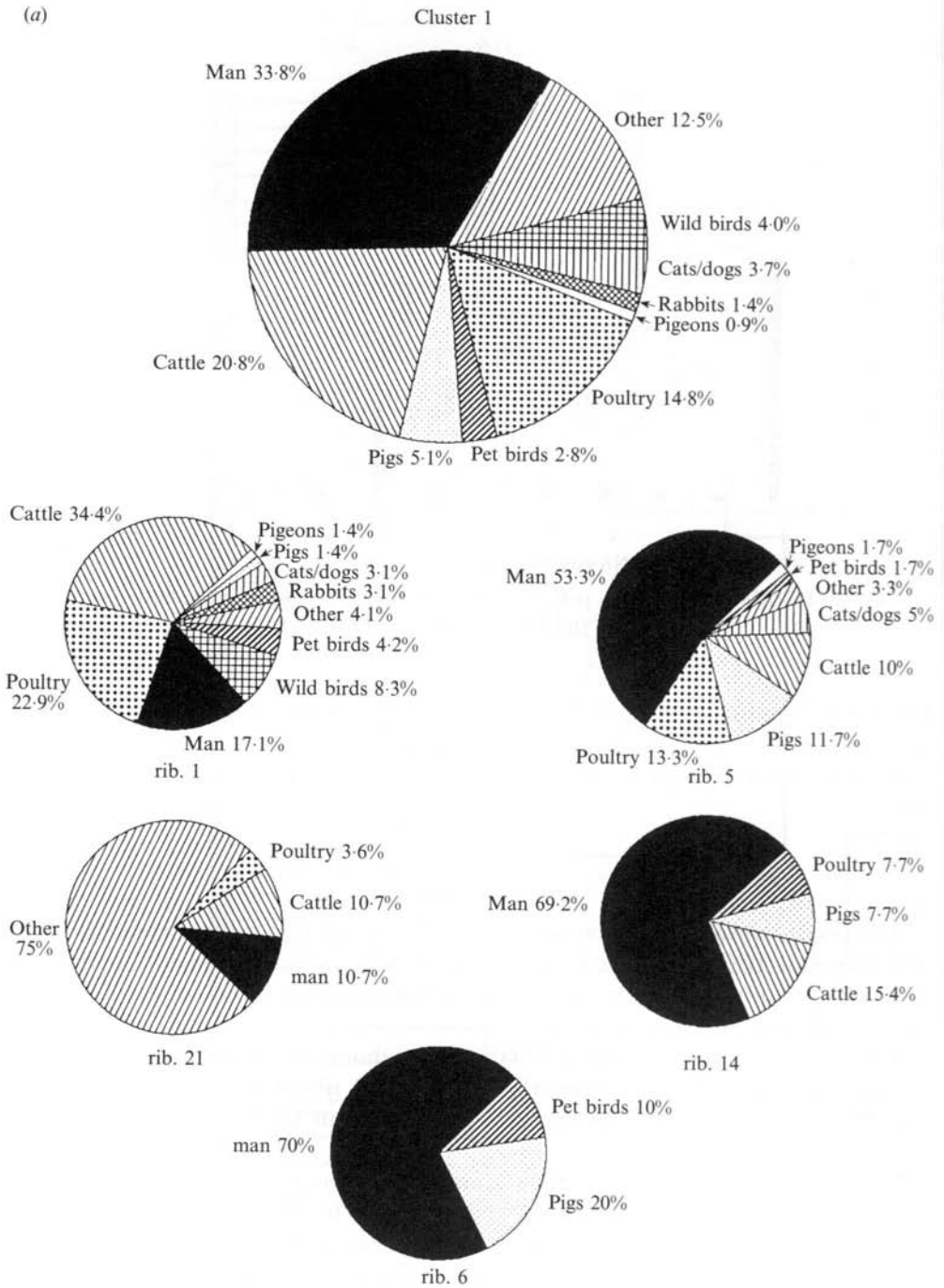
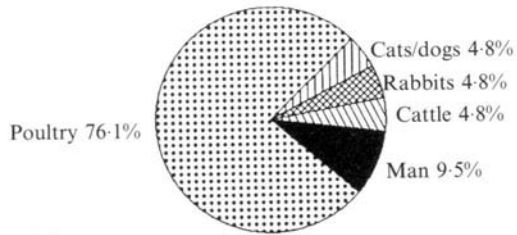
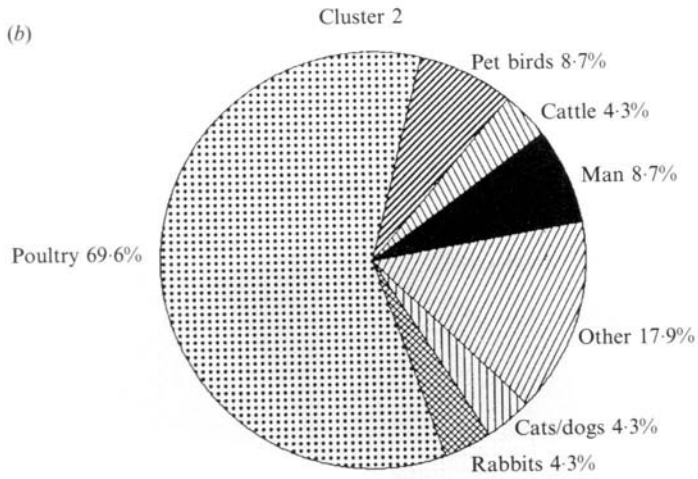
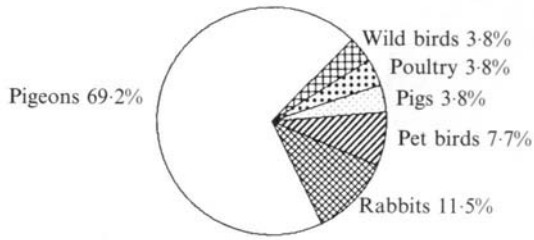
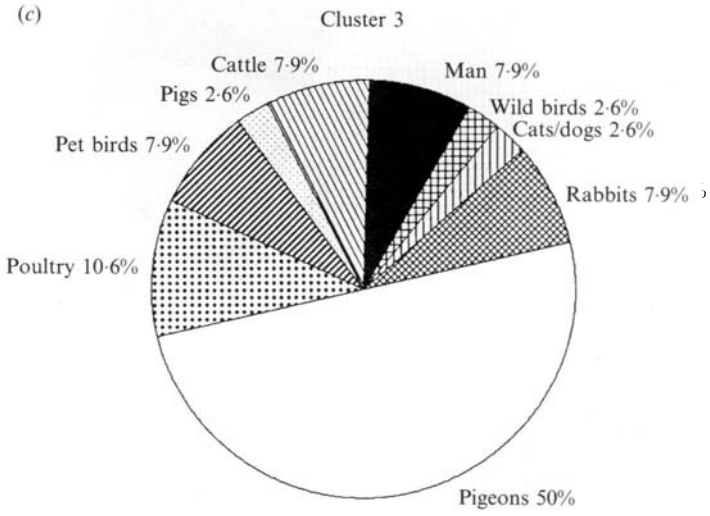


Fig. 4a. For legend see p. 564.



rib. 31

Fig. 4b. For legend see p. 564.



rib. 16

Fig. 4c. For legend see p. 564.

(d)

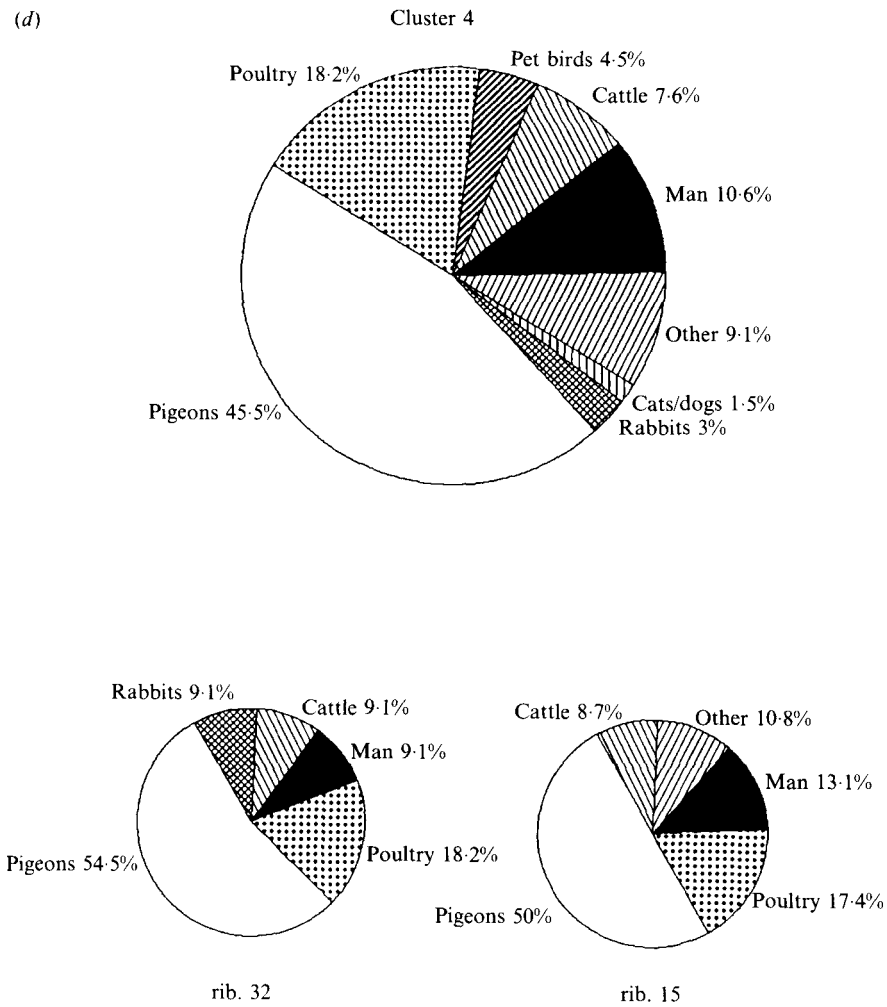


Fig. 4d. For legend see p. 564.

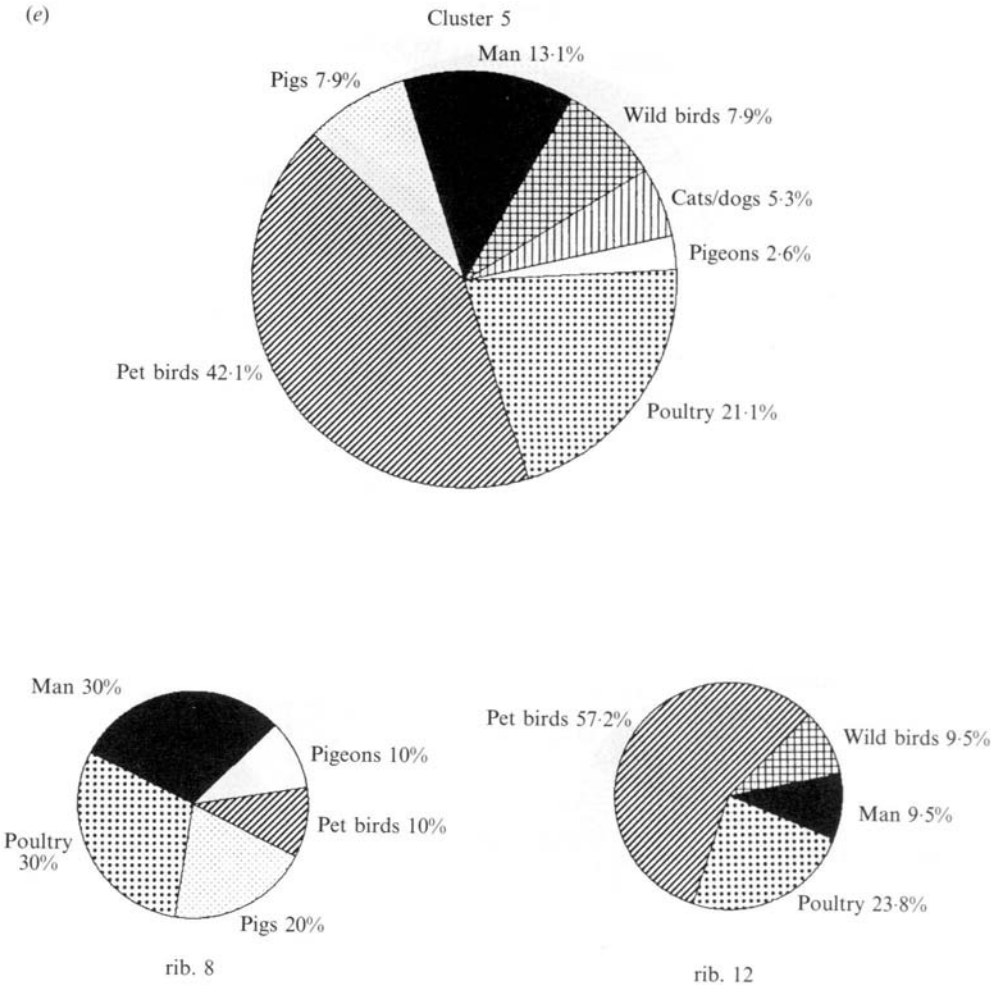


Fig. 4e. For legend see p. 564.

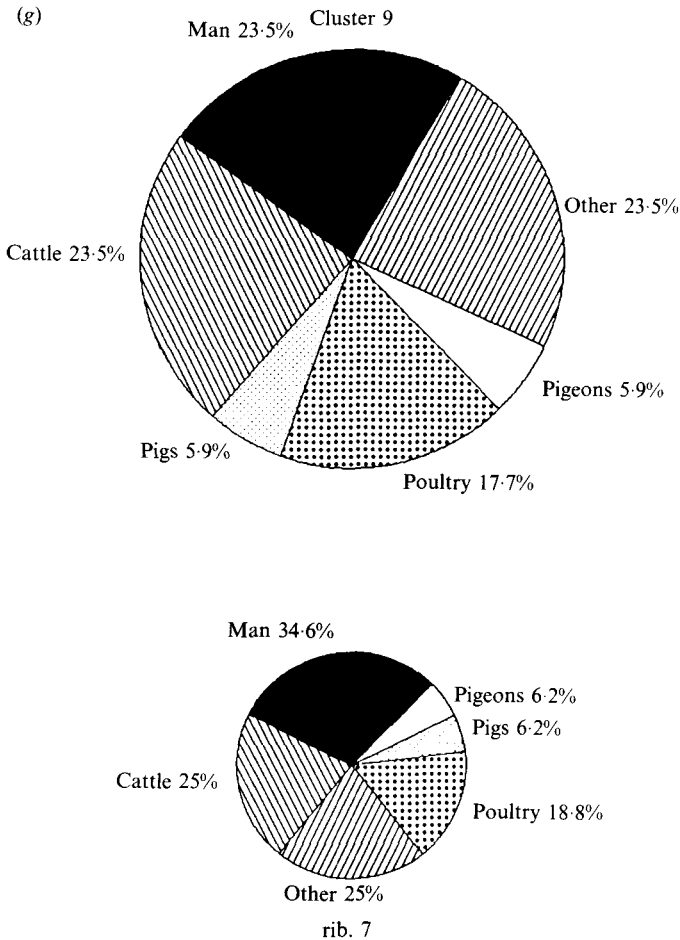


Fig. 4a-g. Distribution of isolates from man and from the main animal sources into each of the most frequently identified rDNA pattern clusters and ribotypes.

some clones of *S. typhimurium*, is compatible with the findings of earlier investigations based on phage and biotyping [4-6] and confirms that they are to some extent host-adapted, only sporadically involving other animal species and humans in their ecological cycles.

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