Bull. Org. mond. Santé
Bull. Wld Hlth Org. 1956, **15**, 299-316

SOME VIRAL AND RICKETTSIAL INFECTIONS IN BOSNIA AND HERZEGOVINA

A Sero-epidemiological Study

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SYNOPSIS

Investigating viral and rickettsial infections in Bosnia and Herzegovina, the authors submitted 115 sera of healthy persons to complement-fixation tests with typhus, Q fever, mumps, rickettsialpox, and psittacosis antigens. The results obtained indicate that the Moslem population tends to show more typhus-positive titres, and at an earlier age, than the non-Moslem. While Moslems under 20 years old tend to develop typhus in epidemic form, an approximately equal number of epidemic and apparently sporadic cases occurs among non-Moslems. On the other hand, Q fever is more frequent, and occurs earlier, in the non-Moslems. An epidemiological explanation of these phenomena is advanced.

From the findings on mumps, it is thought possible that this disease tends to be primarily one of children in Moslems but not in non-Moslems. The rickettsialpox titres suggest the presence of an agent or agents antigenically related to *Ricksettsia akari*, and the psittacosis titres are thought to be caused by contact with organisms of the psittacosis and lymphogranuloma venereum group.

The great majority of the viral infections being more accessible to prevention than to treatment, we found it advisable to investigate the viral and rickettsial " landscape " of Bosnia and Herzegovina by means of serological studies performed on sera of the normal (healthy) population, rather than to perform drawn-out, routine laboratory diagnoses of clinical cases

of these infections. It seemed to us that an investigation of the residual titres in the sera of the normal population, properly selected, might be more profitable than the clinical studies we used to perform previously. The purpose of this paper is to present both the findings and some of the conclusions reached on the basis of the complement-fixation tests performed with five antigens on 115 sera of persons in normal health from Bosnia and Herzegovina, a part of our country still unexplored for viral infections.

Materials and Methods

We chose to investigate the population of hardly accessible villages, which are cut off from the main roads of traffic. Three types of area were selected in order to get samples representing the diversity of the various parts of Bosnia and Herzegovina.

The Fotcha area (52 serum samples) is a region of high mountains covered with forests. The population of the villages consists of poor shepherds and forest workers, mostly Moslems, raising largely sheep, cattle, and poultry. The Fotcha area was known to have endemic syphilis.

The Livno area (36 serum samples) is a plateau region. The population in the villages consists of shepherds and poor farmers, mostly non-Moslems. In addition to sheep and poultry, they raise many pigs and cattle. The Livno area was known to be free of endemic syphilis.

The other areas from which our sera were obtained (altogether 27 serum samples) represent an intermediate mixture of the characteristics listed in connexion with the two areas described previously.

In all louse-infested areas of Bosnia and Herzegovina, the frequency of infestation of Moslems and non-Moslems has been found to be equal.[7]

Serum donors

Sera were taken from persons showing no clinically detectable signs of any illness and no apparent history of any sort of disease (including skin eruptions, headache, chills, temperature, diarrhoea, etc.) for a period of at least 6 months prior to the taking of the blood samples. The healthy donors were further selected according to another criterion: we chose individuals whose place of birth and present dwelling place were at least very close together, if they were not actually the same village. The serum donors were divided into the following age-groups: I, children of pre-school age; II, adolescents below the age for army service; and III, adults above 50 years of age. If not stated otherwise, these are referred to as age-group I, II, or III. Table I shows the composition of the three age-groups in our material for both Moslems and non-Moslems. None of the serum donors had been vaccinated against typhus fever.

TABLE I. AGE COMPOSITION OF POPULATION OF BOSNIA AND HERZEGOVINA STUDIED

Population studied	Age-group	Number of serum donors	Lowest and highest age in group (years)	Mean age (years)
Moslems	children (I)	27	3-7	6.3
	adolescents (II)	23	12-18	16.6
	adults (III)	17	51-83	62.0
Non-Moslems	children (I)	17	5-7	6.7
	adolescents (II)	14	15-19	16.8
	adults (III)	17	52-81	63.9
Total	children (I)	44	3-7	6.5
	adolescents (II)	37	12-19	16.7
	adults (III)	34	51-83	63.0

Sera

We originally obtained samples of serum from more than 130 donors. Those samples which were contaminated or which proved to be anticomplementary in a dilution of 1/4 or more were discarded, and so there remained 115 serum samples which we actually tested with the specific antigens. The sera to be tested were first inactivated in a water-bath at 58°C for 40 minutes at a dilution of 1/4.

Antigens

The following commercial antigens were used: typhus fever (epidemic), soluble type; rickettsialpox, soluble type; and Q fever (Henzerling strain) diagnostic antigen—all three made by Lederle Laboratories in the USA. The psittacosis antigen used was obtained from Dr F. O. MacCallum, Virus Reference Laboratory. Colindale, England. As mumps antigen we used centrifuged allantoic fluids of mumps-infected eggs (10-day-old embryos inoculated in the allantoic cavity and harvested 3-6 days later).

Strains and reference sera

The mumps strain used for making up the antigen was that isolated by M. Milovanović, at the Institute of Hygiene, Belgrade.[5]

The various test sera—used both as positive controls and for testing the typhus, Q fever, rickettsialpox, and psittacosis antigens, we received from Dr C. H. Andrewes (National Institute for Medical Research, London), Dr E. Murray (Harvard School of Public Health, Boston, Mass.), and

Dr J. E. Smadel (Army Medical Center, Washington, D.C.). As reference serum for mumps we used human convalescent sera of clinically and serologically proved cases of mumps.

Reported typhus cases

All the information regarding the occurrence of typhus fever in the course of the last five years in Bosnia and Herzegovina was obtained from the report cards sent by the physicians and health officers in the field to the Central Institute of Hygiene, Sarajevo.

Complement-fixation reaction

The ingredients used and their titration, reading of the results, and the technique used in performing the complement-fixation reaction were as described elsewhere.[6] None of the five antigens used induced more than a 20% decrease in the complement titres. For that reason, titration of the complement in the presence of any of the five antigens seemed unnecessary.

χ^2 values

The methods for constructing both the 2×2 or " fourfold " tables and the 2×3 table, as well as for calculating the χ^2 values are those described by E. G. Chambers.[1] Most of the χ^2 values thus calculated are presented in Table VIII. Since all except one of our calculations are based on fourfold tables (with one degree of freedom) the critical significance value of χ^2 amounts to 3.841 for all our examples, unless otherwise stated.

Results

Of the 115 sera tested in a dilution of 1/4 or higher with the five antigens, only about 10% showed a completely negative result. Table II shows how many of the sera tested once showed fixation with one or more of the five antigens.

Serological specificity

As regards the specificity of our serological findings, the following points may be made:

1. Fourteen samples of sera, all showing fixation with 4 or 5 antigens, as well as 11 additional samples showing a fixation with 3 different antigens, were tested for the presence of syphilitic reagins and found to be negative in both the Wassermann and the Kahn tests. These tests were performed at the Institute of Microbiology at Sarajevo.

TABLE II. NUMBER OF SERA SHOWING COMPLEMENT-FIXATION WITH THE ANTIGENS TESTED

Number of antigens showing fixation with single sera	Children (age-group I)	Adolescents (age-group II)	Adults (age-group III)	All age-groups
0	4 (9 %)	5 (14 %)	3 (9 %)	12 (approx. 10 %)
1	21 (48 %)	7 (19 %)	7 (21 %)	35 (approx. 30 %)
2	10 (23 %)	11 (30 %)	14 (41 %)	35 (approx. 30 %)
3	5 (11 %)	10 (27 %)	4 (12 %)	19 (approx. 17 %)
4	4 (9 %)	3 (8 %)	5 (15 %)	12 (approx. 10 %)
5	0 (0 %)	1 (3 %)	1 (3 %)	2 (approx. 2 %)
Total	44 (100 %)	37 (100 %)	34 (100 %)	115 (100 %)

2. Bosnia and Herzegovina are known to be practically free of brucellosis, which occurs mostly in Slovenia and Croatia,[8, 9] and therefore none of the sera were tested for antibodies against brucellae.

3. All our antigens were cross-tested with monospecific test sera for all five agents and showed no cross-reactions whatsoever.

4. Our confidence in the specificity of the serological results seems to be justified by the fact that in our material, comprising 115 sera, we found 55 samples of typhus-positive sera, of which 40 showed no fixation with the rickettsialpox antigen and 14 showed no fixation with the psittacosis antigen; 65 samples of psittacosis-positive sera, of which 24 showed no fixation with the typhus antigen, 48 none with the rickettsialpox antigen, and 40 none with the Q fever antigen; 23 samples of rickettsialpox-positive sera, of which 8 showed no fixation with the typhus antigen and 6 none with the psittacosis antigen; and 36 Q-fever-positive sera, of which 11 showed no fixation with the psittacosis antigen.

Comparison with findings in Serbia

Table III shows the number of sera showing fixation in a dilution of 1/4 or more and of 1/8 or more, with one or more of the five antigens listed in that table. The latter dilution is given in order to make possible a comparison of our findings for Bosnia and Herzegovina with the findings reported from Serbia,[6] where the serum samples were tested in a dilution of 1/8 or more, but not in a dilution of 1/4.

TABLE III. NUMBER OF SERA SHOWING FIXATION WITH THE FIVE ANTIGENS TESTED IN BOSNIA AND HERZEGOVINA, COMPARED WITH FINDINGS IN SERBIA

Titre adopted as critical		Bosnia and Herzegovina*		Serbia **
		1/4 or more	1/8 or more	1/8 or more
Typhus	Number of sera tested	115	115	317
	Number of positive sera	55	47	61
	Percentage positive	47.8	40.9	19.3
	Highest titre	1/64	1/64	1/128
	Mean titre	1/13.2	1/16.2	1/18.4
Q fever	Number of sera tested	115	115	1049
	Number of positive sera	36	25	108
	Percentage positive	31.3	21.7	10.3
	Highest titre	1/128	1/128	1/256
	Mean titre	1/10.7	1/16.5	1/12.7
Mumps	Number of sera tested	115	115	369
	Number of positive sera	39	35	93
	Percentage positive	33.9	30.4	25.5
	Highest titre	1/128	1/128	1/32
	Mean titre	1/14.4	1/16.6	1/12.7
Rickettsialpox	Number of sera tested	115	115	
	Number of positive sera	23	14	No
	Percentage positive	20	12.8	information
	Highest titre	1/32	1/32	
	Mean titre	1/7.1	1/10.2	
Psittacosis	Number of sera tested	115	115	
	Number of positive sera	65	49	No
	Percentage positive	56.5	42.6	information
	Highest titre	1/32	1/32	
	Mean titre	1/12.1	1/17.4	

*** Tested in 1954**

**** Tested between 1 January 1952 and 31 March 1953**

The material summarized in Table III reveals a somewhat higher percentage of both typhus-positive and Q-fever-positive sera in Bosnia and Herzegovina than in Serbia. The percentage of sera showing fixation with the rickettsialpox and psittacosis antigens could not be compared with other parts of the country, since the present investigations were the first to be performed with these antigens in Yugoslavia.

The incidence of mumps-positive sera does not reveal differences between the material from Bosnia and Herzegovina and that from Serbia.

General results

A tabulation of the results which we did according both to the various areas and to the different age-groups did not reveal any impressive differences in the percentage distribution between the titres with the five antigens tested. The most suggestive data are probably those showing a higher percentage of Q-fever-positive sera and a lower percentage of typhus-positive sera in the Livno area than in the Fotcha area, especially in children. Since the Livno area is populated mostly by non-Moslems and the Fotcha area mostly by Moslems, we have tabulated our findings accordingly in Table IV.

Table IV shows that in Moslems there is a slightly higher percentage of positive sera with the typhus (55.2%) and rickettsialpox antigens (23.9%), and a slightly lower percentage with the Q fever antigen (23.9%) than in the non-Moslem population (37.5% for typhus, 14.6% for rickettsialpox, and 41.7% for Q fever). These data again are not significant enough to warrant any conclusions.

On the other hand, if we analyse the results in Table IV according to the age-groups, we discover a certain number of very suggestive differences.

Before commenting on these differences, it should be pointed out that there still exist some remarkable differences between the customs, habits, and living conditions of the Moslems and non-Moslems in Bosnia and Herzegovina, as a result of which these two population groups are strictly segregated, even when they both live in the same village.[7] These differences may be eliminated in the larger towns and cities (as they are also in other parts of Yugoslavia), but they are still pronounced in the remote villages of those areas of Bosnia and Herzegovina which our sera came from.

Typhus in Moslems and non-Moslems

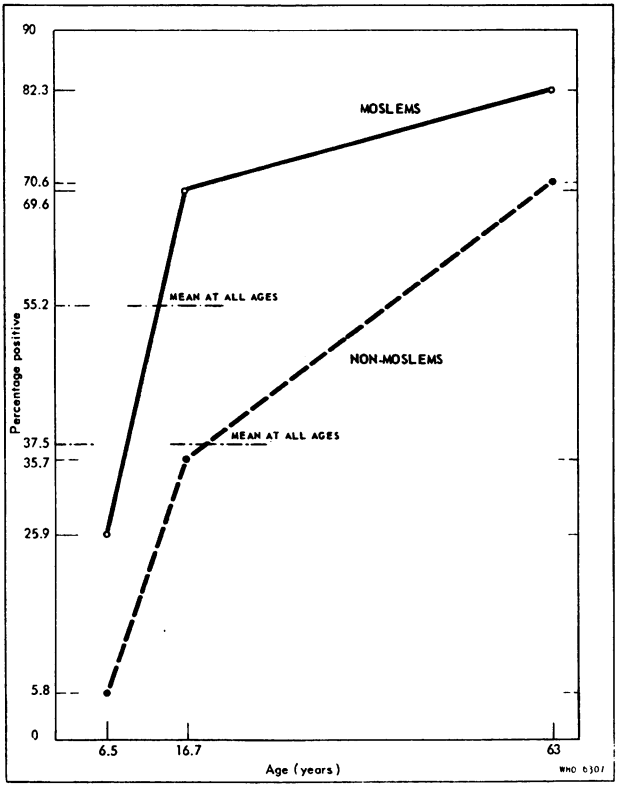
The data in Tables IV and VIII show that in both groups of the population the percentage of typhus-positive sera rises significantly with increasing age.

The difference observed in the percentage of typhus-positive sera between Moslem and non-Moslem children is not statistically significant. However, as shown in Fig. 1, the percentage increase of typhus-positive sera in

TABLE IV. SEROLOGICAL RESULTS OBTAINED IN BOSNIA AND HERZEGOVINA, ACCORDING TO RELIGION AND AGE-GROUP

Population group		Moslems				Non-Moslems			
Age-group		I	II	III	all	I	II	III	all
Number of sera tested		27	23	17	67	17	14	17	48
Typhus	Number of positive sera	7	16	14	37	1	5	12	18
	Percentage positive	25.9	69.6	82.3	55.2	5.8	35.7	70.6	37.5
	Mean titre	1/8.8	1/18.2	1/9.3	1/12.3	1/64	1/18.4	1/12.7	1/15.4
Q fever	Number of positive sera	4	4	8	16	8	8	4	20
	Percentage positive	14.8	17.4	47.1	23.9	47.1	57.1	23.5	41.7
	Mean titre	1/32	1/9.5	1/7.3	1/11.3	1/13.5	1/7.3	1/11.3	1/10.2
Mumps	Number of positive sera	15	6	1	22	11	3	3	17
	Percentage positive	55.5	26.1	5.9	32.8	64.7	21.4	17.6	35.4
	Mean titre	1/16	1/10.1	1/8	1/13.7	1/17	1/20.2	1/8	1/15.4
Rickettsial-pox	Number of positive sera	4	8	4	16	3	2	2	7
	Percentage positive	14.8	34.8	23.5	23.9	17.7	14.3	11.8	14.6
	Mean titre	1/6.7	1/8	1/5.7	1/7.0	1/12.7	1/4	1/8	1/8
Psittacosis	Number of positive sera	11	16	11	38	8	7	12	27
	Percentage positive	40.7	69.6	64.7	56.7	47.1	50.0	70.6	56.3
	Mean titre	1/10.3	1/12.3	1/9.1	1/10.7	1/9.5	1/11	1/19	1/13.4

FIG. 1. PERCENTAGE INCREASE IN TYPHUS-POSITIVE SERA BY AGE-GROUP, IN MOSLEMS AND NON-MOSLEMS



Moslems and non-Moslems of the three age-groups seems to reveal a trend among Moslems to show typhus-positive titres at an earlier age than the non-Moslems. The average percentage of typhus-positive sera in Moslems of all age-groups (55.2%) is higher than that in the non-Moslems. This average is reached by the Moslems in pre-adolescence, while the non-Moslem population does not reach its average (37.5%) until after adolescence.

These findings, as presented in Table IV and Fig. 1, aroused our curiosity and made us anxious to find an adequate explanation. Analysis of the various local habits and living conditions of the Moslem and non-Moslem population revealed certain differences of epidemiological interest which allow us to postulate that the Moslems are more exposed to typhus infection, and at an earlier age, than the non-Moslem population:

(*a*) While the Moslem settlements are usually grouped closely together, the households of the non-Moslems are generally dispersed—a custom retained from the days of Turkish rule.

(*b*) It is a widespread practice among the local Moslem population to visit relatives, especially when they are sick, and to spend several days and nights with them, usually in the same crowded room.[7]

(*c*) The non-Moslem peasants of the remote villages of Bosnia and Herzegovina live mostly in rooms the floors of which are bare during the day and covered with hay overnight; during the winter they often live under the same roof as their livestock. The Moslem population, however, both lives and sleeps in rooms filled with rugs and hides, which offer a good medium for harbouring lice.[7] This, combined with the habit of visiting the sick, also makes for an extensive and repeated exchange of body-lice between members of Moslem families who may not live in the same area.

According both to our findings and to our hypothesis based on those findings, we would expect that typhus cases in Bosnian adult Moslems should be largely sporadic, while among Moslem children the disease should assume a more epidemic character, indicating extensive exposure of this population-group to typhus-infected body-lice from early childhood on.

We have therefore tabulated the age distribution of 568 typhus cases, all confirmed serologically, and all reported during the last five years (1950-54) from the whole territory of Bosnia and Herzegovina (Table V). Two or more cases occurring simultaneously or not more than 30 days

TABLE V. AGE DISTRIBUTION OF 568 TYPHUS CASES REPORTED IN BOSNIA AND HERZEGOVINA, 1950-54

Age-group (years)	Moslems			Non-Moslems			Grand total
	epidemic cases	apparently sporadic cases	total	epidemic cases	apparently sporadic cases	total	
0-2	6 (100 %)	0 (0 %)	6	2 (50 %)	2 (50 %)	4	10
3-7	34 (85 %)	6 (15 %)	40	14 (61 %)	9 (39 %)	23	63
8-11	25 (76 %)	8 (24 %)	33	19 (56 %)	15 (44 %)	34	67
12-19	41 (72 %)	16 (28 %)	57	46 (60 %)	30 (40 %)	76	133
20-50	41 (46 %)	48 (54 %)	89	60 (38 %)	100 (63 %)	160	249
51-83	4 (31 %)	9 (69 %)	13	19 (61 %)	12 (39 %)	31	44
83 +	0 (0 %)	0 (0 %)	0	2 (100 %)	0 (0 %)	2	2
All ages	151 (63 %)	87 (37 %)	238	162 (49 %)	168 (51 %)	330	568

apart in the same village are tabulated as epidemic cases. Single cases and those appearing in the same village but 31 or more days apart are taken as sporadic cases. In fact, no instances were observed in which two cases occurred in the same village with a 21-30-day interval, and consequently all our " two-case-epidemic " cases were separated by 20 days or less. This seems amply to justify our classification of the cases as epidemic or sporadic on the basis of an interval of 30 days.

From Tables V and VIII it is apparent that there is a very pronounced trend for the clinically manifest typhus fever cases in Moslem children and adolescents to appear in epidemic form, while in adult Moslems (20 years of age and older) the tendency is for these cases to occur in apparently sporadic form. Tabulation of the 330 cases of typhus fever in non-Moslems shows no significant differences between the occurrence of epidemic cases and that of sporadic cases in the various age-groups.

Even if the chances of certain individuals' contracting typhus were equal for both Moslems and non-Moslems, the chance of a case's being labelled " epidemic " might, on our criteria, be enhanced by the mere fact that the population-group to which the case belonged was the bigger one in the village concerned. However, the Moslem population of Bosnia amounts to only about 30% of the total population, and therefore it is rather the chance of non-Moslems' being labelled as " epidemic " cases that is, in fact, enhanced—if, indeed, it is affected at all. This would only add to the significance of the opposite finding—that epidemic cases are prevalent among Moslems—for which we obtained a χ^2 value higher than 10 (see Tables V and VIII).

Table VI shows the age distribution of these same cases compared to that of the Bosnian population as a whole.[8, 9] This latter distribution may be taken as valid for the rural population, both Moslem and non-Moslem. It will be seen that in the Moslem population typhus fever has been reported at a fairly even rate for all three age-groups, the observed differences in the incidence of manifest typhus cases between children and adults of the Moslem population giving a χ^2 value as low as 2.7. On the other hand, Tables VI and VIII show that non-Moslem children develop typhus fever at a lower rate than Moslem children (χ^2 value of about 18), while non-Moslem adults tend to have a much higher rate of typhus than non-Moslem children (χ^2 value of about 19). Similar conclusions can be drawn from the data presented in Tables IV and V.

From Table VI, it is also possible to compare the age distribution of Moslem and non-Moslem cases; with a χ^2 test on the 2×3 table we obtained a χ^2 value as high as 18.68 (for two degrees of freedom the critical value of χ^2 amounts here to 5.991).

These high χ^2 values calculated on the basis of data taken from vital statistics and epidemiological reports (a material quite different from the sera we tested) seem to lend strong support to the suggestions made on the

TABLE VI. AGE DISTRIBUTION OF 568 TYPHUS CASES, AND OF TOTAL POPULATION, IN BOSNIA AND HERZEGOVINA

Age-group (years)	Reported typhus cases		Population of Bosnia and Herzegovina (thousands)
	Moslems	Non-Moslems	
0-11	79 (33 %)	61 (18.5 %)	854 (approx. 30 %)
12-19	57 (24 %)	76 (23 %)	564 (approx. 20 %)
20 +	102 (43 %)	193 (58.5 %)	1426 (approx. 50 %)
Total	238 (100 %)	330 (100 %)	2844 (100 %)

basis of our serological results. We may conclude, therefore, (*a*) that the non-Moslem children suffer from typhus fever at a lower rate than the non-Moslem adults, and (*b*) that over the whole of their lives the non-Moslems seem to suffer at approximately the same rate from both epidemic and apparently sporadic typhus.

On the other hand, the Moslems in the Bosnian villages (*a*) seem to be exposed to infected body-lice from their earliest childhood on to a much greater extent than non-Moslems; (*b*) suffer from typhus fever in childhood at a higher rate than the non-Moslems; and (*c*) tend to develop typhus in an epidemic form if young and in an apparently sporadic form if over the age of 20 years.

As regards the officially reported manifest cases (calculated on the basis of the data recorded in Table VI), these cases seem to develop at a comparable rate among both Moslem children and adults (approximately 1 Moslem case per 12 000 inhabitants of all age-groups over the last 5 years).

The two facts that typhus fever in young children tends to have a very mild clinical course and that the Moslems in Bosnia and Herzegovina become infected with *Ricksettsia prowazeki* during their early childhood at a higher rate than the non-Moslem children seem to suggest that the Moslem children might also tend to develop a higher proportion of these mild cases of typhus, which, under field conditions, are neither recognized nor reported as typhus. These mild and unrecognized infections of Moslem children, in a place where there is a permanent exchange of body-lice, might both be conditioned by and condition the supply of rickettsiae for the lice which are transferred back and forth in the families. These mild infections of children seem to be at least as important as potential reservoirs of endemic typhus in Bosnia as the cases of Brill's disease.

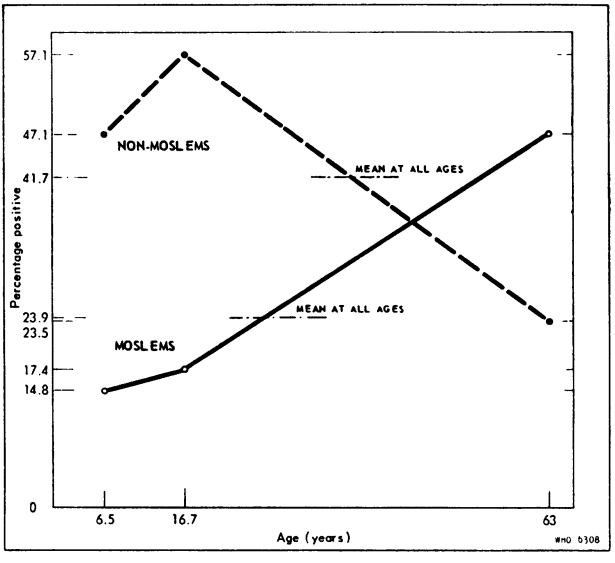
If, in the future, successful isolations of rickettsiae from young, " clini-

cally healthy " children or from those with a " minor illness " from louse-infested families in which typhus has recently occurred should confirm the validity of our hypothesis, that would explain at the same time why epidemiologists in Bosnia and Herzegovina are so often unsuccessful in discovering the source of a typhus outbreak, especially if it occurs in Moslems.

Q fever in Moslems and non-Moslems

The findings presented in Tables IV and VIII and in Fig. 2 seem to suggest that infection with Q fever takes place at an earlier age and at a higher rate in the non-Moslem population than in the Moslem, who show a somewhat lower percentage of Q-fever-positive sera which grows gradually with increasing age.

FIG. 2. PERCENTAGE INCREASE IN Q-FEVER-POSITIVE SERA BY AGE-GROUP, IN MOSLEMS AND NON-MOSLEMS



As mentioned previously, the Moslem population lives and sleeps in rooms completely separate from the livestock, while the non-Moslem peasants of these remote villages in Bosnia and Herzegovina are in much closer contact with their livestock. Sleeping mostly on floors covered with hay, the non-Moslems are exposed to dust soiled with the excretions of these possibly infected animals and of their parasites and, of course, to the bites of the parasites themselves. As we have seen, in winter the

non-Moslems occasionally sleep actually in the same poorly ventilated rooms as their sheep or cows.

These circumstances seem to furnish sufficient explanation of our findings regarding the exposure of non-Moslem peasants in Bosnia and Herzegovina to Q fever infection at an earlier age and at a more intense rate than the Moslem population.

Mumps

The data presented in Tables IV and VIII regarding the percentage of mumps-positive sera in the various age-groups show in both Moslems and non-Moslems a very impressive drop with rise in age. This finding seems to be in good agreement with the well-known fact that anti-mumps titres disappear rapidly from the sera of convalescents, and at the same time to indicate a greater frequency of mumps infections during the earlier period of life. Although the percentages for corresponding age-groups in Moslems and non-Moslems do not show significant differences, the following points are interesting:

Since the Moslem settlements are more closely grouped and their children more exposed to intimate contact with visitors from distant areas, one might expect the Moslems to come in contact with the mumps virus at an earlier age than the non-Moslems. If this were so, the third Moslem age-group should show a smaller percentage of mumps-positive sera than the corresponding non-Moslem age-group. Although that does seem to be the case from Table IV, the data given there cannot be used as an argument since the small number of cases observed deprives them of significance.

In Table VII we have divided our data for mumps in two groups according the height of the titres found; all the sera showing a titre of 1/16 or less we have put in the group of " old infections ", and those showing a titre of 1/32 or more in the group of " recent infections ". When testing the significance of the differences observed in the percentages of " recent " mumps titres

TABLE VII. "OLD" AND "RECENT" MUMPS INFECTIONS IN BOSNIA AND HERZEGOVINA

Population	Age-group	Old infections (titre 1/16 or less)	Recent infections (titre 1/32 or more)	Number of sera tested
Moslems	I	9 (33 %)	6 (22 %)	27
	II	5 (22 %)	1 (4 %)	23
	III	1 (6 %)	0 (0 %)	17
Non-Moslems	I	8 (47 %)	3 (18 %)	17
	II	2 (14 %)	1 (7 %)	14
	III	2 (12 %)	1 (6 %)	17

between the first and the third age-groups in Moslems (Tables VII and VIII), we obtained a χ^2 value which does not permit of any conclusion, but might be considered a value at the borderline of significance. On the other hand, the difference observed in the percentages of " recent " mumps titres in the first and third age-groups in non-Moslems is far below statistical significance ($\chi^2 = 1.13$).

Assuming that our speculations on mumps are valid, they would seem to offer some explanation of how mumps tends to be a children's disease in Moslems but not in non-Moslems—the tendency depending entirely on the specific and distinct living conditions of these two groups of the rural population in Bosnia and Herzegovina.

Rickettsialpox

Although clinical cases resembling those described in the literature for rickettsialpox have been seen in Yugoslavia, not a single case of rickettsialpox has so far been diagnosed either clinically or serologically. This fact makes it difficult to comment upon the findings given in Table IV.

A cross-reaction of the antibodies detected with our rickettsialpox antigen with any of the other four antigens we were using seems to be excluded (see *Serological specificity*, page 302). We have no basis on which to discuss whether the fixations obtained with the rickettsialpox antigen have to be ascribed to residual titres after contacts with *Rickettsia akari*, or whether they indicate an immunological response to other rickettsioses for which no tests were performed and the occurrence of which has not yet been confirmed in Yugoslavia (e.g., boutonneuse fever or some other infections).

The very fact that the rickettsialpox antigen we were using showed no cross-reactions with unrelated antibodies so far tested, and especially the fact that out of the 23 rickettsialpox-positive sera three showed a titre of 1/16 and one even a titre of 1/32, seem to support our presumption that the titres obtained by the rickettsialpox antigen were caused at least partly by an agent or agents antigenically related to *R. akari*. This seems to justify further search for rickettsioses other than typhus and Q fever in Yugoslavia.

Psittacosis

The percentage of sera giving fixation with the psittacosis antigen is surprisingly high and can be compared only with that seen for typhus fever. From the data in Tables IV and VIII one can see no tendency for this percentage to increase with age. No significant differences can be observed in the percentages according to religious grouping or the area from which the samples were obtained. Nor do the data tabulated according to sex suggest any differences between the percentage of psittacosis titres found

TABLE VIII. χ^2 VALUES OBTAINED IN MATERIAL INVESTIGATED IN BOSNIA AND HERZEGOVINA

Material investigated	Variables				Observed frequencies				Total number of observations (N)	Data taken from Table	χ^2 values (with Yates's correction)
	X_1	X_2	Y_1	Y_2	a	b	c	d			
Typhus fever in Moslems	titre < 1/4	titre 1/4 or more	age-group I	age-group III	20	7	3	14	44	IV	13.31 (11.15)
Typhus fever in non-Moslems	titre < 1/4	titre 1/4 or more	age-group I	age-group III	16	1	5	12	34	IV	15.07 (12.45)
Typhus cases reported in Bosnia and Herzegovina, 1950-54	epidemic cases	sporadic cases	Moslems	non-Moslems	151	87	162	168	568	V	11.00 (10.45)
Typhus cases reported in Bosnia and Herzegovina, 1950-54	Moslems	non-Moslems	age-group I	age-group III	79	61	102	193	435	VI	18.70 (17.80)
Typhus cases reported versus total population in Bosnia and Herzegovina	typhus in non-Moslems	population	age-group I	age-group III	61	854 $\times 10^3$	193	1426 $\times 10^3$	2280 $\times 10^3$	VI	19.60 (19.00)
Q fever in Moslems	titre < 1/4	titre 1/4 or more	age-group I	age-group III	23	4	9	8	44	IV	5.47 (4.00)
Q fever in children (age-group I)	Moslems	non-Moslems	titre < 1/4	titre 1/4 or more	23	4	9	8	44	IV	5.47 (4.00)
Mumps in Moslems	titre 1/4 or more	titre < 1/4	age-group I	age-group III	15	12	1	16	44	IV	11.12 (9.08)
Mumps in non-Moslems	titre 1/4 or more	titre < 1/4	age-group I	age-group III	11	6	3	14	34	IV	7.77 (5.95)
Recent mumps infections in Moslems	titre 1/32 or more	titre 1/16 or less	age-group I	age-group III	6	21	0	17	44	VII	4.37 (2.69)
Psittacosis in Moslems	titre < 1/4	titre 1/4 or more	age-group I	age-group II	16	11	7	16	50	IV	4.15 (3.08)

in females and that found in males. (Of the 115 serum samples tested 101 were taken from males and 14 from females.)

As far as we know, only one case of psittacosis (infection after handling pigeons) has so far been diagnosed both clinically and serologically in Yugoslavia.[2] Nothing is yet known about the incidence of this disease in Yugoslavia. However, it is known that in certain areas of the United States of America as many as 40% of the sera of Negroes show a positive complement-fixation reaction when tested with lymphogranuloma venereum antigen.[4] No reliable information for Yugoslavia is available about the frequency of cases of lymphogranuloma venereum because they are rarely recorded, but the disease is known to exist among the population of Bosnia and Herzegovina.

In view of the fact that in cases of latent infections with lymphogranuloma venereum virus, titres as high as 1/40 may persist in the patients' sera for as long as 20 years,[3] we believe it possible that a certain number of our " psittacosis-positive " sera may be ascribed to contact with lymphogranuloma venereum virus.

In view of the fact that syphilitic sera may give false positive reactions with both the psittacosis and the lymphogranuloma venereum antigens, we gave 15 samples of our psittacosis-positive sera to be tested for the presence of syphilitic reagins and found not a single sample showing either positive Kahn or positive Wassermann tests (see *Serological specificity*, page 302).

It should be pointed out that out of the 65 serum samples found to be positive with the psittacosis antigen, 21 sera showed a titre of 1/32.

All these facts seem to support the view that the psittacosis titres we found were caused by contacts with organisms belonging to the psittacosis and lymphogranuloma venereum group or antigenically related to this group.

* * *

Not until this paper had been completed did we have the pleasure of seeing an article by J. A. Montoya et al. (*Amer. J. Hyg.*, 1955, **62**, 255), from which we learned that the authors had obtained data and came to certain conclusions very similar to ours regarding certain aspects of epidemic typhus in Peru. Their conclusions, however, are based on material much larger than ours.

Also since completion of this paper, we have succeeded in isolating two psittacosis (ornithosis) strains from wild pigeons (*Columba livida*), and we have observed serologically proved cases of acute psittacosis in patients from Bosnia. Our findings, which will be published elsewhere, seem to support and complete the serological findings on psittacosis presented in this paper.

ACKNOWLEDGEMENTS

The authors have great pleasure in expressing their thanks to Dr C. H. Andrewes, Dr F. O. MacCallum, Dr E. Murray, and Dr J. E. Smadel for the various reference sera; to Dr F. O. MacCallum again for the generous amounts of psittacosis antigen; to Dr A. Jamnitzky for making available data on the officially reported cases of typhus in Bosnia and Herzegovina; to Dr R. Fried for performing the serological tests for syphilis; and to Dr B. Arsich for valuable information regarding certain epidemiological data in the field.

RÉSUMÉ

Les sérums de 115 habitants de régions reculées de la Bosnie et Herzégovine ont été soumis au test de fixation du complément avec les antigènes du typhus, de la fièvre Q, du Rickettsialpox, des oreillons et de la psittacose. Ces habitants appartenaient à divers groupes d'âge (enfants préscolaires, adolescents, adultes de plus de 50 ans) et étaient tous en bonne santé. Un dixième seulement de ces sérums étaient sérologiquement négatifs pour tous ces antigènes.

De légères différences dans le nombre des titres positifs sont apparues entre la population musulmane et non musulmane, pour certains antigènes. C'est ainsi que, pour le typhus, on a observé des titres positifs à un âge plus précoce chez les Musulmans que chez les non Musulmans. On pourrait expliquer cette différence par le genre de vie différent, dans les populations éloignées des voies de communication, et par une promiscuité plus étroite, chez les premiers, entre malades et bien portants. Les formes bénignes et frustes du typhus chez les enfants représentent probablement un réservoir important du typhus endémique en Bosnie.

La fièvre Q se manifeste à un âge plus précoce et avec un taux d'infection plus élevé chez les non Musulmans que chez les Musulmans. On pourrait, là encore, expliquer cette différence par l'habitude qu'ont les premiers de vivre en hiver dans les mêmes locaux que leur bétail et de dormir sur du foin, étendu le soir sur le plancher de l'habitation.

Les titres obtenus avec les antigènes du Rickettsialpox semblent indiquer la présence d'un antigène apparenté à *R. akari*. Les pourcentages de sérums positifs avec l'antigène de la psittacose sont élevés. On ne connaît pas l'incidence de cette maladie en Yougoslavie, mais on sait que la lymphogranulomatose vénérienne existe dans le pays. Il est possible que la réaction psittacose-positive d'un certain nombre de sérums puisse être attribuée au contact avec l'antigène de la lymphogranulomatose vénérienne.

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