HUMAN TUBERCULOSIS OF BOVINE ORIGIN IN NORTHERN IRELAND

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A survey of the amount of human tuberculosis due to the bovine type of tubercle bacillus in Northern Ireland has been made during the past 5 years. The results seem to show that there has been a marked decline in the incidence of tuberculosis of bovine origin in this area during the past 10 years. This decline appears to coincide with a great increase in the pasteurization of milk, an extensive scheme for the tuberculin testing of cattle and an increased awareness on the part of the public of the risk of contracting tuberculosis from milk.

The pathological material sent into the Central Laboratory of the Northern Ireland Tuberculosis Authority comes from the city of Belfast, and from all areas of Northern Ireland. It is collected from hospitals, chest clinics and general practitioners. Strains of tubercle bacilli have been typed by cultural and inoculation methods. The strains isolated from sputum represent the largest group, but large numbers of strains from other sources have also been typed. Since the introduction of streptomycin the number of strains isolated from cerebro-spinal fluid has increased enormously and these have provided much valuable material for the study of the occurrence of human meningitis due to the bovine type. The number of strains available from tuberculous adenitis is small compared with some of the other groups. This is probably due to the fact that adenitis, particularly cervical, is more easily diagnosed clinically than other forms of tuberculosis and material is not so often sent for examination, and also possibly because adenitis is less common than formerly. Great difficulty has been found in obtaining material from abdominal tuberculosis, again because it is not so common as formerly, and because material can usually only be obtained at operation or by post-mortem examination. Only one strain from lupus was available for typing and this proved to be human in type.

METHODS OF ISOLATION

The methods of isolation of the organism varied with the material examined. Sputum was emulsified with 5% KOH, heated for 20 min. in a water-bath at 37° C., centrifuged and planted without neutralization on tubes of culture medium, using a Pasteur pipette. The KOH was prepared in small screw-capped bottles and autoclaved, to obviate the risk of contamination of the specimen with other strains of tubercle bacilli. For emulsification the sputum is placed in a heavy glass centrifuge tube and closed with a sterilized rubber cork. The emulsification, heating and centrifuging are carried out in the same tube. Fasting gastric residue was centrifuged and the deposit treated similarly to sputum. Lymphatic glands,

portions of infected organs—e.g. granulation tissue from sinuses, organs removed at operation and post-mortem material (including animal material)—were ground up in Griffith's tubes and treated with KOH in the same way as sputum, except that centrifuging was not necessary for small specimens. Urines were centrifuged, and the deposit shaken up with 5 % oxalic acid, heated at 37° C. for 20 min. and centrifuged again. The resultant deposit was then seeded on to tubes of culture medium. It has been found that oxalic acid gives a better deposit in urine than KOH, particularly if the amount of pus, etc., in the urine is small. Throat swabs are frequently sent into the laboratory, having been used for taking material from discharging sinuses and abscesses. These are rubbed up in a few ml. of oxalic acid till as much of the adherent pus and granulation tissue as possible is washed off. The swab tube with contents is then heated in the water-bath for 20 min. and the contained mixture of pus and oxalic acid is centrifuged. The deposit is planted in the usual way on culture medium. Cerebro-spinal fluid is centrifuged and the deposit planted out without treatment, unless a contaminating organism is present. Pleural fluids and pus are similarly dealt with. If other organisms—e.g. staphylococci—are present, the specimen is treated with KOH like a sputum.

Various other methods of eliminating contaminating organisms have been tried, but the two mentioned above, KOH and oxalic acid, have been found to be the most satisfactory.

CULTURE MEDIA

The standard media used were Loewenstein-Jensen and Dorset egg medium without glycerine. For the first 500 strains typed, Griffith's glycerine potato medium, Dorset egg medium with 5 % glycerine and glycerinated serum medium were used as well for subculture. This was to gain experience of the colony characteristics of human and bovine types. Later it was found that eugonic strains could easily be recognized on the Loewenstein-Jensen and Dorset egg media, and where any apparent deviation from the eugonic characteristics occurred the strain was further tested by subculture and rabbit inoculation.

ANIMAL INOCULATION

Most of the samples of cerebro-spinal fluid from cases of tuberculous meningitis are injected into guinea-pigs, particularly if there is likely to be difficulty in repeating the specimen, or if streptomycin therapy is contemplated. Pus from glands, bones, joints, etc., is also inoculated. The method of subcutaneous inoculation in the abdominal wall is that used for guinea-pigs, and the animals are killed after 8–10 weeks. This method is found to give very uniform lesions in the organs of the guinea-pig. The organism can usually be recovered without difficulty from the guinea-pig spleen, for further investigation. For rabbit inoculation the organism used is usually that recovered from the guinea-pig spleen (since it is assumed to be fully virulent), and grown on Dorset egg without glycerine, to avoid any possible alteration of the bacterium by glycerine. The culture is ground in a bacterial mortar and emulsified with saline. Recently it has been found very convenient to subculture from the egg slope into Dubos liquid medium. This gives a very uniform suspension of bacteria in a few days, which can be suitably diluted for rabbit

inoculation. The intravenous injection of 0·01 mg. is the method used. Death of the animal occurs almost invariably within 3–4 weeks, with widespread lesions in spleen, liver and lungs, if the organism is of bovine type. The organisms have been recovered from the rabbit organs in every case tested.

All strains shown to be of bovine origin in this investigation have been proved by rabbit inoculation. A few human strains which appeared to be dysgonic on primary culture were inoculated, and these did not cause any illness in the rabbits, which after being killed showed minimal or no demonstrable lesions. Most of the bovine strains encountered in this survey were very dysgonic. It was particularly noticeable how slowly they appeared in primary culture, and some of them could not be isolated directly by culture and were only recovered by guinea-pig inoculation.

To minimize the risk of missing bovine strains because of their dysgonic character, if the organisms could not be recovered by culture from a specimen which was positive on direct microscopic examination, another specimen was obtained and inoculated into a guinea-pig.

No attempt has been made in this study to select material specially likely to yield the bovine type of organism. For the past five years practically every strain of tubercle bacilli isolated from a new patient has been typed, and it is from these results that the figures have been compiled. The introduction of streptomycin therapy increased enormously the number of strains from miliary and meningeal tuberculosis, as it was in this laboratory that most of the control of treatment (in this area) was carried out.

Table 1

	Human	Bovine	Bovine (%)
Pulmonary cases	1060	1	0·1
Meningeal (including miliary and meningeal)	237	8	3·37
Cervical glands	30	4	13·3
Bone and joint	86	3	3·5
Primary abdominal	2	0	—
Genito-urinary	16	1	6·2

A few typical case histories are here included:

Case 1. The one case of pulmonary tuberculosis due to the bovine type appears to be the first case published in Ireland. This is a girl now aged 20 years. There is no family history of tuberculosis. She has always lived in Belfast, and the only likely source of infection was unpasteurized milk. She first came under observation 6 years ago when she had discharging glands on the right side of her neck. A year later an X-ray photograph showed some infiltration of the left lung with a probable cavity. Tubercle bacilli were first recovered 3 years ago, and they were proved by rabbit inoculation to be of bovine type. She has been in hospital for the last 2 years, but so far there is but slight radiological clearing. Tubercle bacilli have been recovered quite recently from the sputum and the organisms are still of bovine type. No colonies of human type were isolated, though this has been shown to occur in other cases of this kind after the disease has been present for some time (Griffith & Munro, 1943; Jensen & Kiaer, 1938).

Case 2. This case illustrates the risk of infection from milk, even when careful

precautions are taken. This was a child aged 4 years, whose parents were in process of establishing a tubercle-free herd. For the first year the child was fed on boiled milk. After that he drank large amounts of milk 'fresh from the cow', and naturally had frequent contact with cattle on the farm. A few months before he developed tuberculous meningitis, two animals in the herd became positive reactors and were removed. The child died of meningitis of bovine origin. There was no family history of tuberculosis.

Case 3. This case demonstrates the occasional finding of a patient with tuberculosis of bovine origin in a family where there are already cases of tuberculosis of human type. A child of 5 years died of meningitis of bovine type. Three relatives have or have had pulmonary tuberculosis.

Case 4. This case is quoted as an example of 'fastness of type'. A child of 6 years developed meningitis due to the bovine organism. He was first admitted to hospital 1 year ago. The organism was very dysgonic and was originally isolated by guinea-pig inoculation. The child was treated with streptomycin and reacted fairly well. He has, however, had two relapses during the year and on each relapse very dysgonic bovine organisms have been recovered. There is no sign of any alteration of the type characteristics or of a mixed infection. There is no history of tuberculosis in the family.

DISCUSSION

The number of cases of bovine type in this investigation is too small to allow any definite conclusions to be made concerning age, sex, occupation or area. Of the seventeen cases only two came from Belfast; the remaining fifteen all came from rural or urban areas. All the child cases had been fed on untreated milk, at some time. The ages varied from a few months to middle-age. This is in agreement with the findings of investigators in other countries, where cases of bovine type tuberculosis were found in all age groups. An infection may be contracted during childhood and remain dormant for many years, or primary infection may occur quite late in life.

The number of cases of pulmonary tuberculosis included in this investigation seems large compared with the non-pulmonary group. This may be in some measure accounted for by the fact that much of the material sent to this laboratory comes from hospitals and clinics. Yet there must be few cases of tuberculosis, respiratory or non-respiratory, who do not at some time have treatment in hospital or come under the care of a tuberculosis physician. A more important factor is possibly that many of the non-respiratory cases eventually are found to have a pulmonary lesion, and fall into the respiratory group. Of the cases mentioned in this investigation almost all have had the organism isolated several times from their particular lesion, both during their stay in hospital and as out-patients, and it has been possible to trace the course of their disease (bacteriologically) over a number of years.

An extraordinary feature of the investigation here described is the rarity of pulmonary tuberculosis of bovine type in this country. This confirms the observations of previous investigators. Cumming (1935) typed 320 strains of *Myco-*

bacterium tuberculosis from cases of pulmonary tuberculosis in Eire, and found no cases due to the bovine organism. More recently, Kearney, Farrelly & Cronin (1949) typed 500 cases of pulmonary tuberculosis and found no case of bovine-type infection. This survey, the writers point out, was specially directed to cases of known and intimate contact with open cases in cattle. The great rarity of bovine-type organisms in pulmonary cases in Ireland has never been satisfactorily explained. It appears that as a form of the disease it has always been extremely rare, even when other forms of tuberculosis of bovine origin were more common. Various estimates of the amount of tuberculosis in the cattle population show that the incidence does not differ greatly from other countries, and extensive programmes for the production of tubercle-free milk are of fairly recent development.

Table 2. *Types of* Mycobacterium tuberculosis *isolated from* 245 *cases of meningitis, classified according to age of patient*

Age group (years)	0–5	6–10	11–15	Over 15	Total
Type: Human	73	30	22	112	237
Bovine	4	2	0	2	8

The distribution of meningitis according to age follows the pattern of surveys made in many countries, the tendency being for meningitis to develop in the early years or in late adolescence. There were two patients in this series over 50 (both human type). The two bovine-type cases in the over 15 group were aged 21 and 23.

The figures given for the incidence of meningitis of bovine origin are probably the most comprehensive of the non-respiratory group in this investigation. As mentioned previously, the treatment of meningeal and miliary tuberculosis with streptomycin brought an enormous increase in the number of strains of tubercle bacilli from these sources. All ages and areas (rural, urban and industrial) are represented. The history of contact infection was very common, and in cases where a full examination could be made, the large majority were found to have the primary focus in the lung. Frequently the contact is the father, in the case of a child. In this series, many cases of miliary or meningeal tuberculosis in children have been traced to an unsuspected or presumed quiescent lesion in the father. In fact, in some cases the father's disease was only discovered during a search for contacts after the case of meningitis had occurred.

A study of the incidence of meningitis compared with glandular tuberculosis in different countries always seems to show a higher percentage of glandular disease compared with meningeal. Is this evidence of a lower virulence of the bovine organism or is it a matter of dosage? The relation of size of dose to the development of tuberculous infection is a subject of controversy, but it seems obvious that a child living in the same house in contact with an open case of tuberculosis is likely to get a much more frequent and much larger dose of organisms than one drinking infected milk. About 0·5 % of cows excrete tubercle bacilli in their milk (Francis, 1947), but with 'bulking' of milk the number of organisms ingested may be small and the dose infrequent.

The figures given in Table 1 indicate that there has been a marked reduction in the incidence of human tuberculosis of bovine origin in the past 10 years, in Ireland.

McMurray (1941) investigated seventy-two unselected cases of tuberculosis in Northern Ireland, and found that 19·4 % were bovine in type. Out of twenty-nine cases of meningitis, seven (28%) were of bovine origin. Of sixteen cases of adenitis two (16·5 %) were bovine. No pulmonary case due to the bovine type was discovered. In Eire, Mushatt (1940) typed fifty strains of tubercle bacilli from non-pulmonary sources and found three out of twelve cases of meningitis and ten out of fourteen cases of cervical adenitis to be due to the bovine organism.

In the series of cases here described only 3·3 % of tuberculous meningitis and 13·3 % of tuberculous glands were found to be of bovine type.

In England, in 1938, A. S. Griffith (1938) found that 24·6 % of cases of meningitis and 50 % of tuberculous adenitis were of bovine origin. In Scotland, Griffith (1937) found 29·6 % cases of meningitis and 51·6 % adenitis to be due to the bovine bacillus. In France, Gervois (1937) found 7·4 % meningeal and 11 % glandular tuberculosis to be of bovine origin. In Denmark, Jensen, Lester & Tolderlund (1940) found that 23·4 % of meningeal and 46·2 % of glandular cases were of bovine type.

The incidence must vary in different countries according to the amount of tuberculosis in the cattle population and the milk-drinking habits of the people. All these figures relate to surveys carried out 10 or more years ago, and since then extensive programmes for the eradication of tuberculosis in cattle have been started, and it is more than likely that more recent surveys in the countries mentioned would show a reduction in the number of cases of human tuberculosis due to the bovine organism. Blacklock (1947) gives some interesting figures in support of the hypothesis that the incidence of infection with the bovine type has decreased. A table illustrating the relative incidence of meningitis of human and bovine type in all age groups during the period 1907–37 indicates that of 560 cases 35 % were of bovine origin. During the period 1933–44, of 996 cases only 12·4 % were of bovine type.

A recent account of a group investigation in England and Wales (Report to M.R.C. 1949) gives figures for bovine type infection between 1943 and 1945. The report deals with non-pulmonary tuberculosis and shows that in England approximately 26 % of all non-pulmonary tuberculosis during 1943–5 was of bovine type. In Wales the figure was 17 %. In England the proportion of bovine type meningitis was 28·1 % and in Wales it was 10 %. It is pointed out with some reservations that the figures given for England do not differ greatly from those given by Griffith in 1938. The number of cases of bovine-type disease in Wales is considerably lower than in England, and this is explained by the greater proportion of attested herds in Wales, and the extent of pasteurization. The proportion of attested herds in 1944 in Wales was stated to be 21·3 % as against 5·4 % in England. It must be pointed out that this report deals with the period 1943–5. The figures for bovine-type infection are high, but it is probable that in the subsequent years the incidence may have decreased with the more widespread adoption of measures for the production of 'safe milk'.

It seems incontrovertible that the decrease in tuberculosis due to the bovine type in Northern Ireland must be due to the increased amount of pasteurization being

carried out. The scheme for Attested Herds was only started about 2 years ago and represents about 2·5 % of all dairy cows. Before the scheme was introduced there was a small number of grade A herds, but the amount of milk produced by them was an insignificant fraction of the total milk production. Figures kindly supplied by the Ministry of Agriculture (N.I.) show that there has been a marked increase in the production of liquid milk in the province during the past 10 years. In 1939 the figure was 15 million gallons and in 1949 it had reached 41·4 million gallons. In 1939 pasteurization was confined to a few dairies in the larger centres of population, and only represented about 7 % of the total. At the end of 1949 the amount of 'safe milk'—heat-treated and Grade A—amounted to about 90 % of the total liquid sales, and the amount is still increasing. Veterinary observers say that tuberculosis is still common among the cattle population. Kerr, Lamont & McGirr (1949) carried out a series of 600 detailed post-mortem examinations in dairy cows, during the course of an investigation into tuberculin sensitivity in the bovine. Of these 600 cows, 201 had some form of tuberculous lesion. This represents an incidence of 33 %. The period during which the examinations were carried out was 1945–8. Approximately 7·5 % of churn samples of milk are found to contain tubercle bacilli. The figures are very similar to those given for England and Scotland (Francis, 1947). It would appear that ample opportunity exists for infection from raw milk, so a decrease in infection must be due, to a large extent, to increased pasteurization. Another factor that must be considered is the very striking decrease in the number of deaths from all forms of tuberculosis in Northern Ireland during the past 10 years. In 1939 the death-rate per 100,000 was 85, and in 1949 it had fallen to 59. It is sometimes argued (though there are many objections to this argument) that an apparent low rate of bovine type tuberculosis is the result of a high pulmonary rate, the greater opportunity for infection from open cases causing a higher percentage of non-respiratory cases due to the human type. In the face of a falling death-rate for all forms, it would seem that the number of contact cases would tend to decrease.

The pattern of tuberculous infection is changing. In all civilized countries the mortality figures are declining. The argument used to be put forward that infection with the bovine organism conferred a measure of immunity against infection with the human type. In a recent paper, Francis (1950) suggests that the eradication of bovine tuberculosis in cattle may deprive the individual of protection against a more dangerous respiratory infection in later life. There is some evidence that this may happen. It is to be hoped, however, that controlled immunization with B.C.G., improved case finding and the introduction of more therapeutic remedies, together with the elimination of the bovine type of infection should still further lower the mortality from this disease.

SUMMARY

A survey of the extent of bovine type human tuberculosis in Northern Ireland has been made from unselected material during the past 5 years.

Only one case of bovine type pulmonary tuberculosis was found out of more than 1000 cases.

The proportion of bovine type cases in the non-respiratory group was 4·3%; 3·3% of meningitis cases were of bovine origin.

A marked decline in the extent of bovine type infection is shown to have occurred during the past 10 years. This is believed to be due to a great increase in the pasteurization of milk during this period. Milk was a particularly valuable food during the war, and pasteurization facilitated its distribution.

I would like to acknowledge my indebtedness to Dr B. R. Clarke, Director of Tuberculosis Services, who made it possible for me to carry out this investigation and who gave me invaluable help in the preparation of this paper; to Dr H. G. Lamont, M.R.C.V.S., and Dr W. R. Kerr, M.R.C.V.S., for much of the veterinary information; to Dr F. F. Kane for unfailing assistance over several years in the collection of these cases; to the many doctors throughout the province who sent me material; to my senior technician, Mr J. H. McWatters, and the staff of the laboratory for willing and efficient technical help.

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(*MS. received for publication* 22. VII. 50.)