NON-PULMONARY TUBERCULOSIS CAUSED BY THE BOVINE TYPE OF TUBERCLE BACILLUS IN CHILDREN IN EIRE

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Introduction

The object of this paper is to give an indication of the extent to which the bovine type of tubercle bacillus is the causal organism of non-pulmonary tuberculosis in children, 0–14 years of age, living in Eire (formerly the Irish Free State). Only two studies, as far as the writer is aware, have been reported with reference to the subject of human tuberculosis of bovine origin in Eire. O'Kelly (1918) isolated strains of tubercle bacilli from thirty cases of non-pulmonary tuberculosis of all ages in Dublin City hospitals, three of the cases yielding bovine strains. Seven strains were from children under 15 years of age, and consisted of four human and two bovine strains from cervical glands and one human strain from bone tuberculosis. The number of cases was too small to allow any generalization to be made, but "they show that bovine tubercle bacilli do give rise to human tuberculosis in Ireland (i.e. Eire) as elsewhere" (O'Kelly). Cumming (1935) reported the result of typing, during 1931–3, 320 Irish cases of pulmonary tuberculosis in patients of all ages drawn from both rural and urban areas, and partly selected by aiming at the collection of "cattle contacts". All were patients in three Irish sanatoria. Of the 320 cases no less than ninety-seven were described as "cattle contacts". Not one yielded a bovine strain. In a series of 1502 English cases, examined at the same time, thirty-four bovine strains were obtained, thirteen of which came from twenty-nine cases of "cattle contacts" from northern England. Among eleven cattle contacts in the south of England, however, no bovine strains were found. The absence of bovine strains among the ninety-seven Irish "cattle contacts" may possibly be due to regional factors, since the distribution of tuberculosis in cattle varies greatly in the counties of Eire.

There is very little precise official published information available regarding the incidence of tuberculosis in cattle in Eire. During the years 1929–38 the incidence of tuberculosis in cows slaughtered in the Dublin City Abattoir fluctuated between 31 and 33% (*Report of Medical Officer of Health for Dublin for* 1938). The cattle killed were drawn from different parts of Eire, but were not necessarily representatives of the whole country. Moreover, the cattle killed in the abattoir are usually old and unproductive, and since tuberculosis in cattle occurs chiefly in old cattle, the incidence of tuberculosis in the whole milch cow population is almost certainly lower than 31%. In the experience

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of some veterinary surgeons who were consulted the general incidence is probably not more than 25%, except in the province of Munster where the incidence may be 30–40%. In some counties the disease is very infrequent.

The amount of safe milk consumed in Eire is, on the whole, negligible. There are 8000 registered dairies producing milk for sale. There are only thirty-five tuberculin-tested herds in the country and of these thirty-three are in County Dublin, but they supply Dublin with only a fraction of its total daily requirements, namely, a few thousand gallons out of 40,000 used. Only fifteen dairies hold licences to supply Standard, i.e. Accredited Milk, three being in Dublin, eight in Limerick, three in Cork and one in Leix. Pasteurized milk is sold in only five centres in the country. In Dublin it forms about one-third of the city's supply, and in Cork about one-fifth. In the other three places the amount produced is negligible.

As regards the extent of infection of milk in Eire, Bigger (1921) found that four (all from Dublin City) out of fifty milks produced tuberculosis in guinea-pigs. The Irish Government Veterinary Research Laboratory (Interdepartmental Committee Report 1928) reported the isolation of tubercle bacilli from six (2·66%) out of 225 samples of milk, each sample being from the bulked milk of one herd. Cussen (1936) found that ten (6·4%) out of 156 samples of market milk in the County Borough of Cork contained living tubercle bacilli.

RESULTS OF THE INVESTIGATION

Strains of tubercle bacilli were isolated from fifty cases of non-pulmonary tuberculosis collected between January and December 1938 and their types were determined. The tuberculous material was derived from cases of cervical adenitis, bone and joint disease, meningitis and primary abdominal tuberculosis. The majority of the patients appear to have been members of working-class families. Thirty-seven of the strains were found to be of the human type and seventeen (34%) of the bovine type.

The cases examined were selected to the extent that they were limited to children 0–14 years of age. The gland and joint cases were largely confined to those in which abscesses had occurred from which pus was aspirated. Material removed by surgical operation was infrequent because of the modern conservative methods of treatment and not because of scarcity of cases. There was an additional selective factor (Thompson, 1939), namely, that the majority of the cases were children who were in-patients in hospitals. The series did not include a representative proportion of the larger number who attend dispensaries as out-patients.

In Table I, the fifty strains are analysed according to the variety of tuberculosis in which each occurred and according to the age of the patients, in most instances at the time of first appearance of clinically detectable disease. It is recognized that the division of the small number of cases in each variety into quinquennial age groups is not statistically justifiable.

Table 1. *Showing the relative proportion of human and bovine strains of tubercle bacilli in non-pulmonary tuberculosis in children 0–14 years of age in Eire*

Variety of tuberculosis	No. of strains	No. of strains in each age group								0–14 years Percentage of bovine strains
		0–4 years		5–9 years		10–14 years		5–14 years		
		Human	Bovine	Human	Bovine	Human	Bovine	Human	Bovine	
Cervical glands	14	0	4	2	5	2	1	4	6	71·4
Bone and joint	22	7	4	8	0	3	0	11	0	18·2
Meningitis	12	6	1	2	2	1	0	3	2	25·0
Primary abdominal	2	1	0	—	—	1	0	1	0	—

Of the fourteen cases of cervical adenitis from which strains of tubercle bacilli were obtained ten (71·4%) were caused by strains of the bovine type; of the twenty-two cases of bone and joint disease four (18·2%) and of twelve cases of meningitis three (25%). Material from two cases of primary abdominal tuberculosis yielded human strains.

Twenty-five of the cases were from Dublin City; seven cases of cervical adenitis from five of which bovine strains were obtained, six cases of bone and joint disease all caused by human strains, eleven of meningitis from three of which bovine strains were obtained, and one case of primary abdominal tuberculosis caused by a human strain. From rural areas in County Dublin, human strains were obtained from two cases of bone and joint disease. The remaining twenty-three strains, nine of which were bovine, were obtained from twelve counties other than Dublin, namely, Cork, including Cork City (2H, 4B), Wexford (1H, 2B), Mayo (1H), Louth (1H), Tipperary (1H. 1B), Kilkenny (2H), Kerry (1H), Cavan (1H), Kildare (2H), Limerick (1H, 1B), Waterford (1B) and Offaly (1H).

The epidemiological significance of these findings

The statistics recorded in Table 1 merely denote the ratios of bovine to human strains of tubercle bacilli in the varieties of tuberculosis examined. Taken alone, such ratios do not indicate the extent of infection caused by the bovine tubercle bacillus in these varieties of disease, while their full significance can be realized only when they are interpreted in such terms. Unfortunately it is not possible to determine accurately the total infection of bovine origin from these findings, because the number of cases examined is very small and cannot be regarded as representative of Eire, and because there are no exact statistics available in Eire for the separate varieties of non-pulmonary tuberculosis with which they may be correlated. Notifications are unreliable as an index of the number of new cases occurring in a year because only "open" cases are required to be notified, and because official figures for the number of cases of non-pulmonary tuberculosis treated each year under approved schemes

do not include many cases treated in voluntary and other hospitals, which do not come under such schemes. A number of important sources of error arise in collating the ratios with mortality rates, and moreover the latter do not in the slightest degree reflect the total number of non-fatal cases of non-pulmonary disease under treatment in any given period. The incidence of non-pulmonary tuberculosis in children in Eire is high, as indicated by the fact that in 1936 in a child (0–14 years) population of 820,000 there were 2942 cases of non-pulmonary tuberculosis treated under approved tuberculosis schemes (*Report of the Department of Local Government and Public Health for* 1937) and, though the number of cases examined is small, it is justifiable to infer that a considerable amount of suffering and ill-health must be caused in children in Eire by the bovine tubercle bacillus. For reasons stated previously, the number of cases treated underestimates considerably the true incidence of non-pulmonary tuberculosis in children in Eire.

It might be considered desirable to compare the extent of human non-pulmonary tuberculosis of bovine origin in Eire with that in other countries. This, however, has not been attempted, because the number of cases is small and therefore not representative, and because the only relevant figures available for comparison of different countries are ratios of bovine to human strains. These ratios are only of real value for the purpose of comparison when they are considered in relation to the total incidence of non-pulmonary tuberculosis. They then yield comparable estimates of the frequency of infection of bovine origin in the populations considered. The same applies to comparisons between different age groups. This view is mentioned because it is so frequently overlooked in studies on the epidemiology of human tuberculosis of bovine origin. The extent of tuberculosis in cattle with the consequent extent of infection of milk, the amount of unboiled and non-pasteurized milk from infective cattle consumed per head, the method of distribution of milk and the incidence of pulmonary tuberculosis of bovine origin all influence both the total incidence of non-pulmonary tuberculosis caused by bovine strains and the ratio of bovine to human strains. The incidence of pulmonary tuberculosis due to human strains has two effects, namely, it produces a high incidence of non-pulmonary tuberculosis due to human strains, and, as a result, a reduction in the proportion of bovine to human strains in all varieties of non-pulmonary disease. It is difficult to determine the extent to which this lowering of the ratio of bovine to human strains would be accompanied by an absolute reduction in the incidence of non-pulmonary tuberculosis due to the bovine tubercle bacillus. Members of households, in which there is a case of pulmonary tuberculosis due to human strains, may acquire immunity against bovine strains as a result of earlier exposure to infection by human strains. In addition, in pulmonary tuberculosis households the consumption of milk per head may be lower than that in other homes, so that there may be less risk of infection of bovine origin (Nat. Tuberc. Ass. 1933). My view is that these two effects of the incidence of pulmonary tuberculosis due to human strains, which

tend to reduce the incidence of infection by bovine strains, are of small importance. Therefore, I think that, while a high incidence of pulmonary tuberculosis due to the human tubercle bacillus must lower the ratio of bovine to human strains in non-pulmonary disease, it has little effect on the absolute extent of non-pulmonary disease caused by the bovine tubercle bacillus. If this argument is correct, it is obvious that, when comparing the incidence of human infection of bovine origin in various areas or age-groups, the differences shown by morbidity rates may be quite unlike those indicated by mere comparison of ratios of bovine to human strains. Only rates of morbidity due to the bovine bacillus, if they were determinable, would provide a fair comparison between different areas or age groups.

The writer believes that if these views are correct and are applied, it may be found in many places that the bovine tubercle bacillus causes disease in persons over 15 years of age much more frequently as compared with children under 15 than is suggested from a comparison only of ratios of bovine to human strains occurring in different age groups, and that townspeople may not actually be less frequently victims of bovine strains than persons living in rural areas, as is suggested by studies in which the proportions of bovine strains in rural and urban cases are reported. Moreover, by the application of these views, it may be possible to obtain a fairer evaluation of the effects of measures undertaken to render milk safe which apply to urban and not to rural areas, especially when the urban areas have a high incidence of pulmonary tuberculosis due to the human type of tubercle bacillus.

Technique and bacteriological observations

Media. Only three media were used throughout this investigation, namely, plain egg (2 volumes egg + 1 volume normal saline), 5% glycerine egg (5 c.c. glycerine added to every 95 c.c. of the egg-saline mixture used for plain egg medium) and 5% glycerine litmus potato according to formulae prescribed by A. S. Griffith. These three media were found adequate for typing all the strains isolated. Primary cultures were grown on plain and glycerine egg and differential cultures were made on all three media. Plain egg and glycerine egg tubes when used together were invariably made from the same batch of eggs which were never more than 3 days old.

Every specimen examined was planted on egg medium and injected into a guinea-pig. Almost every specimen from glandular disease and bone and joint disease was treated with an equal amount of 5% KOH for $\frac{1}{2}$ hr. at 37° C. and, for culture, sown directly on to medium, but neutralized to litmus with 6% (by wt.) H_2SO_4 before injection into guinea-pigs. Solid material was first ground, suspended in saline, filtered through gauze and the filtrate treated and used. Cerebrospinal fluid was usually tested without treatment or centrifuging.

Criteria for typing of strains. Every strain was typed by the correlation of findings on cultural examination and of virulence for rabbits, and for guinea-

pigs when attenuation might have been suspected. The chief differential cultural criteria were the amount of growth on glycerine egg compared with that on plain egg, and the amount of growth on potato. Broadly speaking strains which grew more luxuriantly on glycerine egg than on plain egg and with luxuriant warty or wormcast growth on potato were classed as human type, provided of course virulence tests supported the classification. Strains which grew less actively on glycerine egg than on plain egg and produced only poor growth or none at all on potato were classed as bovine. Considerable attention was paid to colonial characters. Strains were never typed on primary culture alone. Differential subcultures, and animal inoculation, were always repeated until the type was definitely established. Strains kept in stock were invariably grown on glycerine-free plain egg slopes, glycerine egg being used only for differential subcultures. It was not found necessary to complete the typing within the first four generations as was done by Blacklock (1932) and Park & Krumwiede (1910). Bovine strains did not acquire any striking increase in ability to grow on glycerine egg when kept in stock on plain egg for a long period. Those few bovine strains which grew somewhat better in later than in earlier culture on glycerine egg appeared to do so not because they had acquired the power to utilize glycerine but because they had become better adapted to grow on all artificial media.

Every strain was injected into at least one rabbit. Strains believed from their cultural characters to be human were injected subcutaneously between the scapulae of the rabbits in a dose of 10 mg. (moist wt.), and strains regarded on culture as bovine were injected both subcutaneously in dose of 10 mg. and intravenously in dose of 0·001 mg. The latter dose gave a rapid preliminary diagnosis since only a bovine strain in this dose will cause severe fatal tuberculosis in a rabbit. The criteria followed for differentiation between human and bovine strains by injection of rabbits were those described by Griffith (1911). Rabbits of not less than 1500 g. were used. Cultures used for differential subcultures and for animal inoculation were always actively growing and not more than 6–12 days old.

GENERAL OBSERVATIONS

A. *Cultures*

(1) *Human strains.* On primary culture it was not found possible to identify human strains by the amount of growth on glycerine egg compared with plain egg. Only seventeen strains grew both on plain and glycerine egg in primary culture. In not one of these instances was growth observed earlier on glycerine egg than on plain egg, while in three cases growth appeared later on the former. In these seventeen strains when slopes were examined not later than 6–7 weeks after inoculation, growth on glycerine egg was superior to that on plain egg only in three instances, while in twelve it was inferior both in size and number of colonies. There was no instance of primary growth on glycerine egg with none on plain egg.

In differential subculture a lag phase in growth on glycerine egg as compared with plain egg was very frequently observed, in a number of instances lasting as long as 2–3 weeks, after which period the superiority of growth on glycerine egg became obvious even to the naked eye. It appears that even human strains are unable to utilize glycerine readily when the number of bacilli inoculated is relatively small as in making differential subcultures in the writer's technique, and when the glycerine is present, at any rate in a concentration of 5 %. Small numbers of bacilli of the human type may, in fact, apparently suffer temporary inhibition of growth. In primary culture of three human strains on plain egg green pigment was observed. In two of these cases the pigmentation disappeared after a short period.

(2) *Bovine strains.* Two bovine strains were of interest. Both in the first differential subculture approached Class II bovine strains in their growth on glycerine egg. In the second subculture, the strains grew less readily on glycerine egg, and in the third subculture growth almost entirely failed to appear on glycerine egg and the strains became typically Class I.

(3) *Differentation of types by colonial characters and general appearances of growth.* On primary culture considerable variation was found in the character of the colonies produced by both human and bovine strains on plain and 5% glycerine egg. It was found that while certain features appeared to be more frequent in colonies of one type than in those of the other, colonial appearances on primary culture did not provide constant or reliable differential features. In subcultures on plain egg each type in its colonial characters showed marked uniformity and marked distinctiveness from the other type, so that the appearance of colonies gave a reliable indication of type. This is not intended to imply that rabbit inoculation can be dispensed with if attention is paid to colonial features. Differential subcultures were sown by means of 2–3 drops of a suspension of 1 mg. of growth in 3–4 c.c. saline pipetted on to the slopes. At the end of 4 weeks, in subcultures of human strains on plain egg, slopes were covered with growth dull, grey, dry, frosted and finely granular to the naked eye. With $\times 6$ lens colonies were seen to be closely set, discrete and confluent, low, convex, dry, grey, frosted and with glazed spots. Most had a peripheral zone, flat, dry, grey, frosted. At the end of 4 weeks in subcultures of bovine strains on plain egg, slopes were covered with a shining, translucent, finely granular layer of growth. With $\times 6$ lens colonies were seen to be discrete and confluent, smooth, translucent, conical and pyramidal, and many with ripples arranged radially giving a limpet-shell appearance. Sometimes convex translucent smooth colonies were numerous. Later, in the centre of the conical colony a smooth, convex elevation often appeared, translucent or butyrous and increasing in size until it occupied most of the colony. The characters of growth of each type in subculture on glycerine egg were also found to be fairly uniform, but they gave a much less reliable guide to type than the growth on plain egg.

B. *Animal inoculation*

Human strains. Dose = 10 mg. subcutaneously. Five out of thirty-nine rabbits injected with strains believed from their cultural characters to be human type died spontaneously at 70, 36, 103, 80 and 132 days after injection. The first three of these died from intercurrent infection, the degree of tuberculosis being insufficient to have caused death. The last two, which were injected with the same strain are discussed in detail below as they presented difficulty in typing. The remaining thirty-four rabbits were killed with chloroform, but never earlier than 14 weeks after injection. All the rabbits, except the two discussed later, showed minimal and retrogressive disease characteristic of infection by human strains. Lesions were usually limited to the site of injection, the lungs and kidneys; the lungs were always collapsed and small, the lesions being usually few in number and 1–2 mm. in diameter but sometimes quite numerous or larger. The most frequent lesions in the kidneys were irregular, shallow, purplish pits on the surface, varying from $\frac{1}{2}$ to several mm. in diameter and from one or two to a considerable number in each kidney.

Bovine strains. (i) Dose = 10 mg. subcutaneously. Nineteen rabbits were injected subcutaneously with strains believed from their cultural characters to be bovine, and all were allowed to die spontaneously. The duration of life varied from 38 to 111 days. In all cases death was due to the severity of the tuberculosis. The lungs were always extensively diseased and usually almost completely filled the chest. The kidneys showed from a few to a considerable number of pale cream, almost round foci varying from $\frac{1}{2}$ to 5 mm. diameter and often projecting considerably off the surface of the kidneys. The spleen often was moderately enlarged and showed a variable number of pinhead pale foci. Sometimes it showed no naked-eye disease. There were usually no visible tuberculous foci in the liver. The regional (axillary) lymph glands were diseased, though never severely, on one or both sides in all except three instances. The popliteal lymph glands on one or both sides showed macroscopic foci in all except three rabbits. These glands were enlarged in eight rabbits injected with human strains but they showed no macroscopic disease.

(ii) Dose = 0·001 mg. intravenously. Twenty-two rabbits were injected intravenously with suspected bovine strains. The rabbits injected with the first eleven strains isolated died in 24–59 days, and the remainder in 41–105 days. The two groups of rabbits were obtained from different sources and appeared to be of a different breed. All died from tuberculosis. The character of the disease was similar to that resulting from the subcutaneous injection of 10 mg. of culture of bovine strains, but the spleen and liver showed macroscopic disease more frequently.

Only one strain presented difficulty in typing. This strain, derived from cerebrospinal fluid and typed as human, in two out of six rabbits injected produced changes compatible with the presence of small numbers of bovine tubercle bacilli in the cultures injected, namely:

Rabbit 1. Subcutaneous injection of original strain obtained through guinea-pig. Died in 80 days; loss of wt. $2\frac{3}{8}$ lb.; local lesion present, axillary glands enlarged but no naked-eye foci seen, a few pin-point pits in each kidney, lungs slightly enlarged and studded with cream-coloured nodules 0·5 cm. diameter.

Rabbit 2. Subcutaneous injection of strain isolated on plain egg from lung of rabbit 1. Died in 132 days; loss of wt. $1\frac{5}{8}$ lb.; local lesion present, marked disease of the axillary glands, popliteal gland enlarged but without visible foci, kidneys showed a few pin-point pits with heads of pus in their floor, the lungs almost filled the chest and were extensively diseased with cream-coloured nodules 0·5 cm. in diameter some of which were cavities filled with pus. Smear from cavity—innumerable long acid-fast bacilli.

The strain was characteristically human in type on culture both in regard to the character of the colonies and to the luxuriance of growth on glycerine egg compared with plain egg and on glycerine potato, except for the fact that on one slope of primary culture one colony resembled closely the appearance of one variety of colony seen frequently on plain egg cultures of bovine strains. This colony produced typical eugonic growth in all subcultures except the first, and a subculture from it caused minimal disease in the rabbit, typical of a human strain. The lung lesions of rabbit 2 were fully investigated by culture and rabbit inoculation. All attempts to isolate a bovine strain in pure culture from this strain failed. The strain was of normal virulence for the guinea-pig.

Summary

1. The types of strains of tubercle bacilli isolated from fifty cases of non-pulmonary tuberculosis in children, 0–14 years of age, in Eire have been determined. In cervical gland disease ten (71·4%) out of fourteen strains were bovine, in bone and joint disease four (18·2%) out of twenty-two were bovine and in meningitis three (25%) out of twelve were bovine. Only two cases of primary abdominal tuberculosis were examined. Both yielded human strains.

2. The difficulties in assessing the true significance of the results recorded are briefly discussed.

3. The technique used and some bacteriological observations made are described.

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