

## HUMAN PULMONARY TUBERCULOSIS OF BOVINE ORIGIN IN GREAT BRITAIN

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### INTRODUCTION

At the International Congress on Tuberculosis held in London in 1900 Koch announced his conclusion that infection of human beings with bovine tubercle bacilli was a very rare occurrence. He estimated the extent of the infection by the milk and flesh of tuberculous cattle as hardly greater than that of hereditary transmission, and he therefore did not consider it necessary to take any measures against it. The results of the official inquiries into the relation of human and bovine tuberculosis which were at once instituted in Great Britain, Europe and the United States, and of those which have since been made by individual investigators, have shown how greatly, one might say how tragically, mistaken Koch was in his opinion that bovine tuberculosis was not a danger to man. In numerous instances in this country, in America and on the Continent bovine tubercle bacilli were obtained from cases of human tuberculosis, many of which were fatal. There were, however, wide differences in the proportional frequencies of bovine infection in the different countries and even in different parts of the same country. In Britain, where investigations have been practically continuous since the appointment of the Royal Commission on Tuberculosis in 1901, the proportional frequency of bovine infections was much higher than in any of the continental countries or in the United States of America, while in Scotland the incidence was even higher than it was in England.

In the earlier inquiries investigators naturally gave preference to those cases in which the portal of entry of the bacilli could be presumed to have been the alimentary canal. Thus it came about that for some time little attention was paid to the more prevalent and epidemiologically more important pulmonary forms of the disease, and in 1908, at the International Congress on Tuberculosis held at Washington, in response to Koch's demand that cases of pulmonary tuberculosis due to bovine tubercle bacilli should be brought forward, only two possible instances of the kind could be quoted.

Arloing gave details of a case which he had himself investigated. From the contents of a cavity in the lung he cultivated tubercle bacilli which possessed the cultural characters and the virulence for the calf of typical bovine bacilli. A case, reported by de Jong-Stuurman in a peasant woman, aged 27 years, with numerous tubercle bacilli in her sputum, was also mentioned. A glycerin serum culture of the bacilli was luxuriant but produced fatal general tuberculosis in three calves, one inoculated intravenously. Only one specimen of sputum was investigated and no rabbits were tested. The results of the experiments on calves are evidence that bovine tubercle bacilli were present in the sputum, but the fact that the culture was eugonic points strongly to the presence in it of human tubercle bacilli also. A single finding of bovine tubercle bacilli in the sputum of a phthisical person was, therefore, not proof of a causative relationship with the pulmonary disease. This objection, advanced by Koch, was certainly valid in cases where the bovine bacilli were present in the sputum in small numbers and demonstrable only after injection into

animals but not, as in the majority of our cases, where the sputum showed numerous tubercle bacilli microscopically and yielded a pure culture of bovine bacilli. In a case of the latter kind a single demonstration of bovine type bacilli alone in the sputum of a case of pulmonary tuberculosis would be sufficient ground for concluding that they were the cause of the disease, since it would clearly be impossible for so many bovine bacilli to be contributed accidentally by milk or its products to the sputum.

#### INVESTIGATIONS IN GREAT BRITAIN

The inquiry in Great Britain was begun in 1907 by one of us (A.S.G.) when working for the Royal Commission on Tuberculosis and falls into two distinct periods. The first, lasting about 15 years, was notable for the small proportion of persons found to be expectorating bovine tubercle bacilli with the sputum, namely, only four out of 266 cases, two from the south of England (out of 164 cases) and two from Scotland (out of 102 cases). The second period started about the year 1921, when one of us (W.T.M.) began to make for serological purposes a collection of 100 strains. Of these strains two were of the bovine type (both attenuated) and one was a mixture of human and bovine tubercle bacilli; he subsequently found bovine tubercle bacilli in the sputum of three cases of bone tuberculosis of bovine type and in consequence determined to type the tubercle bacilli in the sputum of all cases admitted to Glenlomond Sanatorium. Later, Griffith (1930 and after), and Griffith & Smith (1940) began the survey of other regions in Scotland, and Cumming *et al.* (1933) and Llynn & Cutbill extended the work which was then being done by one of us (A.S.G.) on a small scale in England. Cumming (1935) also investigated cases in Wales and Eire.

The results of these separate investigations have been published in different reports together with clinical particulars of all the cases, except those examined by Munro in Scotland and by Griffith in England which are now for the first time summarized. Outstanding features of this last series are the numerous cases giving a history of previous glandular tuberculosis and the high proportion of young people, especially females.

The numbers of cases investigated by the different observers are given in Table 2.

#### TYPE DETERMINATION

The methods employed at the several laboratories for obtaining the tubercle bacilli in culture from sputum and ascertaining their bacteriological characteristics have differed to some extent and are fully described by the various authors.

The main reagents for destroying organisms in sputum other than tubercle bacilli have been sodium and potassium hydroxide in 4 or 5% solution (2–2.5% in the sputum mixture). Antiformin was employed exclusively at first, and sulphuric acid was used occasionally in recent investigations, especially when the sputum was grossly contaminated. It should be mentioned that the alkaline mixtures were sown on the egg media without neutralization.

In Cambridge cultural characters were determined in primary culture on egg and glycerin egg and in sub-

cultures on these media as well as on bovine serum and glycerin potato. At other laboratories the number of media used was fewer. According to their cultural characters the strains have fallen into three main groups—eugonic human, dysgonic human and dysgonic bovine. Five of the bovine strains were found to be associated with human tubercle bacilli and another came from a patient who was also expectorating a novel kind of tubercle bacillus which has so far defied repeated attempts to cultivate it on the usual and special media (Griffith, 1940).

In the early part of the investigation all strains, whether eugonic or dysgonic, were tested as to their virulence for the rabbit. After a time, as the scope of the work widened, it became impracticable to test all the eugonic strains, and virulence tests were restricted almost entirely to strains which produced dysgonic growths on glycerin media, that is to say, to strains typically bovine in cultural characters and of the dysgonic human variety. Eugonic strains were, however, occasionally tested, particularly those which were peculiar in any way, for example in showing a large proportion of short or branched forms in the sputum, or in forming rather flatter colonies than usual in primary cultures. The result of this procedure has been that all the bovine strains, most of the dysgonic human strains and a relatively small number of eugonic strains have been tested on the rabbit. A few of the bovine strains were also tested on the calf and (or) the goat (Griffith, 1911, 1914).

*Dysgonic bovine strains.* The number of persons found to be expectorating tubercle bacilli of the bovine type with the sputum was 241. In order to furnish satisfactory proof from the examination of sputum that a case of ulcerative pulmonary tuberculosis is caused by the bovine tubercle bacillus it is necessary to show that this organism is the only type of tubercle bacillus present and that it can be found on repeated examination. We were not able to repeat the investigation in every case, owing either to the death of the patient before there was any suspicion that the bacilli were bovine or to the cessation of expectoration due to artificial pneumothorax, but except in these few cases one or more further specimens of sputum have been examined. All the strains exhibited the cultural characters of bovine tubercle bacilli and were tested on the rabbit, either by intravenous inoculation of 0.01 mg. or less or by subcutaneous inoculation of 10 mg. or less of culture, some being tested by both methods.

Almost all the bovine cases from the Glenlomond, Grassington and Cheshire Joint Sanatoriums were also investigated at Cambridge by A. S. Griffith—cultures as well as sputum being sent to him for examination—where the almost invariable rule was to inject at least one strain from each case subcutaneously into a rabbit. At the other institutions the usual method of test was the intravenous inoculation of 0.01 mg. Thus it has come about that the virulence of most of the bovine strains has been tested on the rabbit both by subcutaneous and by intravenous inoculation. With comparatively few exceptions (p. 234) all the patients yielded strains which were fully virulent for the rabbit. The strains inoculated intravenously in a dose of 0.01 mg. or less caused the death of the rabbit from severe general

miliary tuberculosis. After 10 mg. (or less) subcutaneously the rabbits died of generalized tuberculosis identical with that produced by similar inoculation of bovine strains of direct bovine origin.

As previously stated the different strains from the same patient were identical with one another and with standard bovine bacilli, both in cultural characters and in virulence. For the most part the intervals between the first and last specimens of sputum in each case were comparatively short but sometimes they were long. We quote two examples of long intervals:

Female, aged 27 years, with weak chest since school days. A culture from the sputum was dysgonic and fully virulent for two rabbits. Twenty-two months later a specimen of sputum again yielded a fully virulent bovine strain. The tubercle bacilli which had resided many years in the patient before the first strain was obtained were typically bovine after a further period of residence of 22 months.

Male, aged 17 years, cervical glands at 12 years. A culture from the sputum was dysgonic and fully virulent. Subsequently several cultures were obtained, the last 10 years after the first. This was as dysgonic as the first strain and fully virulent for rabbits.

The identity in virulence of the last with that of the first strain in each of these cases was in great contrast to what has been noted in lupus. In seven instances of that disease the virulence of the bovine strains, originally full, became lowered as a result of further residence in the skin tissues for periods of from 1 to 6 years.

*Attenuated bovine strains.* The sputum of nine patients yielded strains which were identical in cultural characters with bovine strains, but in virulence for rabbits and for guinea-pigs (where these animals were tested) fell in varying degree below that of standard bovine strains.

*Munro 1.* C.T., male, aged 21 years. Virulence of culture for rabbits slightly below 'standard bovine' (Griffith, 1930, case 8, p. 1160).

*Munro 2.* I.W., female, aged 29 years. Virulence of culture for rabbits distinctly below 'standard bovine' and not increased by passage through rabbits (Griffith, 1930, case 9, p. 1160).

*Munro 4.* Male, aged 31 years. Ten days before death patient coughed up matter, supposed to have come from a dorsal spinal abscess, containing tubercle bacilli in abundance. Their virulence for rabbits was distinctly below 'standard bovine' but higher than 'standard human'. Their virulence for guinea-pigs was distinctly below that of 'standard bovine' or 'standard human' (Griffith, 1930, case 6, p. 1158).

*Munro 28 (540).* Male, aged 29 years. This patient, like the last, had tuberculosis of the lower dorsal spine of long duration. Two strains from sputum (interval 83 days) were moderately attenuated for the rabbit and one strain also for the guinea-pig (Griffith, 1940).

*Munro 29.* Mrs T., aged 28 years. Six specimens of sputum were examined; they contained numerous tubercle bacilli, beaded, vacuolated and for the most part long. Six strains of culture were obtained, all dysgonic bovine (class I). Virulence was tested for the goat, rabbit, guinea-pig and vole. Two goats were inoculated intramuscularly (20 and 10 mg.) and killed 147 and 153 days later. Each showed a local lesion,

caseo-calcareous glandular tuberculosis and a few minute calcareous foci in the mediastinal glands; the lungs of one goat (10 mg.) showed a moderate number of miliary calcified tubercles. Eleven rabbits were inoculated. Seven were given 1.0, 0.1 or 0.01 mg. intravenously and died in from 67 to 103 days, and two were given 2.0 and 15.0 mg. subcutaneously and died in 63 and 65 days, both showing chronic general tuberculosis. The last rabbit, inoculated subcutaneously, had a local abscess, large and caseous adjacent and popliteal glands, moderate miliary tuberculosis of lungs and extensive caseation of epiphyses of both knee-joints. A culture from a popliteal gland of this rabbit injected in a second rabbit (10.0 mg. subcutaneously) caused death in 182 days with moderate tuberculosis, the left knee-joint and end of femur being extensively caseous (the disease having extended to the joint through the condyles) and an eye showing a caseous nodule in the sclerotic. A culture from the second rabbit injected into a third rabbit (10.0 mg. subcutaneously) caused death in 152 days, with a local abscess and slight tuberculosis of knee-joints and femur marrow. There was, therefore, no increase of virulence of the strain after passage through two rabbits. Joint and ocular tuberculosis have never been observed in a rabbit after subcutaneous inoculation of tubercle bacilli of human type. Six guinea-pigs were inoculated, one, given 0.1 mg. intraperitoneally, died in 73 days of chronic atypical generalized tuberculosis and five, given 0.1 mg. each subcutaneously, died or were killed in 137-312 days, and showed slight generalized tuberculosis, atypical or obviously retrogressive. One had indefinite lesions except locally. Vole 72 was given 1.0 mg. subcutaneously and killed 54 days later. Post-mortem there was an ulcerated local lesion; the inguinal and iliac glands were large and caseous; all other lymph glands were enlarged and either wholly or partially (t.b. + + +) caseous. The spleen was enlarged and one minute caseous tubercle (t.b. +) and a few glassy foci were found in the lungs. Cultures from the liver and spleen showed moderately numerous colonies.

*Munro 30.* Mr T., aged 35 years, husband of last patient. Five specimens of sputum examined, and five strains obtained, morphologically, culturally and in pathogenic properties like those of Mrs T. One goat given 10.0 mg. intramuscularly and killed 153 days later, showed retrogressive local tuberculosis and a few calcareous foci in mediastinal glands. Seven rabbits given 1.0, 0.1 and 0.01 mg. intravenously died 19-94 days later of generalized tuberculosis, one of severe general miliary tuberculosis (19 days, 0.1 mg.), the others of chronic general miliary tuberculosis; the disease in some of the latter was widespread, affecting severely the joints, eyes, nasal cavities, testes and skin, mainly of the back where it was grasped when handling the rabbit. Of three rabbits given 3.0, 3.0 and 10 mg. subcutaneously, two were killed after 92 and 103 days respectively, one showing local tuberculosis, the other local tuberculosis and slight tuberculosis of lungs and kidneys; the third died after 126 days of moderate generalized tuberculosis, the knee-joints and one elbow-joint being affected. Four guinea-pigs given 0.1 mg. intraperitoneally died of atypical generalized tuberculosis in 34-39 days and nine with similar doses subcutaneously

died or were killed in from 90 to 298 days of mild generalized tuberculosis, which in one or two was very slight. Vole 69 given 1.0 mg. of strain 5 subcutaneously died 39 days later. Post-mortem there was a local caseous ulcer; the right inguinal gland was very large and caseous; the left inguinal, iliac and sacro-iliac glands showed partial caseation; the ventral, mediastinal and other glands minute caseous foci; liver scattered minute foci; the lungs four or five glassy tubercles; the kidneys one minute opaque focus. Microscopically, glands showed numerous tubercle bacilli and the organs small numbers. Cultures from liver yielded a moderate number of colonies.

*Comment on Munro 29 and 30* (Griffith & Munro, 1935). The strains (three more than were reported in 1935) from the two cases were identical with bovine tubercle bacilli in cultural characters but fell distinctly below the standard of the type in virulence for the goat, rabbit and guinea-pig. The results of the experiments on guinea-pigs taken in conjunction with those on rabbits, namely, pathogenic effects intermediate between those of standard bovine and standard human strains, led to the conclusion that the strains, whose cultural characters were unmistakably bovine, were 'attenuated'. The results of the experiments on voles supported the conclusion that the tubercle bacilli were of the bovine type.

*M. 903.* Tests for virulence were made on four rabbits. One given 0.001 mg. intravenously died in 47 days of general miliary tuberculosis, moderate in lungs, slight elsewhere: Two given about 5 mg. subcutaneously were killed 62 and 185 days later and showed slight tuberculosis of lungs, and a kidney of one was rather severely affected. The bacilli recovered in culture from the affected kidney and inoculated subcutaneously into another rabbit showed no increase in virulence. In the three rabbits inoculated subcutaneously the adjacent glands were caseous, and in one the tuberculosis was a little more widespread and in another the lesions in a kidney more severe than after inoculation with bacilli of the human type.

*Burgess.* Three rabbits were given 5.0, 10.0 and 10.0 mg. subcutaneously and died in 99, 37 and 108 days respectively; the first and third showed moderate generalized tuberculosis, intermediate between that produced by standard bovine and human tubercle bacilli; the second showed slight generalized tuberculosis. Two rabbits were given 0.01 and 0.001 mg. intravenously; the first died in 27 days of typical severe general miliary tuberculosis, the second in 122 days of chronic generated tuberculosis. A culture from the last rabbit was fully virulent, two rabbits inoculated subcutaneously each with 10.0 mg. dying of typical severe general tuberculosis in 58 and 68 days.

998. A rabbit given 0.01 mg. intravenously died in 77 days of general atypical tuberculosis. A culture was obtained from this rabbit of which 0.01 mg. was inoculated intravenously into another rabbit. The second rabbit died in 89 days of general tuberculosis, fairly extensive in the lungs, slight elsewhere. The virulence of the strain for the rabbit fell below the standard of bovine tubercle bacilli. Five guinea-pigs were inoculated subcutaneously with culture, two with 0.1 mg. and three with 1.0 mg. They died of general tuberculosis in from 37 to 110 days. One, which died 70 days later,

showed typical caseo-necrosis of liver and spleen, but the glands were hyperplastic with little caseation. In the remaining four the disease was milder, the liver and spleen showing grey lesions and no necrosis and the glands hyperplasia and fibrosis with little caseation. The virulence of the strain for the guinea-pig, as for the rabbit, was rather lower than that of standard bovine tubercle bacilli.

*Eugonic human strains.* The vast majority of the strains exhibited the well-known cultural characters and, where this was tested, the usual virulence of the eugonic human type of tubercle bacillus. The primary colonial characters of this type are usually in strong contrast to those of typical bovine strains. The main features distinguishing human from bovine primary colonies are elevation from the outset and pigmentation in the later stages, the bovine colonies being flat and transparent, later becoming translucent. All these strains grew well in subculture on egg and glycerin egg and produced cream-coloured or canary yellow growths on bovine serum and thick wrinkled or warty layers usually pigmented (creamy white, buff or brownish) on glycerinated potato.

The virulence of some of these cultures was tested on the rabbit and generally proved low, but some caused rather severe disease, occasionally acute general miliary tuberculosis after intravenous or extensive disease of the lungs, though not of other organs, after subcutaneous inoculation. Cultures recovered from the internal lesions of these rabbits were identical with those inoculated (1911). All the eugonic strains tested on the guinea-pig produced progressive generalized tuberculosis.

The colonial characters in primary cultures usually enabled a provisional sorting out of human from bovine strains, but occasionally these were atypical. Excluding the mixed strains two notable examples of divergent colonial characters were encountered. In these the colonies on egg resembled those of the bovine type, that is to say, they were shiny, flat, transparent at first and later the margins developed surface markings like those on colonies of the bovine type. On other media the growths were eugonic like that of human type and one strain exhibited the virulence of that type for rabbits and guinea-pigs. The two strains from the sputum of the other case (C.S. 118) were each moderately virulent for the first rabbit, but on recovery were found to be eugonic and slightly virulent for rabbits. The bacilli were virulent for the guinea-pig.

Other cultures which caused some initial uncertainty were those which showed two types of colony, one relatively large and generally creamy and granular, the other small with raised, opaque, rounded centre and dull grey margin. Both produced typical eugonic growths on glycerinated potato and exhibited the virulence of the human type for rabbits and guinea-pigs. On glycerin egg the primary colonies in these instances were raised and uniform in character.

Three other strains which departed conspicuously from the normal showed beautiful acid-fast branched forms in the sputum. The cultures had the cultural characters and the pathogenicity for rabbits and guinea-pigs of eugonic human bacilli. Branched forms were found, though scantily, in film preparations of the lesions of rabbits inoculated with these strains.

*Dysgonic human strains.* This variety of the human group of tubercle bacilli has been found altogether in fifty-two instances. The relative frequency of its occurrence was carefully determined in four series only, namely, those bacteriologically investigated by A. S. Griffith, who systematically tested all the strains on glycerinated potato, a solid medium which differentiates dysgonic from eugonic human strains. Another such medium is 5 % glycerin agar.

The distinctive features of these strains, which have occurred proportionally less frequently in all four series than bovine strains, are the dysgonic characters of their growth on glycerin agar, potato and broth but their identity with the eugonic human type in other characters, such as canary yellow growths on bovine serum and in virulence for the rabbit, guinea-pig and vole.

The most interesting cultural characters are those on glycerinated potato and on glycerin agar. On the potato a thin grey or granular layer is produced which attains its maximum thickness in 3-4 weeks. Subsequently secondary colonies, from one to a large number, make their appearance in the basal layer. These appear successively over a period of many months, and in some instances the whole surface of the potato is covered with an eruption of colonies of various sizes, shapes and colours, from pearly white to buff, often tinged with pink due to litmus. If one of these colonies is removed and separately subcultivated on egg and then transferred to potato the strain is found to be eugonic, producing layers on potato indistinguishable from those produced by eugonic bacilli.

In primary culture there is generally a distinction between eugonic and dysgonic human strains, the growth of the eugonic strains, especially on glycerin egg, being flat and white with thickened edges and bossy surface.

The virulence of the strains with these cultural characters is that of the eugonic human type and is unchanged by the cultural modification on glycerinated potato.

#### MIXED INFECTIONS

As stated previously, in six instances bovine tubercle bacilli were not the only type of tubercle bacilli present in the sputum. The following are brief records of the facts in these cases:

*Case 3* (see Table 1). Male, aged 7½ years. The culture from sputum was eugonic and produced progressive general tuberculosis in two rabbits from which dysgonic virulent strains only were obtained (Griffith, 1930, 1937).

*E.C.* (Cumming's case). Female, aged 34 years, with chronic pulmonary, following glandular tuberculosis. Three specimens of sputum yielded pure cultures of bovine, and three specimens yielded pure cultures of human tubercle bacilli. The patient died and both types of tubercle bacilli were found in lungs and a bronchial and a mesenteric gland (Cumming, 1935; Griffith, 1937).

A. 339. Male, aged 66 years. The first specimen of sputum (24 March 1938) yielded in direct cultures on egg numerous dysgonic colonies but subcultures were eugonic in appearance. Three rabbits given respectively 0.001 and 0.01 mg. intravenously and 10.0 mg. subcutaneously died of typical general tuberculosis in 21,

19 and 86 days respectively. Cultures from the second rabbit showed two kinds of colonies, one small, flat and transparent, the other large, raised and cream-coloured. The first kind was dysgonic and fully virulent, the second eugonic but still virulent for a rabbit, which yielded, however, only dysgonic strains, the eugonic element having been eliminated. Direct cultures on glycerin egg showed a single colony only, late in appearing, which became large, cream-coloured and wrinkled. The colony was eugonic in subculture, slightly virulent for a rabbit given 30.0 mg. subcutaneously, and of human-type virulence for guinea-pigs.

The second specimen (2 May 1938) yielded a eugonic culture which was not tested on a rabbit. Third and fourth specimens (27 June and 11 July 1938) were negative microscopically and culturally. The last specimen, however, produced tuberculosis in a guinea-pig from which a eugonic strain of human-type virulence was isolated. Two further specimens were negative (Griffith & Smith, 1940).

*C.S. 537.* Female, aged 36 years. One specimen only of sputum was obtained, the patient dying a few days later. This specimen yielded on plain egg small, filmy transparent and larger raised cream-coloured colonies; the former were dysgonic and fully virulent for three rabbits given 0.001 mg. intravenously and 10 and 10 mg. subcutaneously. The rabbits died of typical generalized tuberculosis in 45, 69 and 69 days; the larger colonies were eugonic and of low virulence for a rabbit given 20 mg. subcutaneously. The animal was killed 231 days later and showed small local lesions and five caseating nodules in lungs from which eugonic cultures were obtained. Both types were virulent for guinea-pigs, the dysgonic more than the eugonic (Griffith & Smith, 1940).

*C.S. 567.* Female, aged 36 years, the wife of a farm servant. First specimen of sputum (13 July 1939) yielded numerous filmy colonies on egg and none on glycerin egg. A rabbit inoculated intravenously with one-hundredth part of the growth from one tube died of generalized tuberculosis in 23 days. A subculture made with an emulsion of the original culture produced on glycerin-egg a moderate number of shiny colonies and two large opaque colonies. A subculture of one of the large colonies was eugonic and exhibited typical human-type virulence for the rabbit and guinea-pig. A second specimen of sputum (22 August 1939) yielded a dysgonic culture only, of which 10 mg. inoculated subcutaneously caused the death of a rabbit from typical generalized tuberculosis in 45 days. Human tubercle bacilli were therefore very sparse in the sputum of this patient (Griffith & Smith, 1940).

*C.S. 455.* Male, aged 37, fishmonger. Although from this patient only one strain of tubercle bacilli was cultivated, and that of the bovine type, the case was not a simple one of bovine infection alone. Three specimens of sputum were sent. The second (11 November 1938) yielded directly on one egg tube two filmy colonies, two other egg and a glycerin-egg tube remaining sterile. The direct culture and one through the inguinal gland of a guinea-pig, which died in 16 days, showing no macroscopic lesions, were dysgonic (class I) and each caused typical severe general tuberculosis in a rabbit. The rabbit inoculated with about 1 mg. of the

direct culture subcutaneously died in 89 days, and the other, which received about 3 mg., in 91 days.

The first (16 August 1938) and third (8 February 1939) specimens, though they contained acid-fast bacilli which produced typical tuberculous lesions in guinea-pigs and rabbits, consistently failed to produce growth on the egg media, which ordinary strains of tubercle bacilli found very suitable for their multiplication. These strains (*a* and *c*) are being propagated and have already been passed through upwards of twenty-four guinea-pigs and two rabbits, none of which has yielded a culture (Griffith & Smith, 1940).

#### AUTOPSIES

Autopsies have been performed on fourteen persons whose ages ranged from 11 to 41 years. Accounts of the findings in these cases have been recorded in full in various journals, and it is unnecessary for us to repeat them here. We have made instead an analysis of the distribution of lesions with a view to determining the usual portals of entry of the bovine bacilli causing human pulmonary tuberculosis.

eous or wholly calcareous lesions, obviously older than any other glandular lesion in the body.

In four cases there were no caseous or old calcareous lesions in the glands directly draining the alimentary tract, and the anatomical evidence for entry of the bacilli by inhalation was inconclusive. One case, no. 16, had small shotty glands in the neck, which, however, were not examined, and no disease of the mesenteric or tracheo-bronchial glands. Another case (Ramsey) showed fibrosis of the ileo-caecal glands and what appeared to be recent tuberculosis of an inter-tracheo-bronchial gland. In a third case (case 34) the mesenteric glands were enlarged and not apparently tuberculous; the condition of the tracheo-bronchial glands was not described but one sent to Cambridge showed no sign of tuberculosis; the tuberculous lesions in the lungs of this case were considered by the pathologists to be older than some ulcers in the intestines.

In the fourth case (case 29) the glands directly draining the alimentary canal were normal; the præ-tracheo-bronchial and the definitely interpulmonary glands of the posterior lobes were anthracotic but were not caseous. The inter-tracheo-bronchial glands, how-

Table 1. *Autopsies on fourteen cases of pulmonary tuberculosis due to bovine tubercle bacilli*

No. of case	Sanatorium	Date of autopsy	Made by	State of lymphatic glands			Reference
				Cervical	Mesenteric	Tracheo-bronchial	
(1) S.M.	Glenlond	14. iii. 29	Munro	Small palpable	Partly caseous	—	Griffith, 1930 (case 12)
(2) McN.	"	20. xii. 28	"	Small palpable	0	0	Griffith, 1930 (case 16)
(3) Gilc.	East Fortune	ii. 32	Cameron	—	—	—	Griffith & Summers, 1933 (case 27)
(4) M.D.	Glenlond	12. v. 34	Munro	0	Calcified	0	Griffith & Smith, 1940
(5) Ramsey	East Fortune	?	Cameron	—	Fibrosed	?	
(6) Mrs Reid	Clackmannan	vii. 34	Stevenson	0	3 calcified	0	Griffith, 1935
(7) Jean Watt	Glenlond	18. iii. 34	Drennan	0	? one caseous	Partial	
(8) C.B.	Grassington	19. v. 32	Cumming & Girdwood	0	Several calcified	Slight calcification	Griffith, 1933
(9) E.C.	"	26. v. 32	"	Calcified	Do.	A few calcified or caseous	
(10)	"	—	Gibb	0	Enlarged not caseous	? 0	Griffith, 1935 (case 34)
(11)	Lochmaben	25. xi. 32	Elder	0	0	Partial inter-tracheo-bronchial	
(12) B.G.	Glenlond	v. 33	Munro	Caseous	Caseous	Caseous	Munro & Walker, 1935
(13) J.L.	East Fortune	5. ix. 34	Macgregor	0	Caseous and calcified	0	
(14) Mrs Little	Lochmaben	7. vi. 38	Griffith	0	One calcified	0	Griffith & Smith, 1940

— not examined; 0 no lesions.

Table 1 states when, where and by whom the autopsy in each case was performed, the reference to the paper containing the full details and the condition of those glands—cervical and mesenteric and tracheo-bronchial—which directly drain the alimentary canal and the lungs respectively.

In nine cases the anatomical evidence indicated that infection with bovine tubercle bacilli had taken place through the mucous membrane of the alimentary tract. In two of these most of the mesenteric glands (cervical also in one) were extensively caseated, and in the rest one or more mesenteric glands contained caseo-calcar-

ever, showed partial caseation but no great enlargement. We do not think that the appearance of the lesions in these glands was sufficient evidence of a respiratory origin of the infection in this case.

In the remaining case (case 27) the examination was confined to the lungs, which were extremely diseased.

In all but one patient, who died of pneumothorax with disease limited to one apex, the pulmonary tuberculosis was very severe. In these thirteen cases all the lobes were converted into abscesses or riddled with cavities, or the greater part of the lung was in a condition of caseous pneumonia, with acute softening and

recent cavities, the result of aspiration from an old apical cavity. Except in one of these patients, a child of 11 years, the tuberculous disease was practically confined to the lungs, lesions elsewhere, if any, as for example in the mesenteric glands (or the brain, case 13) being small and obsolescent. The child showed, besides extensive ulcerative pulmonary tuberculosis, numerous ulcers in the intestines, great enlargement and caseation of the abdominal, cervical and thoracic lymphatic glands and minute tuberculous lesions in the kidneys. The child was infected through the alimentary tract before she was 2 years old and the disease had slowly progressed, causing death 9 years later.

Pure cultures of bovine tubercle bacilli were obtained from the sputum and from the tissues in nine cases (lungs in seven cases, bronchial glands in five, mesenteric glands in three, and cervical gland, peritoneal tubercle and intestinal ulcer in one case each). In the

separately. We see from the diagram that bovine pulmonary tuberculosis in its ulcerative stage has its inception in the great majority of cases below the age of 25, especially in women. Of eighty-seven women 68 % were under 25, and 67 % of the men were under 30 when the first pulmonary symptoms were noted.

The latent period of bovine phthisis, that is to say, the period between the original infection and the breakdown of the pulmonary lesions, can be estimated in only a small proportion of the cases.

In fifty-two cases a history was given of previous cervical gland or abdominal tuberculosis. On the assumption that these conditions were due to bovine bacilli and dated approximately the first infection with that bacillus, we find that the latent period ranges from a year or two to twenty-six years. In sixteen cervical gland cases the period ranged from 4 to 17 years (average 8.5 years).

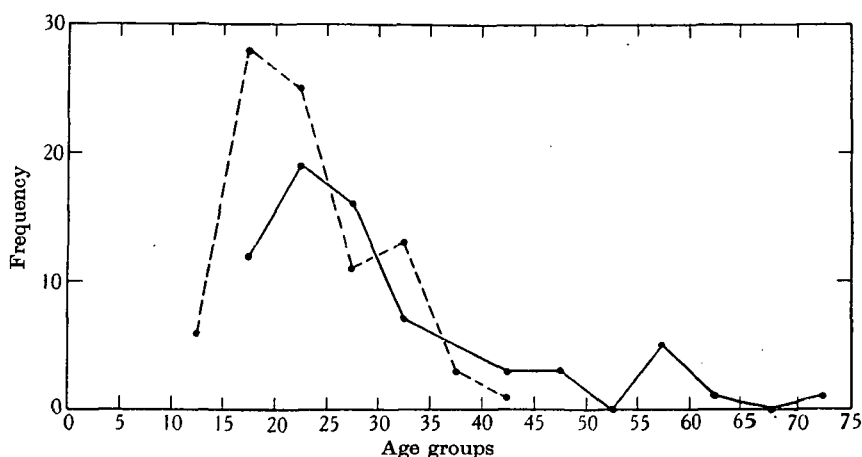


Fig. 1. Showing the approximate ages at which clinical signs were noted in cases of pulmonary tuberculosis due to infection with bovine strains. — Males. - - - Females.

tissues of one case (9) the human was admixed with the bovine type of tubercle bacillus, the sputum having yielded each type of bacillus separately.

The findings in these autopsies show that bovine tubercle bacilli can produce in the lungs of human beings destructive tuberculous disease of the utmost severity, indistinguishable from that set up by human bacilli.

#### AGE INCIDENCE

The ages of the patients when the first specimen of sputum was collected for examination ranged from 7 to 72 years. Unfortunately, it has not always been possible to obtain a history which would date, even approximately, the breakdown of the pulmonary lesions and the first appearance of bacilli in the sputum. Information regarding the duration of clinically detected pulmonary tuberculosis, which varied from a few months to 16 years, was, however, given in 157 of the cases, and in these instances we can state the approximate ages when the first clinical symptoms were noted. These are plotted in 5-year groups in Fig. 1, males and females

#### SEX INCIDENCE

Of the 241 persons 109 were males and 132 were females. There is thus a preponderance of females over males in the combined figures for England and Scotland. But on comparing sex incidence in the two countries one finds that in England there were more males than females, namely 49 to 32, and that in Scotland there was an excess of female over male patients, namely 100 to 60, the ratio of females to males being 5 to 3, whereas in England this ratio is practically reversed. What is the explanation of this wide difference between the two countries? It is not that females in Scotland are more susceptible than males to bovine tuberculosis because in childhood in other forms of bovine tuberculosis the sexes are about equally represented. The features associated with bovine tuberculosis in Scotland, especially in the series investigated by Munro, namely, a relatively high proportion of the attacked persons belonging to the ages of childhood and to the female sex, are those which Hill & Mitra (1936) have suggested may characterize a milk-borne epidemic.

**DISTRIBUTION OF CASES OF  
BOVINE INFECTION**

The surveys were made to determine the proportional frequency of bovine infections in unselected series of cases of pulmonary tuberculosis in various regions of Great Britain. In addition, Cumming (1933) examined groups of selected cases of pulmonary tuberculosis in persons who might be expected to be infected with bovine bacilli more frequently than other members of the community, namely, persons who had suffered from previous cervical or mesenteric glandular tuberculosis, children under 16 and cattle contacts. He found twenty-one bovine infections among 245 such cases and four among an unspecified number. Altogether eighty-one

among 6963 patients were found to be expectorating tubercle bacilli of bovine type with the sputum, seventy-five times alone, five times together with those of human type and once with a novel form of the bacillus which could not be cultivated.

The cases of bovine infection were distributed unequally in the different regions in gradually decreasing frequency from the north of Scotland to the south of England. No instance was found (by Cumming) in Eire. The Orkneys gave the highest proportion, namely, 25.8%, then the country districts of north-east Scotland with 9.1%, the rest of Scotland following with about 5.2%. The north midland region of England comes next with just under 2%. Cumming, Page and Griffith found 1% or less of the cases in the south-east and south of England and in Wales to be of bovine origin.

Table 2. *Statistics*

Authors	Region	No. of cases	Eugonic human	Dysgonic human	Bovine	Percentage bovine
<b>Scotland</b>						
Griffith & Wang	Edinburgh, Glasgow and Lanark (early cases)	102	100	—	2	2.0
Griffith & Smith	N.E. counties	540	468	23	49	9.1
	Aberdeen city	432	399	14	19	4.4
Griffith & Munro	Middle and south	515	476	8	31	6.0
	Additional	15	14	—	1	6.6
	East	1165	1107	—	58	5.0
		2769	2564	45	160	5.7
<b>England, Wales and Eire</b>						
Cumming	North	888	874	—	14	1.5
Lynn & Cutbill	Middle	1000	977	1	22	2.2
Lynn & Cutbill	Middle	315	305	—	10	3.1
Griffith & Menton	Middle	230	228	—	2	0.8
Griffith	South	694*	684	6	4	0.5
Pagel	South	100	99	—	1	1.0
Cumming	South	195	194	—	1	0.5
Cumming	Various parts (selected)	245	224	—	21	0.8
Cumming	Various parts (selected)	Unspecified number (4+)	—	—	4	2.0
Cumming	Wales	203	201	—	2	2.0
Cumming	Eire	320	320	—	—	—
		4194	4106	7	81	1.9

\* The 694 cases are made up as follows:

Author	Cases	Eugonic human	Dysgonic human	Bovine
Cobbett, 1907	2	2	—	—
Griffith, 1911	29	27	—	2
Bullock, 1911	23	23	—	—
Griffith, 1914	105	105	—	—
1916	5	3	2	—
1920	17	16	1	—
1930	146	145	—	1
1930-7	363	359	3	1
Since 1937	4	4	—	—
	694	684	6	4



SOURCES OF BOVINE TUBERCLE BACILLI  
WHICH CAUSE PULMONARY TUBER-  
CULOSIS IN HUMAN BEINGS

We have analysed the clinical information supplied with the cases with a view to ascertaining how the persons were infected with bovine bacilli. These organisms may be conveyed to human beings by the milk or the products of the milk of tuberculous cows, the bacilli reaching the lungs after passing through the mucous membrane of the alimentary canal, or directly by the air inspired in the neighbourhood of (a) persons suffering from 'bovine' pulmonary tuberculosis or (b) tuberculous cattle.

The clinical or post-mortem evidence was in favour of the alimentary tract as the portal of entry of the bacilli in the following cases. Fifty persons suffered from cervical gland tuberculosis in childhood or adolescence. We refer here only to those persons who had gross primary enlargements of the glands which were operated on long anterior to the development of clinical pulmonary tuberculosis, leaving scars on the neck. The cases of pulmonary tuberculosis which showed chains of tiny indurated glands in the posterior triangles are not included in this category. Small glands in the neck can be felt at some time in every case of pulmonary tuberculosis (human or bovine) and probably indicate general infection since tubercle bacilli have been cultivated from them. Several patients, including a few of those with cervical gland tuberculosis, had symptoms in early life which suggested abdominal tuberculosis and others showed calcareous abdominal glands on radiological examination. In four other patients the pulmonary tuberculosis was preceded by bone and joint tuberculosis which, like cervical gland tuberculosis when due to bovine tubercle bacilli, is almost invariably of alimentary origin. In two instances (case 4 in this report and case 540, 1940) the infection of the lungs was probably due to spread by contiguity with abscesses which formed in connexion with tuberculosis of the dorsal spine. Thus in seventy cases the clinical evidence indicated the alimentary canal as the portal of entry of the bacilli. This route of infection was established beyond reasonable doubt in nine out of fourteen persons on whom autopsies were made by finding caseous or calcareous lesions in the mesenteric glands older than those in the lungs. Two of these had given a history of preceding glandular tuberculosis but in seven there was no clinical clue to the channel of entry of the bacilli. The anatomical evidence from autopsies suggests the probability that many of the persons without clinical evidence of preceding infection by the digestive route may have been infected in this way particularly as the great majority, especially of the Scottish patients, were declared drinkers of raw milk in childhood and later, and did not come directly into contact with tuberculous cattle or human beings infected with bovine bacilli.

The foregoing analysis shows that out of 241 cases of 'bovine phthisis' seventy gave clinical or post-mortem evidence of alimentary infection. Of the seventy cases, fifty or about a third of the 160 Scottish cases and twenty or a quarter of the eighty-one English cases came into this category.

We will now consider whether or not any of the 171 cases of pulmonary tuberculosis without clinical evidence

of preceding infection might have been infected through direct contact with human beings who were expectorating bovine tubercle bacilli or with tuberculous cattle.

Evidence clearly indicating human transmission of bovine bacilli is extremely difficult to secure. The first necessity is, of course, to obtain a culture from the persons suspected to have transmitted or to have received the infection. The great importance of this was seen in the review by Walker in 1934 of the recorded instances to that date where in a household containing one case of bovine phthisis one or more of the other members of the family suffered from tuberculosis. It was possible in two families, each containing two cases of phthisis, to type the bacilli. In each family one case was due to bovine and the other to human bacilli. Thus the presumptive evidence of familial infection with bovine bacilli was disproved in these instances by bacteriological investigation.

*Human to human infection.* It is certain that tuberculosis due to the human type of bacillus is spread among human beings almost exclusively through the agency of the sputum of persons suffering from pulmonary tuberculosis. When bacilli of the bovine type, which, as we have shown, are certainly not less virulent for man than bacilli of the human type, are similarly disseminated it would be very surprising if these bacilli were not sometimes transmitted to healthy persons through the inspired air.

Since 1934 five families have been examined, two by us (Griffith & Munro, 1935) and three by Lynn & Cutbill (not yet published) in each of which two cases of bovine pulmonary tuberculosis occurred. We concluded that in one of the two families examined by us both cases of pulmonary tuberculosis were of alimentary origin and in the other infection was probably transmitted from one patient to the other by aspiration. In the remaining three families all the strains were fully virulent and here also there was strong presumption of human to human infection. Proof of familial infection by inhalation in the last four families was, however, lacking as an autopsy was not made on any of the contact cases.

Until recently there has been a remarkable absence of evidence of transmission of bovine bacilli to children who were in contact with the bovine pulmonary cases. In 1939 Munro reported that he had obtained cultures of bovine bacilli from a child of a male patient who was expectorating bovine tubercle bacilli. The child, aged 2½ years, showed clinically tuberculosis of an ankle-joint and radiologically a small lesion in the right upper lobe and marked enlargement of the hilar shadow. Bovine strains were obtained from gastric lavage and urine. An older child had tuberculous iritis but cultures were not obtained from gastric lavage or urine. This is the first occasion on which bovine bacilli were cultivated from a parent and a child. The father was careless in his habits and took few if any precautions to avoid infecting his children. The mother was emphatic that the children had had no other than pasteurized milk from a supply which, when tested, has always been found free of living tubercle bacilli, and there seems little doubt that the bovine bacilli were transmitted from the parent to the child. In this instance evidence as to portal of entry of the bacilli, which might be forthcoming from an autopsy, is lacking.

*Bovine to human infection.* The same difficulties of interpretation arise with the so-called cattle contacts as with the human to human infections. Here, likewise, a case of pulmonary tuberculosis in one who has had much to do with cattle may or may not be of bovine origin. In Scotland, for example, 122 farmers and farm servants were phthisical but of these only twenty cases were caused by bovine tubercle bacilli and the rest by human tubercle bacilli. If due to bovine bacilli the infection may have been of alimentary origin as farmers and farm servants probably regularly consume more milk than other people.

Since cows in their byres, however, are infected by inhalation of dried sputum or infective dust we see no reason to suppose that human beings exposed to the same risk, though for shorter periods, may not occasionally contract tuberculosis in the same way. Only two of the twenty affected persons have however been examined after death. In one case there was no evidence of alimentary infection and the bronchial glands were affected but not in such a way as to leave no doubt of primary respiratory infection. In the other the condition of the thoracic glands was not stated, but one gland, which was sent to Cambridge, was found to be free of macroscopic lesions. There were lesions in the intestines which appeared to the pathologist to be more recent than in the lungs.

In the case of pulmonary tuberculosis due to respiratory infection in adult life with bovine tubercle bacilli the condition of the bronchial glands is important. We should expect these glands to be greatly enlarged and caseous on one or both sides, as in primary infections in children with human tubercle bacilli.

*Human to bovine infection.* One of the persons with 'bovine' pulmonary tuberculosis was in charge of a herd from which all reactors to the tuberculin test were being eliminated with a view to establishing a tubercle-free herd. The plan was abandoned when with every test some previously negative cow gave a positive reaction. The cause of the infection was not suspected. Tubercle bacilli of the human type can also be transmitted by phthisical persons to cattle and cause them to react to the tuberculin test. It is imperative therefore that all persons in attendance on tubercle-free herds should themselves be free from tuberculous infection due to tubercle bacilli either of the human or of the bovine type.

#### DISCUSSION

It has been known for a long time (Griffith, 1911, 1914) that the bovine tubercle bacillus can cause in man ulcerative pulmonary tuberculosis indistinguishable from that due to the human type of tubercle bacillus. But only in the last 15 years or so have widespread inquiries shown that pulmonary tuberculosis is much more frequently of bovine origin than was at one time supposed. Up to 1924 only four cases of such infection were published. Since then, as Table 2 shows, as many as 237 further instances have been reported. This disparity could be accounted for by the regions investigated. At first the cases examined were mainly from the south of England, relatively few coming from Scotland.

In the second period the inquiries covered Scotland and were extended to the northern half of England, Wales and Ireland. The results of the investigations have shown that the proportional frequency of bovine infections in pulmonary tuberculosis is higher in Scotland than in England and higher in the rural districts of the north-east than in those of the rest of Scotland (1940). In England pulmonary tuberculosis due to bovine bacilli occurs more frequently in the northern (2.0%) than in the southern half (0.6%) of the country.

There is no doubt that the great majority of these persons were infected through cows' milk. About a third of the cases in Scotland and a quarter of those in England showed clear indication of previous alimentary infection. Of the others a large number gave no clinical sign of previous alimentary infection and had not been in contact with persons who were expectorating bovine tubercle bacilli or in attendance on tuberculous cattle. Most of these persons had consumed large quantities of raw milk in childhood and, when it is recalled that nine of the fourteen autopsies showed clear evidence of alimentary infection, four in persons without clinical evidence of previous glandular disease, it is reasonable to assume that the great majority of the cases in this last group were infected by feeding.

If, as we think, bovine pulmonary tuberculosis in human beings is in the main due to infected milk and rarely contracted by contact with human cases or with tuberculous cattle, the avoidance of the consumption of milk containing living bovine bacilli would lead to the virtual disappearance of this form of pulmonary tuberculosis.

#### SUMMARY AND CONCLUSIONS

1. This report summarizes the results of investigating 6963 cases of pulmonary tuberculosis in Great Britain.

2. The tubercle bacilli in the sputum of each case were obtained in culture and their types determined.

In Scotland out of 2769 cases 2609 yielded strains of human type (2564 eugonic and 45 dysgonic) and 160 (5.8%) yielded strains of bovine type.

In England tubercle bacilli of the human type were demonstrated in 3592 cases and of the bovine type in seventy-nine cases. Dysgonic human strains were found in seven cases, four of which occurred in the only series of English cases, namely 680, which were systematically examined for strains of this variety; dysgonic human strains were therefore proportionately less frequent in England than in Scotland. Of the seventy-nine bovine cases fifty-four occurred among 3422 unselected cases and twenty-five among a series of selected cases.

In Wales 203 cases were examined and two were found to be bovine infections.

In Eire no bovine infections were found in a series of 320 cases.

3. The total number of cases of pulmonary tuberculosis shown to be expectorating bacilli of the bovine type in the sputum was 241, but twenty-five of them, occurring as they did among selected cases, are not used in the following percentages.

The proportional frequencies of bovine infections were higher in all regions of Scotland than in England, the percentage being highest in the Orkney Islands (25.8 %). The rural districts of the mainland of north-east Scotland follow with 9.1 % and then those of the rest of Scotland with 5.2 %. The City of Aberdeen gave 4.4 % of bovine infections, but many of these had been infected in the country. In England the highest percentages were recorded in the north and middle regions, namely 2.0 %, the southern part yielding only 0.6 %.

4. The strains from 232 of the 241 cases were fully virulent and from nine they showed varying degrees of attenuation.

5. In six cases the bovine bacilli were associated with tubercle bacilli of another type, five times with eugonic human strains and once with a strain which could not be cultivated.

6. The anatomical evidence (previous cervical and abdominal glandular and bone and joint tuberculosis) in about a third of the cases in Scotland and in a quarter of those in England was strongly in favour of the digestive tract as the channel of entry of the bacilli.

7. Autopsies have been made on fourteen cases. In one case the lungs only were examined. In nine

autopsies the anatomical evidence indicated the alimentary canal as the route of infection. In four autopsies the anatomical evidence was inconclusive.

8. A history of tuberculosis was obtained in seven families in each of which two cases of pulmonary tuberculosis occurred. But bacteriological investigations in each of two families showed human in one affected person and bovine tubercle bacilli in the other and therefore disproved human to human infection. All the ten patients in five families yielded cultures of bovine tubercle bacilli. We concluded from the evidence that in one family both cases were of alimentary origin. Human to human infection was presumptive in the remaining four families. No autopsies were made in the last cases.

9. Twenty-five patients were associated in their employment with cattle. Autopsies were made on two of them but the anatomical evidence as to the channel of entry of the bacilli was inconclusive.

10. Of the 241 persons, forty-eight were known to be married and had 120 children. Bovine strains were obtained from two children (two families). Bacteriological evidence disproved infection from the parents in one case but was in favour of it having taken place in the other.

11. One probable instance of infection with bovine bacilli spreading from man to cattle is quoted.

12. A case of tuberculosis of the lungs due to bovine tubercle bacilli is indistinguishable clinically, radiologically and by post-mortem examination from one due to human tubercle bacilli.

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