

INOCULATION AND IMMUNITY EXPERIMENTS ON CALVES WITH THE VOLE STRAIN OF ACID- FAST BACILLUS

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THE acid-fast bacilli which are the cause of an epizootic disease in field voles in this country are not virulent for the guinea-pig, that is to say, when injected parenterally into this species, in large dose subcutaneously or in small dose intraperitoneally, the bacilli do not produce progressive disease. At the seat of injection they form an abscess and are disseminated over the body but the lesions they produce are of a retrogressive nature and eventually heal completely. On the other hand, when injected intraperitoneally in relatively large doses they may set up in the guinea-pig a fatal generalized disease with lesions which resemble tuberculosis macroscopically and microscopically.

Guinea-pig experiments

These pathogenic properties, so similar to those which follow injection of B.C.G., led one of us to test the immunizing power of the vole strain in the guinea-pig against an experimental infection with virulent tubercle bacilli. The vole strains used were L.V. 285 and L.V. 286 which were obtained by him from two naturally infected voles sent by Dr A. Q. Wells. Two experiments are described.

(1) Ten guinea-pigs each received during the period from 8 December 1937, to 2 February 1938, 10 mg. of culture of the vole strains subcutaneously in nine doses (eight of 1 mg. and one, the last, of 2 mg.), the intervals between consecutive doses being 1 week.

Four weeks after the last dose of vaccine the immunized animals, together with five controls, were each injected subcutaneously with 0.001 mg. of virulent bovine bacilli.

The controls died of typical, severe general tuberculosis in from 67 to 111 days, average 87 days.

One of the vaccinated guinea-pigs was killed after 68 days to compare the progress of the disease with that in the control which succumbed first. This

guinea-pig had severe local tuberculosis but only slight generalized disease. The rest of the vaccinated guinea-pigs died in from 109 to 225 days, average 152 days. The tuberculosis was severe in all these guinea-pigs and was typical of infection with virulent bovine bacilli. The effect of the vaccine was simply to delay the development of tuberculosis.

(2) Eight guinea-pigs received during the period from 22 April to 16 June 1938 (8 weeks) two doses each of 1 mg. and five doses each of 2 mg., or 12 mg. in all, of the vole strains subcutaneously.

One day after the last dose the vaccinated guinea-pigs and four controls were each injected with 0.01 mg. of the bovine strain no. 34.

The controls died in from 51 to 93 days (average 71 days) of severe general tuberculosis.

Two of the vaccinated guinea-pigs died in 65 and 66 days respectively, one of volvulus, the other from an unascertained cause. Besides local tuberculosis, one showed slight enlargement of the portal gland only and the other one tubercle in the spleen and a few fibrous nodules in the portal gland. The rest of the guinea-pigs, six in number, were killed 94 and 95 days after the resistance test in order to compare the progress of the disease with that in the controls, which were then all dead. One of the guinea-pigs had moderate general tuberculosis; four showed slight generalized disease and in one the tuberculosis was limited to the site of inoculation and the inguinal and iliac glands on the same side.

Again, all the vaccinated animals were found to be more resistant to tuberculosis than the controls. They all, however, had living bovine tubercle bacilli in their tissues and there is little doubt that these bacilli would have caused progressive fatal disease when the immunizing effect of the vole bacilli had faded.

CALF EXPERIMENTS

Having found that the vole strain is not virulent for guinea-pigs and after inoculation increases their resistance against infection with virulent bovine bacilli, we determined to test the pathogenicity and the immunizing value of the strain for calves.

The Joint Committee on Tuberculosis agreed to our suggestions and consented to our obtaining bull calves from the Agricultural Research Council's Field Station, Compton, and Mr G. W. Dunkin was good enough to supply us with fifteen calves in all. These calves came to the Field Laboratories at different times and were injected tentatively with different doses of the strain. The first few calves received smallish doses and one of them (calf 4) died of an intestinal disorder with diarrhoea 19 days after the injection, showing no lesions that could be attributed to the vole strain. It seemed a pity to kill the other animals, which had shown no sign of illness, and they were therefore kept for immunity experiments. Later obtained calves received ascending doses of the vole strain which, had the strain been as pathogenic as bovine strains are,

would have caused generalized and fatal disease. None of the calves, however, died of disease produced by the vole bacillus. A few of the animals were killed in order to ascertain whether or not this organism produced tuberculosis-like lesions. The rest were used in an immunity experiment.

INOCULATION EXPERIMENTS

Calf 2

Injected intravenously with doses of 0.01 and 2.0 mg., the interval being 116 days, died $8\frac{1}{2}$ days after second injection. Death was due to consolidation of the lungs, the result of allergic reaction. Cultures of the vole strain were obtained from the liver, five colonies on three tubes. Cultures from spleen and popliteal gland were negative.

Histological report. Lungs: a few small nodules consisting of alveoli filled with desquamated cells, giant cells of the Langhans type and some hyaline material. One giant cell showed a trace of an acid-fast rod. Liver: some small areas of necrosis; several small tubercles with giant cells—no caseation; lymphocyte and mononuclear infiltration of the portal sheath; no acid-fast rods detected.

Calf 4

Inoculated intravenously with 0.05 mg. of the vole strain (L.V. 285); died of non-specific pulmonary and intestinal disease (diarrhoea) 19 days after injection.

Autopsy. Lungs: both caudal lobes, particularly left, showed many lobules, dark red, fleshy and airless. No tubercles were visible in the lungs or other organs. Intestines congested.

Smears. No acid-fast bacilli found in bronchial or iliac glands, spleen or liver.

Cultures. Bronchial gland, contaminated. Iliac gland, four colonies of vole strain. Spleen, eighteen colonies on one tube. Liver, two colonies on two tubes.

Histological report. Lungs: similar changes as in calf 2, but smaller number of giant cells and no fibrinoid or hyaline exudation; acid-fast rods in fair numbers in one focus.

Calf 13

Killed 44 days after intravenous injection of 10.0 mg. of the vole strain.

Clinical. For a week or 10 days after injection calf was seedy and was not well for about 3 weeks, breathing a little faster than normal; appetite, however, good.

Autopsy. Lungs a little fuller than normal, speckled with petechial haemorrhages throughout substance (traumatic due to shooting); some irregular areas of red hepatization around roots of lungs and in anterior and right middle lobes.

Thoracic and all other glands normal in appearance. Heart: on right side

endocardial surface above valves showed some grey translucent granules, projecting only slightly from surface; suspicion of grey speckling on endocardium of right ventricle. Spleen: normal. Liver: through capsule a few minute yellow foci (? fatty). Kidneys: cortices speckled with 1 mm. pale grey foci of doubtful nature. Suprarenal bodies: pale grey, cortical foci of doubtful nature.

Smears. A mesenteric gland (two smears) showed three or four typical acid-fast bacilli in one smear; none found in other organs or glands.

Cultures. Colonies in moderate number from mesenteric gland and in small number from lung, spleen, thoracic glands and submaxillary and popliteal gland (one colony on each of two tubes from latter).

Histological report. Spleen: a few typical epithelioid cell tubercles with giant cells; no necrosis or caseation; no acid-fast elements. Lungs: no foci; one or two acid-fast elements (? bacilli) found in purulent contents of small bronchi. Heart muscle (right auricle): small nodules of mononuclear cells and lymphocytes with slight fibrinoid necrosis; scanty number of acid-fast elements therein. Lymphatic glands: mediastinal and mesenteric, small epithelioid cell nodules and occasionally typical Langhans giant cells; bronchial, many typical tubercles, but no necrosis or acid-fast bacilli; popliteal, no foci. Suprarenals: numerous mononuclear cell nodules with slight necrosis in the cortex; no acid-fast bacilli. Kidneys: epithelioid and mononuclear cell nodules in the cortex; no acid-fast bacilli. Liver: typical tubercles but no necrosis or acid-fast bacilli.

Comment. On the whole, changes very slight in the calf. Noteworthy that apart from non-characteristic areas of necrosis there are typical tubercles in various organs of several animals. Content in acid-fast elements scanty throughout.

Calf 12

Killed 89 days after intramuscular injection of 80·0 mg. of the vole strain.

Clinical. 14 days, elongated tumour in muscles, 14 × 7 cm.; prescapular gland enlarged, 7 cm. or less in length. 21 days; tumour and gland much diminished in size.

Autopsy. Elongated local lesion, 10 × 3 cm., tapering at each extremity; it was a cyst with fibrous wall, lined internally with a thin layer of vascular granulation tissue, and thick creamy purulent contents. Left prescapular gland, 6 × 3 × 1·5 cm., showed at one extremity a nodule, 1 × 1 × 0·8 cm., translucent and beset with soft whitish foci or streaks 1 mm. thick. Right prescapular gland, 5 × 2·5 × 1·2 cm., normal on section. Left prepectoral gland, size of small pea, composed of brownish translucent tissue surrounding a caseous nodule 1·5 mm. in diam. There was no sign of disease in any other organ or gland.

Smears. Local pus, acid-fast bacilli + + + +; prescapular gland nodule, acid-fast bacilli + +.

Cultures. Five colonies were obtained on two egg tubes sown with local pus. Five colonies on each of two egg tubes from the left prescapular gland. Tubes sown from popliteal gland and spleen remained sterile.

Comment. The visible lesions were limited to the seat of injection and the nearest prescapular and prepectoral glands. Acid-fast bacilli were very numerous in the local pus and moderately numerous in the prescapular gland but only few of these were viable, as was shown by cultures.

Conclusion

Inoculation experiments show that the vole strain is able to excite in a calf a tuberculous reaction, chronic in nature, which tends to disappear without leaving many traces. There is no proper caseation but scanty necrosis in some places. The longest period after inoculation that the organism has been recovered from the tissues is 89 days but this is evidently not the extreme limit of survival.

It should be borne in mind that the vole bacillus elicits typical tubercles also in guinea-pigs, rabbits and hamsters but not in voles.

IMMUNITY EXPERIMENTS ON CALVES

The rest of the calves were used in an immunity experiment. As the reception of the calves was over a long period, the animals varied widely in age. The controls, however, were not the youngest animals (these were nos. 10 and 11). There were altogether nine calves, which had been vaccinated in various ways, available for testing resistance to tuberculosis. Four were vaccinated once, three by intravenous and one by subcutaneous inoculation, the intervals between the vaccinations and the resistance tests being 65, 65, 166 and 175 days. Five calves were vaccinated twice, two each receiving two intravenous injections, one an intravenous and a subcutaneous injection and two each two subcutaneous injections. The resistance to tuberculosis of the nine treated and two untreated calves was tested by the oral administration of culture of virulent bovine tubercle bacilli, the dose in each case being 7.5 mg. of culture given by pipette. The methods of vaccination and the results of the tests are summarized in the table. The calves were killed at periods ranging from 208 to 223 days after the resistance tests.

Controls. One control, killed 208 days after feeding, showed widespread glandular lesions, two large foci in the lungs, several subcapsular lesions in the liver, some peritonitis and twelve lesions in the diaphragm, ranging in diameter from 2 to 2.5 cm. The glands affected were the left submaxillary, left retropharyngeal, all the thoracic and the abdominal; the lesions were caseating and slightly calcareous. The other control was killed 223 days after feeding and showed generalized disease less widespread than in the first control. There were no lesions in the head glands but the glands bordering the intestines were extensively tuberculous, the lesions showing calcification. The gastric, left bronchial and portal glands were also affected, but less severely.

678 *Inoculation and immunity experiments on calves*

Vaccinated calves. Five calves showed trivial lesions in the glands of the alimentary tract. In each of two of these calves, both of which had been vaccinated twice, there was one focus only, one in a mesenteric and one in a retro-pharyngeal gland. In each of three of the calves (one vaccinated once intravenously and two twice subcutaneously) the mesenteric glands contained a few small lesions, and in one a caseous tubercle was found in a colic gland.

Table giving details of vaccinations of calves with the vole strain and results of resistance tests with bovine tubercle bacilli

On 24 June 1939, the calves were fed by pipette each with 7.5 mg. mixed bovine cultures

No. of calf	Date of first injection of vole strain	Method and dose mg.	Date of second injection of vole strain	Method and dose mg.	Interval in days between last injection and resistance test	Duration of life of calf after resistance test (in days)	Result
1	23. xi. 38	Intraven. 1.0	28. ii. 39	Intraven. 3.0	116	212	One focus in mesenteric gland
3	23. xi. 38	" 0.1	28. ii. 39	" 1.0	116	221	One yellow tubercle in retro-pharyngeal gland
8	9. i. 39	" 2.0	3. vi. 39	Sub. 85.0	21	215	No lesions
5	23. xi. 38	Sub. 20.0	25. ii. 39	Sub. 50.0	119	209	Six mesenteric glands affected with small lesions
6	24. xii. 38	Sub. 50.0	14. iv. 39	Sub. 75.0	71	222	Three foci in mesenteric glands and one in a colic gland
9	9. i. 39	Intraven. 3.0	—	—	166	214	No lesions
10	20. iv. 39	" 5.0	—	—	65	222	No lesions
11	20. iv. 39	" 10.0	—	—	65	214	A few foci in mesenteric glands
7	31. xii. 38	Sub. 75.0	—	—	175	223	No lesions
15	Control					208	Tuberculous lesions in neck, abdominal and thoracic glands. Two nodules in lung and several lesions in liver (subcapsular) and on diaphragm
14	Control					223	Glands bordering intestines extensively involved. Bronchial glands affected, portal glands also

The remaining four calves showed no macroscopic tuberculous lesions in any gland or organ. Three of these calves had had single injections of the vole strain, two intravenously and one subcutaneously. The fourth calf had had two injections, one intravenous and one subcutaneous, the resistance test being made only 3 weeks after the latter injection.

Abscesses were present at the sites of the subcutaneous injections of cultures of vaccine in calves 5, 6 and 7. Cultures made by Dr J. Young from the pus of these abscesses remained sterile.

RESULTS OF INOCULATION INTO GUINEA-PIGS OF EMULSIONS OF LYMPHATIC GLANDS FROM VACCINATED CALVES

Alimentary and thoracic gland emulsions from each of two calves (1 and 9) were mixed before inoculation into guinea-pigs. They produced general tuberculosis and therefore contained living tubercle bacilli but whether these were present only in the glands bordering the alimentary tract cannot be stated.

A mesenteric gland from calf 1 was emulsified separately and inoculated into two guinea-pigs, one of which developed tuberculosis and one remained healthy.

From one calf (no. 11) an emulsion of mesenteric glands only was inoculated into two guinea-pigs, both of which became tuberculous.

Thoracic glands only from each of two calves were examined. A bronchial gland of calf 3 did not infect either of two guinea-pigs but a mediastinal gland from this calf caused tuberculosis in one of two guinea-pigs, the other remaining healthy. The bronchial gland of calf 5 produced tuberculosis in one guinea-pig and nothing in the other.

The experiments just summarized, where only one of two guinea-pigs inoculated with an emulsion became infected, showed that living tubercle bacilli were very scanty in the glands.

Two calves (6 and 7), one of which had no macroscopic lesions, harboured living bovine bacilli in the mesenteric glands but none was demonstrated in the bronchial glands.

From each of the remaining two calves (8 and 10), neither of which showed macroscopic lesions, emulsions of mesenteric or of thoracic glands did not produce tuberculosis in guinea-pigs.

TUBERCULIN TESTS

The first five calves were not tested with tuberculin before injection with the vole strain. This was not done because the calves were from a tuberculin-tested herd and were too young when received to have developed allergy to a chance infection with tubercle bacilli. The autopsies on these animals showed no sign of spontaneous tuberculosis. The other calves were tested with tuberculin and did not react.

All the calves were tested again with tuberculin after injection with the vole strain and gave definite reactions, some being very pronounced, the skin thicknesses on the third day ranging from 22 to 36 mm. and the adjacent preescapular glands from twice to thrice their normal size.

The shortest interval between the injection of the vole strain and the tuberculin test was 13 days, two calves, each of which had received an intravenous injection of vole strain, reacting well. Of six other calves, one was tested 21, four 24 and one 28 days respectively after inoculation with the vole strain; all reacted. It would appear from these observations that sensitiveness to tuberculin following injection of the vole strain develops very early.

The vaccinated calves (except calf 8 which only three weeks previously had been vaccinated a second time) were tested with tuberculin two days before the administration of bovine tubercle bacilli. They reacted well, the skin thicknesses on the third day ranging from 14 to 27 mm.

The two controls reacted strongly 38 days after feeding with bovine tubercle bacilli and again a few days before their slaughter. The nine vaccinated calves also reacted, but on the whole not so strongly as the controls, the skin thicknesses ranging from 10 to 20 mm., the controls giving 20–25 mm. Good reac-

tions were obtained in the four calves which at autopsy showed no tuberculous lesion, a second injection of tuberculin not being necessary in one of the calves (calf 8).

Summary

Both controls became infected, the glands of the alimentary tract showing widespread lesions and some dissemination, slight in one, a little more extensive in the other.

Of the nine vaccinated calves, five were found to have trivial tuberculous lesions in the glands adjoining the alimentary tract and four showed no macroscopic lesions, either in the glands of the alimentary tract or elsewhere.

Living bovine tubercle bacilli were not present in emulsions of mesenteric or thoracic glands of two of the four no-lesion calves and were demonstrated in the mesenteric glands but not in a bronchial gland of a third. The pooled glands of the fourth no-lesion calf caused general tuberculosis in two guinea-pigs.

Living bovine bacilli were very sparse in the thoracic glands of two of the calves with trivial lesions in the head or mesenteric glands and were not present in a bronchial gland of a calf which showed a few foci in the mesenteric glands.

Living bovine bacilli were present in lymphatic glands of one calf, but which were the glands that contained them could not be stated, as these were pooled before injection. The mesenteric glands only of the remaining calf were tested and proved infective.

No lesions were found after single vaccinations in three instances (two intravenous and one subcutaneous) and after two vaccinations in one instance, the periods between the single vaccinations and the resistance tests ranging from 2 to 6 months. There seems no advantage, therefore, in giving more than one dose of vole bacilli. The intravenous is, however, preferable to the subcutaneous method, because the former method does not produce an unsightly local lesion.

CONCLUSION

The results obtained in calves with the vole strain of acid-fast bacillus were unexpectedly good and better than those which followed the use of B.C.G. as a vaccine. We think, however, that the immunizing power of the vole strain against tuberculous infection in the calf should be further tested. We suggest using one form of injection only for the vaccination, namely, intravenous, and one dose, for example 5 mg., and testing the resistance of the calves after six months. The expenses incurred in this work were defrayed by a special grant from the Agricultural Research Council.

[Since this paper was submitted for publication A. Q. Wells and W. S. Brooke have published (1940, The effect of vaccination of guinea-pigs with the vole acid-fast bacillus on subsequent tuberculous infection, *Brit. J. exp. Path.* **21**, 104) the results of experiments on guinea-pigs to determine whether in this species vaccination with the vole acid-fast bacillus confers an immunity to experimental infection with *M. tuberculosis*. They conclude that the vole bacillus "gives a degree of protection which apparently is far greater than has been recorded by other means".]