

Laboratory diagnosis of smallpox: role of the Virus Reference Laboratory, Colindale, 1947–70

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SUMMARY

The Virus Reference Laboratory, Colindale, first embarked on laboratory investigations for smallpox early in 1947. From then, in conjunction with the Department of Bacteriology, University of Liverpool, it provided a complete diagnostic service throughout England and Wales until 1962, after which the service became available regionally until eradication was effected. Up to 1970 it had investigated 2696 specimens from suspected cases of smallpox and had recovered 108 strains of variola and 248 of vaccinia virus. These last were from persons suffering the complications of vaccination. Some outbreaks following smallpox importation are discussed but infection among laboratory staff during this period was not demonstrated.

INTRODUCTION

After 1935 smallpox had ceased to be endemic in England and Wales but during the next decade, except for 1941 and 1943, small numbers of cases were reported annually. These developed from importation of the disease which, by definition, consisted of a case or cases occurring in incoming passengers or crew from ships or aeroplanes, or its appearance in patients indirectly via imported material carrying the infection. With the end of the 1939–45 war and coinciding with large-scale repatriation, importations increased so that in 1946 there were 15 separate episodes all due to the Asiatic variola major. Early identification and close surveillance of contacts plus vaccination restricted the total number affected to 55 persons though 14 of these died (Bradley, 1947). By contrast, in 1947 there were 79 confirmed cases, again due to variola major, with 15 deaths. These arose from only two importations. The first was presumptively introduced by sea, the disease being diagnosed in Grimsby on 13 February, the source unknown though suspected to be from France. The second was in a soldier who returned from India by air with onset of illness shortly afterwards on 6 March (Murray & Bradley, 1948).

This combined outbreak, which covered a period of 5 months represented the highest incidence of smallpox since 1934. It was due to delays in containment from missed diagnosis in persons partially immune and who had therefore developed only sparse and atypical lesions. From experience in dealing with this situation Murray & Bradley (1948) concluded that, for the future effective containment of

smallpox, positive laboratory reports on material from doubtful cases ought to override hesitant clinical opinion. This concept was a validation of the important new role of the laboratory in providing early, accurate identification of the infecting organisms. It stemmed from the application of the pioneering work by Downie (1943, 1946, 1947) on laboratory diagnosis in smallpox, in which he combined the serological vaccinia-variola group reaction (Craigie & Wishart, 1936) with viral culture on the chorioallantoic membrane of the developing chick embryo (Downie, 1939; Irons *et al.* 1941). This amalgam formed the basis of laboratory investigations of smallpox by the Public Health Laboratory Service in England and Wales.

The Virus Reference Laboratory of the Public Health Laboratory Service (PHLS), established at Colindale, London, in 1946 had been directed, as one of its functions, to provide facilities for the laboratory diagnosis of smallpox which could be readily available throughout England and Wales. A short announcement in the January 1947 issue of the *Monthly Bulletin of the Ministry of Health and the Public Health Laboratory Service* stated that smallpox diagnosis, hitherto provided only by Professor A. W. Downie at Liverpool University, would henceforth be available from Colindale also. At the same time medical officers who acted as smallpox consultants were reminded about the collection of material suitable for laboratory examination.

From then until the disease was eradicated in 1978 the Virus Reference Laboratory provided a continuously accessible smallpox diagnostic service. Up to 1962 it was the one public health laboratory besides Liverpool University offering this complete service, though at the Cardiff and Manchester Public Health Laboratories preliminary serological tests were available (Report, 1953). From 1962 the diagnostic facilities were gradually extended to include the Bristol, Cardiff, Leeds and Newcastle Regional Laboratories of the PHLS and the Department of Bacteriology, Birmingham University (Report, 1972).

METHODS

Characteristically in smallpox, material from the skin lesions throughout the disease contained soluble group antigen and infectious virus particles.

For demonstrating the group antigen, the complement fixation test, used originally, was effective but required 18–20 h for a result to be available. It was replaced in 1960 by the agar gel precipitation test (Dumbell & Nizamuddin, 1959) which would provide an answer on vaccinia-variola within 1–2 h though it was less useful for chickenpox. The value of electron microscopy in demonstrating the presence of poxviruses or other viruses, established by Peters, Nielson & Bayer (1962) and Nagington & Macrae (1965), was extensively confirmed (Cruickshank, Bedson & Watson, 1966) in the Birmingham outbreak of variola minor during that year. This technique, as a suitable alternative to the agar gel test, did not become available in the Virus Reference Laboratory until early in 1968 (Macrae *et al.* 1969). From that time its most effective use was in the differential diagnosis of vesicular skin rashes, particularly for distinguishing the herpesvirus particles of chickenpox.

For culture of variola virus the chorioallantoic membrane of the 11–13 day developing chick embryo was used. This required 60–72 h for growth of the

distinctive pock lesions. By distinguishing between variola, vaccinia, cowpox and herpes simplex viruses it provided the final diagnosis.

Initially, though the types of material needed for investigation had been described (Downie, 1946; Murray & Bradley, 1948), there were problems in getting specimens to the laboratory, not only from different parts of the country but also from passenger ships *en route* to England. A sterile collecting outfit was therefore devised, complete with instructions and ready packed in a container suitable for air, passenger train or postal transmission (MacCallum, 1952). Such outfits, commercially obtainable (Report, 1953, 1972) were carried by Health Departments and replaced from laboratories when used.

RESULTS

During the years 1947–70 (Table 1) 2687 specimens were investigated and 103 strains of variola virus, 246 of vaccinia virus and 22 of cowpox virus isolated. Particularly in the early investigations virus could not always be recovered even from undoubted cases. Due to the wide scatter of the disease, laboratory staff were not themselves in a position to collect material and the need for adequate specimens was not always fully understood by others. With experience, the procedures proved their worth. The demand for vaccination fluctuated but usually increased with the publicity which accompanied smallpox outbreaks. The vaccinia viruses isolated were from patients suffering the complications of vaccination. This reinforced the view that, increasingly, the risk from vaccination often exceeded the risk of acquiring smallpox. The cowpox viruses came from sporadic infections in a number of farming communities.

1947 proved an eventful year, not only because of the incidence of smallpox but because the Virus Reference Laboratory was soon involved in diagnosis. The distribution and source of the viruses which were recovered are shown in Table 2. They stemmed from two importations, each of modified smallpox (Murray & Bradley, 1948). From the first importation, there occurred 48 cases, nine fatal. These occurred between 15 February and 11 July starting with 15 in Grimsby, two in Stepney and seven in Scunthorpe. Further spread occurred to Barnsley with 18 cases, Sheffield with three, Doncaster, Wakefield and Bermondsey with one each before the outbreak ceased. The second importation commencing on 6 March resulted in another 30 cases, six fatal, the last one having its onset on 6 July. This outbreak was less widespread, being contained within an area of the West Midlands. Those initially affected had modified disease and it was not till the third generation of cases that classical smallpox was observed. From the considerable mortality both outbreaks were ascribed to variola major.

The 1949 outbreak was confined to one ship with no secondary spread.

Between 29 November 1950 and 22 January 1951 an outbreak occurred in Brighton with 29 laboratory-confirmed and another five clinically suspect cases. It stemmed from missed modified smallpox in a serving officer who returned from Pakistan by air at a time when the disease was prevalent there. This man then travelled to Brighton from Scotland by rail on 28 November to stay with friends. Ill on arrival, confined to bed and mistakenly treated for malaria, the modified skin rash which later developed was not investigated and he recovered and

Table 1. *Isolation of pox viruses 1947-70*

Year	No. of cases (deaths) England and Wales*	No. of specimens examined	No. positive			No. negative
			Smallpox	Vaccinia	Cowpox	
1947	79 (15)	101	22	3	5	71
1948	0	34	0	3	0	31
1949	12 (5)	82	9	3	0	70
1950	4 (1)	74	4	3	3	64
1951	25 (9)	171	8	9	2	152
1952	135 (1)	157	11†	4	2	140
1953	38 (8)	96	5†	3	0	88
1954	0	63	0	1	2	60
1955	0	34	0	5	0	29
1956	0	29	1‡	4	0	24
1957	6 (2)	138	3	18	1	116
1958	6 (1)	91	0	6	0	85
1959	1	51	0	5	1	45
1960	1	56	0	12	0	44
1961	5 (1)	78	0	9	1	68
1962	68 (26)	469	10	87	0	372
1963	0	85	0	3	0	82
1964	0	63	0	5	2	56
1965	0	78	1‡	8	0	69
1966	62	319	22§	21	1	275
1967	2	156	12	10	0	136
1968	1	88	0	9	0	79
1969	0	98	0	9	2	87
1970	0	83	0	8	0	75
Total	445	2696	108	248	22	2318

* Compiled from DHSS figures. † Denotes strains of variola minor; ‡ denotes two strains from the Middle East; § comprises 17 strains of variola minor from an outbreak in the Midlands and five variola viruses from Ethiopia and || includes ten strains from Kuwait.

returned to his unit. The two members of the household, daughter and father, where he had stayed, became severely ill within a few days of each other and had to be admitted to hospital, first the daughter as a probable septicaemia, then the father with a severe haemorrhagic rash. This unusual circumstance, communicated to the Medical Officer of Health, led to immediate investigation and the diagnosis of smallpox was made, soon confirmed by laboratory tests. The daughter worked at the Brighton telephone exchange and two other staff there in turn developed the disease. Bedlinen from the household had been sent to a laundry before the illness was recognized and six members of the sorting staff were infected, requiring hospital admission. Indirect contact with the index case through a hairdressing establishment resulted in three cases. The main spread, however, involved 15 of the hospital's nursing and domestic staff, 12 of whom were unvaccinated. This outbreak underlined both the risk of spread through indirect contact and the vulnerability of unprotected hospital staff.

The Virus Reference Laboratory was only marginally involved in the outbreaks

Table 2. *Isolation of variola virus in 1947*

Patient no.	Sex	Date of confirmation	Place	Importation*
1	M	24. ii. 47	Grimsby	First
2	M	18. iii. 47	London†	First
3	M	24. iii. 47	Grimsby	First
4	M	24. iii. 47	Grimsby	First
5	M	24. iii. 47	Grimsby	First
6	F	17. iv. 47	Bilston	Second
7	M	2. v. 47	Stafford	Second
8	M	12. v. 47	Bilston	Second
9	M	19. v. 47	London†	First
10	M	21. v. 47	Bilston	Second
11	F	21. v. 47	Bilston	Second
12	F	21. v. 47	Sheffield	First
13	M	24. v. 47	Bilston	Second
14	M	31. v. 47	Barnsley	First
15	F	31. v. 47	Barnsley	First
16	M	4. vi. 47	Barnsley	First
17	M	11. vi. 47	Barnsley	First
18	M	25. vi. 47	Bilston	Second
19	M	26. vi. 47	Bilston	Second
20	F	1. vii. 47	Bilston	Second
21	F	3. vii. 47	Barnsley	First
22	M	8. vii. 47	Bilston	Second

* First by sea via Grimsby and second by air from India. † Stepney.

of alastrim in North-West England during 1952 and 1953. It became involved in 1957 in a small outbreak affecting six persons with two deaths which occurred in Tottenham (Hogben, McKendrick & Nicol, 1958). This outbreak surfaced in mid-summer (27 June), an unusual circumstance because smallpox commonly appeared in winter. The first recognized victim was a six-year-old boy admitted to hospital and operated on as an abdominal emergency. Shortly afterwards he developed a discrete, mainly peripheral, papular rash, went downhill and died. Smallpox was diagnosed, confirmed by virus isolation. This boy had no obvious links with any person from abroad. His infection came from his grandmother who was found to be in the late stage of a modified illness with rash. Originally thought to be chickenpox, this was identified from virus isolation as smallpox. She was a hospital employee working as a cleaner in the pathological laboratory. This fact, plus a search of the records, led to a man who had died of an unexplained acute febrile illness 4 hours after hospital admission and on whom a necropsy was done. His illness had developed shortly after a visit to his brother, newly returned from Nigeria. The brother and his daughter, both vaccinated, had been suffering from a mild illness with rash which had been diagnosed as chickenpox. Though no firm diagnosis was possible, it was considered likely that all three had suffered smallpox. The sixth person to develop smallpox had nursed the grandmother.

During the winter of 1961–62 in a four-week period from 16 December there were five importations of smallpox, all by air from Pakistan and dispersed between London, West Bromwich, Bradford, Birmingham and Cardiff (Report, 1962). Viruses were recovered from patients in London, Birmingham and Cardiff. The

Table 3. *Smallpox viruses from Ethiopia during 1966*

Patient no.	Age	Duration of disease	Type of specimen	Date	Gel precipitation	Egg culture
1	Adult	Late peeling stage	Scabs on exfoliated skin	30. xii. 65	Pos. (1 h)	Confluent lesions
2	Adolescent	Advanced pustular stage	Moist tops of lesions	15. i. 66	Pos. (1 h)	Numerous pocks
3	Infant (sib)	Not known	Tiny scabs	3. vi. 66	Pos. (1 h)	Numerous pocks
4	Infant (sib)	Not known	Single tiny scabs	3. vi. 66	Neg.	2-3 pocks only
5	Adult	Late peeling stage	Scabs on exfoliated skin	21. vii. 66	Pos. (1 h)	Numerous pocks

Table 4. *Immune status of V.R.L. staff - 1966*

Virus used in neutralization	Neutralizing antibody titre		
	16	32-128	≥256
Vaccinia	—	13	4
Variola	1	11	5

Bradford outbreak was investigated by Leeds Public Health Laboratory. In London, only three cases were confirmed, the original importation on 25 December, one at Woolwich on 13 January and one at Hornchurch on 22 January. No connexion between them was ascertained. Despite this, many specimens required examination, partly from suspected cases and partly as a result of vaccine complications, with 87 strains of vaccinia virus isolated.

In 1966, 22 viruses were isolated, 17 being from the Midlands outbreak of variola minor centred on Birmingham (Cruickshank *et al.* 1966). The remaining strains came from Ethiopia (Table 3) and demonstrated the relative stability of smallpox viruses during transit, their persistence at all stages of the disease and the sensitivity of tests used in their identification.

Investigation of material from importations came to an end in 1967 with the isolation of smallpox viruses from a mother and child newly returned by air from Pakistan. There was no secondary spread. During 1967, however, an outbreak of smallpox in Kuwait with 41 cases and 18 deaths occurred (Report, 1967). Material from 21 patients was received and 10 strains of variola virus and two strains of vaccinia virus were recovered.

During and beyond the period under review, smallpox did not affect laboratory staff. Not all were directly involved in the smallpox work, but regular revaccination annually was mandatory for all grades. This insistence on vaccination did not include the families of staff or the many short-stay visitors to the laboratory. Neither did it apply in other laboratories at Colindale. Specimens were handled in a safety cabinet, but air extraction was not done in view of a potential risk of aerosol spread. In 1966 immunity in staff, as shown in Table 4, was found to be adequate, based on neutralization tests on CAM against vaccinia and variola virus.

CONCLUSIONS

Smallpox had had an unenviable reputation as a scourge of mankind for centuries. Yet its recent eradication, regarded as a possibility since Jennerian vaccine was shown to protect against the disease, was relatively sudden. It arose from a combination of factors. There was better understanding of the epidemiological situation with curtailment of spread by segregation and ring vaccination of contacts. Advances in virology identified one main serological type of variola virus, showed that despite similarities to a number of animal pox viruses variola had no reservoir other than man and that accurate laboratory diagnosis was practicable at all stages of the disease, even when its pattern was considerably modified.

In England the disease ceased to be endemic after 1935, though periodic

reintroduction from abroad initiated a number of discrete but limited outbreaks over the years. Once the disease and its source had been identified, co-operation between clinician, epidemiologist and laboratory led to control. The provision of a country-wide laboratory diagnostic service marked a significant step forward and the benefits were noted soon after its inception. The events over the next 24 years have been outlined and features relating to some outbreaks mentioned. With improved control the incidence dwindled, though it is uncertain to what extent this was envisaged when the service was first made available. Some unwanted effects from vaccination, mainly in the face of outbreaks, have been recorded. These provided support for the ultimate decision to stop routine vaccination.

The two outbreaks of smallpox in England, which occurred in London in 1973 and in Birmingham in 1978, were not due to importation of the disease and have not formed part of this survey.

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