

Antibody studies in natural bovine cowpox

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SUMMARY

Serological studies on cows recovered from natural cowpox indicated that Haemagglutinin-Inhibiting (HAI) antibody persisted for at least 27 weeks, and Virus Neutralizing (VN) antibody persisted for at least 98 weeks.

INTRODUCTION

Cowpox is reported only when many cattle are involved or when human cases occur. Human infection is sufficiently severe for medical aid to be sought, and the reported incidence of approximately 1 case per year is probably an accurate measure of the total incidence of human cowpox in England and Wales (Baxby, 1977*a*). Bovine cowpox is not notifiable and little information is available about its incidence.

One of us has recently reviewed all outbreaks of human and bovine cowpox reported in England and Wales during 1965–76 (Baxby, 1977*a*). Human cases occurred without contact with infected cattle and it was suggested that cowpox was not enzootic in cattle, and that the virus reservoir was some other, as yet unidentified, animal (Baxby, 1977*a, b*). This conclusion was based, in part, on a serological survey of 1076 samples of bovine serum, only 7 of which had antibody and that in very low titre. It was known that some of the animals from the most recent outbreak had antibody (outbreak 12, Baxby, 1977*a*) but nothing was known about the persistence of antibody in convalescent animals.

We have now been able to monitor antibody titres in some cows from this outbreak, and so are able to present for the first time data, albeit fragmentary, on the persistence of antibody following natural bovine cowpox.

MATERIALS AND METHODS

Serum samples

Serum samples from infected and uninfected animals were collected 6 weeks after the outbreak and on 3 subsequent occasions over a period of 98 weeks. Sera were inactivated at 56 °C for 20 min and stored at –70 °C before test.

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Table 1. *Antibody response in cows following natural cowpox*

Animal	Infected?	Time after infection (weeks)	HAI*	Antibody titre	
				Virus neutralization†	
				Vaccinia	Cowpox
81	+	6	64	80	35
21	+	6	16	1,100	350
50	-	6	< 4	< 10	< 10
123	-	6	< 4	10	10
81	+	27	16	60	25
82	?	27	16	40	20
147	-	27	< 4	10	10
81	+	66	< 4	48	20
21	+	66	< 4	320	85
26	+	66	< 4	60	20
87	+	66	< 4	110	40
63	-	66	< 4	15	NT
71	-	66	< 4	< 10	NT
26	+	98	< 4	60	20
87	+	98	< 4	40	10

* Figure is reciprocal of serum dilution which completely inhibits haemagglutination by 4 units of antigen.

† Figure is reciprocal of serum dilution allowing 50% virus survival after contact for 2 h at 37 °C.

Gel-diffusion tests (GD)

Precipitating antibodies were detected by a micro-double diffusion test (Dumbell & Nizamuddin, 1959). The antigen used was the virus-free supernatant from extracts of cowpox-infected chorio-allantoic membranes (CAM).

Haemagglutination-Inhibition tests (HAI)

Sera were tested for HAI antibodies by a standard method (McCarthy & Helbert, 1960). The antigen used was that prepared for the GD tests above.

Virus neutralization tests (VN)

For reasons still not clearly understood, vaccinia virus is often a more sensitive indicator of cowpox neutralizing antibody than cowpox itself (Baxby, 1972). All the sera were therefore initially tested for neutralizing antibody by using vaccinia virus. They were then retested using the strain of cowpox virus isolated during the outbreak. A standard method was used in which serum dilutions and virus were mixed and incubated together at 37 °C for 2 h, at which time residual infectivity was detected by inoculation onto the CAM of 12-day-old chick embryos (Boulter, 1957).

RESULTS

The outbreak

The outbreak occurred in December 1976 in a herd of 30 cows in Somerset. The owner reported that about 10 animals were infected, but the source of infection was not traced. The outbreak came to our attention when one of the farm workers became infected, and cowpox virus was isolated from the lesion on his hand. By the time the animals were inspected by one of us (A.D.O.) they had almost fully recovered and suitable specimens for virus isolation could not be obtained. No further cases, human or bovine, occurred.

Serological investigations

The results obtained are shown in Table 1. Unfortunately we were not able to bleed all the infected animals, nor could we bleed the same animals on each occasion. However we were able to obtain specimens from 4 infected animals and 1 which was thought to have been infected. Samples were obtained from cow 81 on 3 occasions, and from cows 21, 26 and 87 on each of 2 occasions. We obtained specimens on 4 separate dates ranging from 6 to 98 weeks after the outbreak.

Previous experience has shown that virus neutralization titres of 20 and above can be regarded as specific. All the animals known to have been infected developed neutralizing antibody. The titres in different animals varied and were, on the whole, rather low. Neutralizing antibodies were still present after 98 weeks. As expected the sera had higher titres against vaccinia virus than cowpox virus.

Significant HAI antibody titres were produced by all infected animals. Although present after 27 weeks they were not detected in samples taken at 66 weeks and after.

None of the sera reacted in the gel-diffusion test. Some complement-fixation tests were attempted but high titres of non-specific fixation in control negative sera made the results impossible to interpret.

Antibody was not detected in the sera of animals which had shown no clinical signs of infection. This indicates that sub-clinical antibody-stimulating infections had not occurred.

The clinical diagnosis of cowpox in cow 82 was not certain. However the serological tests on a sample of serum taken 27 weeks after the outbreak indicated that it had been infected.

DISCUSSION

At the start of this investigation no information was available on the persistence of antibody in cows recovered from cowpox. It had been assumed that the absence of such antibody in cattle indicated that cowpox had not recently circulated in them. It was known, for example, that bovine herpes mammillitis virus (BHM) stimulated antibody rises which persisted for 2-4 years (Rweyemamu, Johnson & Laurillard, 1969; Gibbs, Johnson & Osborne, 1972), and there was no reason to believe that the antibody response after cowpox would be unusual.

The results obtained indicate that neutralizing antibodies can be detected for about 2 years, but that HAI antibody declines between 27 and 66 weeks. The value of HAI antibody as an indicator of recent infection is thus upheld (McCarthy, Downie & Bradley, 1958; Downie & Kempe, 1969). Cow 21, which was not sampled after 66 weeks, still had a high neutralizing titre at that time, and so it is possible that some animals may retain significant amounts of such antibody well after 2 years.

The failure to detect precipitating antibody is not surprising. Such antibody is rarely found after human vaccination and is not always present after smallpox (Downie *et al.* 1969*a, b*).

Bovine cowpox is less severe than BHM (Gibbs *et al.* 1970), and probably creates less constitutional disturbance than smallpox or vaccination in humans. This probably explains the relatively low titres and their persistence for a relatively shorter time. Nevertheless these results do indicate that bovine cowpox can be diagnosed retrospectively up to 2 years after the infection. Consequently they validate the suggestion that absence of antibody in the bovine population indicates that cowpox is not enzootic in that population.

This investigation could not have been carried out without the co-operation of Mr W. E. Tibbs who kindly gave us access to his animals, Professor C. S. G. Grunsell who obtained the final serum samples and Dr P. Higgins who supplied the cowpox isolate.

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