THE SURVIVAL OF FOOT-AND-MOUTH DISEASE VIRUS IN MEAT AND OFFAL

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(With 1 Figure in the Text)

It has long been known that the virus of foot-and-mouth disease can survive in certain animal tissues at the temperatures used for the chilling and freezing of meat. The importance of this point need not be stressed in any consideration of the epizootiology of the disease, and in most countries the regulations relating to the control of animal disease embody orders framed to minimize the risk of introduction or spread of foot-and-mouth disease by dissemination of infection in products of animal origin.

The Reports of the British Foot-and-Mouth Disease Research Committee contain the results of work on this particular problem. Stockman, Minett, Davies & Watt (1927) recorded survival of virus in the carcasses of infected guinea-pigs stored at 2–7° C., they found that residual blood in bled carcasses was still infective after 21 days and remained so for at least 35–46 days in unbled carcasses, and that virus could be detected in bone marrow for periods of from 21 to 87 days in bled carcasses and up to 96 days in unbled carcasses. Similar observations were made on the carcasses of cattle and pigs, bone marrow being infective for at least 42 days in the case of bacon carcasses stored at freezing or chilling temperatures or treated by wet or dry salting processes. In the cases of a beef and a bacon carcass stored at −9 to −13° C. virus was recovered from the bone marrow after 76 days. They further reported that the disease could be transmitted to pigs by the feeding of crushed bones known to contain infective marrow. Andrews, Dobson, Bannatyne, Davies, Simmins, Watkins & Evans (1931) continued this work under conditions resembling, as closely as possible, those of the imported meat trade. Bovine carcasses were kept at chilling temperatures, −1 to −2° C., and offals were frozen and stored at −8 to −9° C. The following periods of survival at −1° C. were recorded: up to 33 days in the tongue, cheek and the washings from an intact quarter; up to 40 days in meat-wrapping cloths soaked in virulent blood and up to 80 days in bone marrow and in blood clot.

These results establish beyond doubt the long periods for which the virus may survive in bone marrow or in blood at temperatures ordinarily used in the meat trade. Recovery of virus from muscle, however, was irregular and, as shown later by Hof (1933) in experiments with guinea-pigs, appeared to be closely associated with the reaction of the tissue, the acidity of rigor mortis being found to inactivate the virus.

During the 1939–45 war some changes took place in the methods of handling meat for importation into Great Britain from South America. In order to save shipping space, frozen boned meat was imported. Although the bulk of this meat was frozen after rigor mortis had developed, one marked change from previous practice adopted with a small percentage of the meat was the 'quick-freezing' of boned quarters in special moulds before the development of rigor mortis (Brewster, 1944). As this might produce conditions favourable for the survival of foot-and-mouth disease virus, further research work on this point was necessary. In addition, recent quantitative studies of the virus had shown the possible limitations of using test-animals of a species different from that providing the infective material. It is necessary to use cattle as the test-animals for the detection of minimal quantities of virus if material of bovine origin is being examined. A method of titration using cattle has been evolved for this purpose (Henderson, 1945). The large animal experiments of Andrews *et al.* (1931) had all been done by testing material of bovine origin in swine and, with this newer appreciation of the relative sensitivity of cattle and swine to virus of bovine origin, it became necessary to reinvestigate the survival of virus in those tissues which their work had shown to be non-infective.

As the hydrogen-ion concentration is of great importance for the stability of foot-and-mouth disease virus, parallel determinations were made of the pH values of samples tested for the presence of virus. Neglecting other factors, prolonged survival of virus is likely only if the pH value of tissues does not fall below about 6·2. Thus, as has been noted above, the change associated with rigor mortis is sufficient to inactivate the virus in muscle. In other tissues production of acid after death is much smaller, and in those cases pH change is probably an insignificant factor in the inactivation of virus. In muscle, the change to an acid reaction may be delayed by the quick-freezing process. Those con-

cerned with research in the meat trade have made observations on the effects of quick-freezing on storage qualities, ease of transport and palatability of the meat (Bate-Smith, 1944; Meara, 1947). Incidentally, it has been shown (Bate-Smith) that the pH of meat, frozen before rigor mortis commences, remains at something greater than 6·5 until the meat is allowed to thaw. Thus quick-frozen meat is comparable with offal rather than with frozen or chilled meat when considered as a medium for the survival of virus. However, in the present experiments, it has been found that the rate of onset of rigor mortis in quick-frozen meat after thawing is much greater than in freshly killed meat held at 4° C., and this would, perhaps, lessen the risk of dissemination of the virus by such meat.

METHODS

Strains of foot-and-mouth disease virus. Strain no. 119, Vallée A type, isolated from an outbreak in cattle in Great Britain in 1932; strain WA, Vallée O type, a German strain received from the German Foot-and-Mouth Disease Research Institute, Insel Riems, in 1939; strain ASJ, Vallée O type, an Argentine strain received from Drs Schang and Rossi in 1944, isolated from an outbreak in cattle in 1935 on the 'San Justino' ranch, Buenos Aires. At Pirbright, these strains have always been passaged in cattle.

Origin of meat and offals. For each experiment one Devon steer was inoculated on the tongue with a suspension of virus. Forty-eight hours later, generally at the height of infection, the animal was shot with a captive bolt humane-killer and bled. Portions of the tissues or, as in one experiment, intact quarters, were then removed and stored at the required temperature as soon as possible after collection.

Conditions of storage. The samples of tissue were stored in metal boxes or waxed cardboard cartons at a temperature of 4° or from − 10 to − 20° C. The size of the tissue samples varied but in every case those stored below freezing-point were frozen throughout within 6 hr. of collection. The offals were all frozen in refrigerator cabinets, whereas some samples of beef were frozen in model moulds immersed in the brine tank (− 22° C.) of a cold-storage plant. The greatest thickness of the meat in these moulds was the same as in the moulds described by Brewster (1944) for the quick-freezing of beef in the Argentine. On some occasions the even temperature of storage was interrupted by breakdown of the refrigerating plant which prevented observations being continued for as long a period as desired. Quarters of beef were stored in cold-rooms at 4° or at − 10° C.

Determination of the virus content of tissues. All the material examined was of bovine origin and, for this reason, only cattle were used in tests for the presence of virus. So far as possible these tests were quantitative. The titration method of simultaneous multiple inoculation of different dilutions into the dermal tissue of the bovine tongue (Henderson, 1945) was used, and calculations of the 50 % positive end-point were made by the method of Reed & Muench (1938). A weighed portion of the tissue was minced with scissors, ground with sand and sufficient M/25 or M/10 buffered phosphate solution, pH 7·6, to give a 1 in 4, a 1 in 6 or a 1 in 10 suspension. This suspension was then centrifuged for 15 min. at 2000 r.p.m. and the supernatant used for the preparation of further dilutions using M/25 buffered phosphate solution. All the dilutions cited are in terms of the original tissue. Small portions of frozen tissue were thawed either by placing them in a beaker immersed in a water-bath at 37° C. or by putting them straight into M/25 or M/10 buffered phosphate solution at 37° C. Whole organs or quarters were thawed by leaving them at room temperature (about 20° C.).

Determination of the hydrogen-ion concentration in the tissues. pH values were determined on minced tissues using a glass electrode and portable pH meter. When observations were made on chilled meat or offals the pH values were taken at 15–20° C. With quick-frozen meat it was found necessary to make the determinations with tissue in the process of thawing in order to study the rapid pH change in such material.

RESULTS

Six experiments have been done to study the survival of virus using six donor cattle for the provision of meat and offal and eighty-six test-cattle for determination of the virus content of these tissues. In addition, one experiment has been done in which infective material was fed to swine. This involved the use of a further three cattle and thirty swine. The virus content depends on whether the tissue concerned is an affected site of predilection for multiplication of the virus, for example, the tongue, feet, non-papillated pillars of the rumen, etc. or whether the virus is simply that which may be present in the blood or tissue fluid contained in the material at the time of slaughter.

Exp. 1. Virus strain WA. Donor animal no. C/1 Q. Lesions at the time of slaughter: unruptured vesicles on the tongue and three feet, temperature 103° F. Samples of beef, liver and the non-papillated pillars of the rumen were collected. The virus content and the pH of these tissues were determined at the time of slaughter and after 3 weeks', 8 weeks' and 4 months' storage. No virus was detected in beef stored at 4° C. when tested at 3 and 8 weeks. Nor could virus be detected in beef frozen, stored at − 20° C., and allowed to thaw before preparing a suspension for a test. But when a portion of the frozen beef was thawed

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by immersing it in buffered phosphate solution at 37° C. virus was detected at 3 weeks, 8 weeks and 4 months. It was not necessary to thaw liver or rumen in the buffered solution in order to detect virus, and after 4 months' storage a portion of each tissue was thawed and kept at room temperature for 24 hr. before preparation of a suspension for titration; both samples contained active virus, there being no significant drop in the comparatively high virus content of the rumen. The results are shown in Table 1.

Exp. 2. Virus strain ASJ. Donor animal no. C/82R. Lesions at time of slaughter: unruptured vesicles on the tongue and four feet, temperature 104° F. Samples of defibrinated blood, beef, liver, kidney and the non-papillated pillars of the rumen were collected. The blood and the tissues were tested at the time of collection and after 6 weeks' storage; the liver, kidney and rumen were also tested again after 7 weeks' storage, in this case being thawed and stored at room temperature for 24 hr. before test. The results, Table 2, confirm those obtained with strain WA in Exp. 1.

Exp. 3. Virus strain ASJ. Donor animal no. C/28T. Lesions at time of slaughter: unruptured vesicles on the tongue and four feet, temperature 103·8° F. A sample of beef was collected and the rate of inactivation of the virus was studied during storage at 4° C. As a control a sample was also stored under conditions favourable for virus survival, −20° C. The results are shown in Table 3, where it will be seen that active virus was recovered from the

Table 1. *Strain WA. Virus content and* pH *of bovine tissues at time of slaughter of the animal and after storage*

Tissue and temp. of storage	Initial		Duration of storage: 3 weeks		8 weeks		4 months	
	50 % end-point	pH	50 % end-point	pH	50 % end-point	pH	50 % end-point	pH
Beef	10^{-1}	6·9	—	—	—	—	—	—
4° C.	—	—	Negative	5·6	Negative	5·6	—	—
−20° C.	—	—	(*a*) Negative	5·7	(*a*) Negative	6·0	—	—
			(*b*) $10^{-0·8}$	6·7	(*b*) $>10^{-0·6}$	6·6	(*b*) $>10^{-0·6}$	6·6
Liver	$10^{-2·1}$	6·7	—	—	—	—	—	—
−20° C.	—	—	(*a*) $10^{-2·4}$	6·8	(*a*) $10^{-2·9}$	6·9	(*c*) $<10^{-0·6}$	6·8
Rumen	$10^{-2·8}$	7·5	—	—	—	—	—	—
−20° C.	—	—	(*a*) $10^{-3·2}$	7·2	(*a*) $10^{-3·8}$	7·1	(*c*) $10^{-2·5}$	6·8

(*a*) Allowed to thaw before preparation of suspension. (*b*) Thawed in buffered phosphate solution. (*c*) Allowed to thaw and stored at room temperature for 24 hr. before preparation of suspension.

Table 2. *Strain ASJ. Virus content and* pH *of bovine blood and tissues at time of slaughter of the animal and after storage*

Tissue and temp. of storage	Initial		Duration of storage: 6 weeks		7 weeks	
	50 % end-point	pH	50 % end-point	pH	50 % end-point	pH
Defibrinated blood	$10^{-2·4}$	7·7	—	—	—	—
4° C.	—	—	$10^{-1·8}$	7·9	—	—
−20° C.	—	—	(*a*) 10^{-3}	7·8	—	—
Beef	$10^{-0·7}$	6·4	—	—	—	—
4° C.	—	—	Negative	5·9	—	—
−20° C.	—	—	(*b*) $10^{-0·6}$	6·6	—	—
Liver	$10^{-1·5}$	6·5	—	—	—	—
−20° C.	—	—	(*a*) $10^{-1·3}$	7·2	(*c*) $<10^{-0·6}$	6·4
Kidney	$10^{-1·7}$	7·0	—	—	—	—
−20° C.	—	—	(*a*) $10^{-2·1}$	7·1	(*c*) $10^{-1·5}$	6·9
Rumen	$10^{-0·7}$	6·9	—	—	—	—
−20° C.	—	—	(*a*) $10^{-0·6}$	7·3	(*c*) $10^{-1·2}$	6·8

(*a*) Allowed to thaw before preparation of suspension. (*b*) Thawed in buffered phosphate solution. (*c*) Allowed to thaw and stored at room temperature for 24 hr. before preparation of suspension.

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Table 3. *Strain ASJ. Rate of inactivation of the virus in beef stored at* 4° C.

Tissue and temp. of storage	Duration of storage: Initial 50% end-point	Initial pH	6 hr. 50% end-point	6 hr. pH	24 hr. 50% end-point	24 hr. pH	3 days 50% end-point	3 days pH	9 days 50% end-point	9 days pH	11 days 50% end-point	11 days pH
Beef	$10^{-2\cdot1}$	6·6	—	—	—	—	—	—	—	—	—	—
4° C.	—	—	$10^{-0\cdot7}$	6·6	$10^{-0\cdot6}$	5·6	Negative	5·3	Negative	5·3	—	—
−20° C.	—	—	—	—	—	—	—	—	—	—	(*b*) $10^{-1\cdot7}$	6·8

(*b*) Thawed in buffered phosphate solution.

Table 4. *Strain no.* 119. *Survival of virus in bovine tissues after* $4\frac{3}{4}$ *and* $5\frac{3}{4}$ *months' storage*

Tissue	Initial 50% end-point	Duration and temperature of storage: $4\frac{3}{4}$ months, 4° C.	$4\frac{3}{4}$ months, −10° to −20° C. Thawed	pH	Partially thawed	pH	$5\frac{3}{4}$ months, 4 °C.	$5\frac{3}{4}$ months, −10° to −20° C. Thawed	pH
Beef	(i) $10^{-1\cdot2}$	—	—	—	—	—	Negative	Negative	5·5
	(ii) $< 10^{-0\cdot8}$	Negative	Negative	5·5	Negative	6·5	—	—	—
Liver	(i) $10^{-2\cdot2}$	—	—	—	—	—	Negative	Positive	6·6
	(ii) $10^{-1\cdot8}$	Negative	Negative	6·4	Negative	6·5	—	—	—
Rumen	(i) $10^{-2\cdot7}$	—	—	—	—	—	Negative	Positive	6·4
	(ii) $10^{-2\cdot4}$	Positive	Positive	6·5	Positive	7·1	—	—	—
Lymph node	(i) $10^{-2\cdot3}$	—	—	—	—	—	Negative	Positive	6·7
	(ii) $> 10^{-1\cdot8}$	Negative	Negative	6·8	Positive	6·8	—	—	—

(i) Donor animal C/AB 14. (ii) Donor animal C/AB 39.

beef sample 24 hr. after slaughter but not at 3 days, even although the tissue at 24 hr. was markedly acid (pH 5·6).

Exps. 4 *and* 5. Virus strain no. 119. Donor animals C/AB 14 and C/AB 39. Lesions at time of slaughter: C/AB 14, unruptured vesicles on the tongue, lip and three feet, temperature 102·2° F.; C/AB 39, unruptured vesicles on the tongue, temperature 101·4° F. These two experiments can be considered together as in each case the same tissues were collected, namely beef, liver, rumen and lymph node, and the even temperature of storage was interrupted by breakdown of the refrigerating plant which resulted in complete or partial thawing of the samples for a short period. All the tissues were titrated at the time of collection, but after $5\frac{3}{4}$ months' storage in Exp. 4 and $4\frac{3}{4}$ months' storage in Exp. 5 the samples were tested only for presence or absence of virus. The results are shown in Table 4. In general they confirm those of the earlier experiments in that survival may be expected for comparatively long periods in those tissues that do not become more acid than about pH 6·2. The record of survival in the lymph nodes after $4\frac{3}{4}$ and $5\frac{3}{4}$ months at a freezing temperature is of interest as, although the virus in muscle may be rapidly inactivated, it might be possible for some to persist in the carcass lymph nodes.

The main findings on pH have been included in the tables with those of the survival of virus. A further point, which has been referred to in connexion with the survival in muscle, is the rapid development of rigor mortis during the thawing of quick-frozen meat. Commonly, samples of meat taken from a freshly killed animal and left at 4° C. show relatively slight pH change during the first 5 to 6 hr. In fact, 6·4 was the lowest pH value found in tests on the animals involved in the experiments described and it was recorded at 6 hr. When 5 g. of a sample of quick-frozen meat, stored 4 weeks at −20° C., were allowed to thaw in air at 22° C. the results shown in Table 5 were obtained. Thus in 17 min. the pH was such that survival of virus would be unlikely to be prolonged.

The study of the pH of the tissues and fluids of the bovine carcass, besides providing the results already quoted, has shown that a medium, favourable for the survival of virus, may exist where it might not otherwise have been suspected. For example, the

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Table 5. pH *change during the thawing of quick-frozen meat*

Time in min. after removal from refrigerator	...	3	5	6	11	17	28	42	49	62
pH	...	6·8	6·4	6·3	6·3	6·1	5·9	5·7	5·5	5·4

Table 6. *Strain no.* 119. *Survival of virus in tissues of an intact quarter stored at* $-10°$ C. *after the onset of rigor mortis*

Successive periods and temperatures of storage	Tests	Tissue: Beef	Blood	Lymph node
At collection	50 % end-point	$10^{-0·6}$	$10^{-3·6}$	$10^{-3·4}$
	pH	6·8	7·7	6·7
24 hr., 4° C.	pH	5·8	—	—
6 weeks, $-10°$ C.	pH	5·5	—	—
24 hr., about 20° C.	50 % end-point	Negative	Negative*	$10^{-0·4}$
	pH	5·5	6·0	6·5
24 hr., 4° C.	50 % end-point	—	—	$10^{-0·6}$
	pH	—	—	6·4
24 hr., 4° C.	50 % end-point	—	—	$10^{-0·6}$
	pH	—	—	6·5

* Another sample stored under similar conditions, having a pH of 6·8, was found to be infective, see text.

pH of a lymph node in a carcass with beef of an acidity of 5·6 may be as high as 6·5, and the residual blood in a large blood vessel may remain sufficiently near neutrality to allow of the survival of virus should such a carcass be infective at the time of slaughter. The next experiment was designed to determine whether active virus could, in fact, be recovered from a lymph node and from residual blood in a vessel of a quarter of beef frozen after the onset of rigor mortis.

Exp. 6. Virus strain no. 119. Donor animal no. C/AO 35. Lesions at time of slaughter: unruptured vesicles on the tongue, lips and four feet, temperature 103·4°F. A forequarter and a hindquarter were kept at 4° C. for 24 hr. by which time the pH of the muscle was 5·8. They were then stored at $-10°$ C. At the time of slaughter samples of beef, of blood and of lymph node (the prescapular from the other forequarter) were collected for determination of the initial pH and virus content. After 6 weeks' storage at $-10°$ C. the forequarter was taken from the cold-room and allowed to thaw. Twenty-four hours later the prescapular lymph node was removed. By this time, it and the surrounding tissue were completely thawed although the central portion of the quarter was still partially frozen. The node was then stored at 4° C. to provide samples for subsequent tests for the presence of virus. A small quantity of blood was obtained from the brachial vein and a portion of thawed muscle was collected. The results of the various tests are shown in Table 6. It is noteworthy that the acid formation in a lymph node is insufficient to cause total inactivation of the virus even when such a node is from a quarter in which rigor mortis is complete and the reaction of the neighbouring muscle is as acid as pH 5·5. The possibility suggested by the results of Exps. 4 and 5 and by pH studies, that carcass lymph nodes may act as reservoirs of infective material, is fully substantiated. As anticipated, no active virus was detected in the stored beef. The pH of the residual blood, however, was more acid than previous determinations had led us to expect, and it was not surprising that the result of the test of its infectivity was also negative.

As this acid reaction of the blood might have been due to the inadvertent collection of some muscle 'drip' as well as blood, this part of the experiment was repeated using the hindquarter, now stored for 7 weeks at $-10°$ C. Twenty-four hours after the quarter had been removed from the cold-room, about 1 c.c. of blood was collected from the femoral vein when it and the surrounding tissue were thawing. When thawed the pH of the blood was 6·8, beef 5·5 and the popliteal lymph node 6·4. Two cattle were inoculated on the tongue with a 1 in 2 dilution of the blood in M/25 buffered phosphate solution. One animal reacted at two out of ten sites of inoculation and the other reacted at one out of ten sites, thus demonstrating the presence of active virus although in quantity much less than the initial content of the blood at the time of slaughter.

The demonstration of the persistent infectivity of offals and other parts of the carcass suggested in-

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vestigation of the risk to animals of contact with such material. Earlier observations (Stockman *et al.* 1927; Andrews *et al.* 1931) showed the irregularity with which infection followed the feeding of swine with infective tissues. In most of these experiments crushed bone was included, since traumatic injury of the buccal mucosa was considered by Stockman *et al.* to be of great importance in providing a portal of entry for the virus. Even in these circumstances, these workers failed in two out of seven instances to produce the disease by feeding material of proved infectivity, while Andrews *et al.* had four failures in five attempts. We have found it difficult to infect cattle by feeding virus in glass capillary tubes which were masticated by the animals. Groups of three and six swine fed with beef and pork respectively, from freshly killed infected animals failed to develop the disease. Only one of six fed with virulent bovine lingual epithelium became infected although in all these experiments a strain of virus was used with which no difficulty had been experienced in infecting swine by inoculation.

In the experiments quoted, however, the numbers of swine used were small. It was with this in mind that Exp. 7 was carried out, using as large a group of swine as practicable to estimate the risks of feeding infected offal.

Exp. 7. Virus strain ASJ. Donor no. C/AO 81. Lesions at time of slaughter: ruptured vesicles on tongue and unruptured vesicles on all four feet, temperature 105° F. The liver, kidneys and parotid, retropharyngeal and prescapular lymph nodes were collected and stored at −10° C. from 1 hr. after slaughter of the animal.

Eleven days later rough estimates of the virus titre of liver and lymph node were made, a total of sixteen observations on each being obtained by inoculation of the tongues of two cattle. The estimated 50 % end-point infective dilution of liver was $10^{-1.6}$ and of lymph node $10^{-2.5}$. After 14 days at −10° C. the tissues were kept at room temperature for 18 hr., by which time they were almost completely thawed. They were then minced and the semi-fluid mixture smeared on the feeding troughs in four communicating loose boxes in which were housed thirty healthy swine. These animals had already been fed with the bran and barley meal mixture on which they were maintained during the experiment. The greater

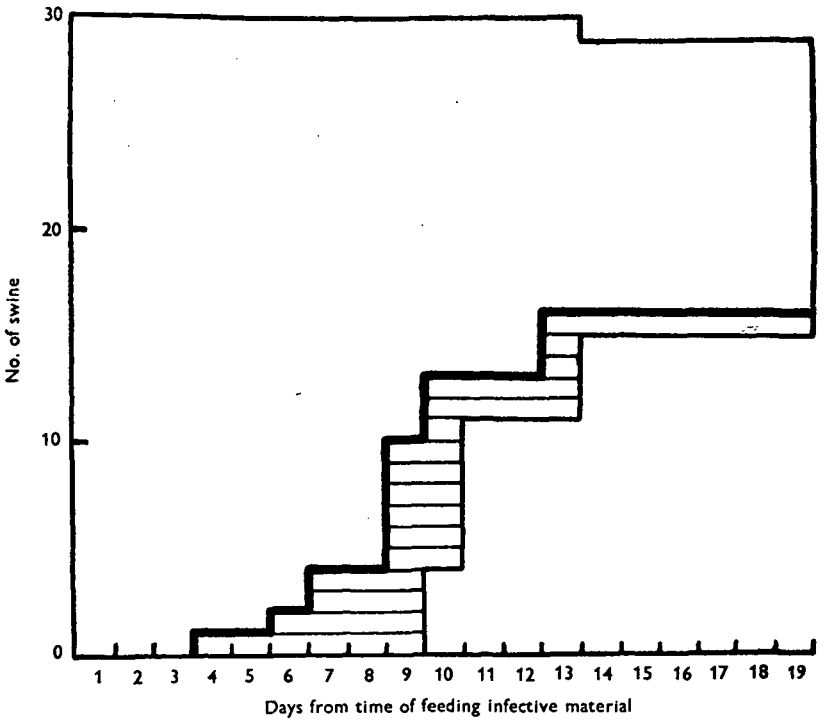

Fig. 1. Feeding of infective offal to swine. The thick line shows the number of swine which had developed foot-and-mouth disease on each day of the experiment. The period for which each diseased pig was kept in the experiment is indicated by the rectangle extending to the right from the thick line. The top line shows the total number of swine in the experiment. The drop at the 13th day resulted from the removal of an injured pig with no lesions.

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part of the minced offal was, nevertheless, eaten rapidly although after an hour some was still present in and around the feeding troughs. The temperatures of the swine were taken daily and frequent examinations made to detect lesions of foot-and-mouth disease. The occurrence of the disease in the group is represented graphically in Fig. 1. It is probable that of the group of thirty, only the first two or perhaps four of the sixteen to become diseased were infected directly from the offal and after this, spread from pig to pig took place. The importance of the number of animals exposed to infected material is thus emphasized.

The susceptibility of the swine which did not develop lesions was tested on the 19th day after feeding. They were inoculated intradermally on the tongue and snout with a suspension of epithelium collected from some of the swine reacting earlier in the experiment. Of the thirteen animals tested, seven remained free of the disease and six developed lesions confined to the sites of inoculation. In earlier experiments with this virus strain a total of twenty-one swine were inoculated with various suspensions of virulent epithelium. All developed lesions at the sites of inoculation which, in twenty animals, were followed by the appearance of secondary lesions on all four feet. The comparative insusceptibility to inoculation of the non-reactors in the feeding experiment is, we think, more an indication of immunity developed as a result of contact with infection during the experiment rather than that these particular animals had, initially, such marked resistance. This development of immunity with 'inapparent infection' is not new in our experience of foot-and-mouth disease.

Through the courtesy of Mr R. Bremner, we were given the opportunity of visiting the cold-storage warehouse at Deptford of Messrs Thomas Borthwick & Sons, Ltd., and determining the pH of samples of beef and offal imported from South America. The results, Table 7, showed that the pH of the tissues used in our experiments was the same as the pH of similar tissues brought into this country under the routine conditions of handling in the imported-meat trade.

Table 7. pH *values of imported meat and offal determined in a London cold-storage warehouse*

Tissue	pH
Beef (forequarter)	5·6
Prescapular lymph node from above forequarter	6·5
Beef (hindquarter)	5·4
Liver	5·9
Kidney	6·3
Tripe	6·3

DISCUSSION

The results of the present experiments confirm those obtained by other workers on the rapid inactivation of virus by the acidity developed during rigor mortis of muscular tissue, and new data are provided upon the quantitative aspects of the virus content of tissues. Our records of survival in offals such as liver, kidney and rumen stress the necessity of considering the host specificity of foot-and-mouth disease virus. These positive results were obtained using cattle as the test-animals for cattle strains. Andrews *et al.* (1931), using swine in attempts to detect virus of bovine origin, obtained consistently negative results in their tests of beef offal, although by guinea-pig inoculation they had demonstrated survival of virus after 2–3 months' storage at $-2°$ C. in liver, kidney and other tissues from guinea-pigs infected with a guinea-pig adapted strain.

The initial virus content of tissues in which multiplication of the virus can occur may be high owing to the presence of lesions. The non-papillated pillars of the rumen are frequently the site of small vesicles that might escape notice, but would account for the relatively higher titres obtained with this tissue. It is important to note, however, that our observations on the prolonged survival in rumen follows less severe treatment than rumen receives in the preparation of tripe. An essential feature of this process is the loosening and removal of the mucous membrane, usually by immersing the washed organ in water at a temperature of 60–65° C. Most of the virus in an infective sample, therefore, will be removed before the offal is frozen. Where such a procedure is adopted the risk will obviously be much less than the infectivity of the untreated tissue would suggest. The position with regard to the other offals is different. Here the virus content seems to depend on the quantity of infective blood or tissue fluid remaining in the organ. The persistence of active virus in these tissues is favoured by a lack of acid production on a scale equal to that in muscle. Thus, when liver or kidney is stored frozen, as in the imported-meat trade, it may be shown to have a high degree of infectivity at 4 or more months and the virus remains active for at least 24 hr. after thawing.

An observation of considerable importance is that the pH of a lymph node does not become sufficiently acid to inactivate the virus even although such a node may be from a carcass with beef of an acidity about pH 5·5–5·6. A number of the lymph nodes of the ox, including the prescapular and the popliteal, are imbedded in fat. In the preserved state this tissue does not have much acid formation and it may effectively isolate such nodes from the more acid muscle. Even in the absence of surrounding fat, however, the fascia, the perinodular tissue and the fibrous tissue capsule of the node may be an adequate

barrier between muscle and node. The fact that the reaction of blood in the lumen of a vein in acid meat may still be near neutrality suggests that the similar barrier of fascia, perivascular tissue and the vessel wall will prevent sufficient diffusion to affect the pH.

There is no doubt that the lymph nodes must be classed with bone marrow as possible sources of infection in an otherwise 'safe' carcass rendered apparently non-infective by the acidity of rigor mortis. The results of tests for the survival of virus in the residual blood of frozen carcasses are less demonstrative, but the one positive finding shows that here too, under certain favourable conditions, is a possible source of danger.

The earlier work on this problem in which survival of virus was most frequently found in bone marrow has, perhaps, laid too much emphasis on the risk attached to bones, whereas it is obvious that there may be an equally good chance of other tissues remaining infective for long periods.

The danger of quick-frozen meat as a medium in which virus survives may be overestimated. In the first place, in a year in which 800,000 tons of meat were imported into Great Britain, while the bulk had been boned to save shipping space, only about 1 % had been quick-frozen. Further, we have shown it to be difficult to demonstrate virus in quick-frozen meat without resorting to the technique of thawing in buffer solution. It should be remembered, however, that such meat may not be completely thawed even when it leaves the retailer. Up to this time any contaminating virus will remain active. Although after thawing, the danger from such meat is likely to be the same as that from chilled or frozen carcasses, there will be more risk attached to the quick-frozen product so long as it remains frozen. The wrapping cloths of such meat are also likely to be a greater danger than the cloths from chilled or frozen carcasses.

Our observations on survival of virus in frozen meat and offal were usually made following storage at a temperature of $-20°$ C. which is lower than the normal temperature of cold-storage warehouses ($-10°$ C.), but our experience has shown that there is no detectable difference in survival of virus at -10 and $-20°$ C.

We have made no observations on the survival of virus in porcine tissues, but pH studies showed that the acid formation of rigor mortis was not sufficiently different from that of beef and beef offal to necessitate separate consideration.

It will be appreciated that all these observations were made on the tissues and fluids of cattle killed at the height of infection in order to have material sufficiently infective for the experimental study of virus survival. Cattle killed earlier in the course of clinical infection would provide tissues of lower virus content. It is known, also, that virus may be recovered from the blood of cattle even before the development of detectable lesions.

Although we trust every effort is made in the country of origin to avoid exportation of meat from infected animals it must be admitted that the distribution of unprocessed meat and offal from animals in the infective stages of foot-and-mouth disease constitutes a risk of dissemination of infection. The risk is obviously less with some tissues than with others, and it may be mitigated by certain control procedures. For example, the Diseases of Animals (Boiling of Animal Foodstuffs) Order (Great Britain), 1947, directs that all waste products of animal origin be boiled before being fed to live stock. Furthermore, so far as is practicable, allocations of imported meat may be made to towns and cities where there are fewer susceptible animals and where central processing of kitchen waste can be more readily undertaken. It must not be supposed, too, that all susceptible stock coming in contact with infective material will develop the disease. In our experiment, two or perhaps four of a group of thirty swine became infected directly from the offal fed. Nevertheless, the appearance of the disease in even one animal of a flock or herd may lead to disastrous results in a country with a highly susceptible animal population.

SUMMARY

Quantitative studies have been made of the survival of foot-and-mouth disease virus in beef and beef offals after storage at temperatures employed in the imported-meat trade.

The survival of virus is closely associated with the hydrogen-ion concentration of the tissue; thus the acidity of rigor mortis of muscular tissue rapidly causes inactivation. Quick-freezing of beef suspends acid formation and active virus was demonstrated for so long as the meat was kept frozen.

Thawing of quick-frozen meat initiates the suspended acid formation at an accelerated rate and rapidly produces a medium unsuitable for virus survival.

Liver, kidney, rumen, lymph node and blood from diseased cattle have all been shown to be highly infective and to remain so if stored frozen. Acid formation in these tissues and in blood is not on the same scale as in muscle, and prolonged survival of virus is more likely even with delay in freezing and after thawing. This remains true of lymph node and of residual blood in vessels of a carcass in which the development of rigor mortis is complete.

Feeding of infective offal to swine under experimental conditions resulted in the appearance of the disease.

The significance of these observations is discussed in relation to the distribution of these products constituting a risk of spreading foot-and-mouth disease.

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