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Persistence of Foot-and-Mouth Disease Virus in Animals, their Products and the Environment

by

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Animal health authorities are constantly faced with problems involving Foot-and-Mouth disease virus (FMDV) in relation to the importation of live animals, meat, dairy and other animal products. They are also concerned with survival of FMDV on various objects.

General reviews on various aspects of this subject were made recently by MÖHLMANN (1), RÖHRER (2), ZATOCIL and GILKA (3), NAURYZBAEV (4), WITTMANN (5) and HESS (6). There have also been many brief discussions as well as important new data reported on the subject since the reviews. Nevertheless, much of the more important portions of this information have not been compiled in tabular form.

This report includes a brief review of the above problems, giving in tabular form the extremes for the earliest detection of FMDV and its longest reported persistence in living animals. Similarly, virus survival in animal tissues and fluids and on various objects is also tabulated. New data is presented on the persistence of FMDV in certain organs and fluids and on the survival of virus in bone marrow and lymph and hemal nodes from infected cattle.

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MATERIALS AND METHODS.

Viruses.

The FMDV strains used were : O-CANEFA-9, O_1-CANEFA-2, A_{12}-119, and SAT-1/2 Rhodesia 11/37. The history of these virus strains, the method of preparing virus suspensions from FMD-infected bovine tongue epithelium, and the methods for virus titration were previously reported (7).

Experimental animals.

Grade Hereford steers were used and their management in isolation rooms has been previously reported (8). The cattle were 14 to 24 months old and weighed about 700 lbs. They were inoculated in the tongue epithelium with about $10^{5.0}$ bovine ID_{50} units of virus.

At various times from 12 hours to 8 days postinoculation (HPI, DPI), cattle were killed to obtain tissue samples for virus isolation. Samples of bone marrow and lymph and hemal nodes were obtained from other cattle killed at 48 HPI during the acute clinical stage of disease. These samples were titrated for virus content at the time of slaughter and after storage for 4, 7, 12 and 24 months at 1-4° C.

Virus detection and diagnostic tests.

Bovine kidney cell cultures were used for virus detection in samples, as previously described (7, 9, 10).

The constant serum/variable virus dilutions neutralization test was used to identify the virus isolates (11).

RESULTS.

The persistence of FMDV in some of the tissues and fluids of infected and convalescent cattle is given in Table I. Samples of thyroid, adrenal and rumen contained FMDV from 12 HPI to as long as 8 DPI. In samples of kidney, and bone marrow, virus was found for only 6 DPI. In samples of blood, spleen and liver, the limit of virus detection was 4 DPI. Similar results were obtained with FMDV Strain O-CANEFA-2 (not included in Table). All cattle had clinical signs and lesions of FMDV within 24 HPI.

TABLE I

Persistence of Foot-and-Mouth Disease virus O-CANEFA-9 in tissue of infected cattle (*).

	Days Postinoculation							
	0.5	1	2	4	6	6	8	8
Blood	4.2	4.2	5.6	1.5	N	N	N	N
Thyroid	3.3	3.0	6.0	5.0	3.7	1.2	N	1.2
Adrenal	2.5	3.4	6.2	6.6	4.8	3.6	1.1	3.2
Pancreas	2.8	3.7	6.4	5.9	3.6	3.4	0.9	N
Kidney	3.1	3.5	4.0	2.6	N	2.4	N	N
Spleen	2.5	3.0	3.1	2.6	N	N	N	N
Liver	2.2	2.6	3.6	1.5	N	N	N	N
Rumen	2.8	4.8	6.3	6.4	5.2	2.0	1.5	1.2
Bone marrow	3.9	4.3	5.9	4,6	1.0	N	N	—

(*) Cattle inoculated in tongue epithelium with 10^5 bovine ID_{50} units of virus. Titers of virus are in bovine kidney cell culture $\log_{10}$ PFU/gm. of sample ; N = negative ; — = not done. Samples tested at 10 DPI were negative.

In samples of bone marrow taken from cattle during the acute stage of infection (48 HPI), FMDV survived at 1-4° C for as long as 210 days (Table II). In similar samples of lymph nodes and hemal nodes, virus persisted for 120 days.

TABLE II

Survival of Foot-and-Mouth Disease virus in tissues of infected cattle during storage (*)

Samples	Viruses	Months storage at 1 to 4° C				
		0	4	7	12	24
Bone marrow	A-119	3.8	2.0	1.2	N	N
Bone marrow	SAT-1	4.0	3.1	1.5	N	N
Lymph nodes	A-119	3.6	1.2	N	N	—
Lymph nodes	SAT-1	3.8	0.9	N	N	—
Hemal nodes	A-119	3.5	0.8	N	N	—

(*) Cattle inoculated in tongue epithelium with 10^5 bovine ID_{50} units of virus. Titers of virus are in bovine kidney cell culture log 10 PFU/gm. of sample. N = negative ; — = not done.

DISCUSSION

Eclipse phase of FMDV.

When susceptible cattle are inoculated in the tongue epithelium with FMDV, virus replication may begin very rapidly and viremia may be detected within 2 hours in some cases (12) (Table III). The length of time that viremia precedes the advent of clinical signs and lesions may vary from about 8-40 hours, depending on the virus dose and route of inoculation (12) (Table IV). Likewise, virus often may be found in saliva, semen, urine, lymph nodes and other tissues and fluids several hours before clinical manifestations of diseases (Table IV).

TABLE III

Time of appearance and persistence of Foot-and-Mouth Disease virus in fluids and discharges of infected animals after inoculation.

Samples	Hours to days	References
Blood	2 - 5 *	12, 18
Semen	12 - 10	22
Urine	12 - 7 *	22, 18
Milk	13 - 4,5	45, 46
Saliva (not O.P. fluid)	9 - 11	47, 48, 49
Synovial	12 - 5	50
Peritoneal	24 - 6	**
Pleural	24 - 6	**
Cerebrospinal	24 - 5	10
Nasal discharge	24 - 7	47, 49, 51
Feces	5 - 4,5	45
Expiration (aerosol)	18 - 14	16

(*) Waldmann *et al.*[18] reported finding FMDV in blood for as long as 58 days and in urine for 246 days.
(**) Unpublished results of Cottral (G.E.) and Gailiunas (P.).

When cattle are infected with FMD by contact or aerosol exposure, the eclipse phase may be lengthened to days (Table IV). Following contact exposure, virus may be found in the pharynx of cattle from shortly after exposure to as long as 9 days before clinical signs and lesions appear (13) (14).

It could be assumed that after replication and release from the cells of the throat, the virus may then spread to the sites

where clinically recognizable gross lesions are more often found in the oral cavity and on the feet (15) (16) (17). A further discussion of the pathogenesis of FMD has been reported previously (17).

Presence and persistence of FMDV in live animals.

The reported extreme times of appearance and persistence of FMDV in various fluids, discharges, and tissues of infected animals (primarily cattle and swine) are given in Tables III and V. Many other studies dealing with virus detection within

TABLE IV

Time variations from first detection of Foot-and-Mouth Disease virus until lesions appeared in cattle infected by inoculation and by contact.

Samples	Time	References
From inoculated cattle	*Hours*	
Saliva	0 - 2	52
Blood	8 - 40	12
Semen	2 - 12	22
Urine	2 - 12	22, 53
Lymph nodes	4	54, 9
From contact cattle	*Days*	
Pharynx	0 - 9	13, 14
Saliva	1 - 7	14
Blood	1 - 6	13, 14, 12 *
Semen	1 - 4	14
Milk	1 - 4	13
Vagina	1	13
Rectum	1 - 6	13, 14
Prepuce	2 - 4	13

(*) With aerosol exposure, the time lapse was 48 hours [12].

the specified ranges were not referenced. The long periods of time for finding FMDV in urine, kidneys and urinary bladder reported by Waldmann *et al.* (18) have not been confirmed by other workers ; however, their exact technique was not duplicated. Likewise, the demonstration of FMDV in feces of infected animals has been inconsistant and sometimes unsuccessful (19).

TABLE V

Time of appearance and persistence of Foot-and-Mouth Disease virus in tissues of infected animals after inoculation.

Tissues	Hours to days	References
Bone marrow	12 - 6	Table I
Pituitary	12 - 6	10
Pineal	48 - 8	10
Thyroïd	12 - 8 *	Table I
Adrenal	12 - 8 *	Table I
Pancreas	12 - 8	Table I
Lymph nodes	8 - 15	9
Liver	12 - 4	Table I
Kidney	12 - 94	18
Spleen	12 - 4	Table I
Spinal cord	12 - 8	10
Testicles	24 - 11	18
Urinary bladder	24 - 94	18
Rumen	12 - 8 *	Table I
Skin	12 - 7	23
Muscle (lesions)	12 - 14	20
Heart (lesions)	4 - 14	20
FMD tongue lesions	8 - 9	9,47

(*) Virus probably persists longer than 8 days postinoculation.

When muscle tissue (skeletal and heart) has gross lesions of FMD, virus persistence may be much longer than that reported for the normal-appearing muscle tissue of infected animals (20).

The longest interval reported for the persistence of virus in tissues that are of current interest in carrier and natural transmission studies is given in Table VI. The dorsal surface of the soft palate seems to be the tissue area where virus can be found for the longest period in cattle slaughtered after recovery from FMD (21). Further studies may show that virus persistence in parts of the digestive and genito-urinary tracts as well as skin, also may have an important role in natural transmission (17) (22) (23).

The FMD-carrier studies demonstrate that the virus is easily established in the throat area of cattle, sheep and goats (15) (24) (21) (25) (26) (27).

TABLE VI

Persistence of Foot-and-Mouth Disease virus in tissues of convalescent and recovered animals.

Tissues	Days	References
Tongue	23	21, 47, 49
Glosso-epiglottic space	31	21
Pharynx	75	21
Soft palate, dor. surf.	196	21
Soft palate, vent. surf.	75	21
Tonsillar sinuses	31	
Tonsils	21	21
Oesophagus	29-31	21
Trachea	23	21
Muzzle	8	47
Foot lesions, cattle	12	47, 49
Foot lesions, pigs	10	55
Hoof, cattle	34	56

Carriers may be detected by virus isolation from œsophageal-pharyngeal fluids collected with a cup probang (15) (25). The duration of the FMDV-carrier state in the 4 most susceptible species, as currently reported, is given in Table VII. These data indicate that cattle apparently remain FMDV carriers longer than sheep, and goats while by similar techniques, pigs have not been shown to be carriers. When FMDV is inoculated in the throat or nasal passages in vaccinated or passively immunized cattle, they may become carriers without showing clinical signs or lesions (26) (28).

FMDV in animal tissues during storage.

When the tissues of animals infected with FMDV are frozen, virus may survive for years, depending on conditions of freezing. Bovine tongue epithelium containing FMDV, strain A-119 lost very little virus titer during storage for 11 years at — 50° C (29).

Semen from an FMD-infected bull maintained its virus titers for 320 days at — 50° C (22). Lyophilisation also will preserve FMDV for long periods of time (30). Thus, since virus survival

is expected when tissues are frozen or lyophilized, reports dealing with those techniques are not included in the tables.

TABLE VII

Duration of carrier state in various species of animals as determined by isolation of Foot-and-Mouth Disease virus from oesophageal-pharyngeal fluids.

Species	Months	References
Cattle	7 - 24	15, 55, 57
Sheep	1 - 9	57, 27
Goats	1+	27
Pigs	Negative	55

The reported extreme survival times for FMDV at 1 to 7° C in the tissues, fluids and organs of infected animals are given in Tables VIII and IX. The virus in bone marrow and lymph and hemal nodes survived longest. Muscle tissues not involved as a lesion area with FMD or contaminated with other tissues provide the poorest conditions for virus survival. Postmortem lactic acid formation and enzymatic changes probably are the main factors in bringing about virus inactivation in muscle tissue (8) (31). Additional work is required to assess more completely virus survival in the internal organs (Table IX).

FMDV in salt-cured products.

In salt-cured beef, virus does not survive in the muscle tissue, but may be found in lymph nodes, bone fragments or large blood clots (8) (Table X). Various techniques have been used in attempts to inactivate FMDV in meat (32). In ham, virus may be found in the bone marrow and fat (33) (34). The grinding and mixing processes used in the manufacture of sausages and similar products reduce the chances of virus survival, even before smoking and heating (33). Salt-cured hides may harbor FMDV for long periods of time (35).

Purified FMDV, A-119, was inactivated at specific isoelectric points, i.e. the salt concentration, as well as *p*H, determine the point at which the virus is inactivated (36).

TABLE VIII

Survival of Foot-and-Mouth Disease virus at 1 to 7° C in tissues and fluids from infected animals.

Samples	Species	Days	References
Blood	Swine	70	58
Blood	Cattle	60	8
Bone marrow	Cattle	210	Table II
Bone marrow	Swine	42	59
Lymph nodes	Cattle	120	Table II
Lymph nodes	Swine	70	58
Hemal nodes	Cattle	120	Table II
Synovial fluid	Cattle	19	50
Muscle	Cattle	3*	31,8
Muscle	Swine	1	33
Muscle (lesions)	Cattle	3	60
Muscle (not bled)	G. Pigs	31	59
Tongue	Cattle	33	38
Tongue	Swine	10	33
Cheek	Cattle	33	38
Intestines	Cattle	6	61
Hides (dried)	Cattle	8	35
Pituitary extract	(Commercial)	30+	62

(*) Virus was found at 60 days in muscle tissue of beef carcass, possibly due to contamination with bone fragments.[8]

TABLE IX

Survival of Foot-and-Mouth Disease virus at 1 to 7° C in internal organs from infected animals.

Organs	Species	Days	References
Brain	Swine	27	58
Parotid	Bovine	8	33
Lung	Swine	42	58
Lung	Bovine	8	33
Stomach	Swine	10	33
Rumen	Bovine	8	33
Kidney	Swine	42	58
Spleen	Swine	42	58
Uterus	Bovine	8	33

TABLE X

Survival of Foot-and-Mouth Disease virus at 1 to 7° C in salt-cured products and tissues of infected animals.

Products & Tissues	Days	References
Beef (lymph nodes)	50	8
Bacon	10	33
Ham (bone marrow)	89	33, 34
Ham (fat)	46	33, 34
Sausages	4	33, 63
Tongues, cattle	14	64
Hides, cattle	352	35
G. pig FMD lesions	(2 years)	65

If these conditions also apply to crude virus situations, virus may survive at lower *p*H levels at a high salt concentration than when salt is not used. Thus, if salt is used, an alkaline *p*H may be more desirable for FMDV inactivation in animal products (17).

FMDV in dairy products.

Dairy products were experimentally contaminated with lymph or suspensions of epithelial lesions from FMD-infected animals (37) (38) (39) (40). Of the fresh products, salted butter seems to provide the most favorable conditions for virus survival (37) (Table XI). Perhaps, as previously mentioned, the *p*H effect on the virus may have been modified by the high salt concentration. Dried milk may harbor FMDV for several years (39). The problem of inactivating FMDV in milk was reviewed by Kästli and Moosbrugger (41) and by Sellers (42). Some of the occasions when milk and other dairy products were suspected to be the cause of FMD outbreaks were reviewed by Hess (6).

In many cheeses, the survival time for FMDV may be reduced to hours. In Emmentaler and Neufchatel cheeses, FMDV was very rapidly inactivated (40). The *p*H and salt concentration of the cheese seem to be the important factors in virus inactivation.

TABLE XI

Survival of Foot-and-Mouth Disease virus in experimentally contaminated dairy products.

Products	Days	References
Milk, skimmed, whole	9 - 12	37
Pre-sterilized milk (held at 18° C)	35	38
Cream	10	37
Butter, plain, salted	26 - 45	37
Buttermilk	14	37
Dried milk	(2 years)	39
Cheeses	*Hours*	
Camenbert	.08	40
Edam	22	40
Limberger curds	14.5	40
Quadrat & Tilsiter curds	5 - 6	40
Cheese whey	20 - 23	40

FMDV contaminated objects.

The survival of FMDV on or within various contaminated objects at ambient temperatures is given in Table XII. The survival time would be shorter for free virus than for virus within cells from epithelial lesions. Also, the amount of protective colloids and tissue debris as found in mucous from the nasal and salivary discharges of infected animals would lengthen the survival time. Sunlight, temperature and *p*H changes, relative humidity and dilution effect of rain and melting snow all would affect virus survival (6) (17). Longer survival times for FMDV on objects would be expected at the low temperatures associated with high altitudes or the arctic regions than at sea level or in the tropic regions. Workers in Germany and the Soviet Union have been interested in this problem and their work provides many of the extremes in virus survival on various objects (1) (4).

In addition to the objects listed in Table XII, many other contaminated items have been suspected as the source of FMD outbreaks. For example, various domestic or wild ani-

mals and birds, man, truck tires, and even postal materials have been under suspicion in outbreaks (6). However, it may ultimately be proved that FMDV may be disseminated over considerable distances by aerosols — adding another important factor to explain the epizootiology of the disease (43) (44) (16) (6).

TABLE XII

Survival of Foot-and-Mouth Disease virus as a contaminant on various objects at ambient temperatures.

CONTAMINATED OBJECTS	WEEKS	REFERENCES
California farm	49	66
Soil, S-W *	1-21	67
Barn dirt ; barn sand	1-10	1
Road sand; garden soil	1.5-4	1, 68
Manure, S-W	1-24	67, 69
Sewage (low ammonia) S-W	3-15	70
Barns (brick, adobe, wood), S-W	2-11	4
Walls, brick & plaster	2-4	38, 71
Soil, water, lichens (Arctic))	4	72
Pasture plants, S-W	1-7	67
Haystack, S-W	4-29	67, 1
Feed sacks ; bran	20	59, 1
Meal	7	1
Vegetables	1	73
Water	3-14	4, 5
Houseflies	10	74
Ticks ; haematin of ticks	15-20	75
Wool, sheep	2	68
Hair, cattle	4-6	1, 68
Glass surface	2 +	38, 1
Clothing & footwear,** S-W	3-9, 14	4, 76
Meat cloth (at 4C)	6	38

(*) S-W, 1-21 = summer 1 week, winter 21 weeks.
(**) Cotton garments, leather shoes, rubber boots.

★★★

SUMMARY

A review is presented on the persistence of Foot-and-Mouth Disease virus (FMDV) in living animals, survival in stored samples of animals tissues, fluids, and products and survival at ambient temperatures on various objects. The material is mainly presented in tabular form. New data are given on the persistence of FMDV in endocrine glands, and internal organs of infected and convalescent cattle. New data are also included on the survival of FMDV in samples of bone marrow and lymph and hemal nodes of infected cattle during storage at 1-4° C.

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