

[ Food Vir. 3 1971 ]

WORLD HEALTH ORGANIZATION

ORGANISATION MONDIALE DE LA SANTE

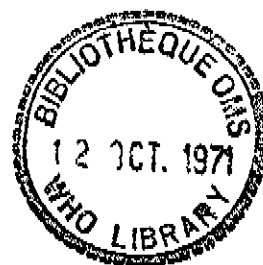
July 1971

Restricted

FOOD VIROLOGY

Report on the Third Informal Consultation

Brno, 21-25 June 1971  
Geneva, 6-10 July 1971



THIRD INFORMAL CONSULTATION ON FOOD VIROLOGY

TOPICS

1. Introduction.
  2. Suggestions of Doc. Dr. Manžák, co-ordinator of the Data Collection Centre on the presence of viruses in raw milk and meat, and his collaborators.
  3. Suggestions of Dr. Cliver, co-ordinator of the Data Collection Centre on the presence of viruses in processed foods, and his collaborators.
  4. Development of the system of collaborating laboratories.
  5. Plans for Food and Water Virology session during the Second International Congress for Virology in Budapest, 27 June-3 July 1971.
  6. Further business in Brno.
  7. The Food and Water Virology session in Budapest.
  8. The final deliberations in Geneva.
- Appendix - INTERNATIONAL COLLECTION OF DATA ON THE PRESENCE OF VIRUSES IN FOODS: guide for reports, with samples of completed reports.

LIST OF PERSONS WHO TOOK PART IN CONSULTATION

Dr D.O. Oliver  
Associate Professor  
Department of Virology and  
Bacteriology  
University of Wisconsin  
Madison  
Wisconsin 53706  
USA

Doc. Dr J. Šteflík, C.Sc.  
Veterinary Research Institute  
Driscova 60  
Brno-Medlanky  
Czechoslovakia

WHO consultant in food virology and  
co-ordinator of the Data Collection  
Centres on the presence of viruses in  
processed foods.

Co-ordinator of the Data Collection  
Centres on the presence of viruses  
in raw milk and meat.

PROF. DR A. Holub, Dr.Sc.  
Director of the Veterinary Institute  
Brno-Medlanky  
Czechoslovakia

Dr J. Černý )  
Dr Z. Dvořáková )  
Dr V. Pícha )  
Dr Z. Pospíšil )  
Dr V. Šteplný )  
Dr B. Štěl )  
Dr I. Štrunc )  
Dr Z. Váňek )  
Dr D. Váňková )  
Scientists of  
the Veterinary  
Research  
Institute,  
Brno-Medlanky

Dr J. Paláček  
Scientist  
Department of Food Hygiene and  
Technology  
University of Veterinary Medicine  
Palackého 1-5  
Brno-12  
Czechoslovakia, at present working  
in the Veterinary Research Institute

Doc. Dr J. Strausz  
Institute of Epidemiology and  
Microbiology  
Črbskova 48  
Prague, Czechoslovakia

SECRETARIAT

Dr Z. Matyas, Food Hygienist, Veterinary Public Health, Division of  
Communicable Diseases, World Health Organization, Geneva

## 1. Introduction

Following the consultations on Food Virology held in Geneva in September 1969 and September 1970, a third consultation was scheduled for 1971. The principal consultation took place on 23 June 1971 at the Veterinary Research Institute, Brno, Czechoslovakia. All of the listed persons were present.

The consultation was opened by Dr J. Menšík and all present were greeted by Professor A. Holub, Director of the Veterinary Research Institute.

Dr Matyas made some introductory remarks concerning the history of the Food Virology effort in WHO. He further said that the main purpose of this consultation was to review the results of the collection on the presence of viruses in foods since the time this programme was established in 1970 and suggest any modifications in the reporting system which could contribute to the improvement of the work.

With regard to the problem of virus transmission through foods, he stated that a good deal of direct evidence already exists and that there is, everywhere, a high incidence of "food-borne illness of unknown etiology", some of which may be caused by viruses. Dr Cliver commented that such "food-borne illness of unknown etiology" was usually recognized because there were gastrointestinal symptoms, but that a good deal of other virus disease might not be recognized as food-borne simply because the symptoms involved parts of the body other than the digestive tract.

## 2. Suggestions of Doc. Dr J. Menšík, co-ordinator of the Data Collection Centre on the presence of viruses in raw milk and meat and his collaborators

The initial efforts of the group in Czechoslovakia had demonstrated that modifications were needed in the form designed during the 1970 consultations. The most obvious problem was that spaces for answers had been left in the question form. In some cases these spaces were too small for the information, and at other times they were not needed because no answer was known. Therefore, the key suggestion of Dr Menšík and his collaborators was that a separate answer sheet be provided, at least for the questions originally numbered 1-6.

Examples of three possible revised systems were presented. The answer sheets for all of these were generally similar: the virus group code and the name of the virus were specified at the top of the page, and the remainder of the sheet was divided into columns for (1) the code number of the question, (2) the answer to the question, and (3) the numbers of any references on which the answer was based. A single report would comprise a number of such answer sheets and an appendix listing the cited references by number. The references would be assigned numbers approximately in the order of their dates of publication.

One of the main differences from the form proposed in 1970 in 1971 was that in the 1970 form the questions were arranged on the kind of answers about geographical areas. Numbers were assigned to each of the species listed in question 1, to permit coding the individual parts of the question. No special key "numbers" would be needed, as any question part which had no known answer would be entirely omitted. Question 3 remained in the form of a two-dimensional table.

The second system differed further in that code numbers were assigned both to the host species and the sites of localisation in question 3. This had the principal advantage of permitting a complete set of reference numbers to be supplied for each of the question parts answered, and it eliminated the leaving of blanks where no answer was known to exist.

The third system included some such more substantial modifications. The content of answers to the parts of question 1 was expanded to permit reporting both natural and experimental infections, specifying the ages of animals studied, and describing the mode of transmission in one or more words. This last would eliminate the need for question 2 of the 1970 form. The new question (what had been question 3) also assigned code numbers for host species and site of localisation and asked that the respondent distinguish between natural and experimental infection. Organ culture was added to the list of possible means of laboratory cultivation in the following question.

Several additional suggestions were offered. Dr Pleva presented a list of additional kinds of information which might be requested in characterising a virus. These would broaden the information content of a report and would make it a more useful future reference for the person who had prepared it. Some of the topics included which had not appeared at all in the 1970 form were cytopathology in the laboratory hosts, pathology in the natural hosts, antigenicity, effects of several kinds of chemicals on the virus, chemical composition of the virus, immunity, and information about the specific strains described in published references and the strains available in the Czechoslovakian collection.

Dr Štěpánek urged that some decisions be made regarding whom the programme is intended to serve. Particularly at the stage of retrieving the data for people's use, it will be important to have anticipated fairly accurately the kinds of questions which are likely to be asked. He also suggested that some of the questions may be too specific: answers are more likely to be gotten, for instance, if one asks about the stability of a virus in the range of temperatures from 50° to 60° than only at 55°.

3. Suggestions of Dr D.O. Gliver, co-ordinator of the Data Collection Centre on the presence of viruses in processed foods and his collaborators

Dr Gliver then reported on behalf of his group. He stated that it had been decided initially to enlist his collaborators on the basis of food groups, while those of Dr Matkík were enlisted on the basis of virus groups. The categorisation and terms of the two seemed so different that

they had even considered using entirely separate forms for their data collections. It actually a single document, comprising questions with spaces for answers and a supplemental set of explanatory notes, resulted. The form containing the questions and the answer spaces was intended to be duplicated for each report, though each worker should need only one copy of the explanatory notes.

Now those working with Dr Mandlik have found it necessary to modify some of the questions and to enter the answers on separate sheets. Those working with Dr Cliver have shown that questions 7-12 of the 1970 form were not clear enough and that the explanatory notes were not serving their purpose adequately. Further, the food group collaborators had encountered the same problem as the virus group collaborators in trying to get their answers into the spaces provided in the form. Therefore, Dr Cliver suggested that the questions be expanded and combined with the explanatory notes, and that a separate sheet also be provided for the answers to questions 7-12.

The combined question and explanation paper might be called a "guide". The five types of reports described in the 1970 form would be defined more fully and coded A, B, C, D, and E. Reports from Dr Mandlik's group would be type A and would use questions 1-6. Reports from Dr Cliver's group would be of any of the other types and would use any of the questions between 7 and 12, as necessary; because of different needs, the answer sheets for these reports would comprise a cover page on which to record some coding information, and additional plain pieces of paper.

#### 4. Development of the system of collaborating laboratories

Dr Matyas described visits that he had made to other laboratories not yet engaged in this project. He said that he had found much interest and that there seemed to be a good likelihood of engaging additional collaborators.

Dr Cliver added that his group would have to be expanded, for it now appeared unreasonable for each food group to be covered by only one person. There should be at least two collaborators per food group, and sometimes more. This would place upon Dr Cliver the responsibility to be sure that no work was duplicated.

#### 5. Plans for Food and Water Virology Session during the Second International Congress for Virology in Budapest

Dr Cliver then mentioned the Food and Water Virology session which was to be held the afternoon of 1 July as part of the Second International Congress for Virology in Budapest. He stated that the meeting would be chaired by him and by Dr Donald Berg, a water virologist from the USA. Virtually everyone known to be working in food or water virology in the world had been invited, although many, of course, would not be able to come. Dr Cliver's presentation at the session was to be devoted to describing the International Collection of Data on the Presence of Viruses in Foods.

Dr. Oliver said that reports of changes in the dates of the Commission's meetings would be welcome and would be passed on to the Commission. Therefore, he said, it would be desirable for the other participants in these consultations to make arrangements of their own as prepared in Budapest. This would help to ensure that each participant's viewpoint would be adequately represented.

Dr. Oliver gave a brief synopsis of the paper he had prepared for the Commission, consisting of a summary of the results of the first and second international symposiums on Food Virology, followed by descriptions of the progress and future of the two working groups. The past accomplishments of the two groups were reviewed and plans for the future were briefly outlined. Working virologists concerned with any aspect of virus transmission through foods were to be asked to make themselves known to the co-ordinators in order that informed of new findings and publications.

As there would be many workers present who would be primarily concerned with virus transmission through water, Dr. Oliver attempted to outline the relationship of water virology to the other collection project. He stated that the obvious areas in which water virology might contribute to the food project were with information about polluted water as a source of contamination of other foods, about polluted water as a component of such foods, and about virus infection as a function of the chemical composition of different waters. The project would not include, at present, work done directly for drinking, nor information about polluted water or sources of which had no clear relation to foods.

#### 5. Further business in Brno

Dr. Maršák began that the project should not be considered only as a source of compiling existing information. He stated his hope that, through the and other efforts of WHO, additional laboratory research would be stimulated and would contribute significant new knowledge. This concluded the main consultation on 23 June 1971.

On subsequent days in Brno, Dr. Oliver was able to discuss some aspects of the work in greater detail. Dr. Džalalović described some of the questions which were being directed to her by Dr. Maršák's collaborators. The principal problem areas could be defined. First, there are some quite philosophical questions about which of the existing knowledge of viruses is truly relevant to transmission through foods. While the general principles which guide such decisions are easily he stated, every specific choice presents some special problems. Second, some difficulties have resulted from the effort to be precise in describing the information included in the reports. Some of the questions which this group would have been phrased quite generally, but which would have been more specific. Some of the questions were by. The participants were permitted to add their own interpretations because they would be used to inform the original investigators.

Dr. Oliver was able to provide some further guidance concerning the project, particularly concerning its location. He stated that he would be glad to discuss the project if the project is directed principally to the study of food viruses of governments, or laboratory workers.

in Food Virology. For instance, a regulatory authority might want to know about all isolations of viruses from a certain food, but he would have little need of detailed information about the techniques which were used in detecting a virus in a food. Dr Cliver pointed out that it was not possible to make such decisions in any scientific way because the emphasis would not be on the food itself. Presently, there are relatively few working food virologists, but there have been real information needs which are not being satisfied in any other way. However, we can hope further that our store of information will help to make regulatory authorities more aware of the tick-borne virus problem. They may then urge other government officials to initiate new laboratory research which will produce additional information. This would result in a new "generation" of laboratory workers who would need the information-services.

#### 7. The Food and Water Virology Session in Budapest

On 1 July 1971, a Food and Water Virology session was conducted as part of the Sixth International Congress for Virology in Budapest. The session was opened by Dr Cliver who outlined the history and proposed conduct of the session.

The first speaker on food virology was Dr T. G. Notzold of the University of New Hampshire, Durham, USA. He told the session of their studies indicating that viruses accumulate in oysters and may persist for long periods. He also gave evidence that these oysters respond actively to the presence of viruses in their tissues, though the virus is not replicated by the oyster.

Dr Kearby Fugate of the US Food and Drug Administration, Dallas, USA described their isolations of enteroviruses from Gulf Coast and Louisiana oysters. Their studies indicated that the bacteriologic quality of oysters and of the water from which they are taken are poorly correlated. No bacteriologic index was found which would predict the occurrence of enteroviruses in these oysters.

Dr E.P. Larkin of the US Food and Drug Administration, Cincinnati, USA, described the work of the Virology Branch of that organization. Inactivation studies measured the effects of cobalt-60 gamma rays and of heat upon viruses. Virus detection methods have been developed and have been applied to testing raw milk and ground beef. Enteroviruses were isolated from two of 57 samples of milk and at least nine of 46 beef samples. Viruses in ground beef were found to be totally inactivated during cooking only if no pink colour remained inside the meat.

Dr Cliver gave the presentation summarized in part 5 above. Several papers on Water Virology followed; none of these was within the province of Food Virology by the criteria listed previously.

The final presentation was by Dr M. Graháková of the Institute of Virology, Bratislava, Czechoslovakia. She described her studies with tick-borne encephalitis virus in milk and milk products. Goats infected with the virus as a result of tick bites were shown to shed the virus in their milk. The same was true of sheep and cows under experimental conditions.

If pasteurization was not done adequately, the virus would escape inactivation. It was then shown to persist for quite long periods of time in the milk or in some products made from contaminated milk.

### 8. The Final Report System in Geneva

The Consultation was continued in Geneva by Dr. Madyès and Cliver. Based upon all of the suggestions received, it was necessary to reach some conclusions regarding the form or guide for preparation of reports.

No specific decisions were reached concerning the retrieval system to be used with the reports generated by this project. This means that the style of the reports will continue to be rather general, for they will not yet be subject to the constraints imposed by a single retrieval system. Some refinements are possible, however.

A single report will generally contain three classes of information:

- (1) that which may provide a basis for retrieval,
- (2) that which (in addition to (1) above) makes up the substance of the report, and
- (3) that bibliographic or reference information which tells the source of (1) and (2) and which directs the report's user to more complete data than could be included in the report. It is most important that the reports and the retrieval system be useful to laboratory workers in food virology, but without excluding others (such as regulatory authorities for food safety) who may need to know about food-borne viruses. It is also important to establish a favourable balance between information of types (1) and (2).

In the case of reports prepared by Dr. Madyès's group, for instance, it is clear that the virus group and virus name which are given at the top of the answer sheet can provide a primary basis for retrieval. However, it may sometimes be more desirable to enter the information collection in quite another way. One might wish to know which viruses have been shown to localize in bovine liver and would hope that the retrieval system would permit him to ask which reports contain an affirmative answer to question 3.2.4 (see questions in the Appendix). If this were possible, one might wish further to know which viruses are stable during a week's storage in the refrigerator (say, at 5°). If the real question being asked is which viruses might be encountered in bovine liver after a week's refrigeration, one would like to be able to retrieve in one operation only those reports which have affirmative answers to both question 3.2.4 and question 6.2.

It is not at all certain that the retrieval system finally chosen, whether mechanical or electronic, will permit complex searches such as that described above. Still, it seems important to preserve as many options as possible at this stage. This is why many questions have been included in the reports and why only a very limited variety of answers to each question may be permitted: narrative answers, or even one or a few words selected freely from the respondent's personal vocabulary, will not be useful for retrieval.

The attempt to limit the permitted answers for many questions to a few possibilities places an extra burden upon the respondent. He will often have to interpret the information he finds in the literature before he answers a question. This is why collaborators have generally been chosen from among working virologists and have been asked to report upon matters about which they have personal knowledge.

These considerations, together with the suggestions related in previous sections of this report, have given rise to the new reporting guide which forms the Appendix. The breadth of the information requested has been limited severely to that which is obviously relevant to food virology, so as not to strain the capacity of the storage system. Inevitably, some of the decisions as to what should be in and what should be out will be regretted in the future. One can only hope that there will not be too many such regrets and that, now that the project is entering a phase of intensive information gathering, the revised guide will prove more of a help than a hindrance to those who must use it.

INTERNATIONAL COLLECTIVE OF DATA ON  
THE PRESENCE OF VIRUSES IN FOODS

This guide contains questions to be used in preparing five different types of reports (called A, B, C, D, E). The reports will be short summaries of different kinds of information about virus transmission through foods. They are to be used ultimately to code data for mechanical or electronic retrieval, and many of the decisions in preparing a report must be made with this in mind.

A type A report will summarize, in a general way, those known properties of a virus which may affect transmission through food. Normally, no food group will be specified on a type A report. Questions 1-6 (only) should be answered as completely as possible on the special answer sheets (see sample). The answers will often be obtained from several source publications, which will be listed by number as "References" at the end of the report. Answers may also be based upon the respondent's personal knowledge, rather than on a specific published study. A type A report will not normally be combined with any other, and all type A reports should be submitted through Dr Mensik.

Reports of types B, C, D, and E will be submitted through Dr Cliver. A single report will normally be based upon no more than one published reference, but one reference may well result in more than one report. A single report may be of more than one of these types, if the reference on which it is based contains more than one kind of information. One virus group and one food group (based upon the instructions given below) must be indicated on each report of type B, C, D, or E.

A type B report is one which presents clear evidence of the presence of a virus in a food. The evidence might be either that virus was isolated from the food or that people who ate the food became infected with virus. If the evidence of virus contamination is less direct, or if the virus was intentionally added to the food for experimental purposes, the report would not be type B. Begin at question 7.

A type C report presents evidence suggesting the association of a virus with food, other than the kinds which would appear in type A or B reports. For example, if it were found that persons consuming a certain kind of food had an unusually high incidence (other than an actual outbreak) of a certain virus disease, or if it were shown that some oyster species could concentrate virus from experimentally-contaminated water, a type C report would be appropriate. Begin at question 9.

A type D report concerns tests of a method for detecting food-borne viruses. Begin at question 10.

A type E report concerns tests of the persistence or inactivation of a virus in a food. Begin at question 10.

An answer sheet may be selected, based upon the type of report that is being prepared, as outlined above. For a type A report, use the type A answer sheets and begin by giving the virus group and virus name at the top of the page. The code for virus group is as follows: ADE = adenoviruses, HE = herpesviruses, CH = chlamydia, KOR = koronavirus, HEE = infectious hepatitis, MYX = myxoviruses, PIC = picornaviruses, PRX = paramyxoviruses, POX = poxviruses, REO = reoviruses, RHA = rhabdoviruses, TOG = togaviruses, or "other". The type A answer sheet has three columns, headed "Code, Description, and Reference No." In the "Code" column, give the number of the question-part which is being answered. Begin with question 1, and answer all parts for which information is available, through question 6. The answers to the questions go in the "Description" column and may be in the form of numbers or words, as the question requires.

If the answer is supported by a published reference, its number is indicated in the right hand column. The respondent is urged to try to answer the question as it is asked. If a published reference answers the question directly, he can just give the referenced number. If he must give an answer based upon his interpretation of some published information (this will be more usual), he should put parentheses around the reference number. The references cited are listed, in order of their assigned numbers, at the end of the report. To allow for continuous updating of type A reports, reference numbers should be assigned approximately in order of publication date. Full editorial information (author(s), year, title, journal or book, volume, pages) should be given.

If the report is not of type A, it should begin with the special cover sheet (see sample). Note that the report may be of more than one of the types B through E, as was explained above. The code for food group is as follows: AMP = animal meat and products, MFP = milk and milk products, PMP = poultry meat and eggs, SSF = shellfish and other seafoods, VFR = vegetables and fruits, WSW = water and sewage, or "other". Where the name of a food has no precise English equivalent, it should be transliterated (if necessary) as accurately as possible, and an approximate translation should be supplied. Information concerning foods of mixed origin may be referred to Dr. Cliver for assignment. If one component of such a food contained virus before the mixture was prepared, the assignment would generally be based upon the contaminated component. Otherwise, duplicate copies of the form might be prepared and filed under more than one food group.

The code for virus group is the same as for type A reports. Only one virus group should usually be mentioned in a single report, so this may require that more than one report be made from a single published reference. If the reference names more than one virus from within a single group, separate reports are necessary only if the findings were significantly different with different virus types. Viruses for which results were similar can all be included in one report. As much reference information as possible should be supplied, whether or not the reference was published.

The questions to be answered for each type of report have been specified above. Plain paper may be used after the cover sheet. The code number of each question-part should be given at the left margin, followed by the

required answer. Questions which are not appropriate to the type of report, as well as questions for which no answer is available, are simply omitted. Examples of such reports are attached. Please note the relationship between the type of report and the questions which are appropriate to answer: type A, questions 1 to 6 only; type B, questions 7 and 8 or 7 and 11; type C, question 9 and possibly 10, 11, and 12; type D, questions 10 and 11; and type E, questions 10 and 12.

### QUESTIONS

1. Summarize the probable natural host range of the virus (+ means animal is susceptible, - means animal is not susceptible; also tell age of animal, if significant). Use your judgement in interpreting the natural implications of experimental results. If very uncertain, enter the information as possible only in question 3 below.
  - 1.1 Pigs
  - 1.2 Cattle
  - 1.3 Sheep
  - 1.4 Goats
  - 1.5 Horses
  - 1.6 Chickens
  - 1.7 Turkeys
  - 1.8 Ducks
  - 1.9 Fish
  - 1.10 Insects
  - 1.11 Man
  - 1.12 Other (specify)
2. The parts of this question request some very general information about the virus. Please generalize freely.
  - 2.1 By what route(s) may natural infections be initiated by this virus - answer: oral, respiratory, or other (specify). Consider broadly (across host species) whether this is probably an oral virus, a respiratory virus, etc.
  - 2.2 Where does this virus probably occur naturally? Use the largest geographic divisions (e.g. continent, subcontinent, country, etc) that will answer the question satisfactorily, combined in any way (such as continents with countries) that will keep the list as short as possible.

3. This question is intended to determine where the virus localizes in the body of the host. The question code will consist of three numbers separated by periods: the numeral "3" (because these are parts of question 3), a host species code (see first list below), and a location code (see second list below). An answer to one part of this question may consist of "NAT" (for natural infection) or "EXP" (for experimental infection) and a symbol to summarize the presence of the virus: "-" for not detected, "+" for detected, "rare" for virus which was only found occasionally or which persisted 15 days post inoculation, and "frequent" for more common or longer occurrence.

Host species code:

- .1 Pigs
- .2 Cattle
- .3 Sheep
- .4 Goats
- .5 Horses
- .6 Chickens
- .7 Turkeys
- .8 Ducks
- .9 Fish
- .10 Other (specify)

Virus location code:

- .1 Blood
- .2 Lung
- .3 Spleen
- .4 Liver
- .5 Kidney
- .6 Heart
- .7 Muscle
- .8 CNS
- .9 Lymphatic system
- .10 Milk
- .11 Eggs
- .12 Skin
- .13 Bone marrow
- .14 Digestive tract
- .15 Other (specify)

4. The parts of this question are intended to summarize the ways in which this virus can be cultivated in the laboratory. Negative results may also be reported, followed by "NCG".
- 4.1 Laboratory animals (give species, age, and route of inoculation).
  - 4.2 Embryonated eggs (give species, age, and route of inoculation).
  - 4.3 Tissue culture (give species, as well as organ of source or strain name).
  - 4.4 Organ culture (give species and organ of source).
  - 4.5 Other (specify).

5. The parts of this question are intended to characterize the virus particle.
- 5.1 Size \_\_\_\_  $\mu$ m (diameter) x \_\_\_\_ nm (length, where appropriate).
  - 5.2 Sedimentation constant \_\_\_\_ S.
  - 5.3 Inherent density.
  - 5.4 Lipid envelope (answer: present or absent).
  - 5.5 Nucleic acid (answer: RNA or DNA and 1 or 2 for the number of strands).
6. The parts of this question ask for general information about the stability of the virus: the answers may be less useful if they are not too specific. The conditions (temperature, pH, and time) selected have some significance in the processing, storage, and distribution of foods; based upon available information, try to tell what the virus would be expected to do under these conditions. The word "stable" should be interpreted to mean that virus infectivity would still be demonstrable after exposure to the specified conditions.
- 6.1 At  $-20^{\circ}$  for 1 month (stable or unstable)
  - 6.2 At  $5^{\circ}$  for 1 week (stable or unstable)
  - 6.3 At  $55^{\circ}$  for 30 min (stable or unstable)
  - 6.4 At pH 3-4 for 1 day at  $5^{\circ}$  (stable or unstable)
  - 6.5 At pH 5.0-5.5 for 6hr at  $20^{\circ}$  (stable or unstable)
7. The parts of this question are to get details about a "naturally" contaminated food which was served to people (1) or from which a sample was taken for laboratory testing (2) - answer: 1 or 2
- Do not answer this question if the food was artificially contaminated for experimental purposes.
- 7.1 Where did this occur? - give name of city and of country.
  - 7.2 Give the date(s) on which the food was served or the samples collected.
  - 7.3 State the most probable source of the virus which contaminated the food (such as food handler, polluted water, flies, etc., or unknown).  
  
Summarize what was said about how the virus got from the source to the food; and, if a food handler was implicated, tell whether he was overtly ill, or had an inapparent infection, or was incubating an infection when he contaminated the food.

For type B reports only, answer just question 8 if there were human illnesses and just question 11 if virus was detected in food.

8. If there were human food-associated illnesses described, give the details in this question.
  - 8.1 Give the name of the disease, or name the principal symptoms.
  - 8.2 How many people were "at risk" (that is, how many probably ate or were exposed to the contaminated food)?
  - 8.3 How many people became infected as a result of exposure to or eating the food?
  - 8.4 How many people became ill as a result of exposure to or eating the food?
    - 8.4.1 What were the ages (in years) of those who became ill (a range may be given)?
    - 8.4.2 How else might the affected group be characterized (e.g., were they students, soldiers, etc.)?
    - 8.4.3 By what route were these people infected (oral, nasal, etc.)?
  - 8.5 If secondary infections occurred in other people as a result of contact with those who had food-associated infections, tell how many persons had secondary infections.
  - 8.6 Was prophylaxis (e.g., with immune globulin) attempted? Give details.
9. This question refers to "type C" reports only. This includes experiments performed which suggest association of a virus with a food, as in studies of virus uptake under experimental conditions. Tell here the kind of study that was done, and give any specific information which cannot be adequately presented in questions 10, 11, and 12. Use questions 10, 11, and 12 to provide details, where possible.
10. The parts of this question are to get details about the experimental inoculation of virus into food. It will be used with type C, D, and E reports only.
  - 10.1 What was the source of the virus (e.g., tissue culture, infected animal tissue, human feces, etc.)?
  - 10.2 How was the virus prepared before adding it to the food? - Briefly describe any dilutions, purifications, or other treatments.
  - 10.3 How much virus was inoculated? - State this in infectious units of virus per weight unit of food, if possible.

If this is a type D report, go on to question 11. If it is a type E report only, pass question 11 and begin again at question 12.

11. The parts of this question are to get details of how a virus was isolated from or detected in a food. It is intended for use in type B and D reports especially, and it should be completed after question 7 or question 10.
  - 11.1 What was the size of a single sample (in grams or milliliters, if possible)?
    - 11.1.1 How many such samples were tested?
  - 11.2 What quantity of fluid, if any, was added to one sample (e.g., as a diluent)?
    - 11.2.1 What was the fluid?
  - 11.3 What other substances were added to a sample and in what quantities?
  - 11.4 If a fluid suspension had to be made from the food sample, tell how this was done (e.g., mortar and pestle, homogenization apparatus, shaker, etc.).
  - 11.5 If the food suspension was clarified or purified to remove food solids or other components, tell how this was done (e.g., centrifugation, filtration, etc.).
  - 11.6 If the sample suspension was concentrated before testing, tell how this was done (e.g., ultracentrifugation, chromatography, etc.).
  - 11.7 Tell what test method was used to detect the virus (e.g., inoculation of tissue cultures, laboratory animals, etc., be specific).
    - 11.7.1 What number or proportion of tests was positive?
    - 11.7.2 If the quantity of a virus in a sample was measured in any way, state the result.
  - 11.8 What method, if any, was used to identify the virus which had been isolated (e.g., serum neutralization, hemagglutination inhibition, etc.)?
12. The parts of this question are intended to get details of a persistence or inactivation experiment with food or a related substance inoculated or contaminated with virus. This will be used in a type E report and will usually follow question 10. Please state, if possible, what principal mode of inactivation (e.g. thermal, irradiation, etc.) was being studied.
  - 12.1 At what temperature(s) was the experiment conducted?
  - 12.2 This part is intended to characterize the environment of the virus in the food or other experimental vehicle.
    - 12.2.1 Give the pH, if stated.
    - 12.2.2 What salts were present, and in what concentrations?

- 12.2.3 What level of proteins was present?
- 12.2.4 What level of fats or lipids was present?
- 12.2.5 What level of carbohydrates was present?
- 12.2.6 What chemical additives or disinfectants were present, and at what levels?
- 12.2.7 Summarize information given about the microbial population.
- 12.3 To what process(es) was the contaminated vehicle subjected (e.g., pasteurization, freeze-drying, irradiation, storage, etc.)?
- 12.4 How long was the virus held in the vehicle under the conditions specified?
- 12.5 This part is intended to summarize the results, which might have been reported in any of several ways. Only one of the following should usually be completed.
  - 12.5.1 Tell whether the virus persisted or was entirely inactivated;
  - 12.5.2 Or, tell what quantity of virus (in infectious units or  $\log_{10}$  infectious units, if possible) was inactivated in what period of time;
  - 12.5.3 Or, tell at what rate (in  $\log_{10}$  infectious units per unit of time) inactivation was found to proceed;
  - 12.5.4 Or, express the results in any other way that has been used by the investigator(s).



INTERNATIONAL ORGANIZATION OF LABOR OF THE

UNION OF AMERICANS IN EUROPE

... of type ...

... (part ...)

... concentrated orange juice

... virus

... (not negligible)

... and Goldman, A.

... infectious hepatitis in a general hospital;

... concentrated orange juice

...

...

...

...

...

...

... (who was an inapparent carrier) working

...

... (infectious detected by liver function tests)

...

...

... hospital staff members

...

...

... to 1345 people who might have

...

INTERNATIONAL COLLECTION OF DATA ON THE  
PRESENCE OF VIRUSES IN FOODS

Cover Sheet for Reports of Types Other Than A

Report type: X B,     C,     D,     E (mark one or more)

Food Group: SSF Food name: Mussels

Virus Group: PIC Virus name: ECHO types 3, 9, and 13

Reference (when applicable)

Author(s): Bellelli, E., and Leogrande, G.

Year: 1967

Title: Ricerche batteriologiche e virologiche sui mitili

Journal or book: Ann. Sclavo

Volume: 9

Pages: 820-828

7. 2
- 7.1 Parma and Bari, Italy
- 7.2 Parma: winter, 1964, and summer, 1965  
Bari: February and March, 1967
- 7.3 Polluted water in which the mussels were growing
- 11.1 3 mussels (no weights given), flesh and shell liquor
- 11.1.1 Parma: 100 samples  
Bari: 100 samples
- 11.2 10 ml
- 11.2.1 doubly-distilled water
- 11.3 10000 u penicillin, 10000 µg streptomycin, and 20 µg amphotericin B per ml of final extract
- 11.4 Method 1: mortar and pestle with quartz sand  
Method 2: homogenization (5 min) in Waring Blender
- 11.5 Let stand 10 min, supernatant collected, centrifuged (3000 rev/min, 15 min), antibiotics (see 11.3) added to supernatant
- 11.7 Primary monkey kidney and primary human amnion cell cultures, 5 tubes of each per sample, 0, 2 ml of extract per tube; a second passage was done if no cytopathic effects were seen in 16 days.
- 11.7.1 Parma: Method 1 -- 0/50  
Method 2 -- 0/50  
Bari: Method 1 -- 0/50  
Method 2 -- 3/50 (ECHO type 9 in monkey kidney and types 3 and 13 in human amnion cells)
- 11.8 Serum neutralization

INTERNATIONAL COLLECTION OF DATA ON THE  
PRESENCE OF VIRUSES IN FOODS

Cover Sheet for Reports of Types Other Than A

Report type:    B, X C,    D,    E (mark one or more)

Food Group:       Food name:   Clams and oysters  

Virus Group:       Virus name:   Infectious hepatitis virus  

Reference (when applicable)

Author(s):    Hays, R.S., Grady, G.F., Chalmers, T.C., Kosley, J.W.,  
Stuart, B.L., and the Boston Inter-Hospital Liver Group

Year:    1957

Title:    Viral hepatitis in a group of Boston hospitals. III. Importance  
of exposure to shellfish in a nonepidemic period

Journal or book:    New England J. Med.

Volume:    276

Pages:    703-710

9.    Statistical-epidemiological study among hepatitis patients in  
Boston (U.S.A.) hospitals.

   Comparison 1: 185 hepatitis patients who had had no recent  
infections, transfusions, or contact with jaundiced  
persons were compared to 185 matched controls with  
suspect to having eaten raw shellfish within the past  
15 to 60 days; answer was "yes" for raw oysters  
(principally    *Crassostrea virginica*) in 25 patients and  
8 controls ( $p < 0.01$ ) and for raw clams (principally  
   *Mercenaria mercenaria*) in 12 patients and 3 controls  
( $p < 0.05$ ).

   Comparison 2: 104 hepatitis patients (defined as above) with  
104 matched controls, compared for eating cooked clams  
15 to 60 days previously; answer was "yes" for steamed  
clams (principally    *Vya arenaria*) in 13 patients and 2  
controls ( $p < 0.01$ ) and for fried clams (principally  
   *Spicula* sp.) in 24 patients and 30 controls (not  
significant).

INTERNATIONAL COLLECTION OF DATA ON THE  
PRESENCE OF VIRUSES IN FOODS

Cover Sheet for Reports of Types Other Than A

Report type:    B,    X C,    D,    E (mark one or more)

Food Group:    SNF Food name:    mussels

Virus Group:    PIC Virus name:    Coxsackie type A8 and poliovirus type 3

Reference (when applicable)

Author(s): Duff, M.F.

Year: 1967

Title: The uptake of enteroviruses by the New Zealand marine blue mussel Mytilus edulis neohausi

Journal or book: Am. J. Epidemiol.

Volume: 85

Pages: 486-493

9. This was a laboratory study of how mussels (Mytilus edulis neohausi) collect enteroviruses from contaminated water. It was shown that virus was removed from the water and (to a considerable extent, inactivated in the mussels, but there was no attempt to prove that the same things would happen under "natural" conditions. Some details are given in questions 10, 11, and 12.
- 10.1 "Stock virus suspensions" were used: probably from infected suckling mouse tissue for Coxsackie A8 and from tissue culture for poliovirus 3.
- 10.2 Each virus suspension was extracted three times with trichloro-trifluoroethane. An extraction comprised homogenization of the virus suspension with an equal volume of chilled fluorocarbon, followed by separation in a centrifuge.
- 10.3 Coxsackie A8:  $10^{5.2}$  suckling mouse LD<sub>50</sub> per 0.03 ml of sea water.  
Poliovirus 3:  $10^{5.1}$  tissue culture ID<sub>50</sub> per 0.2 ml of sea water.  
There were 6 l of sea water in a tank, and these apparently were not changed during an experiment.
- 11.1 3 mussels per sample; shell liquor was rigorously removed
- 11.2 4 volumes (to make a 20% suspension)
- 11.2.1 "standard antibiotic diluent"

- 11.4 "blended on ice"
- 11.5 Suspension was "blended" with an approximately equal volume of chilled fluorocarbon (trichlorotrifluoroethane) and centrifuged at 2000 rev/min for 20 min.
- 11.7 Coxsackie A6 was titrated in mice less than 24 hr old, and poliovirus 3 was titrated in tube cultures of an established human cell line.
- 12.1 18.5 to 22 C
- 12.4 22 days
- 12.5.4 Most of virus (> 98% in 7 days) was removed from water (compared to control suspension in sea water) by mussels, but little of the virus could be recovered from them. Virus was presumed inactivated in the mussels.

Sample

INTERNATIONAL COLLECTION OF DATA ON THE  
PRESENCE OF VIRUSES IN FOODS

Cover Sheet for Reports of Types Other Than A

Report type:      B,      C,   X   D,      E (mark one or more)

Food Group:   VFR   Food name:   tomatoes  

Virus Group:   PIC   Virus name:   Coxsackie type B<sub>2</sub>  

Reference (when applicable)

Author(s):   Oliver, B.O., and Grindrod, J.  

Year:   1969  

Title:   Surveillance methods for viruses in foods  

Journal or Book:   J. Milk Food Technol.  

Volume:   38  

Pages:   421-425  

- 10.1 Primary monkey kidney cell cultures
- 10.2 applied to tomato surface in 0.5 g of sterile human (child) feces
- 10.3 0.5 to 16 plaque-forming units per tomato
- 11.1 one tomato
- 11.1.1 4
- 11.2 100 ml
- 11.2.1 0.6% 14 phosphate buffer, pH 7.2
- 11.3 1 ml, chloroform
- 11.4 rotated in a jar with 20 glass spheres of 1/4" diameter, 100 rev/min for 5 min, to dislodge virus from tomato surface
- 11.5 centrifugation, 7500 rev/min for 30 min
- 11.6 dialysis against polyethylene glycol (mol. wt. 20000) for 18 hr at room temperature, then ultracentrifugation at 50000 rev/min for 2 hr
- 11.7 one primary monkey kidney cell culture flask was inoculated with preparation from one tomato
- 11.7.1 100% positive results at just less than 2 plaque-forming units per tomato; ratio of virus recovered to virus inoculated was 45%

Sample

INTERNATIONAL COLLECTION OF DATA ON THE  
PRESENCE OF VIRUSES IN FOODS

Cover Sheet for Reports of Types Other Than A

Report type:      B,      C,      D,   X   E (mark one or more)

Food Group:   VPI   Food name:   dry banana pudding (modified)  

Virus Group:   PIC   Virus name:   poliovirus type 3  

Reference (when applicable)

Author(s): Cliver, D.O., Kosterbader, K.D., Jr., and Vallenas, M.R.

Year: 1970

Title: Stability of viruses in low moisture foods

Journal or book: J. Milk Food Technol.

Volume: 33

Pages: 484-491

- 10.1 primary monkey kidney tissue culture
- 10.2 diluted to  $2 \times 10^6$  plaque-forming units per ml of fluid suspension
- 10.3  $10^6$  plaque-forming units per 10 g sample
- 12. Thermal inactivation
  - 12.1 5 C
  - 12.2.1 pH 7.6, also modified to 5.5 and to 4 by adding anhydrous propionic acid
  - 12.2.2 "sach" was 3.5% of dry (1.1% moisture) pudding
  - 12.2.3 25
  - 12.2.6 none, or 0.42 ml propionic acid (per 10 g of food) for pH 5.5, or 1 ml for pH 4
  - 12.3 stored in vacuum packages
  - 12.4 4 weeks
  - 12.5.3 Inactivation (in  $\log_{10}$  plaque-forming units per day) was -0.047 at pH 7.6, -0.079 at pH 5.5, and -0.15 at pH 4