Detection of Trichinella Larva in Walrus Meat Harvested by the Sallumiut in Fall 1994

Final Report

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Abstract

Fifteen walruses were harvested by Salluit hunters during the week of October 11, 1994. Five different tissues were sampled per walrus, for a total of 75 samples. The objective of tissue sampling was to conduct laboratory analyses for the presence of trichinella larvae. Muscles from the back were sampled correctly but some shoulder samples were fat rather than the requested muscle tissue. Jaw muscle was collected near the skin rather than near the bone. The diagram showing the location of the sampling site for the diaphragm was unclear because intercostal muscle was sampled twice whereas no diaphragm was sampled. The analytical digestion method was modified to increase its sensitivity. The digestion solution was double centrifuged before larvae were identified using a microscope (n=24 slides/sample). No larvae of trichinella were detected in any of the muscle analysed. Only the analysed muscles were determined acceptable to be eaten raw or prepared as igunak since not all the muscles requested were received. The sampling procedure should be improved before the next harvest season. A more sensitive digestion method, approved by Agriculture Canada and the European Economic Community (EEC), is proposed for use next year.

Acknowledgments

The KRC thanks Willie Keatainak (Mayor of Salluit), Stas Olpinski (Makivik Corporation), Philipe De Pizzo (KRG) and Steven Hodgins (CRSSS) for their time invested in the coordination of the 1994 trichinosis project. CRSSS provided the reagents, vessels and laboratory equipment. Michelle Audy and Josée Archambault, laboratory medical technicians from Ungava Tulattavik Health Centre, provided judicious advice and access to their centrifuge and microscopes.

Dr. Robert Lavoie of Laval Hospital provided the positive control sample and he gave professional advice on the digestion method. Piadli Angotigirk came from Salluit to learn the laboratory procedure. He carried out his work in an eager and conscientious manner. The KRG funded the transport, food, lodging and salary of the trainee.

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Introduction

Outbreaks of trichinosis from eating walrus meat have been reported in Northern Quebec Inuit communities (Ross et al., 1989, Maclean at al., 1989). As a result, a preventive program has been undertaken in collaboration with CRSSS and the KRG to detect the larvae of trichinella in walrus meat since 1992. Meat was analysed at the Kuujjuaq Research Centre (KRC) after the 1994 walrus hunting season. The KRC provided the veterinary expertise to perform the analysis and supervise a trainee. The Kativik Regional Council of Health and Social Services (CRSSS) supplied the reagents, vessels and laboratory equipment required to carry out the digestion method. The Municipal Corporation of Salluit delegated Piadli Angotigirk to learn the method of diagnosing trichinella in walrus meat at the KRC in fall 1994.

The following report evaluates the effectiveness of the sampling and laboratory procedures which had been designed and executed within a short time. Recommendations for future programs are presented at the end of this report.

Materials and Methods

Sampling procedure

Walruses were harvested by Salluit hunters during the week of October 11, 1994. Labels uniquely identifying each walrus were attached using meat skewers to each of the various parts of the butchered carcass. On return to Salluit, five muscles per carcass were sampled (Figure. 1). Meat samples of approximately 4 " X 4 " from the diaphragm, jaw, shoulder, back and rib muscles were requested for analysis. Each sample was placed in a labeled bag (whirlpak) printed with the animal's identification number and the muscle code. Samples were frozen at -20°C then shipped to the KRC.

Laboratory procedure

A digestion method (Proulx and Olpinski, 1993) developed by Dr. Robert Lavoie*, was performed with the following modifications. While being stirred the filtered digestion solution (400 ml) was emptied into eight conical 50 ml tubes. The tubes were centrifuged at 3,000 R.P.M. for ten minutes. The supernatant was decanted leaving approximately 4.5 ml which were transferred with pellets into 12 ml conical tubes. The latter tubes were then centrifuged at 3,000 R.P.M. for ten minutes. The supernatents were then decanted and the pellets resuspended onto three microscope slides (50 µl/slide) for a total of 24 slides per walrus. The theoretical sensitivity is 0.04 larva per gram of meat. The method detected the presence of larvae in polar bear jaw muscles. The positive control was an infected rat which was provided by Dr. Lavoie. A walrus carcass was defined as safe for human consumption if all five target muscles tested negative for the presence of trichinella.

*Robert Lavoie, MD, Clinical Biochemist, Laval Hospital.

Results and Discussion

The KRC received samples from all 15 walruses. The samples were shipped from Salluit during the week of October 18. They were still frozen and well labeled, however, not all the requested muscles were collected according to the KRC's sampling diagram. In some cases, fat had been collected instead of shoulder muscle. Muscles from the lower jaw were collected near the skin which were skin muscle, not jaw muscle. Moreover, intercostal muscles (between ribs) were sampled twice but no diaphragm was sampled. Muscles from the back were sampled correctly in all walruses. Table 1 summarizes the muscles sampled for each walrus.

Table 1. List of five muscle groups sampled from 15 walruses for trichinella analysis in fall 1994.

Walrus Number	Diaphragm	Shoulder	Back	Jaw	Rib
1	NC*	X	X	NC	X
2	NC	X	X	NC	X
3	NC	FAT	X	NC	X
4	NC	FAT	X	NC	X
5	NC	FAT	X	NC	X
6	NC	FAT	X	NC	X
7	NC	FAT	X	NC	X
8	NC	FAT	X	NC	X
9	NC	FAT	X	NC	X
10	NC	X	X	NC	X
11	NC	X	X	NC	X
12	NC	X	X	NC	X
13	NC	X	X	NC	X
14	NC	X	X	NC	X
15	NC	X	X	NC	X

*NC: not collected

Each muscle sample was analysed individually. No trichinella larvae were detected in any of the muscles analysed. However, only the analysed muscles should be eaten raw or prepared as igunak, since not all muscles needed for analysis were collected. On the basis of the analyses, meat from the back and ribs of all walruses and meat from the shoulder of walrus numbers 1, 2, 10, 11, 12, 13, 14, 15 are safe for raw consumption. The rest of the meat should be cooked until the inner part of the meat has changed to uniform grey colour.

The apparent reason why not all requested samples were received for analysis was a confusing sampling diagram (Figure 1). The diaphragm location is too close to the intercostal muscle. Moreover, muscle tissue must be collected near the bone. Transition zones between the fibrous part (tendon or aponevrosis) and the tender part (muscle) are the main area where trichinella larvae concentrate (Directives 77/96/CEE and 84/319/CEE). Hence, the pillar of the diaphragm (near back bone), where the muscle attachs to the abdominal vault, should be collected rather than from other areas. Tagging was correctly performed according to the protocol. To reduce the potential risk of misidentification, individual bags should be grouped in a common bag for each walrus.

Samples were shipped and received frozen, as requested. Biological samples can be transported from Salluit to Kuujjuaq within a reasonable time. Meat analysis was performed at an average rate of three analyses per day. All analyses were completed within six days. The first two days of analysis were used to validate and modify the method. The low daily rate of analysis was associated with the use of two laboratories, individual analysis of each sample, the double centrifugation step and the inspection of 24 slides per walrus.

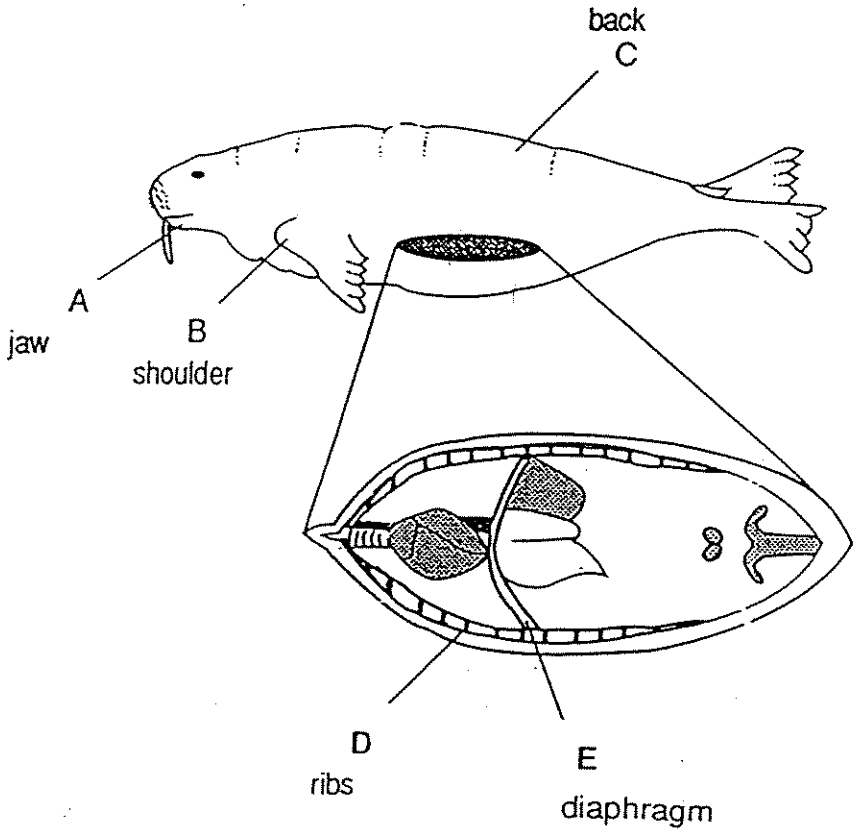

Figure 1. Walrus sampling sites for trichinella analysis 1994

The method has a sensitivity of detecting of 0.04 larva/gram with the following assumptions: 1) No larvae remaining in the residue 2) Observation of all larvae in 150 µl pellet per 50 ml centrifuged tube. The method developed for pooled samples, such as the digestion method (Directives 77/96/CEE and 84/319/CEE)approved by Agriculture Canada and the European Economic Community (EEC), would reduce the time and cost of analysis. This method can process 10 samples per batch and reduce the observation of larvae in two single Petri dishes. However, this method must be validated since the digestion residue of walrus meat is higher than that of terrestrial mammal meat. Walrus meat appears more marbled (diffuse fat) and thus requires optimal digestion to expose embedded larvae.

Training of Piadli Angotigirk was successful. He demonstrated skill when performing the digestion method and diagnosed correctly the positive controls and the samples of polar bear meat. Piadli

showed conscientiousness and consistancy when performing laboratory procedures (Appendix 1). He demonstrated cautiou during manipulation of hazardous chemicals such as hydrochloric acid (35%). Moreover, Piadli was willing to work overtime during the week and weekend days to get the results back to the Sallumiut quickly.

Summary and Recommendations

Muscle tissues sampled from 15 harvested walruses were shipped by Sallumiut for trichinella analysis to the KRC. No larvae of trichinella were detected in any of the muscles analysed. It was recommended that only the analysed muscles could be eaten raw or prepared as igunak since not all of the requested muscles were sampled. The rest of the meat was recommended to be cooked until the inner part of the meat changed to a uniform grey colour.

The sampling procedure must be revised and improved before the next harvest season. A KRC researcher along with Piadli Angotigirk, should accompany the next walrus hunt to ensure that the requested muscles are sampled. The KRC should validate and test other digestion methods for pooled samples since other interested communities will increase the demand for such a diagnostic service.

Literature

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EEC, Directive 84/319, 1984. Modifiant les annexes de la directive 77/96/CEE du Conseil relative à la recherche de trichines lors des importations, en provenance des pays tiers, de viandes fraîches provenant d'animaux domestiques de l'espèce porcine. Journal officiel des Communautés européennes, N° L167/34.

Maclean JD, Viallet J, Law C, Staudt M, 1989. Trichinosis in the Canadian Arctic: report of five outbreaks and a new clinical syndrome. J. Infect. Dis., 160: 513-20.

Proulx JF and Olpinski S, 1993. Appendix 5, meat analysis protocol adapted from Dr. Robert Lavoie, biochemistry department, Laval hospital in: Pilot project for the monitoring and analysis of the parasite Trichinella spiralis in walrus meat harvested by Sallumiut: A local initiative to protect the community against trichinosis. Kativik Regional Council of health and social services, Kuujjuaq, Quebec.

Ross P, Olpinski S and Curtis M, 1989. Relationships between dietary practice and parasite zoonoses in Northern Quebec Inuit communities. Etudes/Inuits/Studies, 13 (2): 33-47.

Appendix 1

Laboratory Procedure

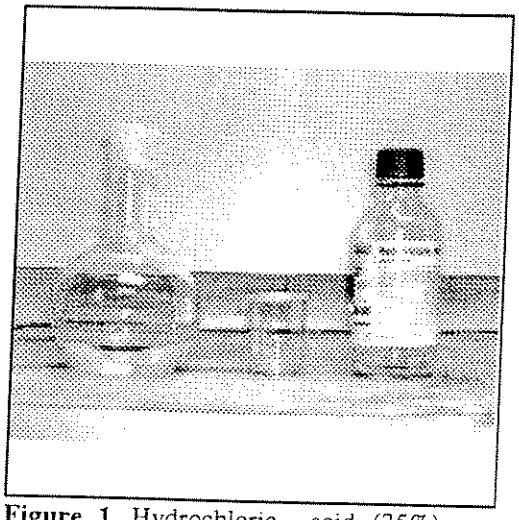

Figure 1. Hydrochloric acid (35%)

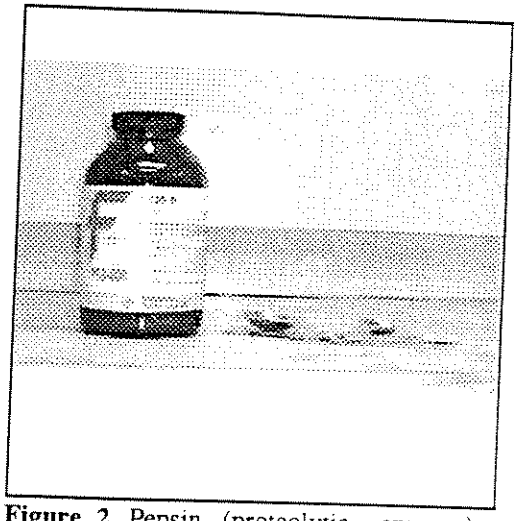

Figure 2. Pepsin (proteolytic enzyme)

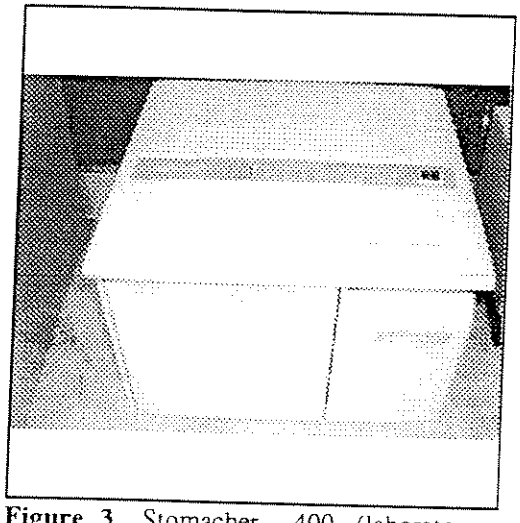

Figure 3. Stomacher 400 (laboratory blender)

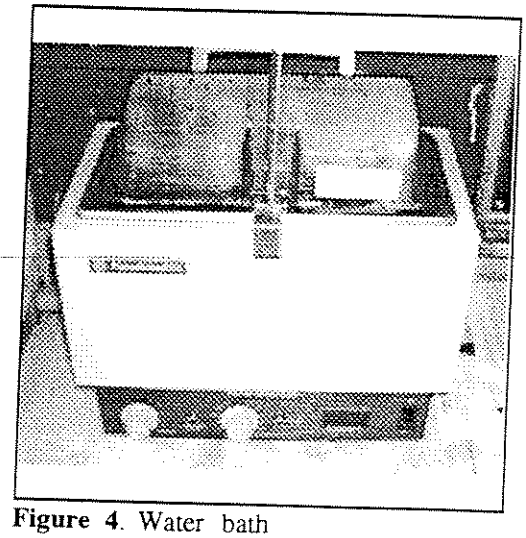

Figure 4. Water bath

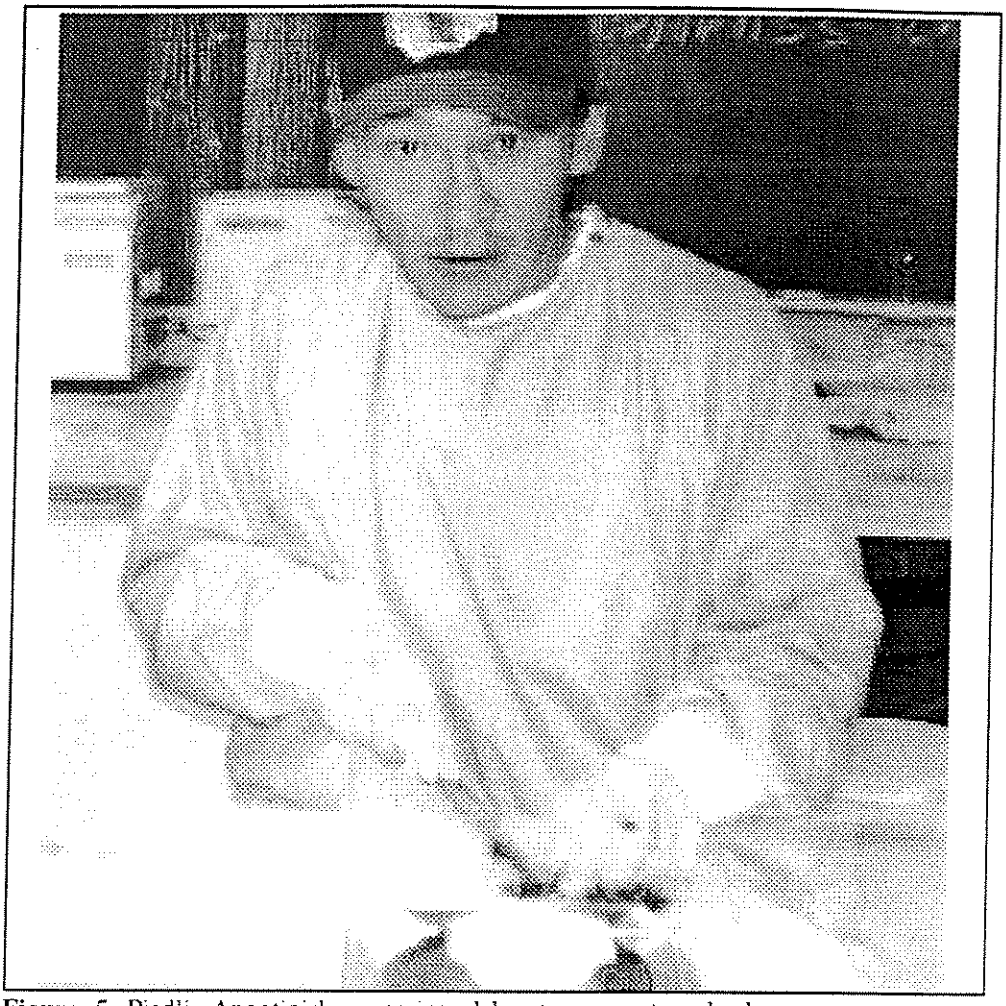

Figure 5. Piadli Angotigirk wearing laboratory coat and gloves

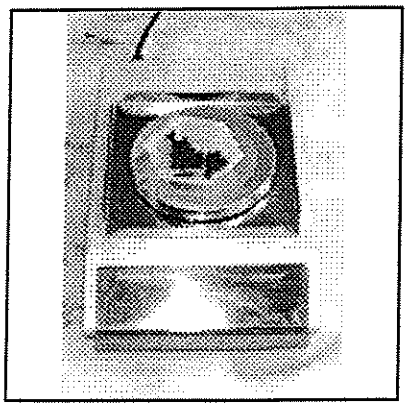

Figure 6. Weighing sample

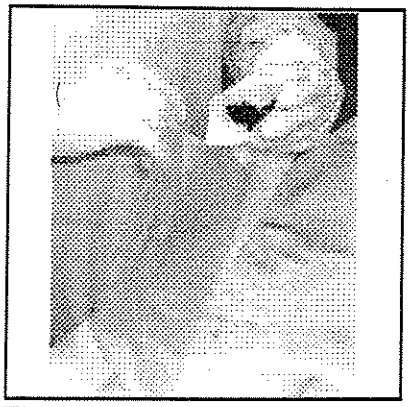

Figure 7. Loading stomacher bag with Walrus meat

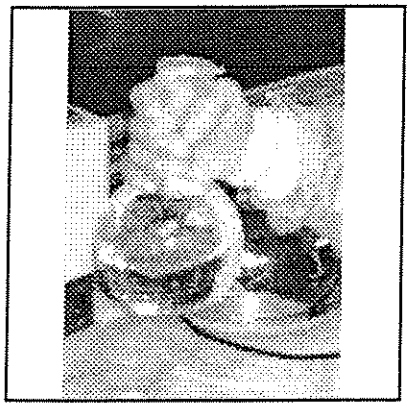

Figure 8. Mixing the diluted acid solution

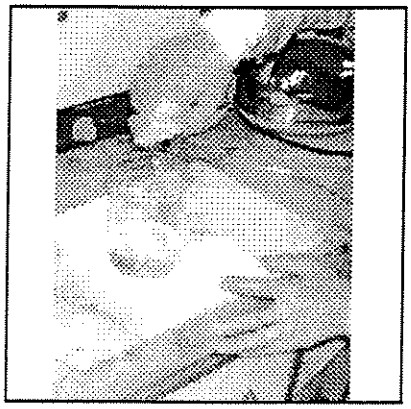

Figure 9. Filling acid solution in a beaker

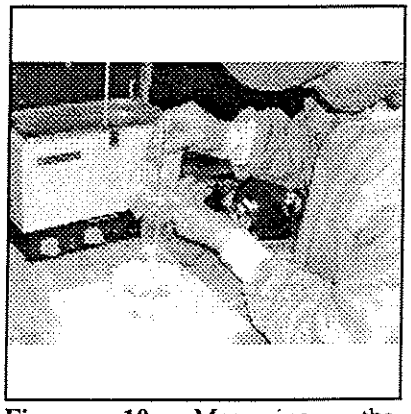

Figure 10. Measuring the temperature of the acid solution

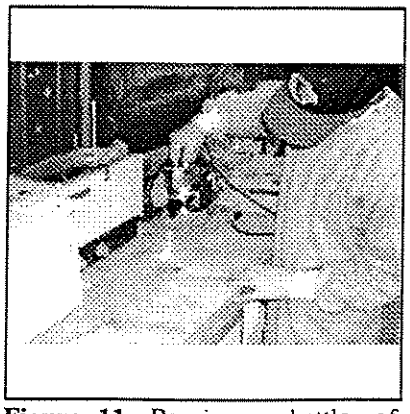

Figure 11. Pouring a kettle of hot water in a beaker

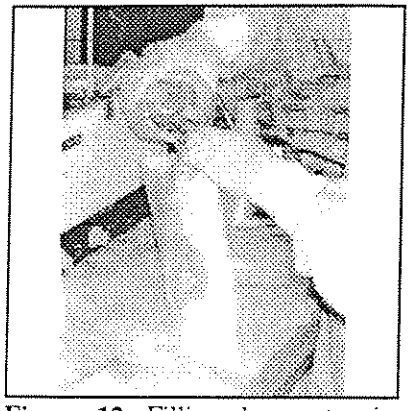

Figure 12. Filling hot water in a stomacher bag

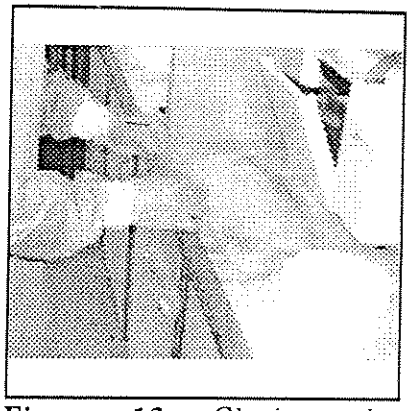

Figure 13. Closing the stomacher bag while removing air

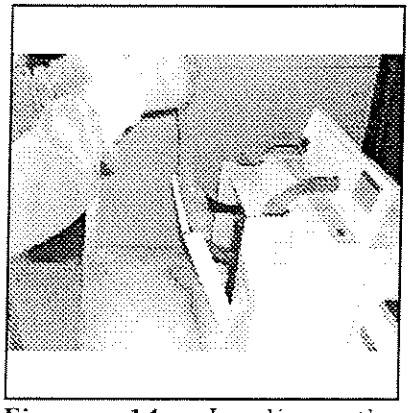

Figure 14. Loading the stomacher with a hot water bag (preheating the stomacher)

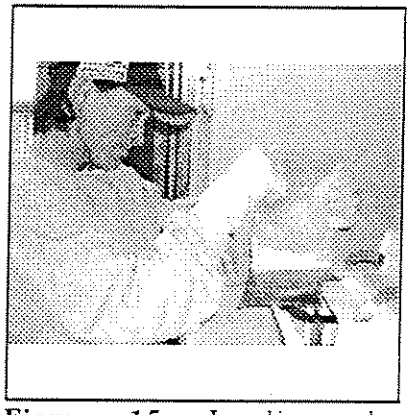

Figure 15. Loading the stomacher with the digestion solution (meat+acid+pepsin)

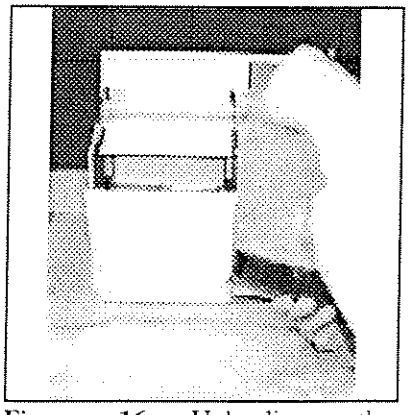

Figure 16. Unloading the stomacher bag

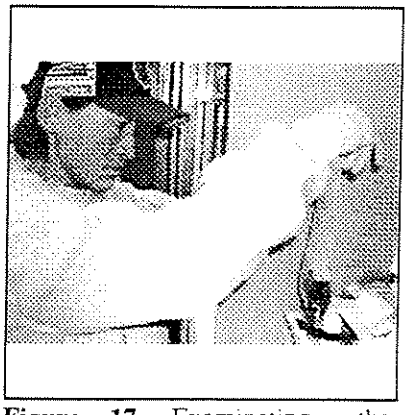

Figure 17. Examinating the digestion solution

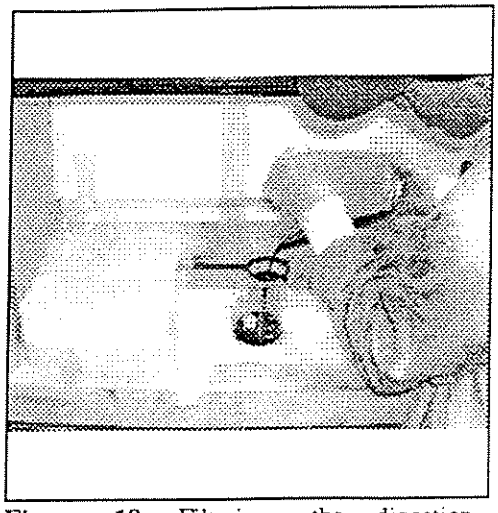

Figure 18. Filtering the digestion solution

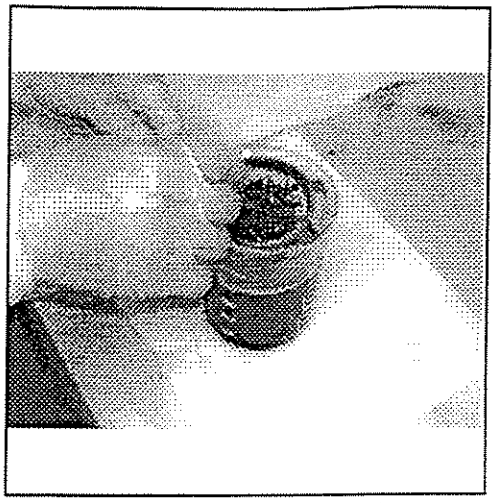

Figure 19. Evaluating the residue

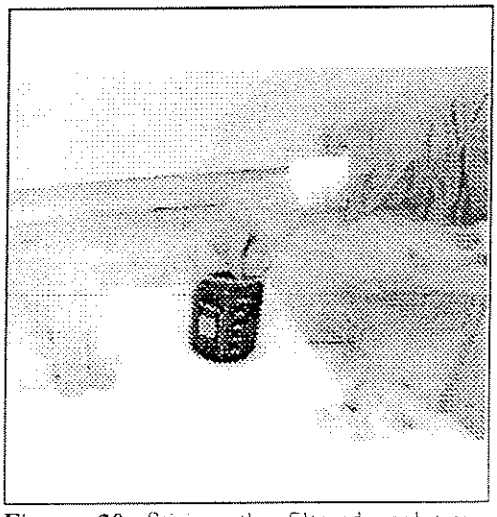

Figure 20. Stiring the filtered solution

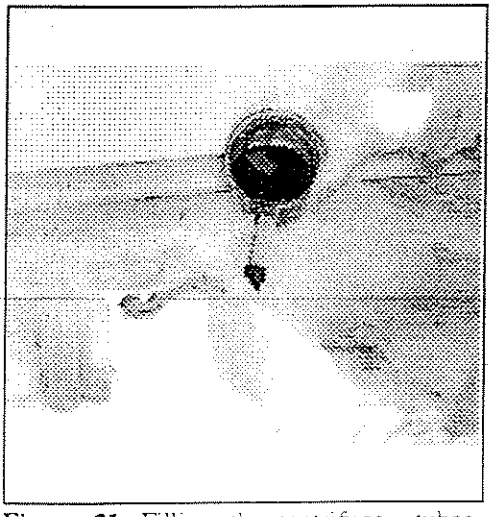

Figure 21. Filling the centrifuge tubes

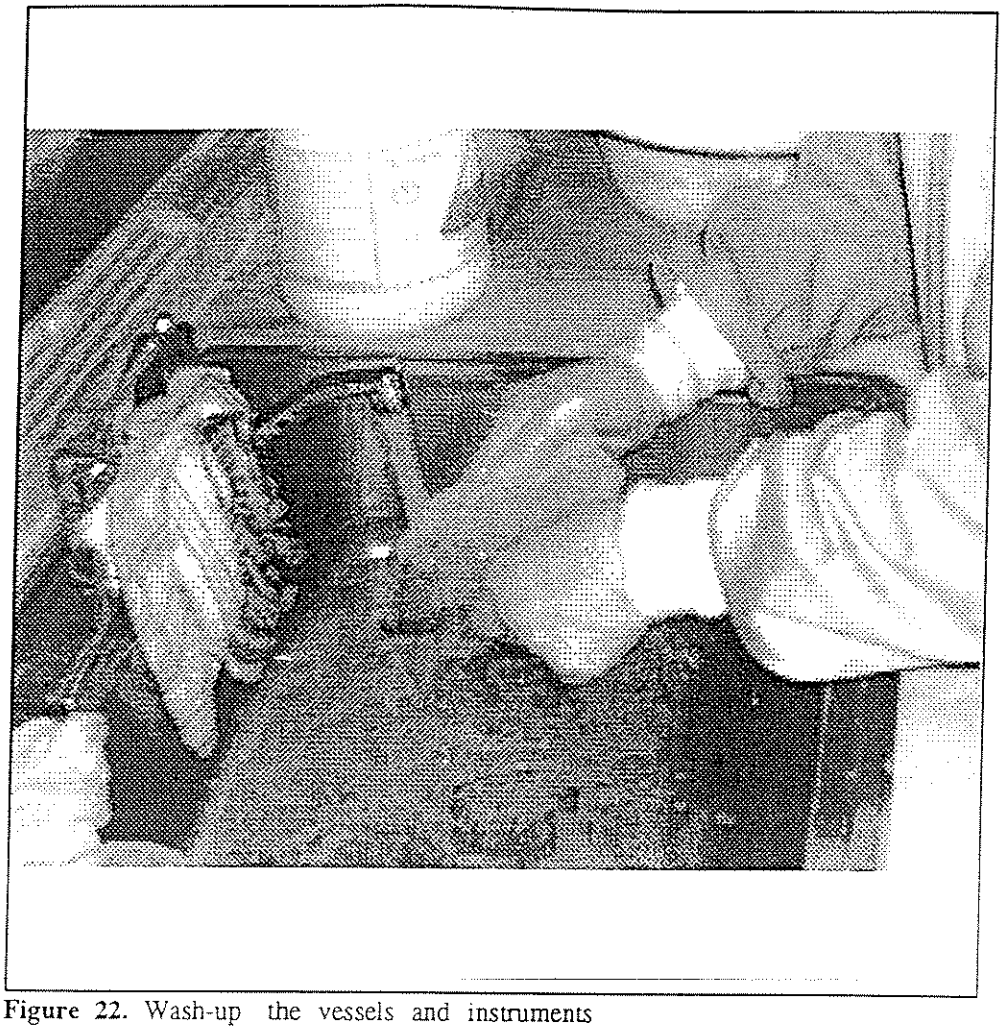

Figure 22. Wash-up the vessels and instruments