NORTH PACIFIC RESEARCH BOARD

FINAL GRANT REPORT

The role of walrus (*Odobenus rosmarus divergens*) in transmission of human trichinellosis in the indigenous people of the Chukchi Peninsula

NPRB Project 641

L. A. Bukina and A. I. Kolevatova

Vyatka State Agricultural Academy, Zoology Department, 133 Oktyabrsky Avenue, Kirov, Russia, 610017

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"Hunting of marine animals is vital for the life, culture and the language of the coastal native communities of Chukchis. We must survive as a nationality, handing down our descendants the experience of a traditional way of life."

- *Indigenous people of the Chukchi Peninsula*

ABSTRACT

Subsistence hunting of marine mammals is one of the primary sources of food for the indigenous Chukchi communities, however, the health risk associated with consumption of *Trichinella* infected marine mammal meat is of concern. The purpose of this project was to study the prevalence of exposure and sources of infection in the Chuckchi Peninsula to develop preventive measures to be used in the future.

Of 361 and 76 individuals tested by enzyme-linked immunosorbent assay in the communities of Lorino and Lavrentiya, 22 (6.1%) and 1 case (1.3%) tested positive respectively. Results indicated that exposure to infection had occurred in the communities. Fourteen individuals had (63.6%) titers of 1:200, seven (31.8%) of 1:400 and one (4.6%) with a high titer of 1:800. Seroprevalence was found to be highest among marine mammal hunters (17.9%) and in schoolchildren (7.4%) compared to the overall population (6.1%).

One of the main sources of trichinellosis in humans is suspected to be through consumption of raw and fermented walrus meat. The prevalence and intensity of infection in various meat types was examined by the compression and artificial digestion methods. Of 257 carcasses from 17 species of mammals and birds examined for *Trichinella* larvae, four species were infected. These included walrus (1.5%), red fox (100%), farmed polar fox (94.5%) and stray (100%) and sled dogs (66.7%). Viable *Trichinella* larvae were found in *kopal'khen* polar fox preparation but were not infective in laboratory rodents and pups. However, *Trichinella* larvae from infected frozen polar fox meat not only remained viable but also resulted in transmission of infection in canine puppies. Results indicated that transmission of trichinellosis from wildlife to domestic animals humans was likely occurring on the Chuckchi Peninsula.

KEY WORDS
Walrus, *Odobenus rosmarus divergens*, indigenous people, traditional diet, trichinellosis, zoonosis, disease transmission, enzyme-linked immunosorbent assay, Chukchi Peninsula, Arctic coast.

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INTRODUCTION

Subsistence hunting of marine mammals is common in Russian Arctic communities (Figure 1-2). As a result of traditional hunting of whales and other marine mammals the native people survived the economic crash during Russia's transition to a market economy. Aboriginal hunting of grey whales (*Eschrichtius robustus*) and the Greenland right whale (*Balaena mysticetus*) has recently resumed and both have become an indispensable food source along the Chukchi Peninsula. Hunting of whale plays an important role in the social and economic structure of coastal villages on the Chukchi Peninsula and in maintaining traditional relations between reindeer-herder and marine mammal hunter families. Seal, walrus and whale meat provided to reindeer-herders decreases the need to slaughter additional caribou and reindeer allowing herders to maintain a larger stock. Traditional ways of storing harvested marine mammals, fish and edible wild plants are common and every community stores traditional meat rolls or *kopal'khen* in ice-storages.

Subsistence hunting of marine mammals is important to both the survival of the Chukchi native people and their culture (Kochnev and Smirnov, 2000). Walrus meat is an important food source for both the Maritime or coastal Chukchi communities and the Reindeer or settled Chukchi population inhabiting the inland and wooded tundra region, making up to 42% and 30% of their diet respectively (Kozlov, 2002). The total consumption of walrus meat by the indigenous communities of the Chukchi Peninsula is estimated to be 690 tons per year. The greatest per capita consumption of meat from marine mammals is among the coastal communities (356.3 gram per day), whereas the consumption among reindeer-herders is less (121.0 gram a day) (Report, 1997).

Consumption of marine mammals poses a threat to human health as muscles from a number of marine mammal species have been found to contain *Trichinella spiralis nativa* larvae. The first cases of trichinellosis in animals were reported in the Canadian Arctic in the 1930's, and in humans in the 1940's (Proulx et al., 2002). In 1947 in Greenland the first human outbreak was linked to the consumption of walrus meat (Forbes, 2004). During that outbreak the incidence of infection was four times greater in people that ate raw or fermented meat compared to cooked meat. Foreign and Russian researchers have since been examining marine mammal meat for the presence of the larvae. In Russia, trichinellosis was first reported in marine mammals in 1966 in a walrus harvested near the Chukchi Peninsula. Since 1966 walrus as well as other harvested marine mammal species have been found to contain the parasite (Delamure et al., 1975; Yurakhno, 1990).

Studies are needed to investigate transmission of trichinellosis to Chukchi natives that consume marine mammal meat to determine if traditional methods of raw or fermented

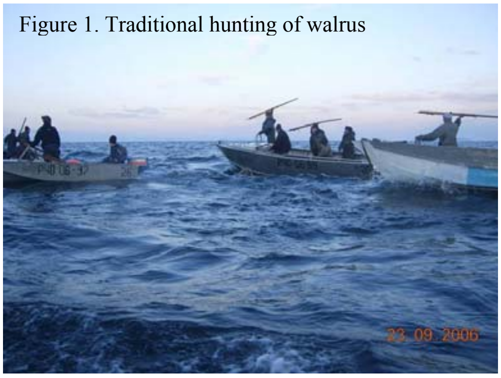

Figure 1. Traditional hunting of walrus

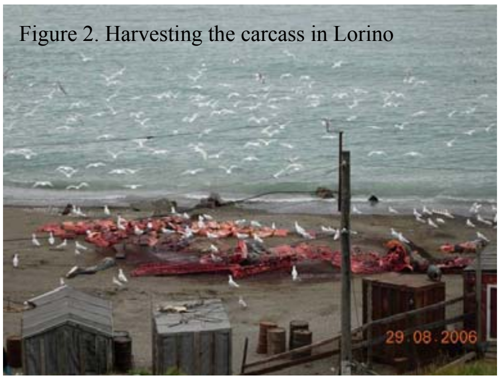

Figure 2. Harvesting the carcass in Lorino

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preparations place the population at high risk for infection. The Arctic *Trichinella* species are known to stimulate a mild immune response (measured serologically) but has a relatively high rate of infection in humans. Clinically, infection results in a long incubation period, mild muscle and abdominal pain and often severe neurologic signs (Ozeretskovskaya, 1968). In 1969, a serosurvey was conducted in residents of coastal Chukchi communities to examine prevalence of exposure to the helminth (Bessonov et al., 1969). Results showed a 27.8% prevalence associated with transmission from local fauna (Bessonov et al., 1969; Wolfson, 1969). Additionally, one woman who had originally come over from the Chukchi Penisula died of trichinellosis on Sakhalin Island (Britov, 1969). The distribution of the seropositive cases varied among communities and Wellen had the highest percentage of *Trichinella*-exposed people. This was thought to be associated with inadequate storage of the walrus meat rolls (*kopal'khen*), therefore likely an important factor in transmission of the parasite. From 1994 to 1997 exposure was detected in inhabitants from six of nine northern Russian regions (Poletayeva et al, 1998). Human outbreaks of trichinellosis have since been reported in the Canadian Arctic associated with the consumption of undercooked, raw, and fermented walrus meat (*igunaq*) (Margolis et al., 1979). Studies investigating sources of trichinellosis on the Chukchi Peninsula revealed high prevalence rates in terrestrial vertebrates and also indicated that meat from marine mammals was one of the main sources of infection, in particular consumption of polar bear and walrus meat (Ovsyukova, 1963; 1965). In Nunavik Alaska, it was estimated that 60% of polar bear meat contained *Trichinella* larvae, compared to 2-4% of walrus. However, the former is usually eaten cooked while the latter is more frequently eaten meat raw or fermented (Proulx et al, 2002).

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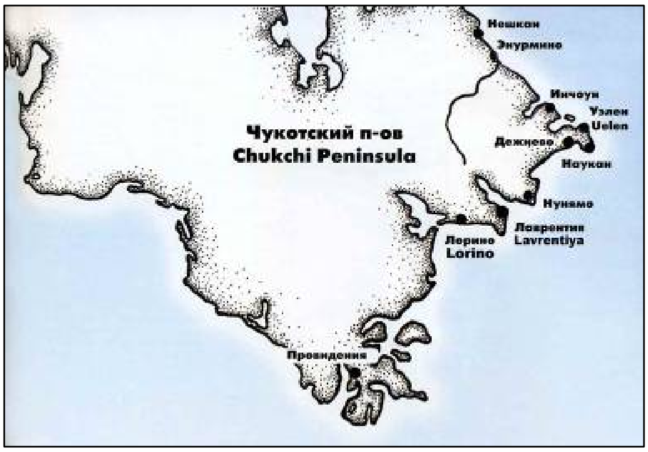
As a result of recent economic and social changes in Russia, consumption of traditional foods by the indigenous northern people has greatly increased highlighting the need to investigate the epidemiology and transmission of trichinellosis in indigenous Arctic communities. In order to meet this need, a research team from the Vyatka State Agricultural Academy initiated a study to examine the role of walrus meat in transmission of human trichinellosis and evaluate the risk of consumption of traditional meat products. The project focused on two coastal communities in the Chukchi Region: the communities of Lorino and Lavrentiya (Figure 3). Residents of these communities had historically been involved in the hunting of marine mammals and inedible meat parts were fed to the animals on the local fur farm.

Figure 3. Map showing the Chukchi Peninsula and the locations of Lorina and Lavrentiya.



GOALS

The purpose of the project was to evaluate the prevalence of *Trichinella* infection in people on the Chuckchi Peninsula associated with consumption of infected walrus meat, investigate the potential for transmission of *Trichinella* infection via traditional preparations of walrus

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meat, and to evaluate the role consumption of other marine mammals and carnivores play in transmission of infection. The overarching goal was to ultimately use these data to develop guidelines to prevent continued transmission of *Trichinella* infection in the indigenous people on the Chuckchi Peninsula.

OBJECTIVES:

1. Determine the prevalence of exposure to *Trichinella* in the local population and association with risk factors such as diet and occupation.
2. Determine the prevalence and intensity of *Trichinella* infection in walrus, other marine mammal and terrestrial wildlife species.
3. Determine the viability and infectivity of *Trichinella* larvae in traditionally cooked walrus preparations.
4. Develop preventive measures to control transmission of trichinellosis.

OBJECTIVE 1:
Determine the prevalence of exposure to *Trichinella* in the local population and association with risk factors such as diet and occupation.

METHODS

i. Sample collection
Prevalence of exposure in the communities of Lorino (n = 361) and Lavrentiya (n = 76) was evaluated by serologic analyses. Random sampling was conducted to obtain serum for analysis. Each person enrolled in the study also completed a questionnaire (Figure 5) to obtain information on:

- age, sex, nationality, duration of residence on the Chukchi Peninsula, occupation
- diet, methods of food preparation, types of meat consumed
- medical history, clinical signs associated with *Trichinella* infection

ii. Sample analysis
Serum was collected (Figure 4), frozen, and transported by air to Martsinovskii Institute of Medical Parasitology and Tropical Medicine in Moscow. Serologic analysis was performed at the Immunodiagnostic Laboratory using an enzyme-linked immunosorbent assay (ELISA) that measured antibodies against the *Trichinella* larvae (Vektor Best, Novosibirsk, Russia). Exposure to trichinellosis was determined by measuring the optical density (OD) at 492 nm using the Multiscan MCC/340 spectrophotometer (Labsystems). Initial screening was done at a dilution of 1:200. Samples were considered to be positive if the OD was 3 times that of the negative control (provided in the test kit). Sera that tested positive at initial screening were two-fold serially diluted to determine the end-point titer.

Figure 4. Collection of blood samples in the community of Lorino.

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Statistical analysis was performed using the statistical package STATISTICA (StatSoft, Russia, 1999). The Fisher exact test was used to compare difference among groups and $P <$ 0.05 was considered to be statistically significant.

Figure 5. Questionnaire for individuals undergoing testing by ELISA.

QUESTIONNAIRE **№________ Date__________**

1. Surname______________________________ First Name______________________
2. Patronymic____
3. Date of Birth_______________ Place of Birth_______________________________
4. Passport: Series_________ Number______________________________________
5. Passport Issued by__________________________________ Date of Issue_________
6. Address____
7. Sex □ Male □ Female (Are you pregnant? □ Yes □ No)
8. Nationality____
9. Place of Employment____
10. How long have you been living on the Chukchi Peninsula?____________________
11. Blood group____
12. How often do you consume meat (routinely, occasionally)?____________________
13. What type of meat do you consume (specify how often you consume this meat type)?

1	pork	□ cooked	□ raw	□ fermented	____________
2	venison	□ cooked	□ raw	□ fermented	____________
3	walrus	□ cooked	□ raw	□ fermented	____________
4	ringed seal	□ cooked	□ raw	□ fermented	____________
5	bearded seal	□ cooked	□ raw	□ fermented	____________
6	whale	□ cooked	□ raw	□ fermented	____________
7	bear	□ cooked	□ raw	□ fermented	____________
	polar	□ cooked	□ raw	□ fermented	____________
	brown	□ cooked	□ raw	□ fermented	____________
8	polar fox	□ cooked	□ raw	□ fermented	____________
9	bird (specify type)	□ cooked	□ raw	□ fermented	____________

14. When was the last time you consumed any meat?_____________________________
15. Clinical illness:

Symptoms____

____

____

(Have you had any abdominal discomfort after consuming meat, pain when eating, eye pain, facial edema, difficulty swallowing?)

When was the first time clinical illness was observed

____

Signature of Interviewee_______________________

ELISA Results:

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RESULTS

i. Prevalence of exposure to Trichinella in the communities of Lorino and Lavrentiya.

Of the 361 individuals tested in Lorino, 22 (6.1%) tested positive. Based on the information obtained from the questionnaires nine different nationalities were represented in the community: Chukchi (86.4%), Eskimo (5.3%), Russian (5.6%), Tabasaran (0.9%), Ukrainian (0.6%) and other (Byelorussian, Kazakh, Mari, Yukaghir) (1.2%). The majority of the

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seropositive cases were of Chukchi (87.0%) origin, followed by Eskimo (8.7%) and Russian (4.3%). Despite this, no significant difference was found among nationality and prevalence of exposure ($P = 0.7$) and may be explained by similarities in dietary habits and food preparation in the various native communities.

Residents not originally from the Chukchi Peninsula generally consume less marine mammal meat and more reindeer, pork and beef. Neither the pork nor beef are produced locally and are therefore transported from other areas. Interestingly, the one Russian that tested positive for exposure to *Trichinella* was a schoolgirl who had lived her entire life on the Chukchi Peninsula. According to the questionnaire, she had likely become infected through consumption of insufficiently cooked ringed seal (*Pusa hispida*) meat.

Of the 76 people tested in Lavrentiya only one (1.3%) was positive. The community of Lavrentiya was represented by 6 nationalities: the majority were Chukchi (61.8%), followed by Russian (13.0%) and Eskimo (13.0%). The other three nationalities were represented by one individual each. The one positive was an Eskimo who had lived his entire life on the Chukchi Peninsula.

Comparison of seropositive individuals by age and sex indicated that in Lorino the highest prevalence of exposure was in men ranging from 31 to 50 years old (46.6%) (Table 1). There was no correlation with seropositivity and age in women. Overall, significantly more men tested positive than women ($P = 0.007$). A possible explanation may be that men are more frequently exposed to raw and inadequately cooked meat as they spend longer periods away from home. The one positive from Lavrentiya was a 22 year old male.

Table 1. Comparison of seropositive individuals from Lorino by age and sex.

	No. Seropositive cases	
Age	Men (%)	Women (%)
< 20 yr	4 (26.8)	2 (28.6)
21-30 yr	1 (6.7)	2 (28.6)
31-50 yr	7 (46.5)	2 (28.6)
> 50 yr	3 (20.0)	1 (14.2)
Total	15	7

When comparing the proportion of people that tested positive by occupation in Lorino, the highest was among marine mammal hunters (17.9%) and retired individuals (13.6%) (Table 2). Additionally, according to the questionnaires all of the retired people had previously been marine mammal hunters.

Overall, the data from the questionnaires in indicated that the residents of Lorino primarily consumed marine mammal meat, and in particular walrus meat (Table 3). Thus, it appears that exposure to *Trichinella* disease correlates with occupation and dietary habits in the native communities. Polar bear (*Ursus maritimus*) was primarily consumed by marine mammal hunters and retirees. The traditional way of cooking bear meat is to slice and cook it for a long time as it is relatively tough. As a result, cooking likely destroys any *Trichinella* larvae. The possibility of legalizing hunting of polar bear for subsistence is under discussion in Russia, therefore there is currently a need to investigate the possibility of disease transmission associated with consumption of polar bear meat.

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Table 2. Seroprevalence of *Trichinella* infection in individuals from Lorino by occupation.

Occupation	No. tested (%)	No. Positive (%)
Fur farm workers	41 (11.3)	1 (2.4)
Traditional marine mammal hunters	28 (7.8)	5 (17.9)
Retired	22 (6.1)	3 (13.6)
Non-manual workers	78 (21.6)	1 (1.3)
Schoolchildren	54 (15.0)	4 (7.4)
Other	138 (38.2)	8 (5.8)
Total	361	22 (6.1)

Interestingly, serologic analysis of samples from schoolchildren revealed a high prevalence of exposure (7.4%) (Table 2). More than 50% of these schoolchildren also indicated that they consumed cooked marine mammal meat. Non-manual workers had the lowest proportion of positives (Table 2), despite the fact that they consumed all types of meat, including traditional preparations.

Table 3. Types of meat consumed by the residents that tested seropositive in Lorino from September to October 2006.

Occupation	Walrus			Whale	Seals	Reindeer	Polar bear	Polar fox
	Raw	Cooked	Fermented					
Fur farm worker	0	1	0	1	0	1	1	0
Traditional marine mammal hunter	5	5	5	5	5	5	5	0
Retired	3	3	3	3	3	3	2	0
Non-manual worker	1	1	1	1	1	1	1	1
Schoolchildren	2	3	2	3	2	3	0	0
Other	6	8	7	8	6	8	6	4
Total	17	21	18	21	17	21	15	5

Surprisingly, a relatively high prevalence of exposure was found in the "Other" group which included the unemployed and people with occupations not related to hunting (off-road vehicle driver, a radio operator, etc.). Fifty percent of these individuals indicated that they primarily consumed farmed polar fox meat. According to the fur farm workers, many of the local people obtain carcasses from slaughtered polar foxes for consumption. The meat from the limbs was noted to be the preferred parts for eating. Examination of the muscles from the limbs revealed a high larval density of 700 larvae per 1 gram of muscle. In contrast, although fur farm workers also consumed polar fox meat, only one tested positive on ELISA. Finally, almost all of those examined, irrespective of their occupation, consumed reindeer meat. Reindeer meat is considered to be free of *Trichinella* larvae, and thus was not considered a source of infection.

The 22 year old male that was the only positive case in Lavrentiya was of Eskimo origin and a fireman by occupation (Table 4). The majority of responders preferred whale meat (93.4%), followed by cooked walrus and reindeer (84.2%), and seal (76.3%) (Table 5). Since the majority preferred cooked walrus meat, the risk of infection was likely greatly decreased. The consumption of polar bear and farmed polar fox meat was much lower in Lavrentiya

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compared to in Lorino.

Table 4. Seroprevalence of exposure to *Trichinella* infection in individuals from Lavrentiya.

Occupation	No. tested (%)	No. Positive (%)
Non-manual worker	38 (50)	0 (0)
Schoolchildren	2 (2.6)	0 (0)
Retired	4 (5.3)	0 (0)
Other	32 (42.1)	1 (3.1)
Total	76	1 (1.3)

Table 5. Meat consumed by the residents of Lavrentiya.

Occupation	Walrus			Whale	Seal	Reindeer	Polar bear	Polar fox
	Raw	Cooked	Fermented					
Retired	0	2	0	4	2	4	0	0
Non-manual worker	6	33	4	35	30	32	12	2
School children	0	2	0	2	2	1	1	0
Other	3	27	2	30	24	27	14	0
Total (%)	9 (11.8)	64 (84.2)	6 (7.9)	71 (93.4)	58 (76.3)	64 (84.2)	27 (35.5)	2 (2.6)

ii. Comparison of titer concentration against Trichinella antigen between the communities of Lorino and Lavrentiya.

All sera with OD values higher than the cut-off (ie. three times the OD of the negative control) were serially diluted to determine the end-point titer. Overall, titers of 1:200 were obtained for 15 (63.6%) individuals, 1:400 for 7 (31.8%), and 1:800 for 1(4.6%) individual. The one positive in Lavrentiya had a titer of 1:200.

Comparison of titer with type of meat consumed showed that those with higher antibody titers consumed more marine mammal meat, although there did not appear to be a correlation with titer and cooked, raw or fermented preparations of walrus meat (Table 6).

Table 6. Range of positive titers measured in individuals consuming different types of meat in Lorino.

Titer	Walrus			Whale	Seal	Polar bear	Polar fox
	Cooked	Raw	Fermented				
1:200	13/14 (92.9)	9/14 (64.3)	10/14 (71.4)	13/14 (92.9)	9/14 (64.3)	10/14 (71.4)	3/14 (21.4)
1:400	7/7 (100)	7/7 (100)	7/7 (100)	7/7 (100)	7/1 (100)	5/7 (71.4)	2/7 (28.6)
1:800	1/1 (100)	1/1 (100)	1/1 (100)	1/1 (100)	1/1 (100)	0/1 (0)	0/1 (0)
Total	21/22 (95.4)	17/22 (77.3)	18/22 (81.8)	21/22 (95.5)	17/22 (77.3)	15/22 (68.2)	5/22 (22.7)

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Polar bear meat was consumed by 71.4% of individuals with a titer of 1:200 and by 71.4% with a titer of 1:400. Farmed polar fox (*Lagopus alopex*) meat was consumed by 21.4% of individuals with a titer of 1:200 and by 28.6% with a titer of 1:400. Despite the lower prevalence associated with consumption of farmed fox meat, this practice is still an important mode of transmission of Trichinella disease as 94.5% of foxes tested were found to contain larvae, and the larval density in muscle varied from 2 to 700 larvae per 1 gram of tissue. Additionally, there was no significant difference among titer concentration and consumption of marine mammal versus polar fox meat.

When correlating occupation with titer, we found that the highest titer (1:800) was measured in the Chuckchi schoolboy (Table 7). Antibody titers of 1:400 were measured in marine mammal hunters (28.6%) and retirees (42.9%), and titers of 1:200 in 14 individuals from the "Other" group.

Table 7. Comparison of antibody titer by occupation in Lorino.

Occupation	Titer (%)		
	1:200	1:400	1:800
Fur farm worker	1 (7.2)	0 (0)	0 (0)
Traditional marine mammal hunter	3 (21.4)	2 (28.6)	0 (0)
Retired	0 (0)	3 (42.9)	0 (0)
Other	7 (50)	1 (14.3)	0 (0)
Non-manual worker	0 (0)	1 (14.2)	0 (0)
Schoolchildren	3 (21.4)	0 (0)	1 (100)
Total	14	7	1

The average age with antibody titers of 1:200 was 31.1 years, 1:400 was 47.9 years, and the child with the titer of 1:800 was 14 years. Examination of the child's medical record indicated he had an increased susceptibility for bleeding but had no other associated hematologic abnormalities or clinical disease.

DISCUSSION

Wolfson (1969) did not find a difference in seroprevalence in Chukchis and Eskimos when comparing exposure to *Trichinella* in indigenous ethnic groups on the Chukchi Peninsula. This finding was attributed to similarities in traditional lifestyle and dietary habits of these northern people. Our recent survey was consistent with this finding as well. Previously, it was believed that *Trichinella* infection in the Russian population was only prevalent only in non-residents who became infected through consumption of brown bear meat (Rausch et al, 1956; Ozeretskovskaya, 1968; Wolfson, 1969). However, our findings show Russian residents of the North do consume marine mammal meat and therefore also acquire the infection through this route.

A high prevalence of exposure to *Trichinella* infection was previously found in marine mammal hunters (Bessonov et al, 1969; 1970). Our serosurvey too found an association between occupation and seropositivity as the highest prevalence was among marine mammal hunters and retirees, who had previously worked as marine mammal hunters. Marine mammal meat was the main component of their diet, thus increasing their risk of infection compared to other occupational groups. Earlier studies did not report severe clinical signs associated with disease in the Arctic indigenous people despite the elevated antibody titers (Rausch et al., 1956; Britov, 1962b), which was consistent with our findings as well. The

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clinical course and resulting immune response has been associated with the number of larvae found in the infection as well as with larval infectivity (Pashuk et al, 1965; Trichinella and Trichinellosis, 1978). Trichinellosis can therefore be classified into four main presentations: clinical, sub-clinical, latent infection and a carrier state, all with varying immune responses. This is further complicated as clinical cases may have a varied response from negative to weak or high positive titers (Pashuk, 1967). Sub-clinical cases test positive but with varying antibody titers, while patients with latent infections have low antibody titers. Similarly carriers test positive but with low titers. In parasitic diseases, the host immunity is thought to be short-term (Ozeretskovskaya, 1968; Pashuk et al, 1965), although the antibody titer remains positive if there are low numbers of infective larvae in the host. If the infection is cleared antibody titers decline over time. Although the indigenous people of Canada, Alaska and the Russian Arctic frequently consume uncooked meat from wild game, they seldom develop edema associated with disease (Klaishevich et al., 1997). This may be due to the high level of immunity in the population.

In 2000, the highest incidence rate of Trichinellosis was recorded in the Chukchi Region (9.8 cases per 100,000) (Onishchenko, Ministry of Health of Russia, 2002). If this rate of infection continued our estimate was that by 2006 this would translate to approximately 6,000 cases per 100,000 people. The overall seroprevalence measured in our study was 6.1% and is three times greater than that reported for the region in 1969 (Bessonov et al, 1969). We think this may be due to changes in dietary habits and a resurgence of ethnic traditions. The 22 patients that tested positive from Lorino all lacked clinical symptoms regardless of their serum titers suggesting we were measuring the presence of latent and carrier forms of the infection.

The prevalence estimate we obtained is likely conservative as we suspect we obtained false negative results in some cases with the ELISA. The low antibody titers may be partly explained by the lack of specificity of the ELISA kit due to a difference in epitopes. The test antigen is from *Trichinella spiralis spiralis* larvae recovered from domestic swine and may have different epitopes than larvae from marine mammal meat. Therefore, it is possible that the kit failed to detect antibodies in people with the early stages of infection with wild strains. The Arctic strain of *Trichinella spiralis nativa* has become adapted to the animals inhabiting the Arctic (Poletayeva and Krasovskaya, 1995). Therefore, a comparison is needed between antigens from larvae from infected farmed fox fed on marine mammal meat and those from domestic swine to better understand the sensitivity of the test in this population. Additionally, Trichinella, like other zoonotic parasites, are adapted to persisting in the host for long periods during which time they can have an immunosuppressive effect (Lysenko, 1994). This too could explain the weak positive results. In conclusion, further work is needed to estimate the prevalence of infection in the communities on the Chuckchi Peninsula and the addition of other tests (e.g. indirect haemagglutination test) may be beneficial as results from the two tests could supplement each other (Poletayeva and Krasovskaya, 1995).

OBJECTIVE 2:
Determine the prevalence and intensity of *Trichinella* infection in walrus, other marine mammal and terrestrial wildlife species.

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MATERIALS AND METHODS

i. Sample Collection

To evaluate sources of exposure to trichinellosis in humans, we examined 257 carcasses from 17 different mammal and bird species (Figure 6). Fourteen were terrestrial species and samples collected were from 7 muscles types (tongue, neck, diaphragm, masseters, intercostals, hind limb, forelimb). Additional samples collected from marine mammals also included tongue (apex) and eye muscle (Figure 7), in accordance with the Directions of Veterinary Department from October 10, 1998, №13-7-2/1428.

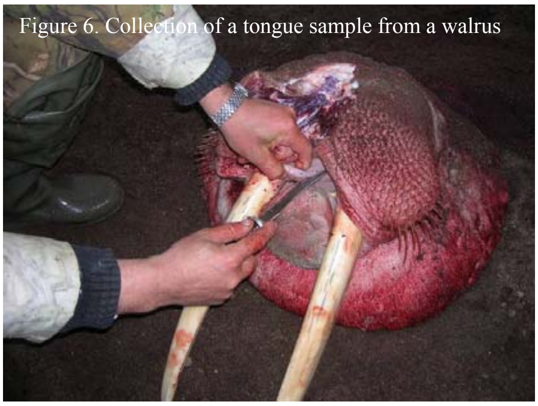

Figure 6. Collection of a tongue sample from a walrus

ii. Sample Analysis

Samples were tested using microscopic examination and biochemical analysis.

1. Microscopic examination (compression method).

The Trichinoscopy method was used to determine the intensity of infection. Three muscle sections (each consisting of 24 tissue cuts) were taken from each tissue type and pressed between two sheets of glass. The total number of larvae were counted in each pressed section with a MBS-10 microscope (Petrolizer). The mean larval density in the various muscles was determined and defined as the number of larvae per 1 gram of muscle tissue (lpg). The sensitivity of this method was found to be low.

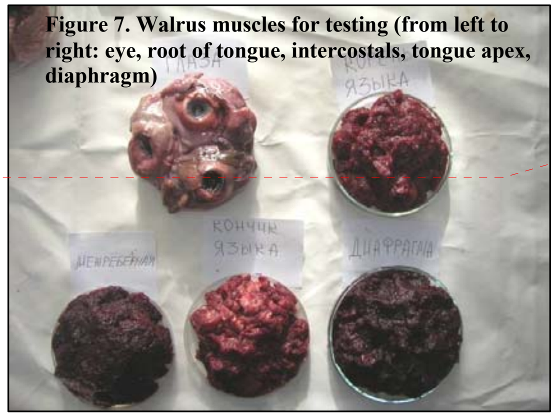

Figure 7. Walrus muscles for testing (from left to right: eye, root of tongue, intercostals, tongue apex, diaphragm)

2. Biochemical analysis.

The artificial digestion method was performed using the AVT apparatus (Gastros model, Petrolizer) (Figure 8). The apparatus contained a thermostatically controlled chamber with an in-built reaction-vessel intended for dissolving the muscle tissue with digestion enzymes. After microscopic examination muscles were digested in 1% hydrochloric acid (1L tap water with 10 ml of HCl) and 10g swine pepsin (according to the All-Union standard 49-53-84). Each sample was stirred for 1 min. Muscle samples (20g each) were collected from 5 marine mammal carcasses and up to 10 grams were collected from individual terrestrial species and pooled (100g). Additional samples tested include head muscle from

Figure 8. Gastros apparatus used for artificial digestion of muscles

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a brown bear (*Ursus arctos*); tongue, neck, diaphragm, masseters and intercostal muscles from reindeer; and pectoral and leg muscles from birds. Tissues were ground (3-4 mm mesh size) and placed into the reaction-vessel for digestion. The fluid was allowed to settle for 15 minutes and the sediment was transferred into a 2ml cuvette for microscopic examination. All glassware and equipment were disinfected between samples to prevent cross contamination of samples.

RESULTS

Trichinella larvae were detected in 4 out of 17 species tested: 2 wildlife - walrus and fox (red (*Vulpes velpes beringiana*) and farmed polar fox) and 1 domestic species - stray and sled dogs (*Canis familiaris*). Of 138 walrus of different sex and age examined, only 2 (1.5%) tested contained *Trichinella* larvae. No larvae were detected in pinnipeds that were potential carriers of the pathogen and included ringed and bearded seals (*Erignathus barbatus nautilus*), and 18 grey whales (*Eschrichtius robustus*, 7 females and 11 males).

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Both red foxes tested were infected and prevalence of infection was 100% and 66.7% in stray and sled dogs respectively (Table 10). The rate of infection in caged polar fox was 94.5%.

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Table 8. Prevalence of Trichinella infection in tissues from wild and domestic species tested in Lorino.

Animal species	No. tested	No. infected	Prevalence (%)
Order *Insectivora*			
Lesser shrew *Sorex*	3	0	0
Order *Carnivora*			
Brown bear *Ursus arctos*	1	0	0
Grey whale *Eschrichtius robustus*	18	0	0
Walrus *Odobenus rosmarus divergens*	138	2	1.5
Ringed seal *Pusa hispida*	3	0	0
Bearded seal *Erignathus barbatus*	7	0	0
Caged polar fox *Alopex lagopus*	53	50	94.5
Red fox *Vulpes vulpes beringiana*	2	2	100
Stray dog *Canis familiaris*	3	3	100
Sled dog *Canis familiaris*	3	2	66.7
Order *Rodentia*			
Arctic ground squirrel *Citellus undulates*	5	0	0
House mouse *Mus Linnaeus*	3	0	0
Norway rat *Rattus norvegicus*	4	0	0
Order *Lagomorpha*			
Mountain hare *Lepus timidus*	5	0	0
Order *Artiodactyla*			
Reindeer *Rangifer tarandus*	1	0	0
Order *Lariformes*			
Glaucous-winged gull *Larus glaucescens*	5	0	0
Order *Passeriformes*			
Common raven *Corvus corax*	3	0	0
Total	257	59	

Deleted: *Eschrichtius gibbosus*

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Differences were found in distribution of larvae between species and muscle types (Table 9). The wild red fox were the most heavily parasitized and forelimb muscles contained the highest larval density (92.3 ± 26.8 lpg), compared to the tongue (81.3 ± 23.1 lpg), and was lowest in the intercostal muscles (47.3 ± 18.4 lpg). Overall, the mean larval density in the striated muscles was 67.2 lpg. Both sled and stray dogs had the highest larval density in tongue (8.9 ± 3.2 lpg; 73.3 ± 29.6 lpg, respectively), followed by the neck and masseters in sled dogs and the diaphragm, forelimb and lower hindlimb muscles in stray dogs. The lowest larval density was found in the intercostals in both. Although both the sled and stray dogs had the highest larval density in tongue, muscles from strays contained 8 times more larvae than those of the sled dogs ($P = 0.0$). Overall, the mean larval density within the striated muscles of the stray dogs (35.3 ± 12.7 lpg) was almost 6 times that of the sled dogs (6.1 ± 1.7 lpg) ($P = 0.01$).

Table 9. Mean density (SD) of *Trichinella* larvae in muscles of mammalian predators (larvae per gram).

Muscle type	Polar fox	Red fox	Sled dog	Stray dog
Tongue	54.7 (18.4)	81.3 (23.1)	8.9 (3.2)	73.3 (29.6)
Neck	47.8 (23.1)	55.0 (29.2)	6.7 (1.9)	29.1 (10.4)
Diaphragm	32.0 (13.9)	55.0 (14.4)	5.8 (1.8)	31.6 (8.8)
Masseters	38.5 (11.3)	76.0 (25.0)	6.6 (2.8)	26.9 (7.6)
Intercostals	34.4 (10.5)	47.3 (18.4)	4.4 (1.7)	25.3 (6.5)
Lower hindlimb	52.8 (15.7)	63.3 (21.7)	5.0 (1.7)	30.9 (8.7)
Forelimb	68.8 (21.9)	92.3 (26.8)	5.2 (14.9)	30.2 (8.8)

The Rusetsky polar fox farm is in Lorino and currently has a breeding stock of 600 animals. The farmed foxes were primarily fed leftover "garbage" or "off-cut" tissues from hunted marine mammals. Our results show that both prevalence and intensity of *Trichinella* infection is high in these foxes. The highest larval density was found in 3 muscles: forelimb (68.8 ± 21.9 lpg), neck (47.8 ± 23.1 lpg), and the lower hindlimb muscle (52.8 ± 15.7 lpg). Overall, the mean larval density in polar fox muscle was 47.0 lpg.

Statistical analysis revealed differences in intensity of infection and distribution of *Trichinella* larvae in muscles between males and females as larval density was significantly higher in all muscle types in females than in males (Table 10).

Table 10. Comparison between mean density (SD) of *Trichinella* larvae in muscles in male and female farmed polar foxes.

Muscle tested	Female	Male	*P*-value
Tongue	71.6 (25.3)	16.7 (5.6)	*0.03*
Neck	61.8 (23.7)	14.4 (4.1)	*0.01*
Diaphragm	41.8 (12.3)	10.2 (2.7)	*0.00*
Masseters	48.6 (15.9)	16.1 (4.2)	*0.02*
Intercostals	43.0 (14.9)	15.3 (3.8)	*0.03*
Lower hindlimb	67.5 (22.0)	20.3 (5.4)	*0.01*
Forelimb	86.6 (31.0)	29.3 (7.2)	*0.03*

Differences were also found by age as the highest larval density in all muscles was in animals aged 5 months old to 3 years, followed by those of 3 to 5 years of age (Figure 9). Overall, the

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larval densities in muscles from individuals aged 3-5 years differed from the most heavily parasitized group (5 mo-3 yr) by an average of 17.0%. Animals under 5 months of age had the lowest larval density in all muscles.

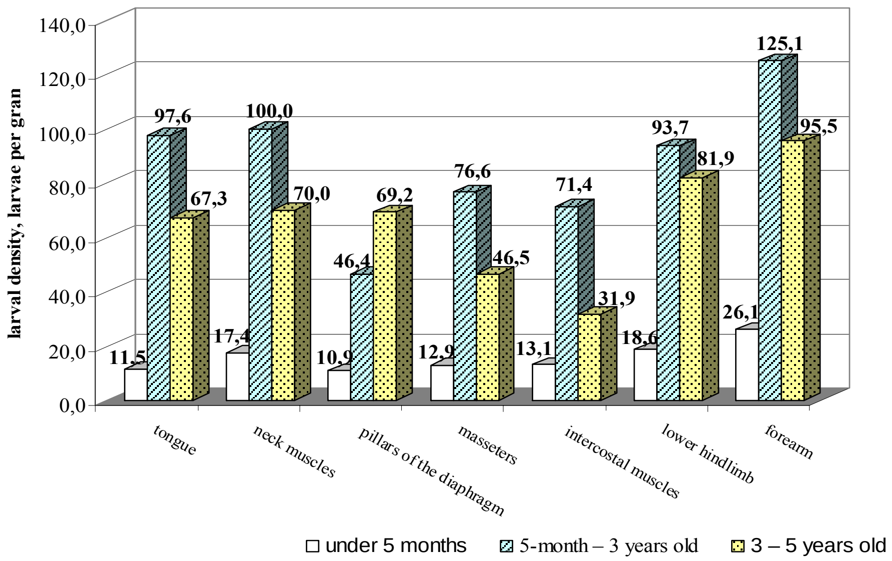

Figure 9. Larval density in muscles among caged polar foxes by age

To summarize, differences in larval density of muscles were found by age and sex in farmed polar foxes. Differences were also found in larval load and distribution by species. This may be associated with adaptation of the animal hosts to different environment and therefore must be taken into account when testing for *Trichinella* infection in different species.

DISCUSSION

Infection of marine mammals with *Trichinella* has been well documented on the Chuckchi Peninsula since the 1960's. Since then infection has also been documented in terrestrial wildlife as well as domestic species (Table 11). However, prior to our current study, infection in the region had not been evaluated for over 30 years. Our data shows a low prevalence of infection in walrus (1.5%), but an alarmingly high prevalence in other species including wild predators, domestic animals and farmed animals. Research in the Canadian Arctic, Alaska and Greenland (Rausch et al., 1956; Fay, 1960; Richard, Campbell, 1988; Margolis et al, 1979; Kapel, 1997; Moller et al., 2005) reported a prevalence of 22% in harvested walrus in Inukjuak in eastern Hudson Bay, with a larval load of between 20 to 44 larvae per 1 gram muscle tissue (Proulx et al, 2002); 2.5% in Quebec between 1982 and 1999; and 7% east of Greenland, in the Norwegian and Barents Seas. These varied result indicate that larger surveys are needed to better determine if the prevalence of trichinellosis in walrus is increasing.

Trichinellosis has been detected in many other species in the Arctic including marine (bearded seals, ringed seals, and Beluga whales *Delphinapterus leucas*) and terrestrial (snow

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Table 11. *Trichinella* infection in terrestrial, marine mammal and domestic species on the Chukchi Peninsula and in adjacent regions.

Year	Location, Species	Authors	Comments
1962	White Sea - seals	Britov V	1% Prevalence (7/722).
1962-1963	Chukchi Peninsula - 47 species (15 carnivore, 8 rodent)	Kozlov D, Ovsyukova N, Radkevich Zh	Prevalence in 31.9% of species tested (15/47): Carnivores - 73.3% prevalence (11/15), Rodents - 53.3% (4/8).
1962-1964	Chukchi Peninsula - 570 pinnipeds (196 walrus, 16 cetaceans)	Ovsyukova N	All negative.
1965	Chukchi Peninsula (Enurmino) - 50 walrus	Kozlov D	Prevalence of 2.0% (1 positive walrus), high larval load detected. First report in walrus on the Chukchi Peninsula.
1968	Chukchi Peninsula, Bering Sea - walrus	Treshchev V, Serdyukov A	Prevalence of 2%.
1975	Chukchi Sea - walrus	Delamure S, Yurakhno M, Popov V	Unknown prevalence, at least one positive.
2006	Chukchi Peninsula (Lorino) - 17 species (138 walrus, 18 grey whales, 53 farmed polar foxes, 3 sled dogs, 3 stray dogs)	Bukina L, Kolevatova A	Infection in 4 of 17 species tested: Prevalence of 1.5% walrus, 94.5% farmed polar fox, 100% free-ranging fox, 100%, stray dogs, 66.7% sled dogs.

hare, Arctic ground squirrel (*g. Sciurus* spp.*)*, brown and polar bear, polar fox, red fox, wolverine) mammals (Rausch et al., 1956; Bessonov, 1972; 2002). In our region in Lorino We found the host range to include sled and stray dogs, farmed polar fox, red fox, walrus, polar bear, brown bear; with potential transient hosts to be invertebrates and marine birds.

Sled dogs are a vital part to everyday life on the Chukchi Peninsula (Figures 10, 11), and dog teams are the most reliable transportation in the winter. Dogs are kept in homes as pets and are free to roam the village and onto the beach during harvesting of marine animals.

Figure 10. Sled dogs on Cape Akkani

Figure 11. Sled dogs on puppies during a harvest

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Killing of stray dogs, both old and young, is not allowed but providing nutrition for 6-8 or more sled dogs is often difficult. Therefore the dogs are fed according do their work load. Working dogs are between 1 to 5 years old and during this time are well fed and taken care of. After that age, however, many become strays and have to feed for themselves. As a result, our studies showed that the infection in stray dogs was considerably higher, likely associated with feeding on carcasses, whereas sled dogs were mostly fed cooked marine mammal meat and were less heavily infected. These data are consistent with other studies (Ovsyukova, 1965) although the mean larval load was three times lower than in our study.

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Fur farming is also integral to everyday life as it provides employment and utililizes inedible meat parts from harvested marine mammals (Figure 12). These animals consume 65-70% of raw inedible meat parts from harvested marine mammals annually (Report, 1997). As a result, these animals have become infected with *Trichinella* as well. This has also been shown to be the cases on many fur farms in Russia (Bessonov, 1972; Yalymova, 1974). It must be noted that the closer to the ocean a fur farm is, the higher the prevalence of *Trichinella* infection in both farmed animals and sled dogs (Ovsyukova, 1964).

Figure 12. Farmed polar fox (Lorino fur farm)

In Lorino, farmed polar foxes are only fed marine mammal meat therefore they can be used as an indicator to monitor trends of trichinella infection on the Chukchi Peninsula. The highest prevalence of infection in wild terrestrial mammals was in red foxes. Ovsyukova, (1964) reported a prevalence of 56.4% in this species. Due to our low samples size direct comparison is difficult, but it appears that prevalence has increased by a factor of six over the last 40 years, suggesting the red fox likely also play a role in transmission of the infection in the region.

Trichinella larvae are known to localize in striated muscles however a number of research studies have emphasized that the distribution of larvae in muscle types varies (Romashov V. et al., 2006; Belozyorov and Zhdanova, 2000; Sapunov et al., 2000). Berezantsev (1963) found tongue to have the highest load in predating mammals (foxes, stray dogs) whereas sled had highest densities in the fore and hind limb muscles (i.e. muscles with the largest workload), followed by the tongue. High larval density in the tongue muscles was attributed the increased blood supply to the tissue due to it's thermoregulatory role, facilitating larval colonization. Our results in red fox and farmed polar fox were consistent with these findings.

Different muscle types have been recommended for identifying infection in different species: wild boars, bears, nutrias (*Myocaster coypus*), horses - tongue, neck; rodents - masseters, tongue muscles; walrus - tongue (Penkova and Seryogin, 1991; Bessonov, 2001). We determined that the walrus tongue (apex) contained 2-6 times more larvae than the pectoral and intercostal muscles. The larval density was found to be between 0.1 and 0.4 larvae per gram, and therefore is in agreement by the recommendation by Leclair et. al. (2004) that the

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minimum sample sizes should be 10g and the optimal size is 20g for testing.

Overall, our findings suggest that marine mammals play an important role in transmission of *Trichinella* in inhabited coastal regions. Circulation occurs between species through diet and therefore can be transmitted to animals of different trophic levels (Bessonov, 1972). In the Arctic, transmission of Trichinella in terrestrial carnivores also occurs through marine mammals through consumption of infected meat. Therefore they are an important link between the terrestrial and marine systems (Figure 13).

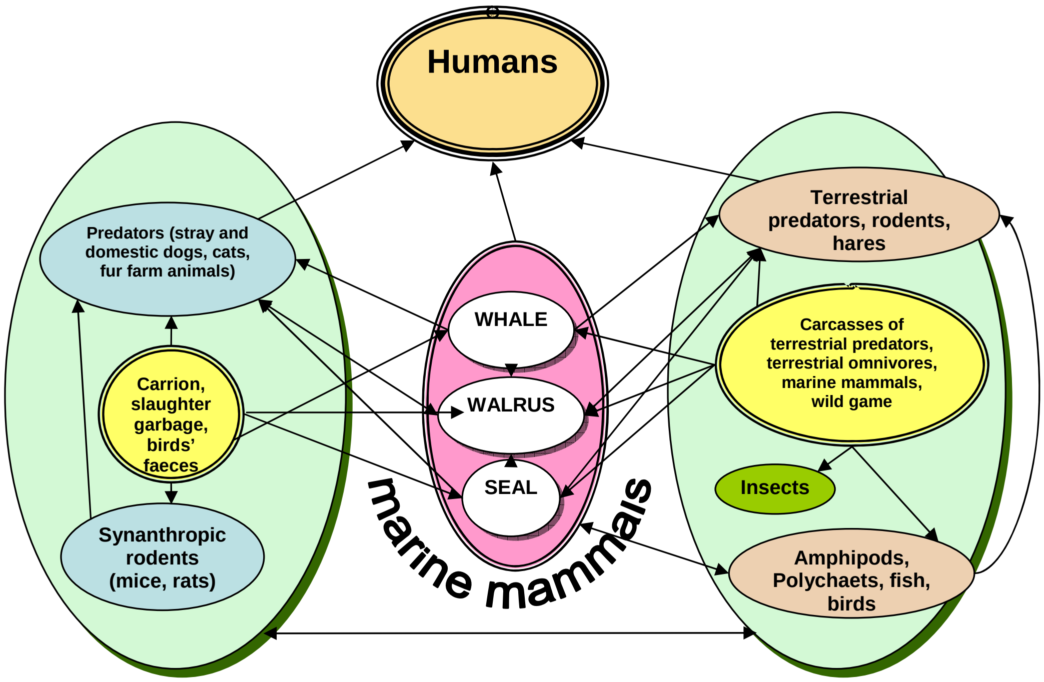

Figure 13. Circulation of *Trichinella* among species on the Chuckchi

Transmission of infection occurs from marine to terrestrial wildlife, wildlife to humans and wildlife to domestic species facilitated by:

1. Consumption of walrus.
2. Traditional dietary habits of the indigenous population in the Chukchi Peninsula.
3. Infection of *Trichinella spiralis nativa* in farmed polar foxes.

It is not clear how walrus obtain the infection but review of scientific literature suggests that this occurs through eating contaminated prey. Walrus can be both carnivorous and benthic-feeders. There are two types of carnivorous walrus, those that feed on meet infrequently when invertebrates are not available or are inaccessible, and those that constantly predate, primarily single bulls (Fay, 1960; Perry, 1976). Stomach contents from walruses have included ringed seal and bearded seal skin, blubber, and marine mammal skeletal remains (Kibalchich, 1986) and predation on narwhals, beluga whales and polar bear cubs as well as scavenging on carcasses and cannibalism has been reported (Cameron,1960, Madsen, 1961; Kozlov and Berezantsev, 1968; Forbes, 2000). Other carnivores (polar bear, brown bear, polar fox, red fox, wolverine) are likely infected when feeding on marine mammals, thus becoming potential sources of infection for humans living in coastal regions.

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So to summarize, there primary tropic linkages allowing transmission of *Trichinella* infection between species on the Chuckchi Peninsula include:
Marine mammals → farmed polar foxes
Marine mammals → farmed polar fox → humans
However, our data also show that transmission to dogs (both stray and sled) plays an important role in maintaining the cycle as they are also eaten in some communities. Red fox is an important wild game species and hunters frequently bring fox carcasses to the villages where they are fed to sled dogs. It is unclear if invertebrates (polichaetes, amphipods, insects) play a role, perhaps serving as transient hosts (Britov, 1962; Penkova, 1972; Uvaliyeva, 1976, Bessonov, 1993), but since they are consumed by walrus they cannot be ignored. Invertebrates (both adult and larval forms) may be important as they feed on marine mammal carcasses and certain species have been reported to ingest and retain larvae (Trichinella and Trichinellosis, 1978). Seals and whales are likely infected through infrequent exposure to infected carcasses, either directly by scavenging or indirectly by consuming amphipods or fish that have fed on infected carcasses, and are therefore incidental hosts (Forbes, 2000). Marine birds and birds of prey likely also serve as transient hosts and can become infected through consumption of invertebrates and fish or feeding on carcasses. Finally, marine mammal meat stored poorly in ice-storages attracts mice, rats and domestic cats and dogs and could result in transmission of infection. The number of rodents in Lorino was very during the time of the study despite the excessive food resources. Their role in disease transmission requires further investigation.

OBJECTIVE 3**:**
Determine the viability and infectivity of *Trichinella* larvae in traditionally cooked walrus preparations.

MATERIALS AND METHODS

i. Samples
Originally the study design included testing traditional preparations of walrus meat (*kopal'khen*). Unfortunately, the study started two months later than planned by which time the local population no longer had these meats available for testing. In order to conduct the experiment, *kopal'khen* was prepared from polar fox meat that was heavily infected with *Trichinella spiralis nativa* muscle larvae (from polar foxes fed solely on walrus meat). The meat was prepared by a professional marine mammal hunter (E. Rypkhyrgin) and stored in an ice store house in Lorino under the same conditions as for traditional preparations of walrus meat (Figure 14). Control samples were frozen (-15ºC) polar fox meat that had not been fermented. Samples were transported in thermal containers to the Zoology Laboratory of Vyatka State Agricultural Academy where the experiments were conducted.

Figure 14. Ice store house for storage of marine mammal products such as *kopal'khen*

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ii. Experimental infection

Experimental infections were conducted using guinea-pigs, white rats, white mice and canine puppies. A newborn litter of puppies was obtained from a veterinary clinic where they were surrendered for euthanasia as they were unplanned. Animals were housed and cared for in accordance with the guidelines of the Federal Law on Protection of Animals from Inhumane Treatment (Clause 17). The artificial digestion method was used to determine *Trichinella* larval count in fresh-frozen meat and the *kopal'khen* preparation and the larvae were counted by light microscopy. Live larvae (coiled or motile) were collected and used for infection trials on the same day. The larval dose was fed to the animals through a tube and animals were observed to ensure they held down the larvae.

Three infection experiments were conducted (Table 11):

1) To determine if infection with *Trichinella* larvae collected from *kopal'khen* and polar fox meat could be established in laboratory animals (*Rodentia*).

2) To determine if infection with *Trichinella* larvae collected from *kopal'khen* and polar fox meat had been established in mongrel pups (*Carnivora*).

3) To determine if infection with *Trichinella* larvae could be established in laboratory animals (rodents) after passage in pups (carnivores). For this experiment, tissues (diaphragm, masseters, hind limb) from infected pup # 1 were obtained following euthanasia, artificially digested and then fed to the laboratory animals (rodents).

Table 11. Experimental infection schemes

Animal	Larval dose		Start	End	Duration	Larvae recovered (lpg)	
	Fresh-frozen meat	*Kopal'khen*				Fresh-frozen meat	*Kopal'khen*
Experiment 1:							
Guinea-pig 1	500		11.25.06	01.31.07	69	0	0
White rat 1	500		11.25.06	01.31.07	69	0	0
Guinea-pig 2		500	11.25.06	01.31.07	69	0	0
White rat 2		500	11.25.06	01.31.07	69	0	0
Guinea-pig 3	Control animal that was not inoculated with *Trichinella*						
White rat 3	Control animal that was not inoculated with *Trichinella*						
Experiment 2:							
Pup 1	800		03.03.07	04.14.07	43	52.9	0
Pup 2		800	03.03.07	04.14.07	43	0	0
Pup 3	Control animal that was not inoculated with *Trichinella*						
Experiment 3:							
White mouse 1	20		04.28.07	06.19.07	52	0	0
White mouse 2	20		04.28.07	06.19.07	52	0	0
White mouse 3	Control animal that was not inoculated with *Trichinella*						
White rat 1	240		04.28.07	06.19.07	52	0	0
White rat 2	240		04.28.07	06.19.07	52	0	0
White rat 3	Control animal that was not inoculated with *Trichinella*						

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RESULTS

Experiment 1: Transmission of infection did not occur in any of the animals through consumption of either the traditional preparation *kopal'khen* or from fresh-frozen polar fox meat as *Trichinella* larvae were not recovered from any of the experimental animals (guinea-pigs, rats).

Experiment 2: Transmission of infection did occur in pup 1 from the fresh-frozen polar fox meat as *Trichinella* larvae were recovered in tissues. The number of larvae recovered (lpg) were: diaphragm - 52.6, root of the tongue - 65.3, apex of the tongue - 47.2, masseters - 62, intercostals - 29.3, hind limb - 61.0, forelimb - 53.3. Mean larval density was 52.9 larvae per gram. The average weight of each muscle tissue in the pup was approximately 1,300g, therefore, the total estimated larval load was 68,770. As was found with the stray dogs in the Chuckchi community, the most heavily infected tissue was the tongue. Pup 2 did not become infected with trichinae recovered from *kopal'khen* as no larvae were detected in any of the muscles. No *Trichinella* larvae were detected in any of the tissues from controls.

Thus, results indicated that *Trichinella* larvae recovered from *kopal'khen* were not found to be infective in both laboratory animals (rodents) and pups (carnivores). *Trichinella* larvae from fresh-frozen muscles from polar fox were however both viable and infective.

Experiment 3: *Trichinella* larvae recovered from the muscles of pup 1 were inoculated into 2 white mice (20 larvae each) and 2 white rats (240 larvae each). Transmission of infection did not occur in any of the four animals as *Trichinella* larvae were not recovered from any of the muscles.

These experiments showed that larvae recovered from fresh-frozen polar fox meat were both viable and infective, and that larvae recovered from *kopal'khen* prepared from polar fox meat in September 2006 and stored until April 2007 were viable. It is unclear why none of these preparations were able to transmit disease in the laboratory rodents and guinea pigs as infection from *Trichinella spiralis spiralis* larvae has been established previously in these species and is widely used in laboratory experiments. A previous study was able to successfully experimentally infect guinea-pigs with *Trichinella spiralis nativa* larvae collected from brown bear meat (the bear was harvested in the southern taiga zone in European Russia, Kirov region) that had been stored at -15 ºC for 3 months (Kolevatova et al., 1999). Further studies are therefore needed to evaluate the infectivity of the Arctic *Trichinella spiralis nativa* species and the suitability of laboratory animals (white mice, white rats, guinea-pigs) for experimental infection with this Arctic species.


Figure 15. Traditional preparation *kopal'khen*

DISCUSSION

Indigenous people living in the Arctic are well adapted to surviving in their environment. Since their diet is rich in protein and fat their physiology and metabolism is geared towards using lipid

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and protein as an energy source, with carbohydrates playing a lesser role. These traits are genetic and appear to also be associated with high immunity (Kozlov A., 2003; Kimstach, Chashchin, 2004). As a result, they are able to eat a primarily raw diet with limited use of heat during food preparation. When food is cooked it is done so by roasting over stones, in sand, on an open fire, or smoked. Common raw food preparations include *stroganina* (long strips of frozen meat and fish) or *dolbanina* and the winter favourite is *kopal'khen* (walrus meat in the form of sausages sewn with a strap of walrus skin allowed to cure, leaven, or ferment, Strogov et al., 2003) (Figure 15). Many outbreaks of Trichinellosis have been associated with consumption of raw walrus (Margolis et al, 1979; Serhir et al, 2001). Despite this eating raw meat continues to be a significant part of the traditional diet of indigenous people hunting walrus (Leclair et al., 2004).

Our data are consistent with findings in Canada (Leclair et. al., 2004) as apparently viable *Trichinella* larvae were collected from both fresh-frozen and fermented (*igunaq*) walrus meat. We however determined that larvae obtained from *igunaq* were not infective as infection was not established in laboratory animals. In contrast, *Trichinella* larvae from fresh-frozen walrus meat were infective. It appears that during the process of fermentation, the larvae were rendered non-infective or less virulent. However some studies showed that in experimental infection fermented walrus meat was infective in cats and mice (Leclair et al., 2004) and work by Forbes (2004) suggested that larvae could be viable in traditional preparations for up to 5 months.

Trichinella are known to have a complex life cycle, and all stages can be completed in a single host. However, the pathogen may only be adapted to surviving in specific hosts. In our experiment, white mice were not infected with Arctic strain of *Trichinella*. Ozeretskovskaya (1969) also found the Arctic strain to have lower infectivity in white mice than in humans. Britov (1982) determined experimentally that *Trichinella spiralis nativa* had high infectivity in domestic carnivores, which was also consistent with our study. Many studies have emphasized the specificity of transmission of some strains between wildlife and humans (Penkova, 1975; Britov, 1982; Kolokoltsev et al., 1987). In some case when a pathogen from a wildlife species infects a domestic carnivore (ie. a new host) it can develop the ability to adapt to the new species (Artyomenko and Artyomenko, 1987). However, in our experiments, this did not occur in the laboratory animals. Further work is therefore needed to understand transmission of *Trichinella* infection among species.

OBJECTIVE 4:
Develop preventive measures to control transmission of trichinellosis.

Preventive measures are needed to control transmission of *Trichinella* infection between humans, and domestic and wild animals. In order to accomplish this is was first necessary to identify the sources of infection. Marine mammals, in particular walruses, have been identified as one of the primary sources and hunting marine mammals provides a link for transfer among species and trophic levels. In accordance with Item 3.2.4. of the Provisional Regulations on Veterinary and Sanitary Expertise of Pinnipedia Carcasses and Organs from December 15, 1989, carcasses from pinnipeds are subject to mandatory examination for Trichinellosis. However in recent times, *Trichinella* examination of harvested marine mammals has become infrequent if at all. The community of Lorino has not had a qualified veterinarian for many years, however once has recently been hired to work at the fur farm in Lorino with whom we now collaborate. Additionally, we trained two employees at the farm to perform sample collection and techniques (compression method, artificial digestion) for

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sample analysis. We have left the microscope for use by the veterinarian to continue testing for *Trichinella*. It will now be possible to test meat both from harvested marine mammals and fox on the farm. Testing for *Trichinella* is still however problematic, as meat from harvested animals is distributed throughout the community immediately after the hunt and harvest. An additional complication is that testing takes 1.5 to 2 hours to complete and people will need to be convinced to not take the meat home before the test results are available. Ideally, hunted animals should not be harvested until testing has occurred. Unfortunately, hunting, harvesting and preparation of *kopal'khen* often occurs in remote locations where marine mammals are abundant. Therefore, timely examination can only occur when a veterinarian is present at the harvest, which in most cases would be difficult.

Nonetheless, we have developed a set of recommendations for the prevention of trichinellosis in Lorino:

1. All the carcasses from harvested and beachcast marine mammals (walrus, whale, different seals) should be tested by trichinelloscopy.
2. Veterinary inspection of meat products imported from neighbouring regions, other Russian territories, CIS states, non-CIS countries should be conducted. Currently, Chukchi Autonomous Region imports pork from 8 countries (Denmark, the USA, Brazil, France, Germany, Austria, Canada, China).
3. Inspection of products, especially marine mammal meat, brought over from the neighbouring communities (between marine mammal hunters and reindeer-herders) should be conducted.
4. All hunters must submit harvested carcasses (wolf, red fox, mustelids, brown bear, white bear, other carnivores) for *Trichinella* testing. Veterinary services must enter results of *Trichinella* tests in the hunting licenses.
5. Increase the number and types of animals tested especially during harvests to include rodents, stray dogs and cats.
6. Better sanitation on beaches is needed following harvests including disposal of discarded meat scraps.
7. A memorandum of understanding should be drawn up between residents consuming slaughtered polar foxes from the fur farm and a representative from the veterinary service so that the risks of consumption of this meat are clearly understood. Mandatory testing for *Trichinella* of 10% of polar fox carcasses should be introduced. Veterinary service must maintain records of the prevalence of infection measured in polar fox to monitor changes over time.
8. Polar fox breeding stock should be fed fresh meat from marine mammals only if they test negative for *Trichinella* or develop a technique to render the meat non-infective prior to feeding.
9. All pigs raised in the community of Lorino must be tested by veterinary service following slaughter to test for *Trichinella* infection.
10. Two methods must be used to diagnose trichinellosis: the compression method (trichinoscopy) and the artificial digestion method (digestion of muscle tissue in artificial gastric juice followed by examination of the sediment for larvae) using "Gastros" kit in accordance with the enclosed instruction to it. Prevalence of exposure should be monitored by ELISA (the enzyme-linked immunosorbent assay) in both people and marine mammals on an annual basis.
11. Sample collection from marine mammal carcasses must include the muscles of the apex of the tongue and the eyelid. The size of a pooled sample from a marine mammal must be a minimum of 100g from one individual. For all other species, samples are collected from the pillars of the diaphragm, masseters, intercostal

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muscles, neck muscles, hind limb muscles, metacarpus flexors and extensors, as well as tongue muscles, larynx muscles, esophagus muscles. The sample size from each muscle group must be a minimum of 5g, and the pooled sample size from one individual animal must be a minimum of 25g.

12. Meat products should be rendered not infective prior to consumption by people. Recommendations include: cuts of meat up to 100 - 200g in weight and 2.5 cm in thickness must be cooked for 2.5 hours. Meat can only be considered decontaminated when the inner temperature reaches 80 ºC. Weak salting, hot smoking, and especially cold smoking does not protect from *Trichinella* infection.
13. Veterinary authorities must conduct regular checks to examine raw materials used in the packing plant in Lorino for processing of marine mammal products. Regular sanitation of the facility must also be conducted.
14. Education and outreach programs should be implemented. People in the indigenous communities must be informed about the life cycle of *Trichinella*, preventive measures for the disease. Educational measures should especially be directed at marine mammal hunters, terrestrial animal hunters, fur farm workers, and schoolchildren. Mass media, photo materials, videos and presentations describing the disease must be widely used to prevent trichinellosis.

CONCLUSIONS

Investigations of trichinellosis on the Chukchi Peninsula were conducted in the communities of Lorino and Lavrentiya in the Chukchi District where the Chukchi and Eskimo make up the majority of the residents and subsistence on wildlife and traditional preparation of food is widespread. Analysis by ELISA detected a prevalence of exposure to infection in 6.1% and 1.3% in Lorino and Lavrentiya respectively. No clinical signs of disease were noted in any people that tested positive. The majority were positive at a low titer (1:200). The highest titer (1:800) was measured in a young schoolboy. More males were seropositive than females and prevalence was the highest in men ranging in age from 31 to 50 years. Seroprevalence also correlated with occupation as marine mammal hunters were most likely to be exposed to infection. Positive individuals consumed meat from marine mammal species (all preparations, including *kopal'khen*), as well meat from farmed polar fox. Results indicated the major source of *Trichinella* infection in humans was walrus meat, although polar fox meat also played a role.

Larval infection and load was measured in 257 carcasses from 17 mammal and bird species revealing infection in walrus (1.5%), red fox (100%), farmed polar fox (94.5%), stray dogs (100%) and sled dogs (66.7%). Larval load in walrus was 1 larva per 1 gram of muscle tissue. Among terrestrial animals, the highest mean larval density was in wild fox (67.2 larvae per 1 gram of muscle tissue). The mean larval densities in dogs were 6.1 lpg (sled) and 35.3 lpg (stray). Farmed polar fox were found to have both a high prevalence intensity of infection. Transmission was thought to occur through consumption contaminated marine mammals meat. The distribution of larvae varied by species in striated muscle. The highest larval densities were measured in forelimb, neck, and hind limb muscles and should be used for testing. The cycle of *Trichinella* is maintained by transmission between marine and terrestrial wildlife, humans and domestic animals through consumption of contaminated meat. The larvae are passed between animal hosts through of predation, scavenging and cannibalism. Transmission is facilitated by the lack of veterinary testing and control of meat products, primitive technologies of storing and processing of feeds on the fur farm and traditional preparation of food products.

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Finally, *Trichinella* larvae were found to remain viable in *kopal'khen* polar fox preparation for 8 months at -12ºC but were not found infective for laboratory animals (rodents) and pups (carnivores). *Trichinella* larvae collected from fresh-frozen polar fox meat were both viable and infective in canine puppies but not laboratory rodents. Therefore, factors affecting transmission of infection requires further investigation.

PUBLICATIONS

Bukina L. A., Kolevatova A. I. (2007) *Trichinella* cycle in coastal areas of Chukchi Peninsula. The contemporary world, nature, and man (Research paper collection, volume 4, issue 2, Tomsk), pp. 101-103

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Manuscripts in preparation:

Bukina L. A., Kolevatova A. I., Vakhlayeva A.V. Issues of fur farming in Chukchi Peninsula (community of Lorino).

Bukina L. A. Traditional subsistence use of lands by Chukchi people.

Bukina L. A., Kolevatova A. I., Bukin V. U. Epizootics of trichinellosis in Chukchi Peninsula (Skryabin All-Russia Helminthology Institute).

Bukina L. A., Kolevatova A. I., Bukin V. U., Poletayeva O. G., Starkova T. V. Epidemiology of trichinellosis in Chukchi Peninsula (Institute of Medical Parasitology and Parasitic Diseases, Moscow)

Comparative characteristics of infectivity of *Trichinella spiralis nativa* larvae collected from *kopal'khen* model and from frozen walrus meat (Skryabin All-Russia Helminthology Institute)

OUTREACH

Conference presentations:

Bukina L. A., Kolevatova A. I. (2007) Traditional diet of the indigenous population in Chukchi Peninsula (community of Lorino). Contemporary problems of natural management, game management, and fur farming (Scientific and Practical Conference, Kirov, May 22-25, 2007).

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Public Lectures :

Bukina L.A. (2006). What is trichinellosis? How can humans become infected with it? Preventative measures. (Lectures for the authorities of the Chukchi District, marine mammal hunters, fur farm workers, patients at hospitals, residents of the communities of Lorino and Lavrentiya).

Presentations at festivals and sporting events:

Bukina L.A., Kuznetsov N.V. (2006) What is trichinellosis? How can an individual living in Chukchi Peninsula become infected with this disease? (Reindeer Herder Holiday, Chukchi District, September 2006).

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Presentations in schools and educational institutions:

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