Evaluation of Protective Clothing for Handling Small Mammals Potentially Infected with Aerosol-borne Zoonotic Agents

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Abstract

The purpose of this study was to describe and compare the protection provided by surgical gowns and coveralls against aerosol contamination. We also sought to quantify the aerosol exposure in terms of particles depositing on the clothing of workers involved in the Montana Longitudinal Hantavirus Study. Prior to sampling, florescent dust was inserted into live rodent traps. Sampling strategies involved two individuals mimicking established rodent handling procedures while wearing protective clothing in the form of tyvek coveralls and/or surgical gowns. A protocol was designed to quantify exposures by counting the number of squares on a pre-drawn grid which were contaminated with florescent particles. This grid covered the front of the tyvek suits worn by the workers, excluding the face, hands and feet, and extended around the cuff of both sleeves. Tyvek coveralls were found to provide a significant degree of protection against aerosolized dust originating from small rodent live traps relative to wearing no form of protective clothing. Surgical gowns provided a significantly greater degree of protection against aerosolized dust than tyvek coveralls. The individual handling the mice (biologist) consistently had greater mean exposure values than the data recorder (technician).

Key Words: aerosol, coveralls, exposure, Hantavirus, *Peromyscus*, surgical gowns

Introduction

Hantavirus pulmonary syndrome (HPS) was first described in 1993 as an acute disease induced by a diverse group of related viral strains in the genus Hantavirus (Nichol et al. 1993, Hjelle et al. 1994). The deer mouse (Peromyscus maniculatus) has been found to be the principal reservoir (Childs 1994) and a single virus isolated from mice in New Mexico has been associated with the majority of documented cases (Childs et al.1995). A variety of rodent species have been shown to possess antibody, and other species of Peromyscus may act as competent hosts (Childs 1994). Virus may be shed in feces, urine, and saliva for several weeks, but the exact duration of shedding and period of greatest infectivity are unknown (LeDuc 1987).

Possible routes of exposure include direct contact with lacerated skin or mucous membranes and rodent bites; however, the primary route of infection to humans is believed to be inhalation of aerosolized virus particles (Mills et al. 1995). HPS is characterized by a febrile prodrome, followed by rapid onset of noncardiogenic pulmonary edema and hypotension, or shock. Approximately 45 percent of identified patients have died. Infection of humans by rodent-borne hantavirus in the United States stimulated a series of longitudinal studies of rodent populations

(primarily *Peromyscus* spp.) in both the southwestern States (Abbott et al. 1999, Calisher et al. 1999, Kuenzi et al. 1999, Mills et al. 1999) and Montana (Douglass 1996 et al.).

Special precautions should be observed to minimize risk of infection because of the high morbidity and mortality associated with onset of HPS, and the possibility of aerosol transmission of viral particles. The United States Centers for Disease Control and Prevention (CDC) published recommendations to assist residents of endemic areas, as well as mammalogists working with potentially infected rodents (CDC 1993, Douglass et al. 1996). These recommendations emphasize the importance of respiratory protection, protective clothing, choice and use of disinfectants, decontamination of instruments and traps, proper disposal of infectious wastes, and preservation and shipment of samples intended for hantavirus testing. Although these guidelines were generated in response to the 1993 HPS outbreak, they are applicable to any study of small mammals potentially infected with a zoonotic agent transmissible by aerosol (Mills et al. 1995).

Mills et al. (1995) advises individuals handling live rodents to wear protective clothing, including a surgeon's gown or coveralls (preferably disposable). The primary function of this precaution is to minimize the probability of worker contact with rodent body fluids, i.e., blood and urine. A secondary purpose is to minimize the amount of aerosolized viral particles anchoring to the workers clothing. Despite the severity of symptoms associated with HPS, no published studies have described or compared the effectiveness of surgical gowns and/or coveralls in protecting against aerosol contamination.

The purpose of our study was to describe and compare the protection provided by tyvek coveralls and surgical gowns against aerosol contamination. We also sought to quantify and compare aerosol exposure in terms of particles landing on clothing of workers involved in the Montana Longitudinal Hantavirus Study.

Six large-scale hantavirus studies are currently in progress within the United States. Each study has developed a unique set of procedures for collecting required ecological and serological data, while observing the safety guidelines established by Mills et al. (1995). Differences between the various studies include, but are not limited to the following: the number of field workers involved, whether or not mice are anesthetized, the specific types of personal protective equipment used, and the work station configuration. Our sampling procedures followed the work practices of the Montana Longitudinal Hantavirus Study (Douglass et al.1996).

The Montana study involved six study sites. The six sites were trapped individually three nights/month from May through October. Each site was composed of three grids, each containing one hundred live rodent traps. Each morning grids were checked and occupied traps were placed in clear plastic bread bags. The bagged traps were then transported back to the truck. Mice were processed on site, using the tailgate of the truck as the processing station. Two large plastic tubs were placed on the tailgate to elevate the working platforms of both biologist and technician. During mice processing, the technician and biologist wear tyvek coveralls, latex gloves, and half-mask negative pressure respirators.

The biologist stands on the left while the technician stands on the right. The technician was responsible for picking up the bagged traps, opening the trap door, and dropping the mouse into the bag. At this time the bedding material, bait, and accumulated dust also drop into the bread bag. The bagged mouse and debris would then be handed to the biologist. The technician recorded the various ecological data as the biologist dictated. The technician would then wash his / her hands with a disinfectant and prepare the next mouse. The biologist, once handed the bagged mouse, would maneuver the mouse out of the bag and secure it by the skin of the neck. The biologist would then inspect the mouse and dictate data on body mass,

sex, sexual status, scars, and ear tag number, if a recapture, to the technician. Newly captured mice were ear tagged. The biologist would then collect a blood sample using the retro-orbital sinus technique. This procedure involves inserting a heparinized capillary tube into the back corner of the right eye and allowing several drops of blood to collect into a plastic cryovial. The mouse would then be released and the blood sample stored on dry ice. The biologist then washed his / her hands, utensils, and the working platform with a disinfectant.

All bedding material, paper towels, used bags, torn gloves, and other trash were deposited into a trash bag under the tailgate in front of the biologist. This cycle would continue until all mice were processed (≥70 mice at times). At this point the biologist would close the trash bag. This involved compressing the bag, to conserve space in the truck bed, and sealing it. Both the biologist and the technician would then remove all personal protective equipment and disinfect their hands. The protocol for this study was approved by the University of Montana Institutional Animal Care and Use Committee (AICUC).

METHODS

Two sampling series were conducted from March through December 2000. The two series varied only by the type of protective clothing worn and sample size. Series one consisted of thirty runs, while series two consisted of twenty-five runs. Each run involved the processing of fifteen mice at one minute/mouse. Mouse-handling procedures mimicked those of the Montana Longitudinal Hantavirus Study with five exceptions: white lab mice were used instead of wild mice, collection of blood samples was only simulated, plastic tubs were placed on a desk instead of the tailgate a truck, mice were not released but placed in a holding container, and the experiment was conducted indoors to minimize the influence of air movements on aerosolized particles. Mice were cared for and treated in accordance with established guidelines (University of Montana Institutional Animal Care and Use Committee 1998).

Prior to each sampling run mice were placed in fully-baited Sherman traps with an ample amount of bedding material. Bait included peanut butter smeared on the back door of the trap and a tablespoon of oats. Bedding material was composed of a handful of synthetic cotton.

Each trap received 1/16 tsp. of dry florescent paint pigment (Palmer Paint Products, Inc., Fluorescent Dry Temp 354017) and was placed in a clear bread bag. The volume of florescent dust placed in the traps was determined during preliminary sampling, and reflected a balance between estimated true values and the amount required to provide observable breakthrough. The fifteen traps were then carried to the sampling room and placed next to the workstation.

Protective clothing worn by the biologist and technician, during series one consisted of an inner and outer Kappler tyvek/pros 3 coverall. A grid was drawn on each set of coveralls consisting of 128 10-cm X 10-cm squares. This grid covered the front of the workers bodies, excluding the face, hands and feet, and extended around the cuff of both sleeves (Fig. 1).

The outer coveralls represented the exposure that may be observed without protective clothing, while the inner set represented the protection provided by wearing coveralls. After the mice were processed, both workers carefully removed their coveralls and placed them in an isolated area. The coveralls were then placed in a dark room and examined under a hand held short-wave ultraviolet light source (Ultra-Violet Prod., Inc. Mineralight Lamp Model H4-S). Each square containing florescent dust, as determined without the aid of magnification, was considered contaminated and recorded accordingly. We also recorded the time required to examine each set of coveralls.

Protective clothing worn by the biologist and technician during series two included an inner pair of coveralls and an

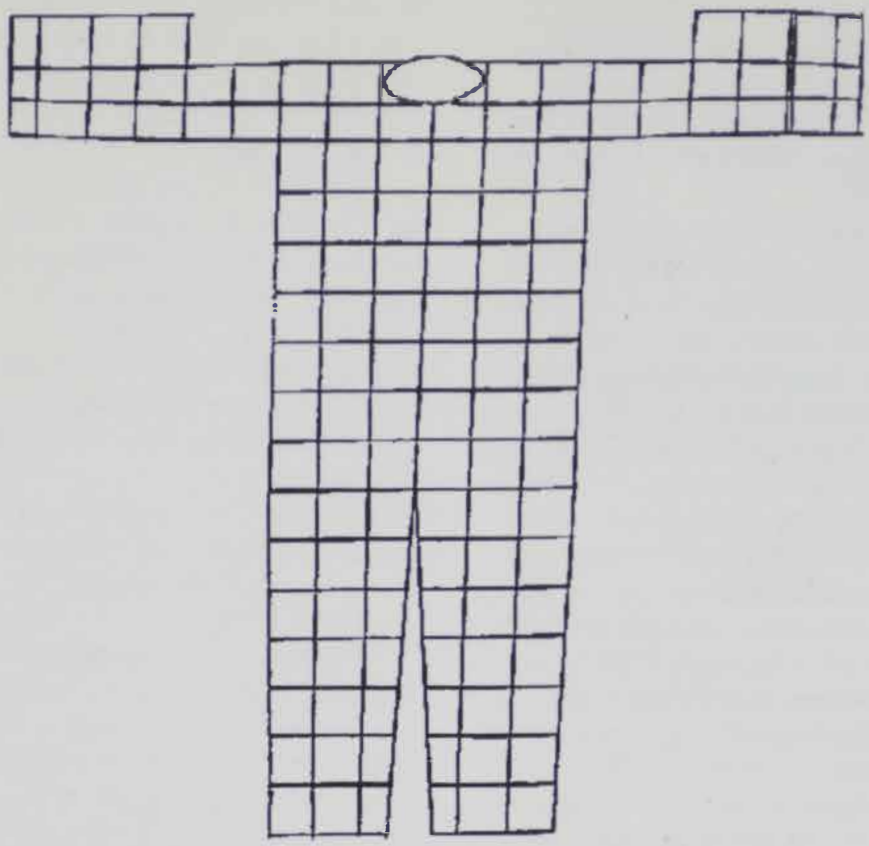

Figure 1. Graphical representation of the grid drawn on each pair of coveralls.

outer Kimberly-Clark ULTRA Surgical Gown. The coveralls were prepared as in series one and represented the protection that may be attained by wearing a surgical gown. The gown and coveralls were carefully removed and the coveralls were processed as in series one.

Quality control measures were introduced at several phases of the experiment. Prior to sampling, traps were thoroughly washed to insure that all florescent dust, peanut butter, and miscellaneous debris from previous sampling was discharged. The coveralls were examined under a short wave UV light after the grid was drawn and then placed in Ziploc plastic bags, where they remained until just before sampling. Ten pairs of coveralls from each series were randomly chosen to be re-examined just prior to sampling. We regressed the number of contaminated squares (c.s.)/pair of coveralls for each exposure group against the order in which samples were collected to determine if a significant amount of florescent dust accumulated on the mice, workstation, or any unidentified mediums.

Mean exposure values reflected the average number of contaminated squares/ 128 squares. We used ANOVA to compare mean time required to inspect coveralls (TRI) and mean exposure values between various groups including: inner biologist vs. inner technician, inner biologist vs. outer biologist, inner biologist vs. biologist with gown, outer biologist vs. outer technician, inner technician vs. outer technician, inner technician vs. technician with gown, and biologist with gown vs. technician with gown. Mean exposure values were regressed against TRI to determine if TRI was an adequate indiction of exposure magnitude. We set α at 0.05 for all statistical comparisons.

Results and Discussion

A significant negative regression between mean exposure values and TRI was

found ($P = 0.005$, $r^2 = 88.2$; Fig. 2). If a negative relationship exists, then TRI may be an adequate indication of exposure magnitude. Further analysis assumes that TRI and exposure magnitude are inversely correlated.

Mean exposure values ranged from 35.6 to 124.9 c.s./ pair of coveralls (Table 1). Significant differences between mean exposures were found in the following comparisons: inner biologist vs. inner technician ($P = 0.018$), inner biologist vs. biologist with gown ($P = 0.006$), inner technician vs. outer technician ($P < 0.001$), and inner technician vs. technician with gown ($P = 0.029$). No significant differences between mean exposures were found in the following comparisons: inner biologist vs. outer biologist ($P = 0.111$), outer biologist vs. outer biologist ($P = 0.353$), and biologist with gown vs. technician with gown ($P = 0.905$).

The mean inner exposure values, for both biologist and technician, were lower than mean outer values, but significant differences were stricted to the technician. Mean gowned exposure values were significantly lower, for both biologist and technician, than those observed while wearing coveralls. Biologist mean inner exposure values were significantly greater than the technician's.

Mean TRI ranged from 51.53 to 373.0 sec (see Table 2). Significant differences between mean TRI were found in the following comparisons: inner biologist vs. inner technician ($P < 0.001$), inner biologist vs. outer biologist ($P = 0.003$), outer biologist vs. outer technician ($P < 0.001$), inner technician vs. outer technician ($P < 0.001$), inner technician vs. technician with gown ($P = 0.029$), inner biologist vs. biologist with gown ($P = 0.003$), and biologist with gown vs. technician with gown ($P < 0.001$).

The biologist and technician TRI were significantly greater for the inner coveralls, which indicates a differences in exspoure magnitude, i.e., inner coveralls required more time to inspect therefore the exposure was not as pronounced. Biologist and technician mean gowned TRI were significantly greater than those observed while wearing coveralls. Technician inner,

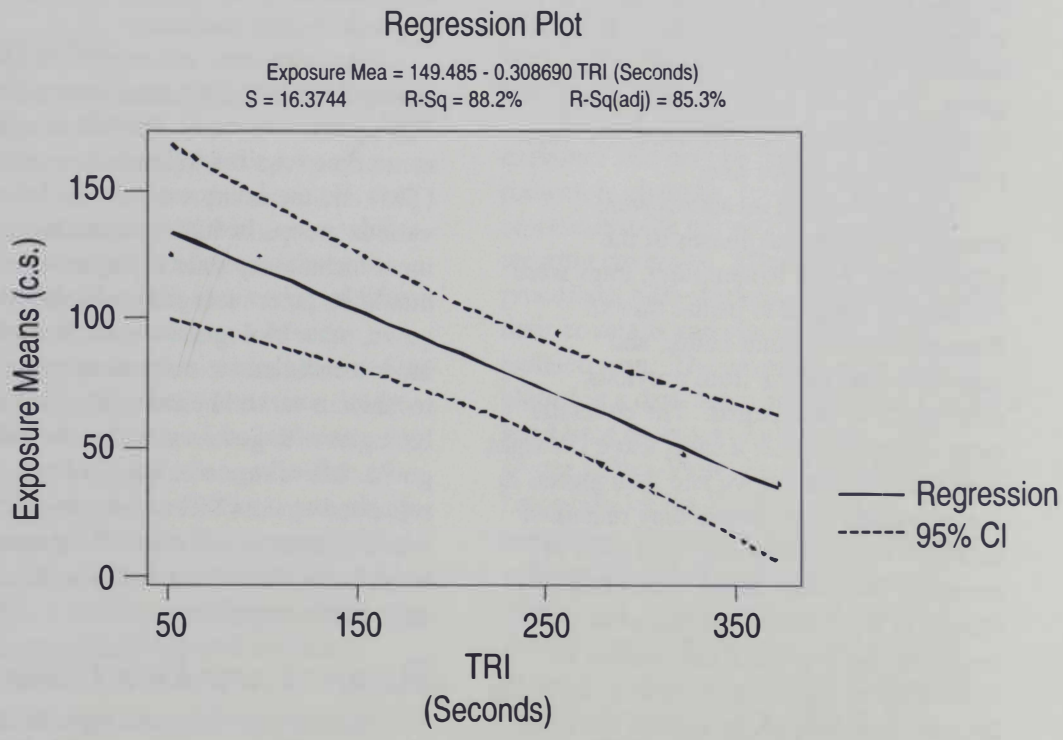

Figure 2. Mean exposure vs. Times required to inspect (TRI) regressional analysis.

Table 1. Summary of mean numbers of contaminated squares (c.s.) per pair of coveralls.

	Variable	N	Mean (c.s.)	St. Dev.
Series I	Inner Biologist	30	94.4	103.1
	Outer Biologist	30	124.9	2.51
	Inner Technician	30	47.3	23.59
	Outer Technician	30	124.3	2.45
Series II	Biologist With Gown	25	35.6	11.1
	Technician With Gown	25	35.96	10.0

outer and gowned TRI were found to be significantly greater than the biologist's values.

No significant regressions between any of the six exposure groups and time were found ($P = 0.401–0.919$, $r^2 = 0.0–2.5$). The lack of a relationship between these parameters is an indication that flourescent dust was not accumulating in significant amounts.

Conclusions

We found that coveralls provide a significant degree of protection against aerosolized dust originiating from small live rodent live traps, relative to wearing no form of protective clothing. Our data strongly suggested that surgical gowns provide significantly greater protection than coveralls against aerosolized dust originating from traps. In our opinion established guidelines should be amended to remove coveralls from the personal protective equipment options. Further investigations should examine the protection provided by coveralls that have been sealed with tape.

We conclude that the biologist was exposed to a greater amount of aerosolized dust originating from traps, corroborating air samples from a previous study (Young 2001). If our experimental process adequately approximated true field exposures, then information of this nature may be applied to the design of future engineering and administrative controls.

It is important to note that applications of our conclusions are limited to unmodified surgical gowns and coveralls of a specific design. Further limitations inherent to this experiment include how accurately: florescent dust approximated the aerodynamic properties of actual trap dust; the amount of florescent dust applied to each trap approximated the quantity of dust that occurs in actual traps; and the semi-controlled experimental environment approximated actual field conditions.

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Table 2. Summary of mean times required to inspect coveralls (TRI).

	Variable	N	Mean (c.s.)	St. Dev.
Series I	Inner Biologist TRI	30	256.3	56.4
	Outer Biologist TRI	30	51.5	12.3
	Inner Technician TRI	30	322.3	101.0
	Outer Technician TRI	30	98.6	38.8
Series II	Biologist With Gown TRI	25	305.6	57.1
	Technician With Gown TRI	25	373.0	55.0

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