

# CONTROL OF ZONOTIC RISKS FOR PREGNANT ANIMAL HANDLERS IN ZOOS

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## Abstract

Pregnant animal workers form a risk group in the zoo because of weakening of their immune system and intensive animal contact. To reduce risks, protocols for personal hygiene and pregnancy are required. In this study control of zoonotic risks for animal handlers by zoos was evaluated. In a Dutch zoo animal handlers were observed during work, hand samples were taken, questionnaires were completed by animal workers and several environmental samples were taken in the zoo. Adherence to the protocol appeared low, most of the animal handlers consumed food with their hands contaminated with *Enterobacteriaceae*, knowledge of the protocols and risks of the animal handlers was found to be insufficient and environmental samples showed an undesirable high level of surface contamination. Lack of knowledge about the protocol hence poor hand washing and adherence to the protocol result in insufficient control of zoonotic risks for pregnant animal handlers.

**Key words:** zoonosis, pregnancy, animal workers, zoo

## Introduction

Animal handlers working in zoos have intensive animal contact. As a great part of the animal handlers are young females, there is a high chance that some of the female animal workers will get pregnant or want to get pregnant sooner or later. During pregnancy the maternal immune system is weakened.<sup>1</sup> This is illustrated by the fact that Listeriosis, a zoonosis, is 18 times more common in pregnant women than in non-pregnant humans.<sup>2</sup> Weakening of their immune system is why pregnant animal workers with occupational contact to feces, urine, blood, parasites and mucosal secretions are at risk of incurring zoonotic diseases that may have adverse effects on their unborn child. A zoonosis is described by the World Health Organization as 'any disease or infection that is naturally transmissible from vertebrate animals to humans and vice-versa'.<sup>3</sup> The risk for animal handlers of acquiring zoonotic infections would be estimated higher than for visitors of the zoo and other zoo staff with less animal contact.

Maintaining high standards of hygiene is important to prevent spread of zoonotic agents. To control zoonotic risks a personal hygiene protocol is required for all animal handlers and a protocol with extra measures is required for pregnant women or women who want to get pregnant. These protocols are based on the Dutch law for working conditions which obliges the employer to provide safe and healthy working conditions by doing risk assessment and making a plan to reduce risks.<sup>4</sup>

Some examples of zoonoses that can be dangerous for the unborn are *Toxoplasma gondii*, *Chlamydia abortus*, tuberculosis, *Listeria monocytogenes* and leptospirosis. These zoonoses may occur in zoo animals. *Toxoplasma gondii* is a coccidium that can lead to abortion, neonatal death or a wide array of conditions in the foetus like encephalomyelitis, retinochoroiditis, intracranial calcifications and hydrocephalus. Infection in general occurs by ingestion of tissue cysts in raw meat or ingestion of material contaminated with feline feces.<sup>5</sup> *Chlamydia abortus* is a zoonotic bacterium that is responsible for the a large part of caprine and ovine abortions. It may cause abortion in women after having contact with aborting or lambing ewes and goats or after contact with materials contaminated with *Chlamydia*

*abortus*. In the past, the genus *Chlamydophila* was split into *Chlamydophila* and *Chlamydia*. The splitting of the genus was not widely adopted and the species in the family *Chlamydiaceae* were merged into one single genus again, *Chlamydia*.<sup>6-9</sup> Tuberculosis is a disease caused by *Mycobacterium tuberculosis* and M. tuberculosis-like organisms which may induce abortion, insufficient weight gain during pregnancy, preterm labour and low birth weight of the neonate. In utero infection is rare and postnatal infection of the newborn occurs more frequently.<sup>10,11</sup> *Listeria monocytogenes* is an intracellular bacterium. It may lead to abortion, stillbirth and preterm birth. It is one of the most common causes of meningitis in neonates.<sup>2</sup> Leptospirosis is a zoonosis caused by bacteria that may transmit through the placenta and induce an increased rate of spontaneous abortion, placental ischemia and placentitis and neonatal icterus.<sup>12</sup>

Despite the recognized risk, little is known about the prevalence of zoonotic infections in zoo staff associated with their work in the zoo. In a study that was carried out in a zoo in Auckland, evidence for some transmission of zoonotic agents from animals to zoo staff was found. The amount of zoonotic infections was almost similar to that of the local population.<sup>13</sup> The purpose of this study was to evaluate if zoonotic risks for pregnant animal handlers are controlled sufficiently by zoos.

## **Materials and methods**

This study was carried out in a zoo with 1500 animals, almost 130 species, 30 permanent animal handlers and a variable number of interns. The zoo attracts about 700000 visitors per year. The animals are divided in seven departments: pachyderms, birds, cats, small mammals, primates, reptiles and insects and ungulates.

The zoo has a personal hygiene protocol and a protocol for pregnant animal workers. The main points in the personal hygiene protocol are about workclothes, personal belongings, hand jewellery, wounds, protective equipment and how and when to wash and disinfect hands. Hand samples and visual inspection of adherence to the personal hygiene protocol were performed because good hand hygiene is important to prevent zoonotic infections.<sup>14-16</sup>

### **Visual inspection**

Observations were performed in each of the seven departments of the zoo for about two hours per department. Observations took place for 15 hours in total. 19 animal handlers were followed during their work and actions seen during encounters with other animal workers were also noted. A checklist based on the hygiene protocol of the zoo was used to see if work was done in adherence to the guidelines and to identify difficulties adhering to the protocol and risk behaviours. The animal handlers were aware of the goal of the study and the fact that they were being observed.

### **Hand samples**

Hand samples of 35 animal workers were taken during the break when the hands were supposed to be clean. Hands were kept in a latex glove filled with 15 ml saline solution with added Tween 80 for a minute, while slightly moving the hand in the glove. One ml of the content was incubated on an agar plate at 37°C for 24 hours to determine the amount of colonies of *Enterobacteriaceae*. If possible, the animal workers were sampled twice and the average amount of colonies was used. 24 attendants were sampled twice. Hands were considered clean if no colonies were found in one ml.

### **Environmental sampling**

36 samples of several surfaces in the zoo were taken during the work day to determine contamination with *Enterobacteriaceae* and total bacterial count. Sampling was done on

surfaces which animal workers are in contact with often in feed kitchens, the canteen, the petting zoo and samples were taken from radiotelephones and a key ring. If those surfaces are contaminated they could form infection risk for the animal handlers. Samples were taken with swabs and agar pressure plates. All samples were converted into colonies per 6 cm<sup>2</sup>. The samples were incubated at 37°C for 24 hours. A qualification was made based on total bacterial count and the presence of *Enterobacteriaceae*. If *Enterobacteriaceae* were present, the qualification was ‘bad’.

**Table 1: qualification total bacterial count**

Number of colonies per 6 cm <sup>2</sup>	Qualification
-	No colonies found in sample
<b>Less than 3 colonies</b>	Excellent
<b>3-9 colonies</b>	Good
<b>10-29 colonies</b>	Moderate
<b>30-90 colonies</b>	Unsatisfactory
<b>More than 90 colonies</b>	Bad

### **Enterobacteriaceae**

*Enterobacteriaceae* were used as an indicator for the level of contamination of the hands and surfaces with potentially zoonotic agents. Contamination with *Enterobacteriaceae* indicate the level of hygiene for they can be found in feces. Transmission of zoonoses often occurs via feces.

### **Questionnaires**

Questionnaires were completed by the animal workers to obtain insight in the level of knowledge on the hygiene protocol and the protocol for pregnant animal workers. All animal handlers who completed a questionnaire were asked when hands should be washed during a work day. Female animal handlers were asked what kind of information is in the protocol for pregnant animal workers regarding hygiene and what other measures should be taken when working while pregnant. 18 women and 14 men completed the questionnaire.

To measure knowledge of risks during pregnancy, female animal handlers were asked which infectious agents are a risk for pregnant attendants. Checking of the questionnaires was based on the two protocols and additions to the personal hygiene protocol, since some important guidelines were missing in this protocol.

The protocol contained a list with moments when the hands should be washed during a work day. Hand washing indications that were missing in the protocol are hand washing before breaks, before going to the toilet, after touching raw fish or meat, after leaving an animal enclosure and before touching the face. The guidelines that described the way the hands should be washed were not complete. It did not prescribe the use of a nailbrush and it only said the hands, underarms and nails should be cleaned. An illustration of the way the hands should be washed so that all the areas of the hands are cleaned would be helpful. The personal hygiene protocol only said that personal belongings should be kept outside forage storages and places where it is handled as much as possible. Actually, personal belongings should always be left in the locker since contamination could also occur during other tasks and at other places. The protocol recommends to not wear hand jewellery during preparation of animal feed whereas contamination of hand jewellery could take place during several other tasks too. Therefore it is recommended not to wear hand jewellery during work at all. Moreover, use of a nail brush is missing in guidelines for hand washing.

## Workclothes

An inquiry was made about where workclothes are washed after they have been used during a work day as it is of importance for the personal hygiene. Taking workclothes home to wash may facilitate spread of infectious agents into homes of animal workers.

## Results

### Visual inspection

During the visual inspection behaviours that could pose direct personal risks were observed especially. Observations started with looking if the workclothes were visually clean. Tainted workclothes at the start of the work day were observed once. Nonadherence especially concerned hand hygiene like no hand washing before hand-face contact for scratching or blowing the nose, no hand washing after touching raw meat or fish and touching materials that are considered clean with tainted hands. After touching those objects animal handlers do not wash their hands since these objects are regarded clean. Objects considered clean that were touched with tainted hands included personal phones, radiotelephones and key rings. Sometimes the hands were cleaned by only using a paper towel. Some of the animal handlers wore jewellery on the hands and the wrists during work. Attendants keeping their face very close to the water jet permitting aerosols to reach the face while syringing an animal enclosure or an animal was seen several times. Eating, drinking or smoking during work was not observed. Hands were often not washed according to instructions in the protocol. Nails and forearms were hardly ever cleaned and regularly no soap was used or no disinfectant was used when prescribed. Nonadherence to the personal hygiene protocol was seen during every observation and by all the animal handlers observed.

### Hand samples

Hands were considered clean if no colonies were found in the hand samples. Only two animal handlers who were sampled twice had clean hands both times. A total of 14 out of the total 59 hand samples was clean (24%).

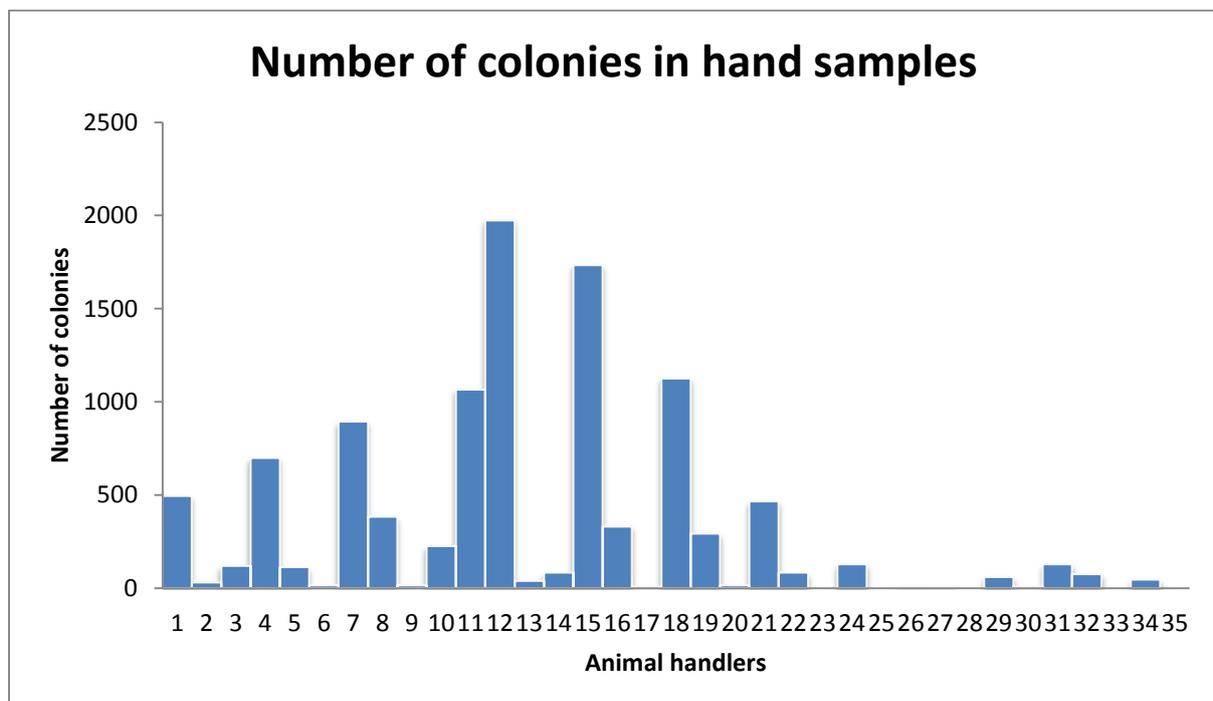


Fig. 1 Number of colonies of Enterobacteriaceae on the hands of the 35 sampled animal handlers

## Environmental sampling

The hygiene of the environment was unsatisfactory. 17% of the samples was qualified as excellent or good. *Enterobacteriaceae* were found in 53% of all samples. 58% of the samples had the qualification bad.

Sample taken of	Enterobacteriaceae	Total bacterial count	Qualification
<b>Petting zoo</b>			
Handgrip of duster	10-29 colonies	30-90 colonies	Bad
Stem of dung fork	-	Less than 3 colonies	Excellent
Handgrip of feed scoop	-	10-29 colonies	Moderate
<b>Kitchen primates</b>			
Handle of knife	10-29 colonies	10-29 colonies	Bad
Tap	Less than 3 colonies	Less than 3 colonies	Bad
Phone	Less than 3 colonies	3-9 colonies	Bad
<b>Place for preparation of raw meat</b>			
Worktable	Less than 3 colonies	Less than 3 colonies	Bad
Door handle	Less than 3 colonies	30-90 colonies	Bad
Soap dispenser	-	3-9 colonies	Good
<b>Kitchen birds</b>			
Administration table	Less than 3 colonies	Less than 3 colonies	Bad
Worktable	Less than 3 colonies	10-29 colonies	Bad
Kitchen counter	Less than 3 colonies	10-29 colonies	Bad
<b>Kitchen nocturnal animals</b>			
Cutting board	30-90 colonies	30-90 colonies	Bad
Clean plastic box	-	-	Excellent
Handgrip of refrigerator	10-29 colonies	30-90 colonies	Bad
Unclean plastic box	3-9 colonies	10-29 colonies	Bad
Worktable	10-29 colonies	10-29 colonies	Bad
Tap	3-9 colonies	10-29 colonies	Bad
<b>Kitchen reptiles and insects</b>			
Kitchen counter	7 colonies	18 colonies	Bad
Tap	-	45 colonies	Unsatisfactory
Handgrip refrigerator	-	6 colonies	Good
<b>Kitchen ungulates</b>			
Hand grip pantry	-	3600 colonies	Bad
Elbow rest of chair	9 colonies	70 colonies	Bad
Marker of whiteboard	-	9 colonies	Good
<b>Kitchen of giraffes</b>			
Wooden work table	50 colonies	50 colonies	Bad
Soap dispenser	-	21 colonies	Moderate
Door handle	-	40 colonies	Unsatisfactory
<b>Canteen</b>			
Door handle	-	35 colonies	Unsatisfactory
Table	-	13 colonies	Moderate

Elbow rest of chair	-	1 colony	Excellent
Handgrip of refrigerator	2 colonies	35 colonies	Bad
Switch of coffee machine	-	11 colonies	Moderate
Table in smoke area	1 colony	20 colonies	Bad
<b>Objects used by animal handlers</b>			
Radiotelephone 1	-	90 colonies	Unsatisfactory
Radiotelephone 2	-	141 colonies	Bad
Key ring	-	18 colonies	Moderate

*Table 2: results of pressure agar plates samples of surfaces*

## Questionnaires

Adhering to the original protocol, 22% of the attendants mentioned three correct moments during a work day when the hands should be washed. When comparing the answers to the question about when hands should be washed during a work day with the personal hygiene protocol including the additions, 50% of the animal handlers who completed the questionnaire mentioned at least 3 correct moments during a work day when hands should be washed. 17% of all women who completed the questionnaire mentioned at least three measures that should be taken by pregnant animal handlers regarding hygiene according to the protocol. In the query about what other measures should be taken during pregnancy according to the protocol 11% of all female animal workers who completed the questionnaire made 3 or more mentions. These measures include extra breaks, limiting bending and lifting, limiting exposure to vibrations, limiting exposure to excessive noise, no mandatory overlabour, the ability to breast feed during work e.g. Agents that could pose a risk to pregnant animal handlers mentioned by the female attendants are toxoplasma, Q-fever, herpes, salmonella, listeria, *chlamydia psittaci*, streptococcus, shigella and bacteria, fungi and worms in general. Four female attendants did not mention any potentially hazardous agent.

## Workclothes

Workclothes are taken home by the animal workers to be washed which means contamination of the houses of the animal workers via workclothes is possible. The shoes need to be kept at work to prevent spread of infectious agents.

## Discussion

The observations made clear that behaviour of the zoo keepers may contribute to transmission of zoonoses. Nonadherence to the personal hygiene protocol was observed very often in all of the departments. However, the circumstances made adherence impossible in several cases since access to hand hygiene was not always directly available when needed. Furthermore, hand washing sites were not always complete. At some places only cold water was available and no soap, nail brush or disinfectant were present.

Animal workers were aware of the observation and the goal of the study. Observation bias may have influenced the results. Since not all of the animal handlers were observed, results may not be completely representative for the whole group. Despite this, the observations clarified the fact that adherence was not performed as often as possible by far. The observations yielded reliable baseline information because most of the animal workers were observed and all animal handlers observed performed nonadherence to the personal hygiene protocol.

Likewise, poor hand hygiene as observed may contribute to transmission of infections. Most of the animal handlers were eating during breaks with their hands contaminated with

*Enterobacteriaceae*, which indicates contamination with possibly several infectious agents and potential infection with a wide range of zoonoses.

No hand washing facility was available in the canteen. Contamination of the hands was possible by entering the building after someone with tainted hands had contaminated the door handles. This is why testing in the canteen may not have been fully reliable to score good hand washing.

The completed questionnaires show that the animal handlers are poorly informed about the personal hygiene protocol and the protocol for pregnant animal handlers. They do not have sufficient knowledge about zoonotic agents. Some of the animal workers did not mention any zoonotic agents in the questionnaire and some only mentioned bacteria, fungi or worms in general and no specific agents. This causes animal handlers that are pregnant or wish to get pregnant to be at risk. Before people know they are pregnant, they should already change their behaviour in order to protect their unborn child before they are aware of their pregnancy. It may take up to weeks until women find out they are pregnant. When pregnant women do not follow the guidelines of the protocols for hygiene and for pregnant animal workers during the period they do not know they are pregnant, they put their unborn child at risk. So even a wish to get pregnant is a reason to be aware of the protocols for hygiene and for pregnant animal workers and to follow the guidelines.

The workclothes are always taken home to be washed. Washing the workclothes in the zoo was financially impossible at the moment of this research. When the clothes are mixed with other laundry and when washed at low temperatures, transmission of zoonotic agents may very well be possible. The shoes that are used during work stay in the zoo to prevent spread of zoonoses to animals and people outside the zoo as much as possible.

Also, it should be mentioned that the personal hygiene protocol was incomplete. This has probably contributed to the fact that animal handlers did not have sufficient knowledge about personal hygiene. Guidelines that were missing are described in 'Materials and methods'. Results from samples that were taken from several surfaces in the zoo that are often touched by animal handlers show that hygiene is poor in multiple places. This may be a threat to pregnant animal handlers and to animal handlers who wish to get pregnant. The poor hygiene in these places may be a result of the incomplete protocol, insufficient adherence to the protocol and little knowledge of the personal hygiene protocol. Insufficient effort is put into keeping these surfaces clean. Encountering *Enterobacteriaceae* in places where animal feed and food for the animal handlers are prepared and consumed is undesirable and may pose health risks.

In a study in an Auckland zoo zoonotic risks were found to be low, which was probably also because of protocols and because the zoo animals are under veterinary supervision. The fact that zoonotic infections in the zoo workers in Auckland were as low as in the local population is not directly associated with a low risk of zoonotic infections in zoological parks. The quality of their hygiene protocols and compliance to them could be above average compared to other zoos. Because disease, environmental and management factors can change, ongoing assessment of risk factors is needed to keep zoonotic risks as low as possible.<sup>13</sup>

## **Conclusion**

Lack of knowledge about the protocol hence poor hand washing and poor adherence to the protocol result in insufficient control of zoonotic risks for pregnant animal handlers.

Improvement of awareness of the protocols and adherence to them is required to increase the level of hygiene and to prevent zoonotic risks for pregnant animal workers as much as possible. Further research is needed to obtain insight in the overall control of zoonotic risks for pregnant animal workers in zoos and to gather more knowledge of the presence of zoonotic agents in zoological parks.

## References

1. Munoz-Suano A, Hamilton AB, Betz AG. Gimme shelter: the immune system during pregnancy. *Immunol Rev.* 2011;241:20-38.
2. Lamont RF, Sobel J, Mazaki-Tovi S, et al. Listeriosis in human pregnancy: a systematic review. *J Perinat Med.* 2011;39:227-236.
3. WHO. Zoonoses and verterinary public health (VPH). Available at: <http://www.who.int/zoonoses/en/>. Accessed February, 2013.
4. Dutch ministry of social affairs and employment. Rights pregnant employees. Accessed February, 2013.
5. Tenter AM, Heckerth AR, Weiss LM. Toxoplasma gondii: from animals to humans. *Int J Parasitol.* 2000;30:1217-1258.
6. Polkinghorne A, Hanger J, Timms P. Recent advances in understanding the biology, epidemiology and control of chlamydial infections in koalas. *Vet Microbiol.* 2013;165:214-223.
7. Stephens RS, Myers G, Eppinger M, Bavoil PM. Divergence without difference: phylogenetics and taxonomy of Chlamydia resolved. *FEMS Immunology & Medical Microbiology.* 2009;55:115-119.
8. Meijer A, Brandenburg A, Vries Jd, Beentjes J, Roholl P, Dercksen D. Chlamydophila abortus infection in a pregnant woman associated with indirect contact with infected goats. *European Journal of Clinical Microbiology & Infectious Diseases;* 2004.23: 6, 487-490.

9. Rodolakis A, Yousef Mohamad K. Zoonotic potential of Chlamydophila. *Vet Microbiol.* 2010;140:382-391.
10. Loto OM, Awowole I. Tuberculosis in pregnancy: a review. *J Pregnancy.* 2012;2012:379271.
11. NhanChang ChiaLing, Jones TB. Tuberculosis in pregnancy. *Clinical Obstetrics and Gynecology*; 2010.53: 2, 311-321.
12. Puliyaath G, Singh S. Leptospirosis in pregnancy. *Eur J Clin Microbiol Infect Dis.* 2012;31:2491-2496.
13. Forsyth MB, Morris AJ, Sinclair DA, Pritchard CP. Investigation of zoonotic infections among Auckland zoo staff: 1991-2010. *Zoonoses and Public Health*; 2012.59: 8, 561-567.
14. Gregory S. How good is your hand hygiene? *In Practice*; 2005.27: 4, 178-182. 11 ref.
15. Anderson MEC, Weese JS. Video observation of hand hygiene practices at a petting zoo and the impact of hand hygiene interventions. *Epidemiology and Infection*; 2012.140: 1, 182-190.
16. Weese JS. Barrier precautions, isolation protocols, and personal hygiene in veterinary hospitals. *Veterinary Clinics of North America: Equine Practice.* 2004;20:543-559.