# MICROBIOLOGICAL FINDINGS OF LEGIONELLAE IN THE MANUFACTURE OF FLAT GLASS: HEALTH RISK ASSESSMENT

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*Legionellosis is an acute respiratory and lung disease caused by bacteria of the genus Legionella. Natural and man-made aquatic environments are the major reservoirs of Legionellae. Transmission to humans occurs through inhalation of infectious aerosols. 104 samples of water intended primarily for the cooling process in flat glass factories were examined. All samples contained varying amounts of the legionellae bacteria which are pathogenic for humans. The most serious finding was Legionella pneumophila sg.1, sbg. Pontiac. Saprophytic strains (L. rubrilucens, L. nautarum, L. worsleiensis) were also found. An increased risk of the disease is associated mainly with the operation, maintenance and cleaning of machines (grinders, washers) and cooling systems. Preventive methods include regular maintenance, the disinfection of cooling water with biocides and the implementation of risk assessment. Medical diagnosis is based on medical examinations of workers supported by specific tests used above all in cases of professionally acquired pneumonia. This will also lead to improvement of the legionellosis surveillance.*

## INTRODUCTION

### Legionellosis as an occupationally acquired disease

Legionellosis is an acute bacterial respiratory and lung disease. The initiators of the disease are bacteria of the genus *Legionella* [1]. The major reservoir of legionellae is the natural aquatic environment [2] but increasingly they can also be found in man-made water systems. Transmission to humans occurs predominantly via the inhalation or aspiration of contaminated aerosols. The risk of infection is increased in buildings equipped with central air conditioning. Industrial cooling towers [3], water distribution systems in buildings [4], spas and thermal treatment facilities [5] also provide major risks. The permanent eradication of legionellae from artificial reservoirs is unrealistic. The only option is prevention and risk minimization.

For epidemiological purposes, cases of legionellosis are generally classified according to the likely source of infection [6], i.e. community-acquired (industrial or domestic sources), travel-associated (overnight stays in hotel, etc.), nosocomial (hospital acquired) or occupational [7]. There is little available data relating to workers' exposure and this disease.

Documented cases of occupational legionellosis are not common. They form 2–3 % of the total number of reported cases in the European database EWGLINET. The real incidence of occupational legionellosis is thought to be much higher. Cases of Legionnaires´ disease have been reported for employees exposed to legionella contaminated water aerosols in industrial premises [9]. In biological wastewater treatment plants of the pulp and paper industry [10]; in the automotive industry via contaminated cutting fluids [11]; for metal workers in the engineering industry [12]; and others. Legionellae can also be found in other sectors such as power plants, and the paper, food and beverage industries. Cooling towers present one of the greatest risks and they continue to be a major source of legionella exposure [3].

### Ecology and epidemiology of Legionella

Legionellae are ubiquitous microbes that are regularly present in natural and man-made aquatic environments. The permanent eradication of legionellae from man-made reservoirs is unrealistic [2]. The only option is prevention and risk minimization. Susceptibility to infection is universal - all humans are susceptible with sufficient exposure to a dangerous strain of legionella, and even a completely healthy person can contract it. The individual risk of infection is multiplied for people with a serious underlying disease such as cancer or chronic kidney disease, those in a post-chemotherapy or post-radiotherapy state, those with immune disorders, and those undergoing immunosuppressive therapy [8].

The aim of this study is to present the results of microbiological analyses of water samples collected in the working environment during flat glass production in the Czech Republic, then to discuss the tangible risk of legionella infection for workers and the need for a special regulatory legislative framework for working environments, and finally to establish the principles and/or requirements for workers' health protection and to propose preventive procedures.

## Related legislation

The basic requirement for the continuous operation of facilities is to ensure cold and, in particular, hot water quality. Water supply companies provide safe water with a low natural incidence of microorganisms, but the risk of an outbreak of legionella occurs when water is heated. Building operators have a direct responsibility for the water quality inside of the building. The limits of water quality indicators are also regulated by the law. The specific quantitative limit for legionella in water is established only for hospitals, medical and accommodation facilities. Government regulations set out conditions for employee health protection at work. A health risk assessment in the workplace and an implementation of measures for employee health protection are required. Legislation which regulates further epidemiological and medical aspects is also available. The Czech Republic has no uniform legislation for accepted levels of legionellae in water used in industrial processing. However, this legislation is present in many European countries (UK, NL, EU Guidelines [3]), and can be applied to basic risk assessment.

## Flat glass manufacturing technology

The production of flat glass, mainly in the manufacturing of automotive glass and windscreens, insulating glass, and glass for buildings and interiors, makes up 45 % of total glass production. The usual production technology is the FLOAT GLASS PROCESS - forming the glass in a chamber with molten tin [15]. The preparation of sand, its transport and introducing it into the furnace is done automatically. Glass production is divided into three main phases: melting of raw materials, refining and controlled cooling. By pouring molten glass onto molten tin at a temperature of about 1000°C a glass strip (6-7 mm thick) is created. Then the solid glass strip is transferred to a "cooling furnace" where it is cooled from 620°C down to 250°C by a controlled thermal regime. This glass is cooled again, its internal stress is removed and it is ready for cutting and machining.

Finally, the glass is refined in CNC machining centres by cutting, breaking, drilling, grinding, milling, etc. The production of windscreens is very specialized: A cutting line is adjusted for the proportions and contours of the windscreens, its edges are ground and this semi-finished product is washed in order to get rid of impurities. The shaping of windscreens into their final forms is done in an electric furnace by continuous heating, and cooling then increases their mechanical resistance. The whole process is finished by lamination and extrusion (application of a polyurethane profile).

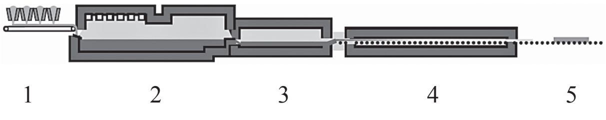


Figure 1. "Float" glass production scheme (source: http://www.izolas.cz/sggs-files/SGS_Memento.pdf); 1 - batch house, 2 - melting furnace, 3 - float tin bath, 4 - cooling furnace, 5 - cutting line.

## The use of cooling and process water and the risk of Legionella colonization

Due to the high temperatures in particular stages of glass production the cooling process is essential. The circulation of water between storage tanks, cooling sources and the line along with temperature changes and the long-term stagnation of water in the system present ideal settings for biofilm formation on surfaces inside the water systems. Biofilm consists of other bacteria, algae,

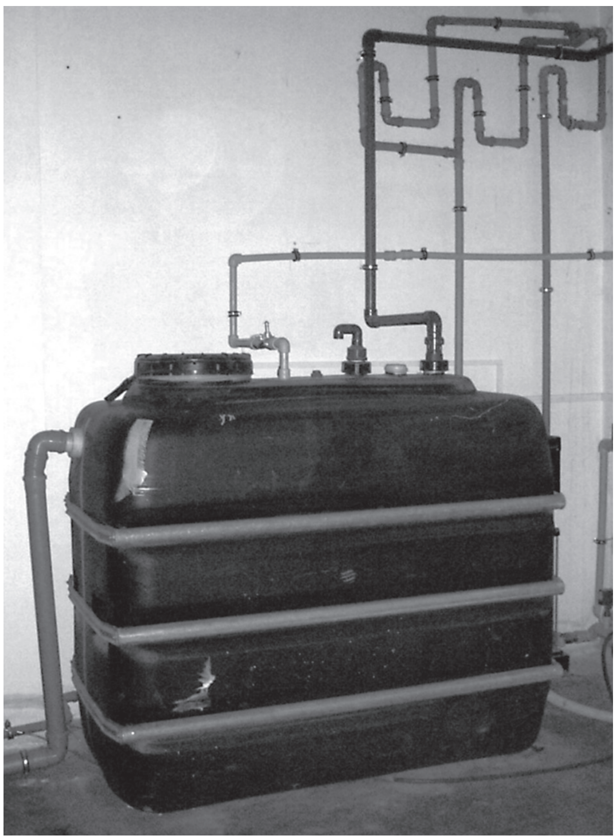

Figure 2. Demineralized water tank (difficult to clean)

amoeba, and protozoans. Biofilm protects legionella from direct exposure to desiccation and the chemicals used to control its growth.

Each type of washing process has its own circuit. Tanks, pipes and hoses are made of plastics towards which legionellae have a special affinity. The water in the circuits tends to have a long retention time and is heated up during its use for cooling. If the cooling circuit is already colonized by legionellae it is not possible to remove these without the use of a biocidal program.

The cooling of grinders is similar. High concentrations of mineral abrasives can be found in the cooling water. The polishing process also uses cooling water to which is added a polishing agent (cerium oxide). For rinses purified demineralised water is prepared, but it is stored in plastic storage tanks which are difficult to clean. The demineralised water is heated before entering the washer. The final washing of the glass is done with purified water prepared by reverse osmosis.

Figure 3. Aerosols in the glass washer.

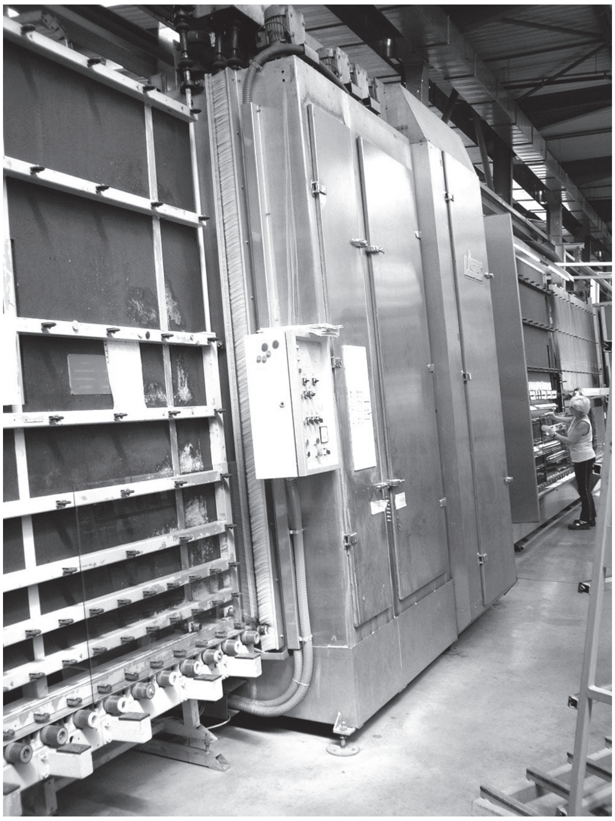

Figure 4. Service of glass washer.

Occupational exposure and health risks

Water containing legionellae can access the washer from the untreated heater tank. In most cases, the actual washing operations are enclosed. The formation

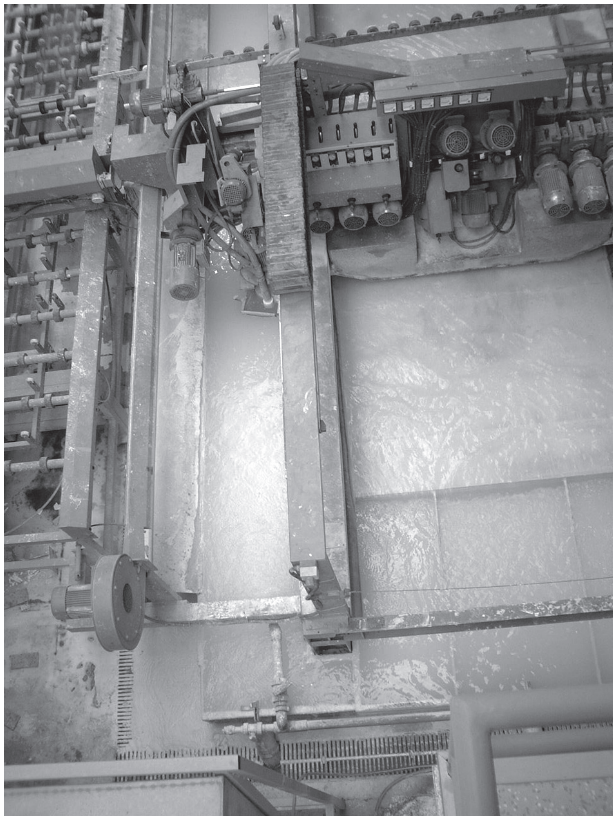

Figure 5. The grinding machine.

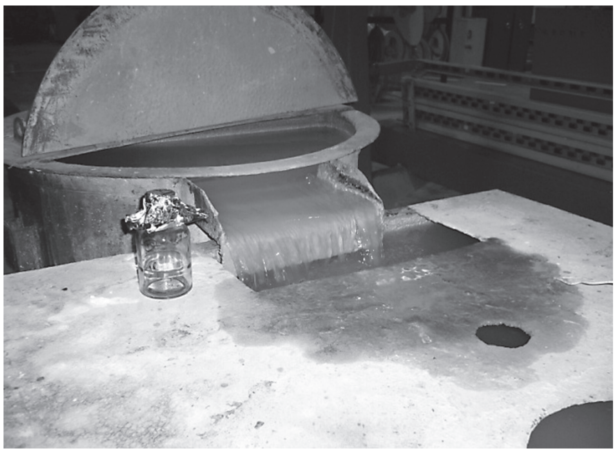

Figure 6. Water from the grinding of glass.

of water aerosols occurs only in the interior of the washer. Operators are exposed to possible infection only during cleaning and sanitation of the machines or at the conveyor belt near the outlet of an automatic washer. The circulating water of the grinders has a lower operating temperature and is heavily polluted with mineral abrasives but contains minimal nutrients. The exposure of grinder operators is possible.

Glass polishing is often done manually using polishing discs. Due to the various sizes of glass, the polishing discs lack a sufficient hood, and operator exposure to emerging aerosols is therefore significant.

## EXPERIMENTAL

### Materials

In 2003-2006 in the National Reference Laboratory for Legionella 104 samples of water used in the technological process from five facilities producing flat glass (mainly for the automotive and construction industries) were tested.

### Water sampling

The sampling of cooling water and subsequent microbiological tests determined the particular types of legionella present in the water system. The number of legionella positive samples along with their quantitative evaluations (CFU/1 ml = the number of colony forming units in the examined volume), the water temperature recorded during sampling and visual assessments of the performance of components demonstrate an overall picture of the situation and the degree of risk. The resulting preventive recommendations are based on these findings.

A microbiological monitoring plan is based on the technical and dispositional layout of the cooling system and on the method of its operation. A draft plan is preceded by an inspection of the site. Water samples of approximately 250 ml are collected at selected locations (after de-sedimentation) into sample containers. In the manufacture of plastics, there are specific locations for water collection. These are storage and cooling tanks, drain valves and connectors of cooled machines. Similarly, in the manufacture of flat glass the monitored areas are storage water tanks, operating cooling water tanks, jets, interiors and outlets of washers, grinder tanks and their wastewater, storage and heating tanks of demineralised water and the processes of extrusion and reverse osmosis. According to the results of repeated investigations, locations with repeatedly negative findings are assessed as risk-free and are excluded from the sampling plan.

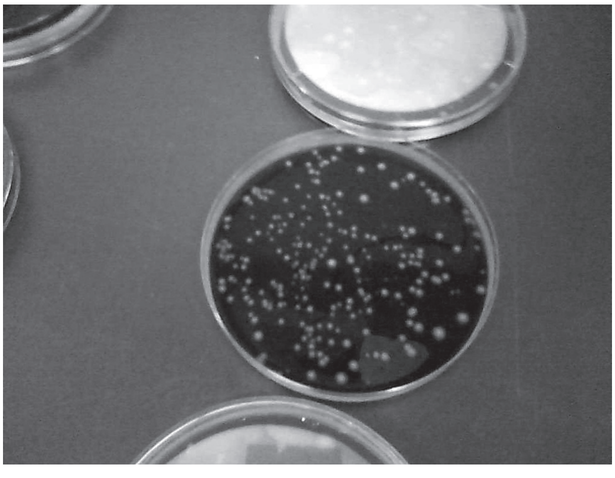

Figure 7. The cultivated colonies of legionella.

### Methods

#### Laboratory sample processing

The determination of the presence of legionella bacteria is performed according to Procedures for the Recovery of *Legionella* from the Environment [16] and, in the Czech Republic, to DIN ČSN ISO11731 *Water quality – Detection and enumeration of Legionella*. This standard describes a culture method for the isolation of legionella organisms and estimation of their numbers in environmental samples. This method is applicable to all kinds of environmental samples including portable, industrial and natural water. For water with low bacterial counts the standard ISO11731-2 *Direct membrane filtration method* can be used. For process water samples the inoculation of 0.1-0.5 ml directly on a culture plate is recommended. As a cultivation medium selective MWY agar of its own production (the National Reference Laboratory for Legionella) and non-selective BCYE agar are used. Incubation is carried out in plastic bags at a temperature of 37°C. The cultivated colonies are identified by standard morphological and biochemical criteria. Confirmed isolates are typed in the two sets of sera of its own production. The first includes *L. pneumophila* serogroup 1-15, and the second includes 48 sera of all known types of legionellae and their serological groups. The whole procedure from the cultivation to the serogroup determination takes two weeks.

## RESULTS AND DISCUSSION

### The overall results of the testing of Czech glass plants in 2003-2006

The samples of water used in technological processes from glass plants labelled A, B, C, D and E were examined. In total, 104 water samples were tested (grinders, various types of washers, extrusion and reverse osmosis). Forty four of them (42.3 %) were found positive for legionellae. The results are shown in Table 1.

Table 1. Test results of testing of glass plants.

| | Type of glass production | Sampling site | Temperature (°C) | Legionella (CFU/1 ml) | Identification |
|---|---|---|---|---|---|
| A | Production of automotive glass | grinders | 21-46 | 0 | – |
| | | washers | 51-52 | 190-350 | L. pneumophila sg.1B<br>L. pneumophila sg.6A |
| B | Production of insulating and anti-fire glass | grinders | 26-35<br>36-40 | 1 × >1200<br>620 | L. pneumophila sg.1C<br>L. cherrii |
| | | washers | | 1 × > 1000 | L. pneumophila sg.1C |
| C | Production of insulating and façade glass | grinders | 23-25 | 5-6 | L. pneumophila sg.1B<br>L. pneumophila sg.5B |
| | | washers | 14-17 | 0 | – |
| D | Production of automotive glass | grinders | 12-19 | 0 | – |
| | | washers | 35-40 | 7-560 | L. pneumophila sg.1A, (subgroup Pontiac)<br>L. pneumophila sg.6B<br>L. nautarum<br>L. gormanii |
| E | Production of building and automotive glass | grinders | 25-30 | 0-24 | L. pneumophila sg.6 |
| | | washers | 25-41 | 2-150<br>1 × > 1000 | L. pneumophila sg.1B<br>L. pneumophila sg.6B<br>L. worsleiensis<br>L. rubrilucens |
| | | technological water from tank | – | – | L. pneumophila sg.1B<br>L. pneumophila sg.6B |

## Spectrum of isolated types of legionellae and their clinical significance

The abovementioned microbiological findings indicate that in all investigated glass production plants legionellae were found in various degrees. Some of them are pathogenic to humans; other strains are common in aquatic environments and are not dangerous to human health. The spectrum of isolated types of legionella and their clinical significance is shown in Table 2.

These results show that washers are ideal settings with optimal conditions for the survival of legionellae because of their favourable temperature and the availability of nutrients in their inner areas especially when rubber or plastic are used on their inputs and outputs. In all the tested plants legionellae were found in the washers or heating tanks, including very serious strains capable of causing severe disease in completely healthy people, particularly *Legionella pneumophila* sg. 1, subgroup Pontiac; sgs. 1B and 1C (subgroups OLDA and Bellingham) and *L. pneumophila* sgs. 5 and 6. Even though the process of flat glass washing took place in an enclosed space, aerosols were formed, particularly at outlets. Operators were exposed to these aerosols intermittently, usually when they were taking the glass out of the washer. The most significant exposure of workers occurred during repairs, cleaning and sanitation of the machines.

Demineralised water does not contain any nutrients or organic carbon. However, if it is stored in bins instead of being prepared continuously, it is colonized by legionellae in most cases, and often above the limit (10 CFU/ml) recommended for sanitary intervention. In such cases, corrective measures should follow. In such mineral poor environments, the source of nutrients for survival and proliferation of legionellae seems to be a complexly organized biofilm nutrition community.

Although the cooling of grinders is in principle similar to that of washers, the discovery of legionellae was surprising. The lower water temperatures, high content of abrasive mineral particles in the grinder cooling water and lack of suitable nutrient factors are a rather inappropriate environment for the nutritionally demanding legionella bacterium. Again, this illustrates the important role of biofilms in the survival of legionella. Cooling water with added cerium oxide is used in the polishing process. However, no significant findings of legionella were detected. Additionally, preparatory and auxiliary operations such as extrusion and reverse osmosis do not seem to cause any problems.

## Proposal for corrective measures

1. Basic corrective measures must be carried out by way of the proper mechanical cleaning of inner and outer surface areas exposed to water used in the

technological process. Particular risk areas for the growth of biofilms are the protective shower screens at the exits of the glass washers and the inner surfaces of the water tanks in the grinders. In practice, often not enough attention is paid to the cleaning of the interior of storage tanks for demineralised water. Their regular maintenance and cleaning are also problematic due to a lack of access to the inside the tanks.

2. The next step is to consider whether it is possible to use oxidizing or non-oxidizing biocides with a certificate of efficiency for every individual procedure during the various technological operations (especially during washing). The chemical composition of any biocide and its effects must not affect the quality of the cleaning process or the appearance of the final product. The most suitable solution seems to be the use of hydrogen peroxide. The other available ones are bromine-based biocides (effective up to pH 9.5), isothiazolins + glutaraldehyde or quaternary ammonium salts. Because all manufacturing operations are certified any change of the matrix (e.g. the addition of biocide) is subject to the approval and eventual validation of those who are responsible for the technological process.
3. A regular inspection for the presence of legionellae in selected processes should be carried out at least once a year. These inspections will also enable an assessment of the dynamics of colonization of different operations during the year and determine the best frequency of monitoring for the next period. An effective risk assessment requires a sequence of data over time and their evaluation including corrective measures if indicated.

Diseases of the workers exposed to aerosol of in the glass factories are not described. It is possible that repeated exposure to low doses of legionella induces cellular immune responses with protection against the disease after a significant exposure. This also corresponds to the finding of protective antibodies in healthy workers. In 2006, Spanish study provides an analysis of aggregations cases of legionellosis in the ceramics factory [17]. They have arisen one summer in Castellon. Microbiological, clinical and epidemiological field methods were employed. Within a few days, there were five cases of legionella pneumonia in workers related to various ceramic industries within an area where a large number of these plants are located. Two constituted a minor outbreak. A possible cause could be contaminated cooling towers or hose with water, the production technology itself have not been investigated. By means of molecular biology techniques performed on the strains, the common origins of three of the cases were ruled out. It is debated whether this episode of an outbreak having arisen within the context of a broader-ranging cluster of cases of a multi-focal origin was a chance event or whether it was the manifestation of a risk related to this industrial activity which might repeat itself.

## Prevention

Primary prevention of biological risks should be considered during the planning and construction of industrial plants. The main issues can be identified as the choice of location, the distance from human settlements, climatic conditions, the landscape relief, the prevailing wind direction, etc. [18] The choice of specific technologies and the setting of the operating temperature are crucial. Creating a good working culture is also important – namely keeping the systems clean, with no deposits, encrustations or biofilms [19]. Biofilms are considered to be crucial for the survival and proliferation of legionellae.

Secondary prevention refers to actions subsequent to finding a problem and during its solution. The number of legionellae in different water environments is not the only important factor. The "infectious dose" for humans and "safe level" of colonization of natural resources has not yet been defined. This fact is doubly true for industrial plants due to the addition of various chemicals into water used in technological processes. The second important factor is therefore the determination of the particular type of legionella present in the environment. More than 50 types of legionellae are known and only 24 of them are capable of causing disease in humans. 50 % of cases of Legionnaires' disease in the Czech Republic are caused by *Legionella pneumophila* sg. 1, and sg. 3-6 (Czech database of infection diseases EPIDAT). In the case of an occurrence of "risk" strains with known clinical effects, it is necessary to introduce remedial

Table 2. Spectrum of isolated types of legionellae and their clinical significance.

| | |
|---|---|
| L. pneumophila sg. 1 | Represents 50 % of all legionella infections in the Czech Republic |
| L. pneumophila (sg. 1A Pontiac) | The most important pathogen, capable of causing disease in healthy humans |
| L. pneumophila (sg. 1 B, 1C) | Opportunistic pathogens, capable of causing disease in immunodeficient individuals |
| L. pneumophila sg. 5, 6 | Rare but clinically significant |
| L. gormanii | Rare in Czech Republic, worldwide: observed only in a few patients |
| L. rubrilucens, L. nautarum, L. cherrii, L. worsleiensis | Common aquatic saprophytic types, clinically insignificant, can lead to technological and operational complications |

measures. A continuous disinfection is expensive, and none of the available disinfection methods is ideal and without an impact on the environment. Therefore it is necessary to choose the right level of sanitation. Findings of other non-pathogenic strains of Legionella in high concentrations reduce the efficiency and increase the energy consumption of the plant. Biofilms reduce cooling efficiency, increase failure rates and increase economic losses; therefore regular inspection and maintenance is required.

Tertiary prevention refers to securing sources (reservoirs) of infectious aerosols in a situation when exposure and disease have already occurred. Usually it is necessary to stop an operation, thoroughly sanitise the environment, apply emergency doses of a suitable disinfectant and permanently disinfect water using biocides. Usually, only a serious epidemiological situation is the impetus for the introduction of mandatory and enforceable procedures, the determination of responsibility and the setting of health limits and requirements.

### Health perspective and obligations of the participants

The employer's obligation is to provide a safe working environment. Working activities with potential exposure to biological agents must be done safely. This means an obligation to provide clear instructions to workers about possible risks, methods of transmission of infections and health protection, and to provide them with personal protective equipment (protective clothing, goggles, gloves, and face masks or respirators to prevent inhalation of bacteria). The prohibition of eating, drinking and smoking at work and strict hand hygiene after work should be self-evident.

Physicians in charge should be familiar with the working environment and during periodic health examinations they should also monitor the anamnestic data of pulmonary and respiratory diseases, especially if they occur frequently in the same worker or group of workers. Positions dealing with the service and especially the maintenance and repairs of machinery and equipment (in cases where the incidence of pathogenic legionellae in the environment is known) should not be held by those with a history of immunodeficiency, cancer, serious chronic respiratory or kidney illnesses and those treated with immunosuppressants.

Every case of legionellosis should be epidemiologically investigated and microbiological tests of the water from all the patients' residences should be carried out. When a concurrence between the patient's microbiological findings and findings at their working environment occurs, an occupational disease may be considered. The consequence for a worker is not only the recognition and reporting of an occupational disease and the resulting compensation, but also a possible loss of their profession for health reasons with its socio-economic impacts. The employer is obliged to rectify the work situation, to shift the worker to a risk-free post and to pay compensation for an occupational disease. Intensive media coverage of companies along with protracted litigation in the case of large epidemics or deaths is almost the rule, as demonstrated by recent cases in England, Germany, France and other countries [13, 14].

## CONCLUSIONS

Pathogenic and saprophytic legionellae were found in various degrees in all the investigated glass production plants. Legionella cannot be completely eradicated from the environment; workers who operate machines and especially those who maintain and repair them are at risk of legionellosis. Corrective measures for industrial technologies consist of regular maintenance, cleaning and flushing of all parts in contact with water, continuous disinfection of cooling water with biocides and the introduction of risk assessment (a technical certification of the equipment and systems for the prevention of microbiological hazards). Medical prevention involves continuous health checks of staff in order to assess their health eligibility and to verify the etiology of pneumonia.

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