A5 - Evaluation of the risk of *Legionella spp.* development in sanitary installations

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Abstract

In order to determine whether it is possible to reduce energy use for domestic hot water (DHW) production and distribution, without increasing the risk of *Legionella spp*. development in sanitary installations, a full-scale test facility was built, consisting of a 200 liters water tank, a circulation system of nearly 40 metres long and 2 draw-off pipes. On a daily basis, a consumption profile corresponding to the DHW use of a single family (4 persons) was applied separately using two tap pipes, one corresponding to a kitchen and the other to a bathroom. *Legionella spp*. was cultivated in a separate water tank and then introduced into the test facility. The DHW production temperature was kept at 45°C with a periodical heating to 60°C for different durations and different frequencies. *Legionella spp.* concentrations were measured, both in the water and in the biofilm. The influence of different parameters was studied: disinfection of the sampling taps, flow rate of sampling, disinfection of the circulation system only or in combination with the draw-off pipes.

This article discusses the first preliminary results of this study, which is still ongoing till mid-2018.

Keywords

Water supply hygiene, *Legionella spp.* development, domestic hot water (DHW), disinfection, biofilm

Introduction

As the energy-use for space heating continues to diminish due to better performances of the building envelope and the use of more efficient heating systems, the energy use for hot water

production becomes increasingly relevant. Since the recast of the Energy Performance of Buildings Directive [1] stipulates that by 2020 all new buildings in the European Union should be almost near zero energy buildings, reducing the energy use for hot water production, whilst maintaining the desired comfort level for the buildings occupants, will become one of the challenges for the future in Europe.

Therefore it becomes ever more important to design hot water production and distribution installations inside our buildings in a more energy efficient way. In certain types of installations, for instance installations with heat pumps and in low temperature district heating [7], an extra pressure to reduce de DHW production temperature exists. A lower temperature is beneficial for the performance (COP) of most heat pumps for instance.

An optimal design [2] of the drinking water system (hot and cold) includes however also other aspects, of which some are even more important such as the hygienic quality of the water at the taps by avoiding for instance the development of *Legionella*, a pathogenic bacterium, which can lead to a severe pneumonia.

Knowing that the *Legionella* bacteria grows between 25°C and about 45°C while it is decimated above 50°C [3,6], the aim of the study was to evaluate whether it is possible to produce and distribute the domestic hot water at temperatures within the growth range of the bacteria - i.e. energy –effective, but still comfortable in use- in combination with systematic in time limited temperatures rises above 50°C in order to ensure hygienic quality.

While several authors reported studies on the influence of the temperature on the growth/death rate of Legionella bacteria in laboratory conditions [3,6] or in a pilot installation [4,5], the full-scale test facility offers the opportunity to study the effect of multiple controlled thermal chocks on the survival of *Legionella*.

At the BBRI, a full-scale test facility was built, consisting of a distribution loop of nearly 40 metres long, fed by a 200 liters water tank (“test tank”) at 45°C and 2 draw-off lines. In this configuration, a consumption profile on a daily basis, corresponding to the DHW use of a single family (2 adults, 2 children), was simulated on the two draw-off lines, one corresponding to a kitchen and one to a bathroom (shower). *Legionella spp*. were, after cultivation in a separate water tank, introduced into the test facility to study the evolution of this contamination when applying a regular, in time limited, thermal disinfection of the tank and the distribution loop .

2 Test facility description

Figure 1 shows a global view of the test facility. There are 2 tanks (a ‘culture’ and a ‘test’ tank), one circulation loop of about 40 m, connected to the test tank and 2 draw-off pipes (with respectively a “kitchen” and a “shower” *consumption profile*).

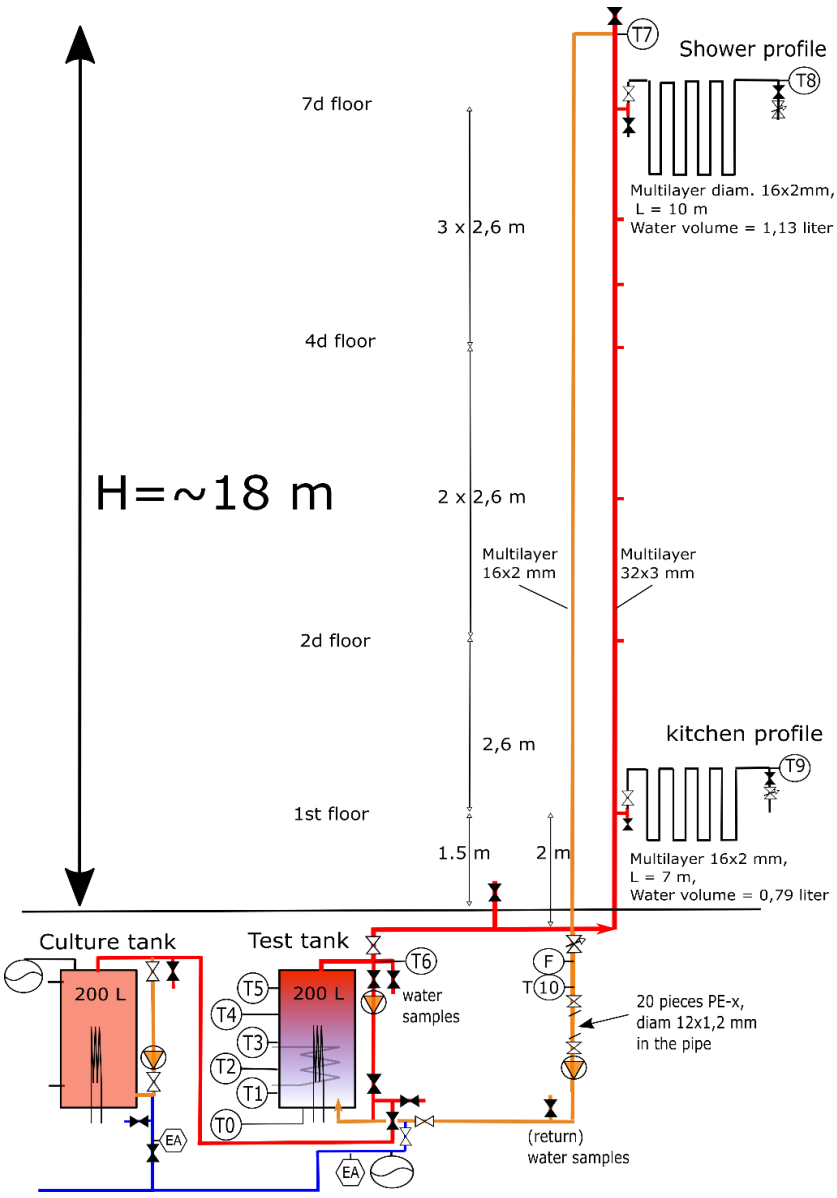

Figure 1 - Schema of the Legionella test facility.

The '**culture**' **tank** (containing a stock solution of *Legionella* bacteria) is a 200 liter steel tank, with an electrical resistance placed in vertical position at the bottom of the tank. The tank was filled with fresh potable water from the district distribution and heated at 37°C. The homogenisation of the water temperature in this tank is obtained by a circulation loop over the tank. Two inoculations with *Legionella pneumophila* bacteria from a hospital facility and a daily draw-off of 127 liters - supplying fresh water - were necessary to obtain a stable stock solution of *Legionella pneumophila* at nearly 2.10^5 cfu/l.

The **"test" tank** (figure 2) is a 200 liters austenic chrome-nickel steel tank. The heating system is an electrical resistance of 6 kW placed in vertical position at the bottom of the tank. The

height of the tank is 136 cm. Temperature probes (thermocouples, precision +/- 0,1°K) are placed on its outside wall (under the isolation jacket): on the bottom of the tank and at different heights (respectively 13.6 ; 40.8 ; 68; 95.2 and 122 cm). Another temperature probe is also placed in the middle of the departure pipe (fig. 3 left).
This tank and its circulation loop was first directly fed with the contaminated water from the "culture" tank (2,64 liters each 30 minutes) during 2 weeks. During the second week, the temperature was changed from 40°C to 45°C over 5 days. After the 2 weeks, the water supply was connected directly to the district distribution of potable water. The DHW production temperature was then kept at 45°C and a realistic consumption profile (see further) was applied at the draw-off taps (= test phase). Samples of the water circulating in the loop were periodically taken via tap points on the depart of the loop and on its return near the boiler, in order to measure the development of *Legionella pneumophila*. After a couple of weeks, a stable concentration of *Legionella pneumophila*. was reached (nearly 5.10^6 cfu/l).

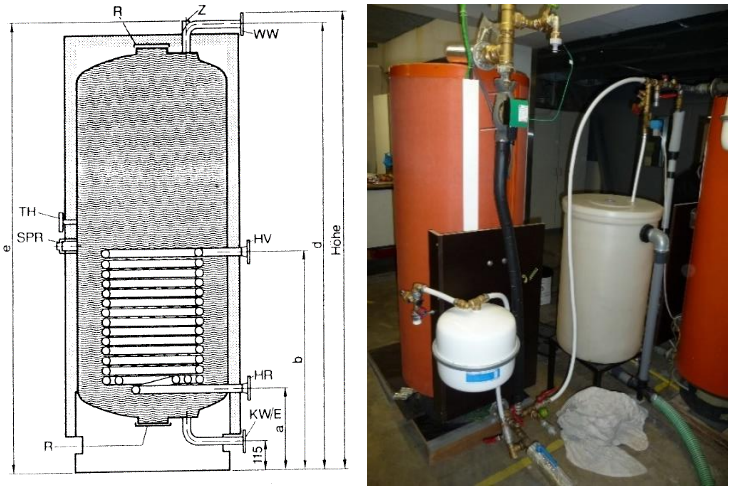

Figure 2 - test tank cross-section (left) and global **view (right)**

At the outlet of the tank, there is a flexible connection between the 'test' tank and the circulation loop. The *circulation loop* connected to the test tank consists of nearly 40 m isolated multilayers pipes (figure 1). The loop starts with a horizontal pipe DN50; the vertical pipe is DN32 with 20 mm insulation. The recirculation pipe is a DN16 with 15 mm insulation. Some temperatures probes were placed inside these DN32 and DN16 pipes. A flow regulation valve and a flowmeter were also placed on the last one. At the bottom of the recirculation pipe (1,5 m before entering the tank), another sampling valve makes it possible to take water samples of the "return" water.

2.1 Sampling facilities

Figure 3 shows some details from respectively a) the temperature probe and water sampling tap on the depart pipe and b) the sampling possibility on the recirculation pipe of the loop. The combination of sampling valves with flow regulation valves makes it possible to differentiate flow rates for sampling. Two flow rates were studied for sampling (0.5 l/min and 2 l/min). As

the aim is to test the global disinfection effect (free water and biofilm) of the thermal shock, the sampling flow rate at 2 l/min was maintained.
In order to avoid every possible contamination in the water samples coming from the flow regulation valves, a chemical disinfection of the valve has been conducted before sampling (using ethylic alcohol and rinsing with sterile water), since week 8. The trapped water volume (~5 ml) between the ball valve and the regulation valve (fig 3 right) has been analysed for *Legionella* (3 repetitions).

Figure 3 – Sampling points near the test tank : (left) temperature probe in the T-piece and sampling tap upstream a flow regulation valve on the departure pipe. (right) Sampling valve upstream of a flow regulation valve on the recirculation loop.

On the recirculation pipe, a section of pipe DN25 (length = 0,8 m) was also inserted (fig 4 left) containing 20 rings of PE-x pipe (ø 12 x 1,2 mm; height of about 29,7 mm) exposed to the recirculation water so that the biofilm can grow on it. When needed, such a ring piece can be taken (fig. 4 right) for the analyses of the biofilm.

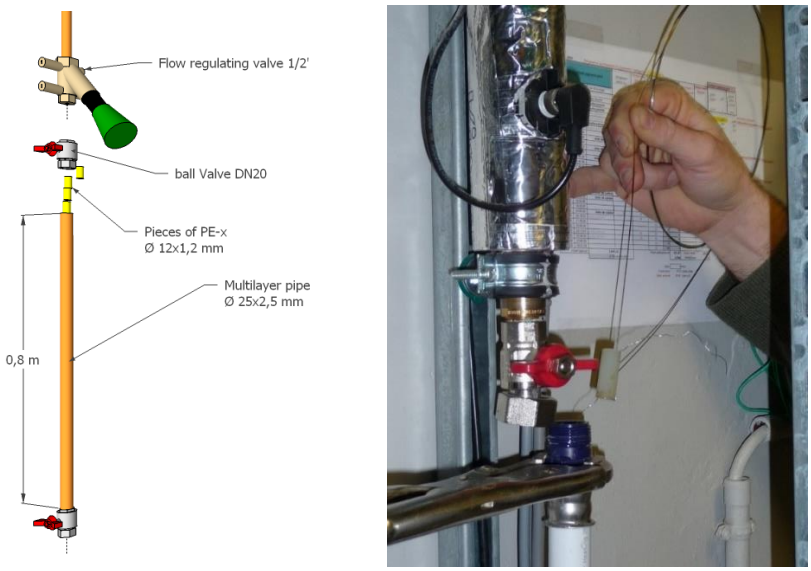

Figure 4 – (Left) Schematic view of the pipe section with rings for the biofilm monitoring. (Right) Extraction of a ring piece in PE-x.

Water samples can also be taken from the two draw-off pipes (fig.1) which are equipped with temperatures probes and regulation valves (fig.5 right). The consumption profiles are applied to these draw-offs with electro valves controlled by electronical timers. The outlets of these electro valves are connected to a closed discharge reservoir avoiding the spread of aerosols in the lab (fig 5 left and center).

Figure 5 - views of the 'kitchen' draw-off (on the left); the 'shower' draw-off (center) and their equipments : temperature probe; electro valve and regulating valve at the reservoir connections (on the right).

2.2 DHW consumption profiles.

According to the tank capacity (200 l), a realistic tap schedule based on the DHW demand of a 4 person- family was established. The tap schedule is given in the table 1.

Table 1 - Consumption profile

Tap schedule		DHW Flow-rate	Tap duration	Tapped DHW volume
Start hour	Type of draw-off	l /min	s	liters
06:59	purge of the shower pipe	6.5	**10**	**1.083**
07:00	Shower n° 1	6.5	355	**38.5**
07:10	Shower n° 2	6.5	393	**42.6**
08:00	Shower n° 3	6.5	296	**32.1**
12:00	Kitchen faucet	5	**6**	**0.50**
12:30	Kitchen faucet	5	20	1.67
13:45	Kitchen faucet	5	30	2.50
18:15	Children's bath (40 L)	6.5	311	**33.7**
19:00	Kitchen faucet	5	6	0.50
19:15	Kitchen faucet	5	3	0.25
20:00	Kitchen faucet	5	30	2.50
	Total tapped daily DHW Volume :			**155,79 l**

According to the temperature setting point of the tank (45°C), the DHW flowrate of the 'shower' draw-off was set on 6,5 l/min (corresponding to a shower with a flowrate of 8 l/min on mixed water at 38°C). The DHW flowrate of the 'kitchen' is calibrated on 5 l /min. The total daily DHW volume consumed is about 156 l/day : ~ 148 l via the 'shower' and ~7,9 l via the kitchen . The first draw-off of the day on the 'shower' (at 6:59) and on the kitchen (at 12:00) gives us the water samples for the analyses of the concentration in *Legionella sp* in the two draw-off pipes (where no circulation occurs during thermal disinfection, *except* when we wanted specifically to test the influence of a disinfection by letting the water flow through it during the thermal disinfection - weeks n° 10 and 11 - see later).

3 Heat shock experiments

As the Belgian Superior Health Council recommends concentrations of *Legionella pneumophila* (in the sanitary water), beneath 1000 cfu/l in high level risk installations, applying thermal shock disinfection of the circulation loop aims to eradicate or stabilise the *Legionella* contamination below this value. A common practice to reduce *Legionella* levels in a sanitary installation is heating up the water in the storage tank to reach 60 °C in the circulation loop for 30 minutes.

In the experimental set-up, the temperature of the boiler was set to 45°C and regulated by the temperature probe placed in the middle of the tank height, while the temperature in the circulation loop remained between 46.8°C (max. on depart) and 42,8°C (min. on return). For a thermal shock, the regulation is then switched to another regulation and when the new set point of a thermal shock is reached, a timer is activated for a chosen time duration. At the end of this period, the regulation is then automatically switched to the usual regulation of the tank.
For the thermal disinfection (thermal shock, during the night), the heating of the tank was modified so that a temperature of 63°C was reached at the outlet of the tank and so that 60°C was obtained at the end of the recirculation pipe. Water samples were taken before and a few hours after (early in the morning) the thermal shock. *Legionella spp.* concentrations were measured, both in the water and in the biofilm on the PEx-rings. All the temperatures as well as the circulation flowrate were monitored every second.

In the first shock disinfection, the temperature rise was maintained during 30 minutes. As the *Legionella* concentration remained present in a unacceptable concentration (>1000 cfu/l) a few hours after the shock, different likelihood sources of *Legionella* (re-) contamination of the test facility, like contamination of the water samples by the biofilm present in the sampling taps or re-contamination of the circulation loop water by *Legionella* bacteria from the 2 draw-off pipes were also investigated.
In order to standardise the sampling protocol, different parameters were studied as a preliminary disinfection of the sampling taps and sampling at different flow rates. Some adaptations were progressively made in the test facility and in the initial thermal disinfection protocol in order to succeed the disinfection in the test facility at 60°C. Different durations (30 min, 1 hour, 2 hour), homogenisation of the water temperature in the tank by activating of a short-loop re-circulation on the tank and different thermal disinfection frequencies were tested (see table 2).

Table 2 - Tested thermal disinfection.

weeks	T production (tank)	T heating (thermal shock)	Heating duration	Frequency	Number of thermal shocks
1 and 2	45 °C	60 °C	Warming up + 30 min	1x / week	2 shocks
3 and 4	45 °C	60 °C	Warming up + 1h	1x / week	2 shocks
5	45 °C	60 °C	Warming up + 30 min	1x / week with extra circulation on tank	1 shock
6 and 7	45 °C	60 °C	Warming up + 1 h	1x / week with extra circulation on tank	2 shocks
8 and 9	45 °C	60 °C	Warming up + 1 h	1x / week with extra circulation on tank. + 30 minutes thermal disinfection of the sampling taps	2 shocks
10	45 °C	60 °C	Warming up +4 x 30 min (for taps disinfection)	1x / week with extra circulation on tank. + 4 x 30 minutes thermal disinfection for each of the sampling taps and draw-off pipes (in the 'circulation' order)	1 shock
11	45 °C	60 °C	Warming up +30 min (for tank) + 4 x 30 min (for taps disinfection)	1x / week with extra circulation on tank. + 4 x 30 minutes thermal disinfection for each of the sampling taps and draw-off pipes (in the circulation order)	1 shock
14-18	45 °C	60 °C	1 h	2x / week with extra circulation on tank	9 shocks
19	45 °C	60 °C	1 h	Daily (7x /week) with extra circulation on tank	7 shocks

Besides the above indicated study on the test rig, temperature experiments were also conducted on water suspensions of *Legionella* in laboratory conditions (in parallel): they were exposed to different thermal shocks profiles (60°C, 65°C, 70°C during 5 min, 15 min, 30 min, 60 min and 120 min) in order to indicate which combination (temperature/time) could lead to success. 2 ml suspensions were transferred into glass tubes, sealed with a knot and placed into a water bath; with 3 replica for each duration.

4 Results

4.1 Sampling protocol

It is very important to standardise the sampling methodology in order to obtain reliable results of *Legionella* analysis. However, no significant difference has been observed between sampling at a flow rate of 0.5 l/min or at 2 l/min. The sampling flowrate of 2 l/min is maintained.
In order to avoid every possible contamination in the water samples coming from the flow regulation valves, a chemical disinfection of the valve has been conducted before sampling since week 8. The trapped water volume (~5 ml) between the ball valve and the regulation valve has been analysed for *Legionella* and presented a very low concentration of *Legionella* bacteria (< 1 bacteria/ml). This contamination will not affect the measured *Legionella* concentrations in the water from the circulation loop.

4.2 Thermal inactivation of *Legionella pneumophila* in laboratory conditions

In laboratory conditions, a sole thermal treatment on a homogeneous stock solution of *Legionella pneumophila* strains from the culture vessel was carried out at 60°C, 65°C and 70°C. No (cultivable) *Legionella pneumpohila* bacteria survived the thermal shock at 65°C or 70°C. The concentration of cultivable *Legionella pneumpohila* dropped from 100.000 cfu/l to beneath the detection limit (< 100 cfu/l) even after 5 minutes. However, several laboratory results indicate a *Legionella pneumophila* resistance up to 60 minutes at 60°C (reduction to 250 cfu/l after 60 minutes for cultivable bacteria).

4.3 Heat shock treatment in the test facility

A heat treatment of the circulation loop once a week (up to 1 hour at 60°C, even with recirculation over the tank) but without disinfection of the draw-off lines seemed to reduce the *Legionella* concentration temporary (in the loop), but the concentration never dropped under 1000 cfu/l. At weeks 8 and 9, the thermal disinfection (30 min, 60°C) of the sampling valves, positioned on the circulation loop, was included in the protocol.
As high concentrations of *Legionella* were reached at the draw-off pipes, at week 10, a thermal disinfection of the each tap point (30 min, 60°C) was also performed. However, the *Legionella* concentration dropped only from ~1.6 10^5cfu/l to ~ 1.1 10^3 cfu/l (2 log reduction) after 30 minutes (tap point at the 7^{th} floor). The lower concentration was observed temporary at the tap point.

At week 11, to optimise the disinfection process, a homogeneous warm up of the tank during 30 minutes at 60°C before starting the thermal shock of the tap points was included in the disinfection protocol. During the disinfection of the tap point at the 7th floor, *Legionella* concentrations dropped from 7.8 10^4 cfu/l to 50 cfu/l (30 min after a continuous water draw-off of 30 min) but 24 hours after the disinfection, the *Legionella* concentration rised to 500 cfu/l and unfortunately, after 2 weeks the initial concentration of *Legionella* bacteria was reached again.
For 5 weeks, a thermal disinfection program of 1 hour at 60°C started two times a week in the circulation loop (to obtain 60 °C at the end of the recirculation pipe, the temperature in the storage tank stays above 60°C for 2 hours). Afterwards, the frequency was raised to a daily thermal disinfection of the circulation loop, for 1 hour at 60°C. However, the *Legionella* concentration in the circulation loop remains above 1000 cfu/l.

4.4 Biofilm monitoring

After several thermal shocks (12 x), a ring piece was collected from the recirculation pipe and analysed for the presence of *Legionella* bacteria and ATP. The results from the ATP measurements shows that the bacterial flora on the ring was not affected by the different heat shocks (average of 4.9 10^5 mean cell counts). Thermal shocks on a weekly frequency show a progressive decrease of the *Legionella* concentration on the rings from 3.3 10^6 to beneath 2.5 10^3 (average reduction of 3.3 log), but after a period of 15 days without any thermal shock *Legionella* bacteria recovered to the initial concentration in the biofilm.

5 Conclusions

By applying a thermal shock at 60°C only on the contaminated water tank and the circulation loop on a regular base, the treatment of the test facility with thermal shocks at 60°C (for 0,5 ; 1 ; 2 hours) does not lead to the eradication neither to a low (<1000 cfu/l) concentrations of *Legionella* bacteria in the loop.
Without thermal treatment of the draw-off lines the concentration of *Legionella* (in these lines) is not affected and remains on a high contamination level, which could be a source of an early recontamination of the loop. We observed that the *Legionella* concentration in the draw-off pipes raised already 30 minutes after the disinfection.

However, applying a thermal treatment to the whole test facility at 60°C (for 1 hour) does not lead to the eradication neither to a low (<1000 cfu/l) concentrations of *Legionella* bacteria. The tests performed in laboratory conditions (heat shock at 60°C up to 1 hour) on a homogeneous solution of *Legionella* bacteria in water confirm the results obtained in the test facility.

Because these results show that eradication of *Legionella spp* from the water and the biofilm, (or even stabilising the concentration beneath 1000 cfu/l) seems to be difficult to achieve and remedial action might only lead to a temporarily and limited reduction of the *Legionella* concentrations, it seems that in a contaminated DHW installation with a permanent production

temperature of 45°C a regular (even daily) thermal shock at 60°C is not appropriate as a curative treatment in hot water facilities.

As the laboratory tests are promising at 65°C and 70°C, different combinations (production temperature, thermal shock temperature, duration and frequency) will be studied in the full scale test facility.

Sampling at higher flowrate (> 2 l/min) seems also to be interesting to be able to evaluate if there is a possibility of releasing biofilm during the sampling.

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7 Presentation of Authors

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