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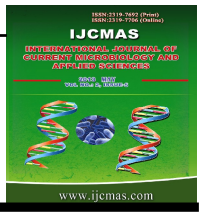

Original Research Article

Insulation test of Listeria in raw milk in Benin

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Listeria; culture; Food poisoning; Milk; Benin.

A B S T R A C T

Milk is a very rich food and favors the development of microorganisms. It is most often implicated in cases of foodborne illness. Therefore, it should be subject to control of all microorganisms of food poisoning in order to preserve the health of consumers. But Listeria, which increasingly is indexed, is not frequently sought in food in Benin. The present work was done to isolate Listeria and identify problems related to that. Thirty (30) samples of raw cow's milk were analyzed at the National Laboratory of the Ministry of Health (Benin). Listeria was isolated in 9 samples, a percentage of 30%. No major difficulties, apart from the problem with the supply of specific media culture of the bacteria were noted. It is possible to isolate Listeria in Benin so this study showed that the research of this organism should be now included in diagnostics in food technology in Benin.

Introduction

The importance of milk in nutrition requires that a particular attention should be given toward its production, its distribution, and especially to its quality. (Luquet, 1985). In fact, milk is a very rich food, favourable to the growing of microorganisms, so many affections and various diseases have been reported after its consumption or its derivate products. In developed countries, it is often implicated in episodic foodborne illnesses. (Goulet *et al.*, 2001). In France for instance, between 1988 and 2003, milk has been implicated in 368 outbreaks of foodborne illnesses. Among the microorganisms implicated in those

periodic diseases, Listeria occupies a prominent place. Between 1992 and 2005, 200 to 250 cases of listeriosis have been yearly recensed in France and many of them suggested that milk products were implicated (AFSSA, 2005). In California, 142 cases of listeriosis were reported in 1985 after the consumption of cheese. It is the same situation in Swiss which has counted 122 cases between 1983 and 1987 (Huhtanen *et al*., 1989).

In veterinary medicine, Listeria especially *Listeria monocytogenes* is implicated in many cases of encephalitis, septicaemia, abortions and mastitis. In human medicine, this bacteria is implicated in pregnant women and new-borns babies' infections, but also adults' especially for immunocompromised people. In Benin, treatment conditions of milk and the lack of hygiene characterizing its sale and its distribution as those of its derivatives on the markets, are factors which can favour the proliferation of Listeria.

Taking into account, on one hand, the great number of cases observed in developed countries despite their level in terms of respect of good livestock habits, and on the other hand the growth of milk sector in our country, it's important, to proceed to the research of Listeria in milk and its derivate in order to protect the health of consumers. That's the reason why the present work is entitled "Insulation test of Listeria in raw milk".

Materials and Methods

Equipment

Thirty (30) samples of raw milk obtained at Cotonou and Parakou were used. Glassware, Petri dishes, materials devices such as Stomacher, autoclaves, baths, culture fields (buffered peptone water, PALCAM, Oxford agars etc.), reactant, etc. were used.

Methods

The used samples, are distributed between the northern region (20) and the southern region (10) of Benin. The pH was determined according to the source.

Isolation methods of *Listera sp.*

On OXFORD Agar, the procedure below has been observed:

Collect and transfer with a sterile pipette 0,1ml of stock solution to the surface of each of the two dishes of OXFORD Agar. Carefully spread the inoculum with a sterile platinum loop the quicker possible at the surface of the medium avoiding to touch edges of the Petri dish with the loop. Let the Petri dishes closed during almost 15min to the lab temperature so that the inoculum is well absorbed in the Agar. Turnover and incubate one of the two dishes at 37°C aerobic condition and the other one in micro aerobic condition during 24 to 48 H. All small colonies of 1.5-2mm of diameter, coloured in green with grey reflections surrounded by a black halo have been considered for confirmation.

Confirmation test

It has been completed with the previously suspicious considered colonies. They were purified on the agar with Soja tryptone and Yeast extract (TSYEA). For the purifying process five suspicious colonies were selected on each one of considered Petri dishes. Each colony was seeded on the agar in order to obtain well isolated colonies. The seeded dishes were

incubated at 37°C during 18 to 24H until a satisfying growth. Only the typic colonies which were convex, uncoloured, translucent and smooth were retained for confirmation tests. The confirmation tests that were used are catalase reaction, Gram stain and mobility test.

Catalase reaction

Put on a slide a drop of peroxide water. Collect and mix up a portion of isolated colony on TSYEA agar. Effervescence appearance indicates a positive reaction.

Gram staining

Collect a part of isolated colony on TYSEA agar. Spread the colony on a slide inside of a drop of sterile distilled water. Let dry and set the smear by passing the back of the slide thrice (3 times) through the flame of Bunsen beak. Cover the slide with violet gentian during 1min. Rinse with water. Cover the slide with lugol solution during 1min. Throw up lugol solution and rinse with water. Cover the slide with 95°C alcohol solution during 30-60 seconds; Rinse abundantly with water. Cover the slide with 1/10 Ziehl fuchsin solution during 20seconds; Rinse the slide with water and dry it; Look at ordinary microscope at immersion objective. The bacteria named *Listeria spp*. are little and thin Gram positive bacillus.

Mobility test

Collect an isolated colony on the TSYEA agar. Dissolve inside a tube containing TSYEA in solution. Incubate at 25°C during 8h to 24h until turbidity is noticed. Put a drop of the previous culture on a clean slide with a platinum loop; Observe on a microscope between slide and lamellae at X40 objective. Listeria is a bacillus animated of pirouette mobility.

Statistical analysis

Data obtained were analysed in accordance to Student t test ($p<0.05$). The Software such as Microsoft Excel and XL Stata were used.

Results and Discussion

Colonies obtained on PALCAM Agar are small-sized, coloured in green with grey reflections surrounded by a black halo. Colonies obtained on OXFORD Agar are small-sized, coloured in grey surrounded by a black halo. After Gram stain, it's observed on stained smears some Gram+ bacillus, coccids, isolated or assembled in short chains (Figure.1&2). Among the 10 samples of the southern region, 5 (50%) were contaminated by Listeria. In the northern region, it has been noticed a contamination rate of 25% (4 positive samples out of 20). pH vary between 6.72 and 6.82. There is no significant difference between averages which have the same letter ($p>0.05$) (Table 1).

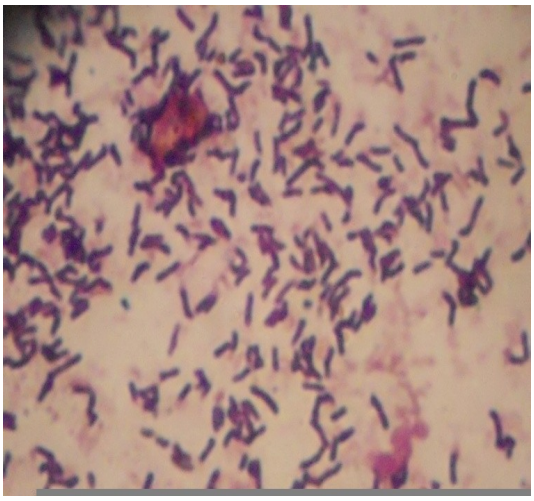

Figure.1 Smear from the reference of *Listeria spp*.

Table.1 pH Variation according to Listeria presence and milk source

Research results	Average Value of pH	Source
Positive	6.76±0.02a	Cotonou
Positive	6.82±0.04a	Parakou
Negative	6.72±0.02a	Cotonou and Parakou

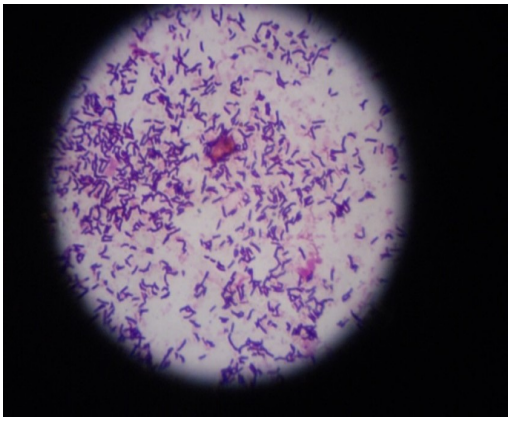

Figure.2 Smear from an isolated strains of a milk samples

New-Interesting microorganisms in Benin, because of its cultural characteristic, Listeria , it has been detected in 9 samples out of the 30 tested with a significant high percentage (30%) compared with the usually signalized prevalence in literature (Monk *et al*., 1994; Jay, 1996 ; Larpent, 2000 ; Lunden *et al*., 2004). Data collected indicate that the prevalence of this germ in European countries such as Netherland (4.4 %), England (3.6%) and Sweden (1%) remain very low compared with those found after this first work on milk samples collected in two great ecological region of Benin, Atlantique (Zongo and Abattoir) and Parakou.

Among the important remarks related to the culture of this microorganism, is the pH in the tested samples is important. Average values calculated on the nine samples containing *Listeria spp*. added to those which don't contain it are (in this order) for Zongo samples (X_{moy} =6.76), for Parakou's (X_{moy}=6.82) samples free from contamination (X_{moy}=6.72). These various pH were in the optimal zone of favouring growth pH of *Listeria spp.* (4.6 – 9.6). Therefore the milk samples pH doesn't affect its contamination by Listeria. The cultural characteristics of this microorganism remain in accordance with those described in literature (Lopes, 1986; Giovannacci, 1999). Isolated colonies shows the same aspects as those described by AFSSA, 2006 and smears that were made with isolated colonies and on which respiratory enzymes tests such as catalase, and mobility were made are similar to those described by Todar, (2009).

If current techniques of PCR (Polymerase Chain Reaction), sequencing and NMR (Nuclear Magnetic Resonance) were not used during these works, the first detection and insulation test of Listeria in raw milk samples in Benin pound the alarm of the risks of forborne illnesses related to the consumption of this food. *L. monocytogenes* wasn't systematically isolated and identified. However, Listeria spp. detection fears a possible presence of pathogens in raw milk and consumed milk products in Benin. Therefore, their isolation and identification should

continue. Difficulties during these works sum up to the low availability of material and reactive needed for a complete identification of isolated strains of Listeria, especially precise identification tests and specific molecular categorizing techniques for more complete identifications.

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